An analysis of ototoxicity in children:
Audiological detection, clinical practice
and genetic susceptibility

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Submitted for the degree of Doctor of Philosophy

University College London (UCL)

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2014
Declaration:

‘I, Ghada Al-Malky, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis. I, the candidate, designed all experiments with assistance from my supervisors. As part of MSc projects that were supervised by the candidate, the following students collected the data for some of the studies reported in this work: Suparna Bali (Theme A: Control Study), Anne Abiodun (Theme C: Study 1) and Miranda De Jongh & Mirijam Kikic (Theme C: Study 2). The remaining testing and all analyses were conducted by the candidate.’
Abstract:

Ototoxicity is the damage to the ear from exposure to medications. The inner ear is the commonest site of damage where cochlear and/or vestibular functions are affected. Ototoxic medications can cause irreversible toxicity, with aminoglycosides (AGs) and cisplatin being the most established agents. A series of studies are reported in this research under three main themes. Theme A focused on audiological assessments and assessment tools; Theme B focused on causation; and Theme C focused on the impact of ototoxicity and current service provision. The main Theme A study was a clinical observational study with a cross-sectional design assessing the auditory status of children with cystic fibrosis (CF) exposed to AGs. Theme B investigated potential risk factors and aspects in genetics that may be associated with increasing patient susceptibility to the ototoxic effect of AGs. Theme C assessed the effect of ototoxicity on the quality of life (QoL) of children surviving cancer. It also included a survey of current UK practice regarding auditory monitoring for ototoxicity.

The novel outcomes of these studies included showing that the prevalence of AG ototoxicity in children with CF is higher than previously reported and evaluating the efficacy of auditory assessment tools. They stressed the importance of choosing appropriate criteria to define ototoxicity and identified potential risk factors associated with it. The genetic studies highlighted a rare case of normal hearing in a child with the m.1555 A>G mutation despite exposure to AGs. They complemented the limited research on the impact of ototoxicity in children on their QoL and on current practice. The latter identified gaps in the provision of ototoxicity monitoring services in the UK, especially due to the absence of nationally agreed guidelines.

This research has generated recommendations for several future studies and has informed the clinical management of patients with CF.
Table of Contents

Chapter 1: **Introduction** .................................................................................................................. 20

1.1 **Overview** .................................................................................................................................. 20

1.2 **Ototoxicity** ............................................................................................................................... 20

1.2.1 Definition of Ototoxicity .............................................................................................................. 20

1.2.2 Overview of ototoxic agents ......................................................................................................... 20

1.2.3 Type of ototoxic agents ............................................................................................................... 21

1.2.4 Aminoglycoside antibiotics .......................................................................................................... 23

1.2.5 Ototoxic chemotherapeutic agents – Cisplatin ........................................................................... 28

1.2.6 Ototoxic effect of aminoglycosides and cisplatin on the inner ear: ........................................... 29

1.3 **Current practice in monitoring for toxicities** ........................................................................... 34

1.3.1 Overview of auditory monitoring for ototoxicity .......................................................................... 38

1.3.2 Criteria for detecting/grading ototoxicity ................................................................................... 40

1.4 **Patients with Cystic Fibrosis (CF) as a study population:** ......................................................... 44

1.4.1 Overview of CF as a disease .......................................................................................................... 45

1.4.2 Controversy in reported prevalence of AG ototoxicity ................................................................. 48

1.5 **Children with cancer as a study population** ............................................................................... 50

1.6 **Common tools used for measuring quality of life of patients** .................................................. 52

1.7 **Genetic susceptibility to AG ototoxicity** .................................................................................... 53

1.7.1 Mitochondrial DNA (mtDNA) ....................................................................................................... 53

1.7.2 mtDNA mutations and sensorineural hearing loss (SNHL) ......................................................... 54

1.7.3 A1555G mutation in the 12S rRNA gene and AG ototoxicity ..................................................... 56

1.7.4 Drug metabolizing genes and ototoxicity .................................................................................... 61

1.8 **Conclusion:** ............................................................................................................................... 65

Chapter 2: **Aims of the research** ....................................................................................................... 68

2.1 **Overview** ................................................................................................................................... 68

2.2 **Themes of the research project:** ............................................................................................... 68

2.2.1 Theme A: Audiological assessment and assessment tools ......................................................... 68

2.2.2 Theme B: Causation .................................................................................................................... 69

2.2.3 Theme C: Impact of ototoxicity and current service provision .................................................. 70

Chapter 3: **Material and Methods** ................................................................................................... 72

3.1 **Study populations** ...................................................................................................................... 72

3.2 **Materials:** .................................................................................................................................... 72
3.3 Methods:...................................................................................................................... 74

3.4 Theme A: Audiological assessments and assessment tools................................. 74
   3.4.1 Clinical observational study investigating aminoglycoside ototoxicity in patients with CF.................................................................................................................. 74
   3.4.2 A control study investigating the short-term repeatability of DPOAE recordings in school-aged normal hearing children................................................................. 80

3.5 Theme B: Causation .................................................................................................. 83
   3.5.1 Investigation to identify potential risk factors that can be associated with ototoxicity in CF children................................................................................................. 83
   3.5.2 Genetic studies investigating susceptibility to aminoglycoside ototoxicity in patients with CF.................................................................................................................. 84

3.6 Theme C: Impact of ototoxicity and Current service provision ......................... 98
   3.6.1 Investigating the effect of hearing loss on the quality of life of paediatric cancer survivors receiving ototoxic chemotherapeutic agents................................................. 98
   3.6.2 Survey of Oncology, Audiology and CF services in the UK to assess current practice of auditory monitoring for ototoxicity................................................................. 102

3.7 Statistical Analyses of studies included within all three themes: ....................... 105
   3.7.1 Theme A: Audiological assessments and tools- Statistical analysis.............. 105
   3.7.2 Theme B: Causation- Statistical analysis.............................................................. 106
   3.7.3 Theme C: Impact and current service provision- Statistical analysis.............. 108

Chapter 4: Results & Discussion of Theme A: Audiological assessment and assessment tools.......................................................................................................................... 110

4.1 Clinical observational study investigating AG ototoxicity in children with CF 110
   4.1.2 Discussion of the audiological observational study of CF children:............ 127
   4.1.3 Conclusions:........................................................................................................... 132

Assessment of DPOAE testing as an audiological tool - rationale behind the control studies:.................................................................................................................... 134
   o Significance of DPOAE as a monitoring tool for ototoxicity in children:......... 134
   o Pilot study on DPOAEs in school-aged children vs. adults:............................. 134
   o Generation of research questions:........................................................................ 135

4.2 A control study assessing the short-term test-retest repeatability of DPOAE recordings in normal hearing school-aged children................................. 136
   4.2.2 Discussion of DPOAE test-retest repeatability control study:.................. 144
   4.2.3 Conclusion:........................................................................................................... 146
Chapter 5:  **Results and Discussion of Theme B: Causation**

5.1  **Potential risk factors associated with AG ototoxicity in CF children**

5.1.2  Discussion of potential risk factors associated with AG ototoxicity in CF children

5.1.3  Conclusions:

5.1.4  Investigating susceptibility to AG ototoxicity in CF children through Genetic studies

5.2  **Analysis of mtDNA A1555G and other variations in the 12S rRNA gene in CF patients**

5.2.1  Outcomes of genotyping for the A1555G mutation:

5.2.2  Further analysis of the two children with the A1555G genotype:

5.2.3  Discussion of outcomes from investigating the A1555G mutation and sequencing the 12S rRNA gene

5.3  **Investigating the association between variants in the TPMT & COMT genes and AG ototoxicity**

5.3.1  Genotyping for the rs4646316 COMT SNP:

5.3.2  Genotyping for the rs12201199 TPMT SNP:

5.3.3  Statistical analysis of the results:

5.3.4  Overview for outcomes of genetic analysis of the children with ototoxicity

5.3.5  Discussion of the investigation of association between TPMT & COMT variants and AG-ototoxicity:

5.3.6  Conclusions from the genetic studies:

Chapter 6:  **Results & Discussion of Theme C: Impact of ototoxicity and current service provision**

6.1  **Investigating the effect of hearing loss on the quality of life of paediatric cancer survivors receiving ototoxic chemotherapeutic agents**

6.1.2  Discussion of the effects of ototoxicity of the quality of life on children:

6.1.3  Conclusion:

6.2  **Survey of UK Oncology, Audiology and CF services to assess current practice of auditory monitoring for ototoxicity**

6.2.2  Discussion of survey study of current UK service provision:

6.2.3  Conclusions:

Chapter 7:  **Summary and General Discussion**

7.1  Recap of research aims:
7.2 Summary of findings ..............................................................................................................228
7.3 General Discussion & Recommendations for future work and practical applications ..................................................231
  7.3.1 Significance of monitoring for ototoxicity in susceptible patient populations 231
  7.3.2 Recommendations for achieving an ideal ototoxicity monitoring protocol: 231
  7.3.3 Role of risk factors and genetic susceptibility in increasing the prevalence of ototoxicity ..................................................................................................................234
  7.3.4 Developing an audiological screening protocol using EHF screening PTA and portable DPOAE ...........................................................................................................237
  7.3.5 Investigating a possible otoprotective role of the CFTR gene mutation against ototoxicity ..................................................................................................................238
  7.3.6 Other recommendations for future work include: .........................................................239
7.4 Conclusion: ..............................................................................................................................239

Chapter 8: Bibliography .............................................................................................................240

Chapter 9: Appendices: ..............................................................................................................261
  9.1 Appendix 1: High frequency measurements made by Otodynamics to generate correction factors for the high frequency DPOAE recordings. ..............261
  9.2 Appendix 2: HUI3 Questionnaire ......................................................................................262
  9.3 Appendix 3: HUI Mark 3 (HUI3) Classification System ..................................................264
  9.4 Appendix 4: Paediatric Quality of Life Questionnaire .....................................................266
  9.5 Appendix 5: PAQL Items ....................................................................................................269
    o A.1. Communication and independence.............................................................................269
    o A.2. Emotional well-being ...............................................................................................269
    o A.3. Peer comparisons ......................................................................................................269
    o A.4. Acceptance by peers ...............................................................................................269
  9.6 Appendix 6: Online survey questionnaire for UK Audiologists ..................270
  9.7 Appendix 7: Online survey questionnaire for UK Oncologists .......................275
  9.8 Appendix 8: Online survey questionnaire for UK CF Clinicians ..................282
  9.9 Appendix 9: Plan for ototoxicity monitoring pathway developed for CF children at GOSH .................................................................284
  9.10 Appendix 10: GOSH Ototoxicity referral form ..............................................................285
  9.11 Appendix 11: GOSH Ototoxicity post-assessment feedback report .................286
9.12 Appendix 12: Ototoxicity monitoring protocol prepared for the CF patients at GOSH

9.13 Appendix 13: Multivariate analysis for potential risk factors related to Theme B study 1:

List of Figures:

FIGURE 1-1: SCHEMATIC REPRESENTATION OF THE TONOTOPIC ORGANIZATION OF THE COCHLEA. .......... 29
FIGURE 1-2: SCANING ELECTRON MICROSCOPY (SEM) PICTURES OF THE SURFACE OF A RAT COCHLEA
DEMONSTRATING DIFFERENT LEVELS OF INNER EAR HAIR CELL DAMAGE FOLLOWING EXPOSURE TO
OTOTOXIC DRUGS .................................................................................................................. 30
FIGURE 1-3: SHOWING THE PERCENTAGE HAIR CELL SURVIVAL ALONG THE LENGTH OF THE BASILAR MEMBRANE
FOLLOWING EXPOSURE TO AMIKACIN ............................................................................. 31
FIGURE 1-4: INTRACELLULAR MECHANISMS OF AMINOGLYCOSIDE AND CISPLATIN-INDUCED CELL DEATH .... 32
FIGURE 1-5: ILLUSTRATION OF DRUG-INDUCED HAIR CELL INJURY AND DEATH AT THE CELLULAR LEVEL ....... 33
FIGURE 1-6: ILLUSTRATION OF THE CHEMICAL REACTIONS OCCURRING WITHIN THE MITOCHONDRIUM WHERE
INITIATION OF APOPTOSIS OCCURS. (FROM (WALLACE, 2005b)) ........................................... 33
FIGURE 1-7: THE ASHA RECOMMENDED OTOTOXICITY MONITORING PROTOCOL FOR ONCOLOGY PATIENTS
(FROM (ASHA, 2013)) ........................................................................................................... 37
FIGURE 1-8: FREQUENCY REPRESENTATION AND APPROXIMATE INTENSITIES OF DIFFERENT CATEGORIES OF
SPEECH SOUNDS .................................................................................................................. 51
FIGURE 1-9: AN ILLUSTRATED DIAGRAM OF THE MTDNA ..................................................................... 54
FIGURE 1-10: THE DISORDER ASSOCIATION WITH DIFFERENT PARTS OF THE MITOCHONDRIAL GENOME .... 55
FIGURE 1-11: MECHANISM THAT IS PROPOSED FOR ENHANCED SENSITIVITY OF THE COCHLEA TO
AMINOGLYCOSIDES ........................................................................................................... 60
FIGURE 3-1: A GRAPHIC REPRESENTATION OF A TYPICAL Audiogram WITH AREAS SHADED TO ILLUSTRATE THE
different Brock’s grades ........................................................................................................ 79
FIGURE 3-2: A SCREEN SHOT OF THE INFORMATION DISPLAYED BY THE NANODROP TEST ................. 88
FIGURE 3-3: SCATTER PLOT OF THE NANODROP YIELD OF THE EXTRACTED DNA ................................. 89
FIGURE 3-4: PROMEGA 100 BP DNA LADDER ................................................................................. 90
FIGURE 4-1: HISTOGRAM SHOWING THE DISTRIBUTION OF CASES ACCORDING TO THE NUMBER OF AG lv
COURSES TAKEN OVER THEIR LIFETIME ........................................................................... 111
FIGURE 4-2: FREQUENCY PLOTS OF THE NUMBER OF CASES (CHILDREN) DISPLAYING THE DIFFERENT THRESHOLD
INTENSITIES AT A RANGE OF FREQUENCIES RECORDED USING STANDARD AND EHF PURE-TONE
AUDIOMETRY .......................................................................................................................... 113
FIGURE 4-3: LINE GRAPH SHOWING MEAN ±SD THRESHOLDS OF AUDIOMETRIC RESULTS OF THE RIGHT AND LEFT
EARS OF THE OTOTOXIC VS. NON-OTOTOXIC GROUPS ....................................................... 114
FIGURE 4-4: AUDIOMETRIC DATA (STANDARD AND EHF) OF FOUR CHILDREN WITH BROCK’S GRADING AND
LEVEL OF AG EXPOSURE STATED AS EXAMPLES TO DEMONSTRATE THE NON-LINEAR RELATIONSHIP
BETWEEN THESE FACTORS .................................................................................................. 117
FIGURE 4-5: THE DISTRIBUTION OF IDENTIFIED NON-OTOTOXIC VS. OTOTOXIC GROUP OF CHILDREN USING BOTH
STANDARD AND EHF AUDIOMETRIC DATA PRESENTED ACCORDING TO THEIR LIFETIME EXPOSURE TO LV AG
courses .................................................................................................................................... 118
Figure 4-6: DP-Gram showing DPOAE mean amplitudes for right and left ears of the two audiometrically defined groups (ototoxic vs. non-ototoxic) and the corresponding noise floor recordings. .................................................................................................................. 120
Figure 4-7: Showing the SNR scores of the audiometric non-ototoxic vs. ototoxic group for the RT and LT ears respectively. .................................................................................................................. 121
Figure 4-8: Distribution of the recruited children according to age (years). .................................................................................................................. 136
Figure 4-9: Mean ±SE DPOAE amplitudes for each f2 frequency for each of the repeated three recordings. .................................................................................................................. 138
Figure 4-10: Mean ±SD of the three repeated recordings of DPOAE amplitudes and its associated noise floor recordings. .................................................................................................................. 139
Figure 4-11: Mean ±SD of the signal-to-noise ratios (SNR) of the 3 repeated DPOAE recordings as a function of the f2 frequencies. .................................................................................................................. 139
Figure 4-12: Scatter plot with trend line showing a positive weaker correlation (R²=0.30) between the 1st and 2nd repeat DPOAE recordings at f2 frequency 0.8 kHz. .................................................................................................................. 142
Figure 4-13: Scatter plot with trend line showing a positive strong correlation (R²=0.77) between the 1st and 2nd repeat DPOAE recordings at f2 frequency 5 kHz. .................................................................................................................. 142
Figure 5-1: Comparison between the number of courses of IV AG received by each of the children with CF and average FEV₁ % predicted score. .................................................................................................................. 150
Figure 5-2: RFLP analysis of the 125 RNA A1555G mutation using HaeIII restriction enzyme. .................................................................................................................. 159
Figure 5-3: Outcomes of audiological and genetic assessments for both child A and child B. .................................................................................................................. 162
Figure 5-4: UV image of 2% Agarose gel electrophoresis of the PCR-amplified COMT gene section containing the rs4646316 SNP. .................................................................................................................. 170
Figure 5-5: UV image of 2% Agarose gel electrophoresis of PCR-RFLP analysis of COMT rs4646316 using XcmI restriction enzyme for 13 samples. .................................................................................................................. 170
Figure 5-6: UV image of 2% Agarose gel electrophoresis of PCR-RFLP analysis of TPMT rs12201199 using MNLI restriction enzyme for 14 samples of CF children (Wells 1-14). .................................................................................................................. 172
Figure 5-7: DNA sequencing chromatogram of TPMT rs12201199 SNP. .................................................................................................................. 172
Figure 5-8: Comparison between the TPMT rs12201199 allele frequencies of the 1958 cohort study population representing good and poor hearing and the study CF group with and without ototoxicity (as defined by standard & EH FPTA outcomes). .................................................................................................................. 177
Figure 6-1: Shows mean with 95% CI error bars for the HUI3 single attribute responses for each of the two normal hearing and ototoxic oncology groups. .................................................................................................................. 192
Figure 6-2: A Boxplot showing the multi-attribute utility scores of HUI3 for both the normal hearing and ototoxicity group of cancer children. .................................................................................................................. 193
Figure 6-3: A Boxplot showing the distribution of scores for all four subscales of the PAQL for each of the two groups of children. .................................................................................................................. 195
Figure 6-4: Regional distribution of respondents from the three professions. .................................................................................................................. 203
Figure 6-5: Distribution of responses of the three professions confirming whether they do or do not monitor their patients’ hearing for signs of o totoxicity. .................................................................................................................. 207
Figure 6-6: Percentage of respondents confirming if other forms of monitoring for toxicities is carried out: ................................................................................................................................. 208

Figure 6-7: Response of Audiology (n=85) and Oncology (n=39) respondents to: “What are the criteria used for referring patients for auditory monitoring”: ................................................................. 209

Figure 6-8: Response of CF respondents (n=23) to: “What are the criteria used for referring patients for auditory monitoring?” ........................................................................................................... 210

Figure 6-9: Professional opinions from audiologists and oncologists in regards to when patients should be referred for monitoring in the case of absence of referral criteria ........................................................................... 210

Figure 6-10: Distribution of responses to the question: “Is there a protocol used to identify when changes in auditory status become clinically significant?” ............................................................................ 215

Figure 6-11: Distribution of respondents’ comments to the question: “What changes in audiological results should prompt consideration or an actual change in medical management?” ................................................................................................. 216

Figure 6-12: Distribution of oncology respondents’ response to question: ‘What changes would be made if auditory monitoring shows evidence of ototoxicity?’ ......................................................................................... 217

Figure 6-13: Distribution of CF clinicians’ comments to the question: “What changes do you implement to your medical management if auditory monitoring shows evidence of ototoxicity?” ................................................................................. 217
List of Tables:

Table 1-1: ASHA 1994 criteria for a significant threshold shift due to ototoxicity from (ASHA, 1994a) ................................................................. 42
Table 1-2: Brock’s grading criteria for ototoxicity from (Brock et al., 1991) .................. 42
Table 1-3: Chang’s grading criteria for ototoxicity from (Chang and Chinorsornvatana, 2010) 42
Table 1-4: The recently proposed and agreed SIOP Boston Ototoxicity Scale from (Brock et al., 2012a) ................................................................................................. 42
Table 1-5: CTCAE criteria for hearing impairment for ototoxicity ................................. 43
Table 1-6: Different classes of cystic fibrosis transmembrane conductance regulator (CFTR) mutations ......................................................................................... 46
Table 1-7: Recommended dosages for antibacterial agents in the management of P. aeruginosa lung infections in CF patients ................................................................. 48
Table 3-1: Primers used for PCR amplification of the DNA segments containing the different target genes or mutations. ................................................................. 90
Table 3-2: Summary of the expected outcomes of RFLP using HaeIII restriction enzyme on the 12S rRNA (MTRNR1) segment of the mtDNA ........................................ 94
Table 3-3: Describing action of the restriction enzyme MnlI used for digestion of the TPMT amplicon and its cutting positions for both ‘A’ or ‘T’ alleles .......................... 95
Table 3-4: Describing action of the restriction enzyme XcmI used for digestion of the COMT amplicon and its cutting positions for both ‘C’ or ‘T’ alleles ..................... 95
Table 3-5: Formula used for preparation of 10 µL/sample reaction mix for real-time PCR using a 96-well plate ......................................................................................... 97
Table 3-6: The thermal cycles conditions used for real-time PCR .................................. 97
Table 3-7: Summary of the structure of the three surveys ............................................. 103
Table 3-8: A priori sample size calculation performed to assess the number of subjects needed to detect an effect of ototoxicity ......................................................... 106
Table 4-1: Characteristics and audiological results of the fifteen children with ototoxicity with comparison of diagnosis based on different assessment tools ............. 121
Table 4-2: A 2x2 contingency table of outcomes using Standard PTA vs. EHF Audiology .... 122
Table 4-3: A 2x2 contingency table of outcomes using DPOAEs vs. EHF Audiology .......... 123
Table 4-4: A 2x2 contingency table of outcomes using DPOAEs vs. Standard Audiology ........ 123
Table 4-5: Correlation between the audiometric thresholds and DPOAE amplitudes at frequency recordings that showed significant changes in outcomes indicating ototoxicity ................................. 124
Table 4-6: Detailed bivariate Correlation analysis with bootstrap corrections between the higher PTA and DPOAE frequencies that previously showed significant differences between the two groups (non-ototoxic vs. ototoxic group of children) ............. 126
Table 4-7: Reliability statistics showing a strong correlation between all 33 tested items (11 DPOAE F2 frequencies, 3 repeats) ................................................................. 140
Table 4-8: ANOVA statistic assessing the significance of the ICC calculations performed.  
Table 4-9: ICC calculations showing the strength of the overall correlations between the repeated recordings of the DPOAE f2 frequencies as single measures (as shown below) and with all the measures averaged together.  
Table 4-10: Outcome of interclass correlation coefficient (ICC) analysis assessing the correlation between the three repeated DPOAE recordings as a function of each f2 frequency tested- corrections for biasing from multiple correlations was performed using the simple bootstrap method.  
Table 4-11: Showing the calculated interclass correlation coefficient (ICC), standard error of measurement (SEM) and minimum detectable difference at 95% probability (MDD 95%) for the repeated measurements at each of the DPOAE f2 frequencies recorded.  
Table 5-1: Assessment of potential risk factors associated with ototoxicity.  
Table 5-2: Contingency table showing a record of patient numbers with and without the A1555G mutation in the two groups (ototoxicity was defined by the audiometric data).  
Table 5-3: Contingency table showing a record of patient numbers with and without the A1555G mutation in the groups defined by the audiometric outcomes when more samples were genotyped.  
Table 5-4: Characteristic features of the two children with CF who had A1555G mutation and history of exposure to AGs.  
Table 5-5: Chi-square analysis showing lack of association between SNPs in TPMT & COMT and ototoxicity.  
Table 5-6: TPMT association analysis for alleles and genotypes under different genetic models.  
Table 5-7: COMT rs4646316 association analysis for alleles and genotypes under different genetic models.  
Table 5-8: Combined effect of TPMT and COMT genotypes on AG-induced ototoxicity.  
Table 5-9: Genotypes for the British 1958 birth cohort samples.  
Table 5-10: Genetic characteristics of the children with ototoxicity.  
Table 6-1: Description of the audiological data retrieved from the records of oncology patients (N=219) assessed in the ototoxicity clinic.  
Table 6-2: Summary of types and distribution of tumours affecting the children.  
Table 6-3: Summary of descriptive and audiological data of all recruited children.  
Table 6-4: Number of children in each group with suboptimal levels of function in each attribute of the HUI3.  
Table 6-5: Descriptive data of the multi-attribute HUI3 utilities scores for both groups (including the outlier).  
Table 6-6: The distribution and statistical analysis of the four subscales and total scores of the PAQL between the two normal hearing and ototoxic groups of cancer children.  
Table 6-7: Clinical and program characteristics of respondents.
TABLE 6-8: ONCOLOGY RESPONDENTS’ ESTIMATION OF THEIR PATIENTS’ EXPOSURE AND MONITORING TO OTOTOXIC CHEMO AND RADIOTHERAPY. .......................................................... 205

TABLE 6-9: CF CLINICIAN RESPONDENTS’ ESTIMATION OF THEIR PATIENTS’ EXPOSURE AND MONITORING TO OTOTOXIC AMINOGLYCOSIDES. ..................................................................... 205

TABLE 6-10: RESPONSES FROM AUDIOLOGY AND ONCOLOGY RESPONDENTS THAT DO PERFORM AUDITORY MONITORING REGARDING THE MONITORING SET-UP. .................................................... 213

TABLE 6-11: DISTRIBUTION OF RESPONSES TO “WHAT AUDIOLOGICAL TESTING IS CONDUCTED FOR OTOTOXICITY MONITORING?” ................................................................. 214

TABLE 5-9-1: MULTIVARIABLE LINEAR MIXED MODELS ANALYSIS APPLIED FOR EACH OF THE AUDIOMETRY AND DPOAE TESTING METHODS IN RELATION TO EXPLORED RISK FACTORS. ........................... 295
### Abbreviations:

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AG</td>
<td>Aminoglycoside</td>
</tr>
<tr>
<td>ART</td>
<td>Acoustic reflex threshold</td>
</tr>
<tr>
<td>A1555G</td>
<td>mtDNA mutation in the 12S rRNA gene at bp position 1555</td>
</tr>
<tr>
<td>bp</td>
<td>Base pair</td>
</tr>
<tr>
<td>CF</td>
<td>Cystic Fibrosis</td>
</tr>
<tr>
<td>CTCAE</td>
<td>Common Terminology Criteria for Adverse Events</td>
</tr>
<tr>
<td>ddH₂O</td>
<td>Milli Q distilled water</td>
</tr>
<tr>
<td>DPOAE</td>
<td>Distortion-product otoacoustic emissions</td>
</tr>
<tr>
<td>dNTPs</td>
<td>Dideoxynucleotide triphosphates</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediamine tera-acetic acid</td>
</tr>
<tr>
<td>EHF</td>
<td>Extended high frequency (audiometry)</td>
</tr>
<tr>
<td>GOSH</td>
<td>Great Ormond Street Hospital</td>
</tr>
<tr>
<td>ICH</td>
<td>Institute of Child Health</td>
</tr>
<tr>
<td>i.v/IV</td>
<td>Intravenous</td>
</tr>
<tr>
<td>LD</td>
<td>Linkage disequilibrium</td>
</tr>
<tr>
<td>mtDNA</td>
<td>Mitochondrial DNA</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>PTA</td>
<td>Pure-tone Audiometry</td>
</tr>
<tr>
<td>rpm</td>
<td>Revolutions per minute</td>
</tr>
<tr>
<td>rRNA</td>
<td>Ribosomal RNA</td>
</tr>
<tr>
<td>TAE</td>
<td>Tris-acetate-EDTA electrophoresis buffer</td>
</tr>
<tr>
<td>TDM</td>
<td>Therapeutic Drug Monitoring</td>
</tr>
<tr>
<td>TEOAE</td>
<td>Transient-evoked otoacoustic emission</td>
</tr>
<tr>
<td>SNHL</td>
<td>Sensorineural hearing loss</td>
</tr>
<tr>
<td>SNP</td>
<td>Single nucleotide polymorphism</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>95% CI</td>
<td>95% Confidence Interval</td>
</tr>
</tbody>
</table>
Acknowledgements:

I would like to first deeply thank my supervisors Prof. David Kemp, Dr. Sally Dawson and Dr. Ranjan Suri. I have been privileged and honoured to have them all for my supervisors. Prof. Kemp has always been an icon for me since I joined Audiology and worked on my Masters degree on Distortion product otoacoustic emissions in Egypt many years ago. Meeting him in person and working with him has increased my respect and admiration for him a million times over and I want to thank him for setting the bar so high for what a true academic should be. I can never thank Dr. Dawson enough for all her support, teaching and endless patience. Sally you have allowed me to join your lab and taught me so much and I apologise for any frustration I’ve caused you along the way. I really have so much respect for you as an academic and researcher and look forward to continuing work with you. It was an absolute pleasure working with Dr. Suri. Ranjan you are a great clinician with an open mind and a drive to implement change. I can’t thank you enough for all your support at Great Ormond Street Hospital (GOSH) and look forward to continue our collaboration in future research.

I would like to thank Prof. David McAlpine, Director of the Ear Institute, for his continuous support to allow me the time to undertake this research. I would like to thank Dr. Tony Sirimanna for all his support in allowing me to work within the Audiology department at GOSH. Tony you are an excellent clinician and a huge asset for Audiovestibular Medicine in the UK and internationally. I would like to thank all the staff members of the Audiology department; especially John Veness, Melanie, Kalpana and Brindha and members of CF unit at GOSH including the Badger ward nurses and phlebotomists; especially Ammani, Den, Charlie, Amy and Ema for their help. I would also like to thank Dr Kaukab Rajput and Dr Penelope Brock for their support with the work related to ototoxicity in the children with cancer. I am very grateful to Dr. Brad Backus for all his help during the control study. I am very grateful to my MSc students Suparna Bali, Anne Abiodun, Miranda De Jongh and Mirijam Kikic for collecting some of the data included in this research.

Last but not least, I want to thank my family. I want to thank my mother and father for always believing in me, my husband for always being my rock and my sole mate, my beautiful children who are my pride and joy, my wonderful sister Rania for proof reading some of my work and the rest of my wonderful brothers and sisters for their continuous encouragement.
Dedication:

This thesis is dedicated to my mother, husband and children
Publications & conference presentations:


3. **The dichotomy in hearing of cystic fibrosis (CF) children following high exposure to aminoglycosides: A study using DPOAEs and extended high-frequency audiometry.** 33rd Midwinter ARO meeting, Anaheim, USA; February 2010 - abstract published in the ARO 2010 abstract book and available online at www.aro.org.

4. **Aminoglycoside antibiotics cochleotoxicity in paediatric cystic fibrosis (CF) patients: A study using extended high-frequency audiometry and distortion product otoacoustic emissions.** Author(s): Al-Malky, G; Suri, R; Dawson, SJ, Sirimanna T; Kemp D Source: International Journal of Audiology Volume: 50 Issue: 2 Pages: 112-122 Published: 2011

5. **Investigation into the Role of Variation in the 12S rRNA, TPMT and COMT Genes to the Incidence of Aminoglycoside Ototoxicity in Children with Cystic Fibrosis (CF) - 35th Midwinter ARO meeting, San Diego, USA; February 2012 - abstract published in the ARO 2012 abstract book and available online at www.aro.org.

6. **High-frequency audiometry reveals high prevalence of aminoglycoside ototoxicity in cystic fibrosis children.** Author(s): Al-Malky G; Dawson, SJ, Sirimanna T; Suri, R. Journal of Cystic Fibrosis (under review)

7. **Assessing the incidence of variations in the 12S rRNA, TPMT and COMT genes in association with aminoglycoside ototoxicity in children with Cystic Fibrosis – 3rd BSA Annual Conference and Experimental Short Papers meeting, Nottingham, UK; 5-9th September 2012 – Abstract published in BSA conference proceedings booklet and in International Journal of Audiology (IJA; Vol. 52 (4), April 2013, pp: 301).

8. **Normal hearing in a child with the A1555G mutation despite repeated exposure to aminoglycosides. Has the penetrance of this pharmacogenetic interaction been overestimated?** Al-Malky G, Suri, R., Sirimanna T; Dawson, SJ. Int. J. Pediatr. Otorhinolaryngol. (2014), http://dx.doi.org/10.1016/j.ijporl.2014.02.015


*Copies of all these publications are available at the end of the thesis under Appendix 14: section 9.14*
Chapter 1: **Introduction**

### 1.1 Overview

Ototoxicity is damage to the ear following exposure to drugs or chemicals. There are hundreds of drugs and chemicals that can cause either reversible or irreversible damage, with some established groups such as the aminoglycoside antibiotics and chemotherapeutic cisplatin. Despite their well-known ototoxic effects there are still many research questions that have not been addressed enough, such as why there is a significant variation in the reported ototoxic effect of these drugs, what are the most appropriate auditory monitoring tools that should be used for early detection of this effect and what genetic mutations or variations affect patients’ susceptibility to this side effect.

This research project will aim to address some of these questions through a series of clinical audiological and laboratory-based genetics studies to further our understanding of this important field and translate these findings into clinical practice to improve patient care and enhance their quality of life.

### 1.2 Ototoxicity

#### 1.2.1 Definition of Ototoxicity

Ototoxicity ("ear-poisoning") is damage to the hearing or balance functions of the ear by drugs or chemicals. The most commonly affected parts of the ear are the cochlea with the associated vestibulo-cochlear nerve and the vestibular apparatus in the inner ear.

Ototoxicity can result in temporary or permanent disturbances of hearing, balance, or both. It is one of the main preventable causes of deafness and one that is most directly influenced by managing clinicians that make vital decisions regarding what, when, how much and how often these medications are prescribed. Many chemicals have ototoxic potential, including over-the-counter drugs, prescription medications and environmental chemicals.

#### 1.2.2 Overview of ototoxic agents

Generally, there are more than 200 medications and chemicals known to cause hearing and balance problems. It has even been reported that at least 743 drugs, 30 herbs and 148
chemicals include known ototoxic substances (Bauman, 2003). The main classes of drugs and chemicals that have been implicated as having ototoxic potential include aminoglycoside antibiotics, chemotherapeutic agents such as cisplatin, non-steroidal anti-inflammatory drugs (NSAID) like aspirin, antimalarial drugs and diuretics. Some of these drugs mainly affect the cochlear function leading to hearing loss and tinnitus and are therefore classified as cochleotoxic drugs; others mainly affect the vestibular function leading to oscillopsia, imbalance or even vertigo and are classified as vestibulotoxic drugs. Some drugs may affect both functions. Ototoxic drugs can also be classified according to whether they cause a reversible or irreversible permanent damage to the ear. Drugs known to have a reversible ototoxic effect may only induce a temporary threshold shift with return to normal thresholds following stoppage of intake. These include salicylates and loop diuretics. Aminoglycosides and cisplatin mainly produce irreversible permanent damage and continue to have an ototoxic effect even after weeks and months of intake (Campbell, 2007).

Cochleotoxicity can manifest as hearing loss in one or both ears, range from a mild to a severe-profound impairment, and have temporary or long-lasting effects. Regardless of the type or severity, drug-induced hearing loss can have devastating effects on communication and can lead to difficulties in educational, vocational, and social settings for both children and adults (ASHA, 1994b). It also represents one of the main preventable causes of deafness, in the sense that it is an outcome that can perhaps be most directly influenced by healthcare professionals (Yorgason et al., 2006).

1.2.3 Type of ototoxic agents

The most well established substances known to cause ototoxicity include:

**Aminoglycoside antibiotics:** include gentamicin, streptomycin, kanamycin, tobramycin, neomycin, amikacin, netilmicin, dihydrostreptomycin, and ribostamycin. All members of this family are known for their potential to cause permanent ototoxicity and nephrotoxicity. They may enter into the inner ear through the blood system or through inhalation by unknown mechanisms, which may include being secreted into the perilymph by the spiral ligament or endolymph by the stria vascularis (Forge and Schacht, 2000, Rybak and Ramkumar, 2007, Schacht et al., 2012, Wu et al., 2002). They may also diffuse from the middle ear through the round window membrane into the inner ear when administered topically for management of middle ear infections or specifically for chemical labyrinthectomy for treatment of unilateral Meniere’s disease (McCall et al., 2010, Perez et al., 2003). They enter the blood stream in largest amounts when given intravenously (i.v).
Aminoglycosides are excreted by the kidney. These are one of the drugs of interest in this research project.

Other possible ototoxic but much less established antibiotics include, macrolides like erythromycin possibly causing reversible hearing loss; vancomycin, which is effective against gram-positive infections such as *Staphylococcus aureus* and reported to be ototoxic and nephrotoxic but with limited evidence (Vella-Brincat et al., 2011, Forouzesh et al., 2009, Shields et al., 2009); and penicillin, sulphonamides and cephalosporins, which may be associated with minor topical ototoxicity when administered in large doses to the middle ear (Brown et al., 1989).

**Anti-neoplastic** (anti-cancer /chemotherapeutic drugs): include platinum compounds such as cisplatin and carboplatin, bleomycin and vincristine. Cisplatin is most known to cause hearing loss with incidence as high as 62%-81% and even up to 100% in some children (Knight et al., 2005, Knight et al., 2007). It has a similar histopathological mechanism of cochlear damage as aminoglycosides. Clinically, it is associated with bilateral high frequency symmetric SNHL, which is usually not reversible and cumulative in nature. Risk factors include age extremes, cranial irradiation, high dose or cumulative therapy and concomitant use with other ototoxic drugs. Carboplatin has been implicated as well but is reported to be less ototoxic so that ototoxicity is usually only associated with intake of large doses (Musial-Bright et al., 2011, Taudy et al., 1992).

**Loop diuretics:** include furosemide (Lasix), bumetanide (Bumex) and ethacrynic acid (Edecrin). They are examples of reversible ototoxic medications with a reported incidence at 6-7%. Clinically a common presentation includes tinnitus, temporary and reversible SNHL and rarely vertigo, possibly occurring within minutes of intake. High doses can cause irreversible SNHL. The highest risk is co-administration of aminoglycosides due to the associated synergistic effect (Bates et al., 2002, Hirose and Sato, 2011).

**Salicylates (Aspirin) and NSAIDs (non-steroidal anti-inflammatory drugs):** here the cochlea is histologically normal with no hair cell loss and is therefore another example of reversible ototoxic medications. The mechanism of ototoxicity is probably due to decreased blood flow and decreased enzymes. Clinically, the commonest presentation is tonal, high frequency (HF) tinnitus (7-9 kHz) with reversible mild to moderate HF-SNHL that is rarely permanent (Brien, 1993).

**Antimalarial quinine products:** these may cause temporary ototoxicity, particularly tinnitus, but may also reduce hearing. The mechanism, which is similar to salicylates, includes decreases in cochlear blood flow through vasoconstriction. Reversible changes within outer
hair cells also seem to play an important role. Also, one of quinine's main actions, which involve antagonism of calcium-dependent potassium channels, has not been confirmed for its possible role in ototoxicity (Jung et al., 1993, Koegel, 1985, Hennebert and Fernandez, 1959).

*Environmental chemicals*, including butyl nitrite, mercury, carbon disulphide, styrene, carbon monoxide, tin, hexane, toluene, lead, trichloroethylene, manganese, and xylene. Most are associated with hearing disturbances that may be permanent; mercury has also been linked to permanent balance problems in addition to evidence showing that it produces peripheral and/or central damage (Hoshino et al., 2012, Hoet and Lison, 2008, Fechter et al., 1998, Counter and Buchanan, 2002). Workplace exposure to some of these organic solvents, such as toluene, was shown to increase the risk of hearing impairment especially when associated with concomitant noise exposure where a synergistic interaction was confirmed (Lund and Kristiansen, 2008, Lataye and Campo, 1997, Johnson et al., 1990).

### 1.2.4 Aminoglycoside antibiotics

As these are one of the ototoxic agents of interest in this current research, aminoglycosides (AGs) will be further discussed in detail in the following sections. They are potent water-soluble antibiotics, with peak concentration-dependent bactericidal activity against many pathogenic aerobic gram-negative bacteria as *Pseudomonas aeruginosa* and including many multi-drug resistant bacilli, *mycobacterium tuberculosis* and to a lesser extent against gram-positive bacteria as *Staphylococcus aureus*. They have a bactericidal effect, i.e. they actually kill the bacteria not just weaken them. AGs probably have more than one mechanism of action on bacterial cells beginning at the plasma membrane followed by internalization and interference with several intracellular processes (Forge and Schacht, 2000). They bind to the 16S ribosomal RNA and the 30S ribosome leading to misreading of the RNA code and subsequent inhibition of essential protein synthesis and cell death (Aronson, 2006).

The common route of administration for systemic therapy is parenteral (intravenously (i.v) or intramuscularly (i.m)). If possible, they are given in localized form to decrease side effects, e.g. as topical ear dressing for treatment of otitis media or inhaled through nebulization for treatment of chest infections. The most significant side effects include trough concentration-dependent reversible nephrotoxicity and more commonly irreversible ototoxicity (Kumana and Yuen, 1994). Generally, ototoxicity is bilaterally symmetrical, but it may be asymmetrical, affecting the basal turn of the cochlea first then progressively extending towards the apex. This leads to appearance of high frequency sensorineural hearing loss (SNHL) first which gradually affects lower frequencies. The usual onset time of ototoxicity is often unpredictable, with reports that significant hearing loss can occur even after a single
dose. In addition, ototoxicity may not manifest until several weeks, months or years after completion and cessation of antibiotic therapy. This is because AGs are cleared more slowly from inner ear fluids than from serum, making continued monitoring for evidence of ototoxicity after cessation of treatment important. (Matz, 1993, Rizzi and Hirose, 2007, Schacht, 1998, Selimoglu, 2007, Waguespack and Ricci, 2005).

1.2.4.1 Types and Epidemiology:

Streptomycin was the first of the aminoglycosides to be discovered in the 1940s by Selman Waksman (Jones et al., 1944). It was a long-awaited cure for tuberculosis (TB) and became widely popular in the 1940s and 1950s and remains a first/second-line treatment choice for this condition to date, particularly in developing countries where the prevalence of TB has increased (Wu et al., 2002). Over the following years, new AGs were isolated or derivatives synthesized including amikacin, gentamicin and tobramycin, kanamycin, neomycin, netilmicin, and dihydrostreptomycin are currently available in most countries. The combination of high efficiency with low cost makes them still one of the most highly used antibiotics. This is especially still the case in developing countries where the prevalence exceeds the 20-25% reported for ototoxicity in developed countries. In certain countries such as China reports that 2/3 (66%) of all deaf-mutism was due to the administration of aminoglycosides to children, highlighting the impact of this condition on the general population (Forge and Schacht, 2000, Lu, 1987). In developing countries of sub-Saharan Africa, diseases such as TB and HIV/AIDS go hand-in-hand, which necessitates the intake of antiretroviral and TB medications simultaneously. It has been reported that HIV-positive multidrug-resistant (MDR) TB patients, requiring 2nd line ototoxic drugs such as streptomycin and kanamycin, who are also receiving highly active antiretroviral therapy (HAART) were more likely to present with ototoxic hearing loss than HIV-negative MDR-TB patients. In addition, treating HIV-associated opportunistic infections and malignancies with aminoglycosides, amphotericin B, and platinum-based antineoplastic drugs, such as cisplatin, increase the incidence of ototoxicity in these populations (Harris et al., 2012).

On the other hand, aminoglycosides’ prominent chronic side effects of nephrotoxicity and ototoxicity have led to a steep decline in their use in developed industrial countries since the 1970s and 80s. Here, prescription of these antibiotics has been restricted to certain population groups where they are most needed. These include children, even neonates, hospitalized within intensive care units (ICU) for serious infections like septicaemia caused by aerobic gram-negative bacteria; cystic fibrosis patients with chronic chest infections; multi-drug resistant TB patients; surgical prophylaxis during operations such as urological, vascular or cardiac surgery where only a single dose of gentamicin is given; empirical
therapy (pathogen unknown) for severe conditions such as intra-abdominal infections, acute cholecystitis, genito-urinary infections, infective endocarditis or hospital acquired pneumonia where gentamicin is always used in combination with other antibiotics for <48 hours; and directed therapy (pathogen known) for conditions such as prosthetic valve infective endocarditis, enteric organism bacteraemia and pseudomonas aeruginosa infections where different types of aminoglycosides are almost always used in combination with other antibiotic groups for prolonged durations (often weeks) (Avent et al., 2011).

1.2.4.2 Differences in ototoxicity between different types of AGs

The ototoxic effect of aminoglycosides occurs either in the form of vestibulotoxicity or cochleotoxicity depending on the type of aminoglycoside used. Gentamicin and streptomycin are known to be mainly vestibulotoxic with an element of cochleotoxicity; whereas others like tobramycin, amikacin, neomycin, dihydrostreptomycin and kanamycin are mainly cochleotoxic.

Damage to the vestibular organ presents with symptoms associated with chronic bilateral vestibular insufficiency i.e. ataxia and oscillopsia and possibly nystagmus, but not vertigo due to its common bilateral symmetrical affect. However, it can rarely cause unilateral damage even when administered systemically where in this case patients may complain of vertigo due to the asymmetrical nature of the damage (Selimoglu, 2007, Waterston and Halmagyi, 1998). Vestibulotoxicity can occur in up to 15% of patients following exposure to AGs (Fee, 1980). Whereas cochleotoxicity first presents as a high frequency sensorineural hearing loss (SNHL) due to the selective damage of outer hair cells (outer layers first) followed by inner hair cells of the basal coil of the cochlea, where tonotopically high frequency sounds are represented, before gradually extending towards the apex.

Several studies have been performed to rank AGs in order of their probability of auditory toxicity including animal (Parravicini et al., 1983, McCormick et al., 1985) and clinical studies (Lerner and Lorber, 1983, Rybak et al., 1999, Lerner et al., 1986). Comparisons of ototoxicity with antibacterial activity have shown that netilmicin is the least toxic and has the highest therapeutic index followed by tobramycin then by gentamicin. A clinical study showed that the average incidence of cochlear toxicity was 13.9% for amikacin, 8.3% for gentamicin, 6.1% for tobramycin, and 2.4% for netilmicin (Kahlmeter and Dahlager, 1984) whereas another study comparing amikacin and gentamicin indicated that amikacin is just as effective against severe infections caused by gram-negative bacteria as Pseudomonas auroginosa and is not significantly more or less ototoxic or nephrotoxic than gentamicin (Smith et al., 1977).
In general, gentamicin is usually considered the agent of choice for most conditions, tobramycin is shown to be slightly more potent against *Pseudomonas aeruginosa* infections, amikacin is the most resistant to break down by bacterial enzymes and therefore is more suitable for use in hospital settings where gentamicin resistance is high, and netilmicin is possibly the least toxic whereas neomycin is considered to be the most toxic (Kumana and Yuen, 1994).

### 1.2.4.3 Risk factors:

Identification of risk factors associated with occurrence of AG ototoxicity is one of the main aims of many research studies. Identifying these risk factors can prove most useful especially if they can be realistically avoided or stopped and consequently decrease the incidence of ototoxicity. Some of these findings are reported below.

Patient susceptibility to ototoxicity depends on a number of factors, such as route of administration (systemic vs. transtympanic perfusion), genotype and existing medical conditions (Guthrie, 2008). Factors related to the drug administration regimens such as intake of larger doses, higher blood levels, longer duration of therapy, or intake of cumulative courses with short intervals between courses that don’t allow for complete clearance of the drugs from the inner ear, are all associated with increased incidence of ototoxicity. Other high-risk patients include elderly patients especially if they have other inner ear disorders such as noise-induced hearing loss or presbyacusis, those with renal insufficiency as this impairs the excretion of the drugs and higher blood levels, those with pre-existing hearing problems, those with a family history of ototoxicity, and those receiving other ototoxic, e.g. loop diuretics, or nephrotoxic medications (Gatell et al., 1987, Moore et al., 1984). The remaining part of this section will present some of the literature that identified some of these risk factors.

The route of administration has been shown to affect the levels of ototoxicity. Transtympanic administration, commonly used for treatment of incapacitating vertigo in Meniere’s disease, manifests with a lower incidence and less severe cochleotoxicity than systemic parenteral administration. Bottrill et al. reported that only one patient developed a profound hearing loss from a total of 83 patients who received transtympanic gentamicin to treat incapacitating vertigo secondary to Meniere’s disease (Bottrill et al., 2003). Wu and Minor also reported one case of profound hearing loss in 34 patients treated in the same way for the same condition (Wu and Minor, 2003). Both authors concluded that the risk of transtympanic gentamicin is minimal and does not outweigh the benefit.
Many studies have suggested that certain individuals may be at a greater risk of developing hearing loss due to the existence of genetic mutations. Molecular studies revealed a mitochondrial DNA (mtDNA) 1555A>G point mutation in the 12S ribosomal RNA gene to be significantly associated with increased susceptibility to aminoglycoside ototoxicity and non-syndromic sensorineural hearing loss. A single parenteral injection of aminoglycosides could lead to a severe-profound hearing loss in subjects with this mutation (Stergaard et al., 2002, Estivill et al., 1998, Gallo-Teran et al., 2004, Kokotas et al., 2009, Shohat et al., 1999). More studies related to genetic susceptibility to aminoglycosides will be discussed in more detail later.

Pre-existing medical conditions are also associated with increased incidence of ototoxicity. Deutsch et al. reported an incidence of hearing loss of 12% in 125 liver transplant children taking aminoglycosides (Deutsch et al., 1998) and Naunton and Ward reported an association between renal dysfunction and kanamycin ototoxicity (Naunton and Ward, 1959). Li and Steyger reported that noise exposure had a synergistic effect even when there is no simultaneous exposure to aminoglycosides (Li and Steyger, 2009). They reported that prior acoustic insult, which does not result in permanent threshold shifts, potentiates aminoglycoside ototoxicity and conversely, exposure to sub-damaging doses of aminoglycosides aggravates noise-induced cochlear damage so that the damage caused is more than the damage caused by each of them individually.

Gunther et al. (1988) reported that treatment of Mg-deficient rats with gentamicin induced a more severe and irreversible hearing loss compared to a milder reversible hearing loss in normally fed rats. They also found that Zn-deficient rats had a stronger yet still reversible hearing loss with salicylic acid. The authors concluded that Mg and Zn deficiencies enhanced ototoxicity of both gentamicin and salicylic acid (aspirin) (Gunther et al., 1988).

Aronson recommended strategies for minimizing aminoglycoside toxicity which all include avoiding risk factors. Therefore advice was given for early bedside detection of hearing and vestibular dysfunction, which should lead to prompt withdrawal, use of shorter durations of treatment, increasing dosing intervals to at least 12 hours, monitoring of serum concentrations, and awareness of potential risk factors, such as renal or liver dysfunction, old age, pre-existing hearing loss, and previous recent AG exposure (Aronson, 2006).

In summary, this wide range of risk factors highlights the importance of identifying, monitoring and accurately recording all possible aspects that may increase the prevalence of side effects such as ototoxicity in patients. Part of the research conducted and presented
within this thesis specifically investigates some of these risk factors in the study population and presents difficulties encountered with accurate recording of the data required.

1.2.5 Ototoxic chemotherapeutic agents – Cisplatin

Platinum group of drugs, including cisplatin, carboplatin and oxaliplatin, are effective chemotherapeutic/anti-neoplastic drugs commonly used for a variety of paediatric and adult cancers. These include germ cell tumours (ovarian and testicular), non-small cell lung cancer, malignant mesothelioma, bladder and cervical cancers, liver tumours, squamous cell carcinomas of the head and neck and some brain tumours including neuroblastomas (NCI, 2011). Carboplatin has slightly more limited use than cisplatin and is used against specific types of cancer of the breast and in combination therapy for treating lung cancer; oxaliplatin is the least known and is used in combination therapy against colorectal cancer. Cisplatin is known to be the most ototoxic but carboplatin is also ototoxic when administered in large doses for example when myeloablative doses are given during bone marrow transplantation or when given in conjunction with other drugs that open the blood-brain barrier (Neuwelt et al., 1998). Both cisplatin and carboplatin are shown to cause oxidative stress to the cochlear hair cells (Ravi et al., 1995, Rybak et al., 2009, Taudy et al., 1992, Saito et al., 1989). As with aminoglycosides, cisplatin is shown to affect the outer layer of the outer hair cells (OHCs) at the basal turn of the cochlea then gradually extends to the inner layers and inner hair cells (IHCs) and apically. However, several animal studies have shown carboplatin to cause species-specific, dose-dependent high-frequency SNHL attributed to primary damage of the inner hair cells instead of the OHCs (Wake et al., 1994). With both drugs, due to the tonotopic organization of the cochlea, high frequencies are affected first followed by the lower frequencies containing the speech frequencies. These platin group of drugs however, tend to be cochleotoxic only and not vestibulotoxic, as some of the aminoglycosides. Other toxicities include nephrotoxicity, which is the most significant side effect. This is especially substantial in older patients but which can also be reversible to some extent through good hydration using saline or mannitol diuresis (Rybak et al., 2009). They may also be associated with neurotoxicity, anorexia, nausea vomiting, thrombocytopenia with bleeding tendencies and hair loss (Schacht et al., 2012). Hearing loss tends to be permanent, bilateral symmetrical SNHL affecting 75-100% of patients especially if they are children or elderly, exposed to head and neck radiotherapy as well, exposed to larger doses of cisplatin (150–225 mg/m²), concomitant use of other ototoxic medications, or exposed to noise (Reddel et al., 1982, Bokemeyer et al., 1998, Kopelman et al., 1988, McKeage, 1995).
1.2.6 Ototoxic effect of aminoglycosides and cisplatin on the inner ear:

As mentioned earlier, aminoglycosides and cisplatin affect the basal turn of the cochlea first and then spread their effect apically. Figure 1-1 shows the tonotopic distribution of frequencies along the cochlear basilar membrane, where the highest frequencies are represented at the basal turn and the lower frequencies are represented more apically. The outer layer of the OHCs is affected first followed by the middle then the inner layer of OHCs with the IHCs being the last hair cells to show damage. This is demonstrated in Figure 1-2, which shows scanning electron microscopy pictures of different levels of cochlear damage to a rat organ of corti following exposure to aminoglycosides. Figure 1-3 shows the rate of hair cell loss along the basilar membrane.

Figure 1-1: Schematic representation of the tonotopic organization of the cochlea. The higher frequencies (starting at 20,000Hz) are represented at the basal turn of the cochlea with the lower frequencies represented more apically. (From: http://quizlet.com/15959363/splh-120-physic-of-speech-midterm-flash-cards (accessed: 18/03/2014).
Figure 1-2: Scanning Electron microscopy (SEM) pictures of the surface of a rat cochlea demonstrating different levels of inner ear hair cell damage following exposure to ototoxic drugs
A: shows normal inner ear with 3 healthy rows of OHCs and one row of IHCs
B: early damage affecting mainly the outer row of OHCs at the basal turn of the cochlea.
i, inner hair cells; o, outer hair cells
C: More significant damage affecting all layers of OHCs
D: Total damage affecting OHCs and IHCs. The red arrow points to the only remaining stereocilia of one IHC. The supporting cells fill up the entire surface where hair cells were lost.
Figure 1-3: Showing the percentage hair cell survival along the length of the basilar membrane following exposure to Amikacin.
The chart shows that the outer layer of OHCs (3) demonstrates the most significant loss followed by the middle layer of OHCs (2) then the inner layer of OHCs (1). The IHCs are the least damaged with the loss only affecting the basal part of the cochlear basilar membrane (from: Hawkins and Johnssen, 1981)

At the cellular level, different reactions lead to cell death through apoptosis following exposure to ototoxic medications. Figure 1-4 and Figure 1-5 illustrate a simplified version of the intracellular mechanisms of cell death from aminoglycosides and cisplatin (Rybak et al., 2000). Both drugs actively cross the blood-labyrinth barrier mainly through the stria vascularis into the endolymph (Steyger and Karasawa, 2008). They then enter the sensory hair cells through the apical mechano-electrical transducer channels (Marcotti et al., 2005, Gale et al., 2001). Aminoglycosides form AG-iron complexes that attract electron donors like arachidonic acid, which form reactive oxygen species (ROS), mainly superoxide anion (O$_2^-$), hydrogen peroxide (H$_2$O$_2$), and hydroxyl radical (’OH) that are commonly also referred to as ‘oxygen radicals’. ROS then activate c-Jun-N-terminal kinase (JNK), which translocate into the nucleus activating cell death pathway genes. These then translocate to the mitochondria activating cytochrome-c, which activates a cascade of caspases causing hair cell death through apoptosis. Another caspase-independent pathway is also identified with aminoglycosides. Cisplatin only follows a caspase-dependent pathway by first forming a cisplatin-monohydrate complex which activates NADPH oxidase (NOX3) forming ROS which activate JNK which then translocate into the nucleus and follows a similar pathway as aminoglycosides (Rybak and Ramkumar, 2007). In addition to targeting the hair cells
(mainly OHCs), these reactions also affect different cochlear tissues including the stria vascularis, spiral ligament and spiral ganglionic cells (Clerici et al., 1995, Clerici and Yang, 1996, Kopke et al., 1997).

Figure 1-4: Intracellular mechanisms of aminoglycoside and cisplatin-induced cell death. AG, aminoglycoside; AG-Fe, AG-iron complex; ROS, reactive oxygen species; JNK, c-Jun N-terminal kinase; Cyt c, cytochrome c. (b) CP, cisplatin; CP-MHC, cisplatin-monohydrate complex; (From Rybak and Ramakumar, 2007)

This ROS-induced apoptosis is counteracted within the cell by mechanisms to detoxify the reactive intermediates causing oxidative stress with the aim of supporting cell recovery and survival. Figure 1-5 (part B) illustrates some of these intracellular antioxidant cellular mechanisms, which involve production of Heat Shock Proteins, reduction of glutathione, utilization of intracellular antioxidants such as Vitamin C & E and trophic factors and targeting genes encoding antioxidant enzymes and detoxifying enzymes such as Superoxide Dismutase (SOD), Peroxidases and Catalases. An imbalance between the ROS pathways and the detoxification processes causes oxidative stress and cell death through apoptosis (Wu et al., 2002).
Figure 1-5: Illustration of drug-induced hair cell injury and death at the cellular level.
A. Shows the formation of damaging reactive oxygen species (ROS) e.g. superoxide (O$_{2}^-$) and hydroxyl anion (OH-) that are initiated by entry of ototoxic drugs into the hair cells.
B. Shows some of the internal antioxidant mechanisms that sensory hair cells use to control ROS-induced damage. These include production of heat shock proteins, reduced glutathione, use of antioxidant factors such as Vitamins C & E and production of antioxidant enzymes.
C. If antioxidant mechanisms fail to balance the reactions towards cell survival, ROS will irreversibly damage cell membranes, mitochondria, nuclear DNA and proteins, which signals cell death through apoptosis. (From: http://www.soundpharmaceuticals.com/technology.html (accessed 18/03/2014))

Figure 1-6: Illustration of the chemical reactions occurring within the mitochondrion where initiation of apoptosis occurs. (From Wallace, 2005b)
To describe this complicated process in more detail, reactions occurring at the level of the organelles, specifically the mitochondria are presented. The mitochondrion is defined as “a cellular organelle of endosymbiotic origin that resides in the cytosol of most nucleated (eukaryotic) cells and which produces energy by oxidizing organic acids and fats with oxygen by the process oxidative phosphorylation (OXPHOS) and generates oxygen radicals (reactive oxygen species, ROS) as a toxic by-product” (Wallace, 2005b). Figure 1-6 illustrates the complex chemical reactions continuously occurring within the mitochondria where the three main functions of the mitochondria are carried out. These functions include: i) energy production through formation of ATP (adenosine triphosphate); ii) formation of Reactive Oxygen Species (ROS); and iii) initiation of controlled cell death through apoptosis from reactions at the mitochondrial permeability transition pore (mtPTP) when an imbalance between the anti-apoptotic and pro-apoptotic reactions occurs. The reactions shown on the right-hand side of the figure are the ones associated with apoptosis. The pro-apoptotic Bax in the mtPTP interacts with the anti-apoptotic Bcl2 and the benzodiazepine receptor (BD) leading to the opening of the mtPTP and release of numerous pro-apoptotic proteins. Cytochrome-c (cyt c) then interacts with cytosolic Apoptotic protease activating factor 1 (Apaf-1) and activates it, which consequently activates caspase-9 that activates a downstream reaction to activate caspase-3 and caspase-2 initiating proteolytic degeneration of cellular proteins. Apoptosis initiating factor (AIF) and endonuclease G (EndoG) are released to the nucleus where they target nuclear peptides and degrade the nuclear chromosomal DNA leading to cell death (Wallace, 2005b) as shown in part C of Figure 1-5.

1.3 Current practice in monitoring for toxicities

There is a widespread variability in monitoring for toxicities. The deficiency in monitoring for both nephrotoxicity and ototoxicity exists even in developed countries, especially for ototoxicity, and is reported in the literature. Al-Aloul et al. reported that the commonly used glomerular filtration rate (GFR) monitoring tool is not effective in detecting nephrotoxicity (Al-Aloul et al., 2007). They investigated different methods for measuring renal function in CF and concluded that equations and 24-hr urine collections were inaccurate and that monitoring of renal function using more sensitive methods and increased vigilance were needed to reduce toxicity. Whereas in regards to ototoxicity, in the UK, Tan et al. undertook a questionnaire study of all UK CF specialist centers to determine prescribing practice and surveillance of AG antibiotics where 23 out of 28 centres responded (82%; 17 paediatric, 6 adult). Of these, only 3/23 (13%) centers reported routinely assessing hearing function, and even then, only standard pure-tone audiometry up to 8 kHz was used to detect the
cochleotoxic aspect of ototoxicity (Tan et al., 2002). Wilkinson & Mora performed another questionnaire survey of just the Paediatric CF units in the UK to audit hearing surveillance in these services. They had a response from 22/27 centers who all administered i.v. AGs to their patients. Despite providing more encouraging results in that most centers provided some form of audiological screening and verbal/written information regarding ototoxicity, they showed that only 9/22 (41%) centers reported having an established protocol for audiological screening with only 2/22 (9%) centers carrying out baseline hearing assessments prior to i.v. AG treatment. They concluded that: “There seems to be a lack of consensus regarding audiological screening procedures and methods of information sharing” (Wilkinson and Mora, 2009).

In the US, Van Meter et al. performed a similar survey where 84 out of 195 (43%) Cystic Fibrosis Foundation-accredited care centers and affiliated programs (CFFACCs) completed the survey of which 43 (51%) were paediatric only centers. They reported that audiometric evaluation was performed routinely in only 22 (26%) centers with annual assessment performed in only 14 (64%) of these 22 centers (Van Meter et al., 2009). In Australia a survey in 1999 by Phillips and Bell showed that despite confirmation from 23/26 (88%) of the CF centers responding to the questionnaire that they undergo creatinine monitoring for nephrotoxicity, only 4/26 (15%) monitored for auditory and vestibular toxicity. Of these centers 19%, 27% and 12% reported evidence of nephrotoxicity, cochleotoxicity and vestibulotoxicity respectively. Soulsby et al. repeated this survey in 2006 where an improvement in the number of centers monitoring for ototoxicity was reported, with 17/27 (63%) confirming that they do monitor yet more detailed questioning showed that the methods of monitoring were varied and crude. They stated that 13 units only used standard audiometry, 3 just asked the patient about their hearing and one unit undertook monitoring only during the conduct of a study. Frequency of testing was again varied ranging from each admission to when considered necessary which invariably would lead to a lower reporting of incidence of these toxic effects (Phillips and Bell, 2001, Soulsby et al., 2009).

There were no available survey studies reporting the current provision for ototoxicity monitoring in oncology patients, yet published clinical research indicates that it is highly variable and inconsistent. The ASHA 1994 recommendations and criteria for ototoxicity monitoring for oncology patients are very comprehensive. They recommend baseline testing before commencement of ototoxic chemotherapy like cisplatin, or within the first 48 hours of the first dose if that was not possible. Auditory monitoring following every cisplatin cycle is advised as well as post-treatment follow-up for late-onset hearing impairment. Figure 1-7 is the flow-chart published by ASHA summarizing the recommended ototoxicity monitoring
protocol for oncology patients (ASHA, 2013). The American Academy of Audiology (AAA) has also published a position statement and clinical guidelines for ototoxicity monitoring in 2009. There it was highlighted that ototoxicity monitoring goes beyond conventional methods used in routine clinical practice so that comprehensive baseline and follow-up assessments, including extended high-frequency audiometry, OAEs, speech audiometry and tympanometry, were required to allow for early detection of ototoxicity. It also highlighted the significant need for audiologists to take the lead in developing ototoxicity-monitoring programs, as they are best placed to achieve the main goals of such programs of both preventing or minimizing hearing loss before speech frequencies are affected and helping the patient to maintain the most effective hearing communication possible if ototoxicity is inevitable (Durrant et al., 2009). One of the aims of the current research presented in this thesis aimed to assess current practice in the UK regarding monitoring for ototoxicity in oncology and cystic fibrosis patients and assessing how well this matches with the recommended ASHA guideline for monitoring.
Figure 1-7: The ASHA recommended ototoxicity monitoring protocol for oncology patients (from ASHA, 2013)
1.3.1 Overview of auditory monitoring for ototoxicity

Review of the literature has indicated that a wide variation exists between the available recommended audiological tools for ototoxicity monitoring and the ones actually used in practice. In general practice, the standard method for ototoxicity monitoring is the baseline and serial measurement of pure-tone hearing thresholds within the conventional standard frequency range, 0.25 to 8 kHz. Research has shown that extended high-frequency (EHF) audiometry and evoked otoacoustic emissions (OAEs) are audiological tests that are more sensitive to initial ototoxic damage (Rybak et al., 2009, Lonsbury-Martin and Martin, 2003, Fausti et al., 1992b, Campbell et al., 2003). They were shown to detect early changes in auditory function before ototoxicity affects hearing at frequencies important for speech recognition when patients actually start complaining of deterioration in hearing. However many studies report only using standard pure-tone audiometry (PTA) instead (Riethmueller et al., 2009, Mulherin et al., 1991, Mulheran et al., 2001). A more detailed review of the literature in support of more detailed audiological assessment, beyond standard audiometry, for ototoxicity monitoring is provided below.

Standard conventional PTA has been the traditional method for monitoring decreased auditory sensitivity due to aminoglycoside treatment (Greenwood, 1959). However, Lancaster et al. reported that despite having high plasma gentamicin levels in 16 patients treated with topical gentamicin for chronic otitis media, pre- and post-treatment audiometric assessments revealed no statistically significant differences. They concluded that conventional audiometric assessments might not be sensitive enough to monitor aminoglycoside ototoxicity (Lancaster et al., 1999). Several studies reported that otoacoustic emissions (OAE) appear to better monitoring tools for early detection of aminoglycoside ototoxicity than conventional audiometry. Stavroulaki et al. performed pre and post-treatment auditory assessment using conventional audiometry and OAEs in 13 children treated with gentamicin (4 mg/kg per day, i.v) for 8–29 days. They reported that audiometry showed no statistically significant pre-post treatment changes while OAEs revealed a significant difference (Stavroulaki et al., 1999). The same authors underwent another study (Stavroulaki et al., 2002) where they tested children with cystic fibrosis (CF) treated with gentamicin (4 mg/kg for 14 days). Again, the audiometric assessments revealed normal thresholds at pre-post treatment recordings whereas pre-post-measurements of transient evoked (TE) OAE, distortion product (DP) OAE-gram and DPOAE input/output (I/O) function showed statistically significant deteriorations compared to a control group. Kathamna et al. assessed both children and adults who received tobramycin (<1250 mg/kg). They also reported that pre-post conventional audiology was normal and similar to the
control group while OAE latency and growth (I/O) function thresholds were significantly different from the controls. Therefore, these authors concluded that OAEs should be recommended as more effective measures for monitoring cochlear function during aminoglycoside ototoxicity than conventional audiometry (Katbamna et al., 1999, Stavroulaki et al., 2002).

Extended high frequency (EHF) audiometry (>8 kHz up to 20 kHz) has also been shown to be effective as early detectors of aminoglycoside and cisplatin ototoxicity. Fausti et al. (1992 and 2003) have shown a high frequency 1/6th-octave protocol to significantly improve the detection of ototoxicity. They identified the highest frequency that a patient can hear and the frequency region of one octave below this to be the most sensitive region to early damage from ototoxic drugs and called it the Sensitive Range for Ototoxicity (SRO). They proposed that identifying this region pre-treatment and then monitoring it at 1/6th octave intervals would be the most sensitive and least time and cost consuming method of monitoring for ototoxicity (Fausti et al., 2005, Fausti et al., 1992b, Fausti et al., 2003). This approach was most appropriate for adults; especially elderly patients that are more liable to show evidence of progressive high frequency hearing loss due to presbyacusis or noise induced hearing loss leading to variability in their limit for high frequency hearing even before exposure to ototoxic agents. Knight et al. reported on ototoxicity monitoring of 32 patients aged 8 months to 20 years receiving platinum-based chemotherapy. Baseline and serial measurements of conventional audiometry and distortion-product (DPOAEs) was performed with extra EHF audiometry recording in 17 of these patients. Their results showed evidence of bilateral ototoxicity in 20 (62.5%) using conventional audiometry, in 26 (81.3%) using DPOAEs and in 16/17 (94.1%) using EHF audiometry. They concluded that EHF audiometry and DPOAEs have the potential to reveal earlier changes in auditory function than conventional frequency audiometry during platinum chemotherapy in children (Knight et al., 2007). The reliability of EHF audiometry especially in younger age groups was considered an important factor to establish before advocating its use in ototoxicity monitoring. Frank et al. and Schmuziger et al. both assessed the test-retest reliability of EHF audiometry at 1/6th octave frequencies ranging between 8 and 16 kHz in normal-hearing adults (Schmuziger et al., 2004, Frank, 2001). The intersubject test-retest variability was shown to be within the accepted ±10 dB range in at least 94% of all the recorded intersession measurements made for these frequencies. They also measured the changes in thresholds against the ASHA 1994 criteria for ototoxicity and indicated that the false positive rates where <3% for all the assessed ears. Beahan et al. performed a study with a similar aim but assessed children at three different age groups (4-6 yrs., 7-9 yrs. and 10-13 yrs.). Good test-retest variability within the ±10 dB range was demonstrated in 89.9%, 93.0% and 97.0%
for the three age groups respectively. They calculated this to equate to false-positive rates when compared to the ASHA 1994 criteria for ototoxicity to 24.6%, 11.0% and 7.6% respectively. This was deemed acceptable for the two older age groups of >7 years of age but not for the younger age group (4-6 yrs.) as the false-positive rate was too high. It was recommended to supplement the EHF audiometry testing with an objective test such as DPOAE to confirm that a genuine threshold shift had occurred especially in this age group (Beahan et al., 2012).

These reports all supported the hypothesis that the inclusion of non-standard assessment tools, such as EHF audiometry and DPOAE testing, for audiological monitoring would be more sensitive to detection of early or mild evidence of ototoxicity. Confirmation of this hypothesis in a population of cystic fibrosis children exposed to aminoglycosides but never monitored for occurrence of ototoxicity was also one of the main aims of this current research project. Results are presented in section 4.1.

1.3.2 Criteria for detecting/grading ototoxicity

It is highlighted from the section above, that despite the presence of evidence in the literature supporting specific audiological assessment tools as effective monitoring tools for ototoxicity, there is no clear ‘gold standard’ protocol of monitoring for ototoxicity. More critically, this section highlights that there is a variation in the criteria used to define the occurrence of ototoxicity. Some define an initial indication of hearing loss as a drop of more than 10 dB at one or more frequencies when assessed by pure-tone audiometry (PTA) (Wright et al., 1998); while others specify a hearing loss of 20 dB at two or more adjacent test frequencies (Forge & Schacht, 2000) and others follow even more stringent detailed criteria such as the ASHA (1994) criteria of a 20 dB or greater increase in pure-tone threshold at a single test frequency, 10 dB or greater increase in threshold at two adjacent frequencies, or the loss of response at three consecutive frequencies where responses were previously obtained (Table 1-1) (ASHA, 1994a). Within the field of oncology several grading systems for defining ototoxicity criteria have been developed and used to identify different classes of hearing loss especially in children with cancer. The grading systems were either based on measuring absolute hearing levels specifically for children, such as Brock’s scale (Table 1-2) (Brock et al., 1991), the more recently Chang scale, a modification of the Brock scale proposed by Chang and Chinosornvatana which took into account the mild degrees of hearing loss (Table 1-3) (Chang and Chinosornvatana, 2010), or the latest new International Society of Paediatric Oncology Boston Ototoxicity Scale (SIOP Boston scale: Table 1-4). The latter is based on the recommendations made by a variety of experts, including international paediatric oncologists, audiologists and basic scientists, at the 42nd
Congress of the International Society of Paediatric Oncology (SIOP) in Boston in 2010 (Brock et al., 2012a). The other grading systems are based on identifying changes in hearing from baseline including National Cancer Institute Common Toxicity Criteria for Adverse Effects (CTCAE) which specify an extensive range of side effects including hearing impairment from ototoxicity (Table 1-5) (Knight et al., 2005), the WHO Common toxicity Criteria (Gallegos-Castorena et al., 2007) and the Children’s Hospital Boston (CHB) scale (Lewis et al., 2009). As seen in the examples provided in the tables below these grading systems are quite variable and may partly account for the differences reported in incidence of ototoxicity and make comparing research outcomes very difficult. The recently proposed SIOP Boston Scale is aiming to provide uniformity between different international groups especially those performing clinical trials in order to compare results appropriately. It was aimed to make it simple, easy to use and avoided disadvantages of other scales such as the Brock’s scale where the mild levels of hearing loss (20-40 dB) were completely ignored. However it still only considered frequencies within the conventional standard audiometry range up to 8kHz and excluded outcomes of EHF audiometry. Brock’s grading was used in the assessment of the study population of cystic fibrosis children included in this current research to evaluate the effect of using these criteria on determination, identification and estimation of severity of ototoxicity.

DPOAEs are faced with a similar hindrance where absence of universally accepted criteria for ototoxicity also exists. Reavis et al. recently highlighted that interpreting DPOAE findings in the context of ototoxicity monitoring requires that their accuracy be determined in relation to a clinically accepted gold standard test. Knight et al. performed regular pre-and post-treatment recordings and considered decreases in DPOAE greater than 8 dB SPL a significant clinical change. This was based on the work of Beattie et al. who reported that differences in DPOAE amplitudes must exceed 7 dB SPL at 1 to 4 kHz to be statistically significant at the 0.05 level of confidence. Reavis et al. defined that DPOAE change criteria in their study as a ≥4 dB reduction in amplitude or loss of response (i.e. reduction in DPOAE amplitude level to below -10 dB SPL) at ≥2 adjacent f2 frequencies, with a false-positive rate of around 5% to be expected. Constantinescu et al. also recommended serial DPOAE measurements to accurately monitor for aminoglycoside ototoxicity (Beattie et al., 2003, Constantinescu et al., 2009, Knight et al., 2007, Reavis et al., 2011, Reavis et al., 2008). Another aim of the current research was to determine the range of intersubject variability of DPOAE recordings in ears of children on repeated testing, in order to determine the limit beyond which a change in DPOAE amplitudes is considered true evidence of inner ear damage and not just a standard error of measurement. Results of this work are presented in section 4.2.
### Examples of different criteria for ototoxicity reported in the literature

**ASHA criteria for ototoxicity (1994)**

<table>
<thead>
<tr>
<th>Grade</th>
<th>Thresholds</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>20 dB or greater increase (worsening) in pure tone threshold at one test frequency OR</td>
</tr>
<tr>
<td>B</td>
<td>10 dB or greater increase at two adjacent test frequencies OR</td>
</tr>
<tr>
<td>C</td>
<td>Loss of response at 3 consecutive test frequencies where baseline responses were previously obtained, signifying a decrease in hearing following treatment</td>
</tr>
</tbody>
</table>

Table 1-1: ASHA 1994 criteria for a significant threshold shift due to ototoxicity from (ASHA, 1994a)

**Brock’s grading criteria for ototoxicity**

<table>
<thead>
<tr>
<th>Grade</th>
<th>Thresholds</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>&lt; 40 dB at 500 - 8,000 Hz</td>
</tr>
<tr>
<td>1</td>
<td>≥ 40 dB at 8,000 Hz</td>
</tr>
<tr>
<td>2</td>
<td>≥ 40 dB at 4,000-8,000 Hz</td>
</tr>
<tr>
<td>3</td>
<td>≥ 40 dB at 2,000-8,000 Hz</td>
</tr>
<tr>
<td>4</td>
<td>≥ 40 dB at 1,000-8,000 Hz</td>
</tr>
</tbody>
</table>

Table 1-2: Brock's grading criteria for ototoxicity from (Brock et al., 1991)

**Chang’s grading criteria for ototoxicity**

<table>
<thead>
<tr>
<th>Grade</th>
<th>Thresholds (Sensorineural hearing thresholds by AC/BC with normal tymmps)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>≤20 dB at 1,000; 2,000 &amp; 4,000 Hz</td>
</tr>
<tr>
<td>1a</td>
<td>≥ 40 dB at any freq. 6,000 - 12,000 Hz</td>
</tr>
<tr>
<td>1b</td>
<td>&gt;20 dB &amp; &lt;40 dB at 4,000 Hz</td>
</tr>
<tr>
<td>2a</td>
<td>≥ 40 dB at 4,000 Hz and above</td>
</tr>
<tr>
<td>2b</td>
<td>&gt;20 dB &amp; &lt;40 dB at any freq. &lt;4,000 Hz</td>
</tr>
<tr>
<td>3</td>
<td>≥ 40 dB at 2,000/3,000 Hz and above</td>
</tr>
<tr>
<td>4</td>
<td>≥ 40 dB at 1,000 Hz and above</td>
</tr>
</tbody>
</table>

Table 1-3: Chang's grading criteria for ototoxicity from (Chang and Chinosornvatana, 2010)

**SIOP Boston Ototoxicity Scale**

<table>
<thead>
<tr>
<th>Grade</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>≤ 20 dB HL at all frequencies</td>
</tr>
<tr>
<td>1</td>
<td>&gt; 20 dB HL (i.e. 25 dB HL or greater) SNHL above 4,000 Hz (i.e. 6 or 8 kHz)</td>
</tr>
<tr>
<td>2</td>
<td>&gt; 20 dB HL SNHL at 4,000 Hz and above</td>
</tr>
<tr>
<td>3</td>
<td>&gt; 20 dB HL SNHL at 2,000 Hz or 3,000 Hz and above</td>
</tr>
<tr>
<td>4</td>
<td>&gt; 40 dB HL (i.e. 45 dB HL or more) SNHL at 2,000 Hz and above</td>
</tr>
</tbody>
</table>

Table 1-4: The recently proposed and agreed SIOP Boston Ototoxicity Scale from (Brock et al, 2012a)
<table>
<thead>
<tr>
<th>Grade</th>
<th>Adverse Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hearing impaired: Adults enrolled in Monitoring Program (on a 1, 2, 3, 4, 6 and 8 kHz audiogram): Threshold shift of 15 - 25 dB averaged at 2 contiguous test frequencies in at least one ear. Adults not enrolled in Monitoring Program: subjective change in hearing in the absence of documented hearing loss. Pediatric (on a 1, 2, 3, 4, 6 and 8 kHz audiogram): Threshold shift &gt;20 dB at 8 kHz in at least one ear.</td>
</tr>
<tr>
<td>2</td>
<td>Adults enrolled in Monitoring Program (on a 1, 2, 3, 4, 6 and 8 kHz audiogram): Threshold shift of &gt;25 dB averaged at 2 contiguous test frequencies in at least one ear. Adults not enrolled in Monitoring Program: hearing loss but hearing aid or intervention not indicated; limiting instrumental ADL. Pediatric (on a 1, 2, 3, 4, 6 and 8 kHz audiogram): Threshold shift &gt;20 dB at 4 kHz and above in at least one ear.</td>
</tr>
<tr>
<td>3</td>
<td>Adults enrolled in Monitoring Program (on a 1, 2, 3, 4, 6 and 8 kHz audiogram): Threshold shift of &gt;25 dB averaged at 3 contiguous test frequencies in at least one ear; therapeutic intervention indicated. Adults not enrolled in Monitoring Program: hearing loss with hearing aid or intervention indicated; limiting self care ADL. Pediatric (on a 1, 2, 3, 4, 6 and 8 kHz audiogram): hearing loss sufficient to indicate therapeutic intervention, including hearing aids; threshold shift &gt;20 dB at 3 kHz and above in at least one ear; additional speech-language related services indicated.</td>
</tr>
<tr>
<td>4</td>
<td>Adults: Decrease in hearing to profound bilateral loss (absolute threshold &gt;80 dB HL at 2 kHz and above); non-servicable hearing. Pediatric: Audiologic indication for cochlear implant and additional speech-language related services indicated.</td>
</tr>
</tbody>
</table>

Table 1-5: CTCAE criteria for hearing impairment for ototoxicity
(Table extracted from published document at: http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf; accessed 12/10/2013)
1.4 **Patients with Cystic Fibrosis (CF) as a study population:**

Several reasons have supported the decision to make children with cystic fibrosis as the study population of choice to investigate certain aspects of ototoxicity. These reasons are briefly presented in this section.

As mentioned earlier, the early confirmation of the chronic complications of AGs led to significant restrictions in the use of these antibiotics, especially in developed countries. CF populations are still one of the few populations that are exposed to a large number of cumulative repeated AG courses, in order to combat pulmonary exacerbations due to chronic *pseudomonas* chest infections, making them an ideal study population for investigating ototoxicity. Research has shown that the pharmacokinetics of antimicrobial drugs in CF patients is abnormal when compared to normal individuals. This is mainly in the form of increased volume of distribution of highly hydrophilic medications such as AGs, as expressed in liters/kg body weight, in addition to an increased total body clearance through renal excretion. The increased volume of distribution is mainly explained by the fact patients with CF are largely undernourished and have a scarcity of body fat leading to an increased amount of lean tissue/kg bodyweight. The justification for the increased renal excretion is not clear but may be attributed to increased glomerular filtration and tubular secretion of AGs in the kidney or to the occurrence of sub-clinical renal toxicity (Touw, 1998). These factors mean that patients with CF have to be prescribed larger doses of AGs that are given over longer durations of time/course in order to achieve the appropriate therapeutic drug levels. Courses are usually given over a period of at least 14 days with tobramycin or gentamicin usually prescribed at a once daily dose of 10mg/kg bodyweight/day and amikacin at 30mg/kg bodyweight/day compared to a dosage of 3-8 mg/kg bodyweight/day over 7-10 days in non-CF patient groups (Doring et al., 2000, Govaerts et al., 1990, Kahlmeter and Dahlager, 1984, Tan et al., 2003). It may be hypothesized that the increased volume of distribution and larger, longer and cumulative intake of AGs in this population may be associated with more accumulation of aminoglycosides in the inner ear and consequently with a higher incidence of ototoxicity.

The other main reason for choosing patients with CF was due to the controversy in the reported prevalence of ototoxicity in this patient group, especially in children. This point is discussed in more detail in section 1.4.2 below. Patients with CF are also regularly reviewed within dedicated CF centers distributed all over the UK with a well-established network coordinated through the UK CF Trust. This may aid in being able to access and retrieve
better documentation of patient management regimens (e.g. information regarding the type, number of courses, duration of intake of AG antibiotics and information regarding any other associated potential risk factors); it may also aid in better distribution of significant outcomes of the research and implementation of recommendations for practice if these are achieved. Currently there are no guidelines to managing or monitoring ototoxicity in CF patients even following repeated exposure to AGs. As overall management of this condition has resulted in improvements in general well-being, through adequate use of antibiotics and respiratory physiotherapy, the patients’ average survival rate has risen in the past decades from around 4 years of age up to around 40 years. This improvement has placed more emphasis on improving the quality of life of these patients. Hearing impairment caused by ototoxicity can have significant deleterious effects on the patients’ health, social, educational, psychological and emotional well-being especially when it affects a young age. Therefore further investigation of ototoxicity in this patient group was warranted.

The final reason for choosing this study population was due to an observation made by some previous researchers that ‘lower-than-expected’ results for acute or chronic ototoxicity were obtained from their study CF patients compared to non-CF patients. Their possible explanation for this observation was either due to the rapid clearance of AGs in CF patients or possibly, secondary to the fact that the CF condition actually bestows some form of protection against AG-induced cochleotoxicity (Mulheran et al., 2001, Mulheran et al., 2006, Pedersen et al., 1987). It was interesting to investigate whether a lower-than-expected prevalence of ototoxicity truly existed in patients with CF and if it did, if factors associated with this possible CF-induced otoprotection effect could be investigated.

1.4.1 Overview of CF as a disease

Cystic fibrosis affects different ethnic groups worldwide but is identified as the commonest life threatening autosomal recessive hereditary disorder affecting Caucasians. The incidence of clinical disease is ~1 in 2500 live births with 1 in 25 being heterozygous carriers of the mutation. There is a prevalence of ~70,000 patients (Ramsey et al., 2011) worldwide including over 10,078 CF patients in the UK (UK CF Trust, 2013). This disease is caused by mutations in a single gene on the long arm of chromosome 7, which encodes the CF transmembrane conductance regulator (CFTR) protein (Bear et al., 1992). The CFTR protein is an epithelial ion channel that is involved in the regulation of liquid volume on epithelial surfaces through chloride secretion and inhibition of sodium absorption in a variety of tissues, including the lung, sweat glands, gastrointestinal tract and pancreas, (Rowe et al., 2005). Abnormal CFTR protein leads to impaired transport of chloride ions leading to depletion of chloride in the pericilliary layer and reduction of the pericilliary fluid volume.
This consequently impairs the movement of water in and out of cells. As a result, the cells that line the passageways of organs such as the lungs, pancreas, and others produce mucus that is abnormally thick and sticky (Davies et al., 2007).

The commonest cause of morbidity and mortality is repeated respiratory infections and consequent respiratory failure. The ‘low volume’ hypothesis is the commonly accepted explanation for respiratory disease in CF where the reduced volume of airway surface fluid leads to a significant reduction in mucociliary clearance and failure to sufficiently clear inhaled bacteria and other airborne harmful viruses or pollutants. There is also a reported abnormally excessive inflammatory response where patients with CF were shown to have 10 times more inflammatory response to a given bacterial load compared to other patient groups with lower respiratory tract disease (Davies et al., 2007). More than 1,700 different mutations have been identified so far (Ashlock and Olson, 2011), however, a deletion of phenylalanine at codon 508, commonly known as ΔF508 (recently known as phe508del), is present in ~66% of all CF patients worldwide. The different mutations produce different gene products leading to formation of different classes of mutation depending on how the protein function is affected. These different classes have a prognostic significance as is shown in Table 1-6 below. This table was presented by Bush and Harcourt who identify classes 1-3 to be the ones with severe mutations. These are usually associated with pancreatic insufficiency and significantly poorer survival (Bush and Harcourt, 2007). Patients with the G551D genotype are the first to be involved in a genomically guided therapy using an FDA approved ‘orphan drug’ called Ivacaftor (Kalydeco) marking a new frontier in the management of CF (Ramsey et al., 2011, Barrett et al., 2012) with CF patients in the UK being also involved.

<table>
<thead>
<tr>
<th>Mutation class</th>
<th>Nature of defect</th>
<th>Example of genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class 1</td>
<td>No synthesis of CFTR mRNA</td>
<td>Nonsense, G542X, Frame shift, 394delTT</td>
</tr>
<tr>
<td>Class 2</td>
<td>Block in intracellular processing of CFTR protein, leading to destruction before reaching the apical membrane</td>
<td>ΔF508 (most common)</td>
</tr>
<tr>
<td>Class 3</td>
<td>Block in regulation of CFTR on arrival at the apical cell membrane</td>
<td>G551D</td>
</tr>
<tr>
<td>Class 4</td>
<td>Altered conductance of the ion channel function of CFTR at the apical cell membrane</td>
<td>R117H</td>
</tr>
<tr>
<td>Class 5</td>
<td>Reduced synthesis of CFTR</td>
<td>A455E, Alternative splicing, 3849+10 kbC → T</td>
</tr>
</tbody>
</table>

Table 1-6: Different classes of cystic fibrosis transmembrane conductance regulator (CFTR) mutations (From (Bush and Harcourt, 2007)
**Respiratory infections and need for repeated intake of AG to combat gram-negative infections:**

The commonest cause of death from CF is respiratory failure secondary to bronchiectasis. Chronic lung infection and inflammation rapidly develops despite the lung being essentially normal at birth. This is mainly explained by the ‘low volume’ hypothesis and the higher than normal inflammatory response, as mentioned earlier. Early on, common pathogens include *Staphylococcus aureus, Haemophilus influenzae, and Burkholderia cepacia*, with chronic infection with mucoid *Pseudomonas aeruginosa* affecting around 80% of patients at a very young age. Infection with *methicillin-resistant S. aureus* (MRSA) is also increasingly common. Aggressive treatment of infection, and airway clearance techniques, are essential. Usually, the child will be doing two sessions of chest physiotherapy per day with antibiotics commonly prescribed prophylactically in addition to active treatment for any positive respiratory culture of pathogens. Infection with *P. aeruginosa* is frequently described as the most opportunistic infection in CF. Currently the recommendation for intravenous (i.v) antibiotic treatment of pulmonary exacerbations with *P. aeruginosa* is a combination of two antibiotics with different mechanisms of action (UK CF Trust, 2009). Combination antibiotic therapy, usually of an aminoglycoside and a β-lactam, has been shown to produce a synergistic effect in vitro (Weiss and Lapointe, 1995), and may limit the emergence of antibiotic resistant strains of *P. aeruginosa* (Cheng et al., 1996). Table 1-7 is cited from Doring et al. which displays the recommended antibiotic treatment for CF respiratory infections (Doring et al., 2000).

As aminoglycosides are considered to be an essential part of the 1st line treatment regimen for *P. aeruginosa* infections and because this infection commonly affects CF patients even at a young age, audiological monitoring for ototoxicity would logically need to be considered as an essential component of management of these patients. On contacting several key clinicians of the UK CF Trust and CF units it was quite clear that there was limited audiological monitoring or even assessment of CF patients, which was also confirmed through review of the UK CF-related literature. This confirmed that a CF population was an ideal one to include as the study population to investigate ototoxicity. CF children were specifically chosen because the reported prevalence of ototoxicity was characteristically low as shown in the following section. Research with this population in the UK would aid in raising the awareness of this aspect of patient management and contribute to the extensive efforts of a limited number of UK clinicians in this field (Mulheran et al., 2001, Mulherin et al., 1991, Mulrennan et al., 2009, Prayle et al., 2010, Prescott, 2011, Smyth, 2010, Smyth and Bhatt, 2012, Smyth and Campbell, 2013, Tan et al., 2003).
<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Route of administration</th>
<th>Dose mg/kg/day</th>
<th>Administrations per day (n)</th>
<th>Maximum daily dose (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin*</td>
<td>i.v.</td>
<td>30</td>
<td>1 or 2</td>
<td>-</td>
</tr>
<tr>
<td>Tobramycin*</td>
<td>i.v.</td>
<td>10</td>
<td>1 or 2</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Inhaled (TOBI)</td>
<td>150±300#</td>
<td>1±2</td>
<td>0.6</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>i.v.</td>
<td>150</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>i.v.</td>
<td>100</td>
<td>Continuously</td>
<td>8</td>
</tr>
<tr>
<td>Cefepine</td>
<td>i.v.</td>
<td>100±150</td>
<td>2±3</td>
<td>6</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>i.v.</td>
<td>150±250</td>
<td>3±4</td>
<td>12</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>p.o.</td>
<td>30</td>
<td>2±3</td>
<td>1.5±2.25</td>
</tr>
<tr>
<td>Colistin</td>
<td>Inhaled</td>
<td>80±160#</td>
<td>1±2</td>
<td>0.320#</td>
</tr>
<tr>
<td>Sulphomethate</td>
<td>i.v.</td>
<td>160#</td>
<td>3</td>
<td>0.48</td>
</tr>
<tr>
<td>Imipenem/cilastatin</td>
<td>i.v.</td>
<td>50±100</td>
<td>3±4</td>
<td>4</td>
</tr>
<tr>
<td>Meropenem</td>
<td>i.v.</td>
<td>60±120</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>i.v.</td>
<td>60</td>
<td>Continuously</td>
<td>3</td>
</tr>
<tr>
<td>Netilmicin*</td>
<td>i.v.</td>
<td>10</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Ticarcillin</td>
<td>i.v.</td>
<td>500±750</td>
<td>4</td>
<td>30</td>
</tr>
</tbody>
</table>

*Dose based on measurements of serum concentrations; #: absolute dose (dependent on age and situation).

i.v., intravenous; p.o, per os (by mouth).

Table 1-7: Recommended dosages for antibacterial agents in the management of P. aeruginosa lung infections in CF patients.
(From (Doring et al., 2000))

1.4.2 Controversy in reported prevalence of AG ototoxicity

There is variability in the reported prevalence of ototoxicity in patients with CF ranging from 0 to 51% (Mulheran and Degg, 1997a, Mulheran et al., 2001, Haddad et al., 1994, Ozcelik et al., 1996, Piltcher et al., 2003, Cheng et al., 2009, Martins et al., 2010) and no clear consensus among clinicians on the diagnostic tests that would be most suitable for monitoring ototoxicity. Mulheran et al. have reported the prevalence of hearing loss in 70 young (10–18 years, n = 27) and adult (18–37 years, n = 43) patients with CF using standard and high frequency pure tone audiometry to be 17% (12/70 including 1 paediatric case). The prevalence of hearing loss in the pediatric population was generally reported to be lower (0–6%), which may be interpreted to reflect less AG exposure and less environmental damage (Mulheran and Degg, 1997b, Mulheran et al., 2001, Mulheran and Degg, 1997a).
However, there was no agreement over a low prevalence of hearing loss in children. Cheng et al. (2009) performed a retrospective study of all CF children seen in the Department of Otolaryngology and Communication Enhancement at Children's Hospital Boston during a period of 13 years. Their aim was to determine the prevalence of sensorineural hearing loss (SNHL) in CF patients and its relationship to antibiotic use. A total of 171 CF patients were identified but only 50 of them had received audiological assessments, which were only performed on the discretion of the physicians with absence of a systematic protocol for follow-up monitoring. Only standard audiometry was performed with criteria of ototoxicity defined as thresholds >15 dB at two or more adjacent frequencies. A 14% (7/50) prevalence of SNHL was reported. The children had received courses of intravenous aminoglycosides, nasal aminoglycoside irrigations or macrolides. They reported a higher significant prevalence of hearing loss when patients received >10 courses of i.v aminoglycosides, >5 courses of nasal irrigations or >5 courses of macrolides. The authors concluded that CF patients receiving aminoglycosides are at a high risk of developing SNHL.

When reporting prevalence of a specific condition it is quite important to identify a control group that can exclude false negative and positive cases. This control group could be healthy, normal and age-matched to the study population or, for example in this condition, have CF but no history of exposure to aminoglycosides. However, this is usually quite difficult to achieve, especially if avoiding any confounding factors, is required. Cipolli et al. conducted a study that actually compared auditory function of 75 CF children (mean age±SD: 9.9±3.6 years) to 50 healthy normal age-matched children (mean age±SD: 9.3±4.2 years). They performed otoscopy, standard audiometry (up to 8kHz), speech audiometry and tympanometry with reflexes to both groups of patients. They reported a 25.4% and 18% prevalence of hearing loss, using an original scoring system, in the CF and normal groups respectively. They concluded this to be statistically non-significant at p>0.05 and stated that CF was not associated with an increased risk of ear disease despite the associated pulmonary disease, radiological sinusitis, nasal polyposis or even the use of parenteral aminoglycosides. However the study does have a few flaws, which may affect this conclusion. First, the ‘original’ scoring system that they used attributed only 20% of the score to the audiometry result of both ears with 50% attributed to otoscopy and tympanometry. This allows for a lot of weighting to be placed on conductive middle ear problems, which may explain the 18% prevalence of hearing loss in the normal healthy group, as it’s a common occurrence in children. They also only used standard conventional audiometry and not extended high frequency audiometry, which has been shown to miss early cases of aminoglycoside ototoxicity and therefore reduced the prevalence of hearing loss in the CF group (Cipolli et al., 1993). This highlights the difficulties that may be faced when conducting similar
research and the need not just to accurately identify the appropriate population but also to have clear measurement criteria and effectively exclude confounding factors. Critical analysis of this article also presents some of the points that have led to possible reasons for under-reporting of ototoxicity where conservative figures of clinical prevalence may generally be reported.

1.5 **Children with cancer as a study population**

Ototoxic chemotherapeutic agents as platinum compounds like cisplatin and high dose carboplatin are primary therapies for a wide range of childhood malignancies including germ-cell tumours, neuroblastomas, osteosarcomas, hepatoblastomas, Wilm’s tumour and brain tumours such as medulloblastomas. Here, ototoxicity is more common and has more significant effects in children compared to adults (McHaney et al., 1983). Due to increased susceptibility to ototoxicity from platinum compounds in children the maximum cisplatin cumulative dosage is limited to 400mg/m² to avoid unacceptable hearing loss. However, this may not always be possible as with neuroblastomas where triple this dose is needed. Increased risk for ototoxicity is also associated with concomitant use of head and neck irradiation or other ototoxic drugs, brain tumours, renal impairment and younger age groups (<5 years) (Walker et al., 1989, Schell et al., 1989).

Hearing loss starts at the high frequencies at the basal turn of the cochlea and then progresses to the lower frequencies to include the speech frequencies. The effect of hearing loss in young children is more detrimental compared with adults as they have a higher need for more audibility to allow speech recognition and comprehension. Prelingual children or even those that have just started developing speech and language do not have an established language base and CNS maturity to allow them to fill in the gaps of missing speech components or to be able to communicate in complex acoustic environments with background noise. Even with hearing loss affecting only the high frequencies, children will miss the ability to distinguish high-frequency fricative consonants (/s/, /ʃ/, /t/, /z/, /θ/, /ð/, /k/, /p/) occupying the 4-8kHz tones as seen in Figure 1-8. These are essential for clarity of speech and discrimination in the presence of background noise. This would also affect essential grammatical aspects of speech e.g. identifying plurals such as /s/ in ‘ducks’ and /z/ in ‘girls’ with verbal errors and delays in speech and language development will occur (Crandell, 1993, Finitzo-Hieber and Tillman, 1978). Stelmachowicz et al. highlighted that even mild-moderate high frequency loss in children will cause delays in acquisition of all phonemes especially fricatives and increased difficulty in understanding female or child speech compared to male speech. They also drew attention to the fact that due to the limited
bandwidth of many of the available behind-the-ear hearing aids, where gain cannot be provided above 5kHz, amplification would not help in restoring audibility of these sounds. They suggested that frequency transposition/compression aids may be useful but are not suitable for all patients, and that cochlear implants do not really have this problem as the high frequency representation is much better due to usually guaranteed basal cochlear turn stimulation (Stelmachowicz et al., 2004). Ototoxic permanent progressive loss in children has been shown to cause significant emotional, educational and developmental difficulties. Gurney et al. showed that the 43/137-neuroblastoma survivors with hearing loss had twice the rate of educational difficulties and need for special education compared to their normal hearing peers in addition to reporting a significantly poorer quality of life (Gurney et al., 2007). There is limited research assessing the effect that ototoxic hearing loss has on the quality of life of paediatric cancer patients. These children already suffer from many other aspects of their disease that significantly deteriorates their health related quality of life (HRQL). Therefore this is an aspect of interest in this research project.

Figure 1-8: Frequency representation and approximate intensities of different categories of speech sounds (From (Hall and Mueller, 1997))
1.6 **Common tools used for measuring quality of life of patients**

The WHO published a position paper in 1995 related to Quality of Life (QoL) assessment. In it they defined QoL as "individuals' perception of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards and concerns" (WHOQOL Group, 1995). Measurements of QoL need to be multidimensional and applicable under all people to include different circumstances in life. QoL is generally defined as a person’s satisfaction or contentedness with life and is therefore affected by multiple factors including emotional well-being, expectations and environment. Health Related Quality of Life (HRQL) represents the domains of QoL associated with a person’s health (Lin and Niparko, 2006).

Measurement of HRQL can be made through two different types of instruments: health index surveys and health utility instruments. Health index surveys contain groups of items organized into scales each measuring a domain of HRQL such as physical, mental, and social well-being domains. Scores are compiled from questions associated with each domain and a single score or domain-specific domain score can be presented. These can be either generic or disease-specific aimed at understanding the health status of patients and assess the impact of a specific disease on the overall QoL of these patients. An example of this disease-specific health index survey is the Paediatric Audiology Quality of Life (PAQL) questionnaire, which was developed by Edwards et al. to assess aspects of QoL impacted by childhood deafness (Edwards et al., 2012). Health utility instruments try to capture a patient’s estimation of well-being by assigning a value to their current health status using a measure ranging between 0 -1 where 0 is equivalent to the death and 1 to perfect health. Health utility is measured through one of three utility metrics, which include visual analogue scales (VAS), time trade off or standard gamble (Froberg and Kane, 1989, Torrance, 1986). Health utilities provide a common metric to allow for comparisons to be made of the impact of different health interventions. A well-established popular measure is the Health Utilities Index (HUI) family of generic health profiles developed over many years at the McMaster University in Canada. Some health utility instruments have been adapted for children such as the EuroQoL EQ-5D and parent-proxy forms of the HUI. One of the studies in this research will utilize these instruments to assess the QoL of children with cancer suffering from ototoxicity and will be discussed in more detail later.
1.7 Genetic susceptibility to AG ototoxicity

1.7.1 Mitochondrial DNA (mtDNA)

The mitochondrial DNA is the only extra-nuclear component of the DNA in eukaryotic cells. It is much shorter than the nuclear DNA, being composed of around 16,600 base-pairs (bps) containing 37 genes and having a circular rather than a double helix structure (Figure 1-9). The other difference between it and the nuclear DNA, where there is only a single copy within each cell nucleus, is that there are 2-10 copies of the mtDNA within each mitochondrion. As there are hundreds to thousands of copies of mitochondria within each cell, there are thousands of mtDNAs in each cell. Mitochondria are the power source of the cell producing energy through a process called oxidative phosphorylation (OXPHOS). They are also involved in multiple cellular activities including regulation of apoptosis. Thirteen of its genes code for enzymes involved in oxidative phosphorylation whereas the remaining genes provide instructions for making transfer RNAs (tRNA) and ribosomal RNA (rRNA), which are needed for protein formation. Variations within the 12S rRNA gene have been repeatedly reported to be associated with non-syndromic deafness in addition to increased susceptibility to aminoglycoside ototoxicity. The most well documented mutation within the 12S rRNA gene is the A1555G (Shohat et al., 1999, Kupka et al., 2002b, Chen et al., 2012, Bottger, 2010, Prezant et al., 1993b). If the mutation is affecting all copies of mtDNA in the cell this is called ‘homoplasmy’ (i.e. present in all mitochondria of a cell and/or tissue), but if it only affects a fraction of these copies it is called ‘heteroplasmy’. mtDNA mutations are transmitted through the mother (matrilineal inheritance) with no father to son transmission.
1.7.2 mtDNA mutations and sensorineural hearing loss (SNHL)

Mitochondrial DNA has been associated with a wide variety of disorders of genetic origin including non-syndromic deafness and increased susceptibility to aminoglycosides leading to ototoxicity. Figure 1-10 shows a display of these associated disorders. Mutations in either the 12S rRNA (also called MT-RNRI) or the tRNA serine 1 (also called MT-TS1 or tRNA-Ser (UCN)) genes are associated with non-syndromic mitochondrial hearing loss and deafness. Mutations encoded in the 12S rRNA genes have been associated with predisposition to AG ototoxicity and/or late-onset non-syndromic SNHL. Mutations in tRNA-Ser (UCN) are usually associated with childhood onset of non-syndromic SNHL (although sometimes they are also associated with other manifestations such as palmoplantar keratoderma in some families). SNHL associated with AG ototoxicity is commonly bilateral, severe to profound, irreversible but not progressive, occurring within a few days to weeks following administration of any quantity of an AG antibiotic even when it’s still within the therapeutic drug levels. The hearing loss can even occur even after administration of a single dose of an aminoglycoside. Association with signs of vestibular ototoxicity is uncommon (Pandya, 1993, Bates, 2003, Bravo et al., 2006).
As mentioned earlier, the commonest 12S rRNA gene disease-causing mutation associated with AG ototoxicity is a homoplasmic single base-pair substitution from A to G at nucleotide 1555. Two further changes in this gene, m.961_962delTinsC(n), T1095C, T1291C, A827G, and a homoplasmic C-to-T transition at position 1494 (m.1494C>T), have also been associated with AG ototoxicity in some populations. The A1555G or the m.1494C>T mutations were reported to form a novel 1494C-G1555 or 1494U-A1555 base-pair (bp) at the highly conserved A-site of 12S rRNA making the human mitochondrial ribosomes more bacteria-like and altering binding sites for AGs (Guan, 2011, Human et al., 2010).

Mutations of tRNA-Ser (UCN) causing mitochondrial nonsyndromic hearing loss and deafness include m.7443A>G, m.7444G>A, m.7445A>C, m.7510T>C, m.7511T>C (Sevior et al., 1998, del Castillo et al., 2002, Hutchin et al., 2000, Pandya, 1993) in addition to other less common mutations.

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Figure 1-10: The disorder association with different parts of the mitochondrial genome. The variant type and position on the mtDNA is presented in association with each disease (i.e. transition/transversion, or insertion/deletion). LHON, leber’s hereditary optic neuropathy; MELAS, mitochondrial encephalopathy lactic acidosis with stroke-like episodes; MERRF, myoclonic epilepsy and ragged-red fiber; T2DM, type II diabetes mellitus. (From Wallace, 2005a).
1.7.3 A1555G mutation in the 12S rRNA gene and AG ototoxicity

Prezant et al. first proposed the connection between this mutation and non-syndromic deafness or increased susceptibility to aminoglycoside ototoxicity in 1993 (Prezant et al., 1993b). Changes in the structure of the 12S rRNA gene due to the A1555G mutation make it similar to the bacterial 16S rRNA gene providing an AG antibiotic binding site (A) and therefore allowing the AG to have its destructive bactericidal effect on the cells by disrupting the OXPHOS and increasing production of reactive oxygen species (ROC) activating apoptotic cell pathways and causing hair cell death. The mutation predisposes aminoglycosides-induced permanent SNHL reaching severe to profound levels that is independent of dose i.e. presenting with the SNHL phenotype from the first exposure to the antibiotic. This mutation is also associated with non-syndromic deafness and increased effect of age-related hearing loss (presbyacusis) even if the patient was not exposed to AGs (Vandebona et al., 2009). However, Bravo et al. showed that even though cochlear alterations, in the form of lowered DPOAE amplitudes, were present in all carriers of A1555G mutation, the phenotypic expression of this mutation was extremely variable. They varied with age of onset and severity of hearing loss, ranging from profound deafness to subclinical presentations of normal hearing (Bravo et al., 2006).

*Prevalence of A1555G mutation:*

The reported prevalence of the A1555G mutation varied mainly due to whether the prevalence was reported in population-based versus pedigree studies investigating the prevalence of this mutation in patients with hearing loss and their families. Prevalence also varied with different ethnic groups. Regarding the reported prevalence in population-based studies; in the UK Bitner-Glindzicz et al. genotyped the A1555G variant in the Avon Longitudinal Study of Parents and Children (ALSPAC) birth cohort, a cohort of 7-9 years old children who were not selected for hearing loss (Bitner-Glindzicz et al., 2009). They found the mutation in 18 children out of 9371 equivalent to a prevalence of 0.19% (95% CI, 0.10 to 0.28) i.e. 1/520. Rahman et al. also found the mutation 19/7350 of the British 1958 birth cohort of 44-45 years old adults reporting a prevalence of 0.26% (95% CI, 0.14 – 0.38%) i.e. 1/385 (Rahman et al., 2012). Vandebona et al. identified a positive genotype in 6/2856 in an Australian population (Blue Mountains hearing study cohort) equating a similar prevalence of 0.21% (95% CI, 0.08 to 0.46) i.e. 1/500 (Vandebona et al., 2009). In a control group of 439 South African subjects representing the major four ethnic groups in South Africa (n=93Afrikaner, n=104 Caucasian, n=112 Black and n=130 Mixed ancestry) a
prevalence of the A1555G mutation of 1/112 (0.9%) of the Black and 1/93 (1.1%) of the Afrikaner ethnic groups was recorded (Human et al., 2010).

On the other hand, pedigree studies involving patients with hearing loss reported a higher prevalence of this mutation. The most extensive research on this topic was reported from China with variability in prevalence and penetrance identified. An example includes research by Dai et al. who examined 16 Chinese pedigrees (a total of 246 matrilineal relatives) with AG-induced hearing impairment reporting a prevalence of 0.4-1.8%, with an average of 0.8% (19/246) but also reported an prevalence of very low penetrance of hearing loss with this mutation yet confirming that aminoglycosides are the major modifier factor for the development of deafness. In addition, Lu et al. identified 69 subjects harboring the A1555G mutation in a cohort of 1642 hearing-impaired Han Chinese pediatric subjects from Zhejiang Province, China with AG-induced and non-syndromic deafness equivalent to 3.96% prevalence (Lu et al., 2010a, Dai et al., 2006). The highest prevalence was reported in Spanish populations where Estivill et al. studied 70 families with SNHL and reported that 19 families had the m.1555 A>G mutation of which 12 had history of AG exposure. Gallo-Terán et al. also reported a frequency of A1555G mutation in 6/21 (29%) patients affected by late-onset non-syndromic SNHL from Cantabria and in 15/72 (20.8%) maternal relatives of subjects with non-syndromic deafness (Gallo-Teran et al., 2002, Gallo-Teran et al., 2003, Estivill et al., 1998). However, the A1555G mutation was not detected in 45 familial and 77 sporadic cases of nonsyndromic hearing loss in an Austrian Caucasian ethnic group and in 3/955 (0.3%) deaf probands ascertained throughout the United States. This confirmed that the prevalence is not generally high in Caucasians as was reported in the Spanish hearing loss population (Ramsebner et al., 2007, Arnos, 2003)

Regarding the prevalence of A1555G mutations in CF patients, Conrad et al. sequenced the 12S rRNA gene in 157 North American adult CF patients and identified two (1.3%) subjects with the mutation exhibiting severe-profound SNHL from non-toxic exposure to tobramycin and identified other known and new variations in the gene which were associated with deafness. They commented that this prevalence was higher than the commonly reported 0.2% (Conrad et al., 2008). As this was the only study identified to actually assess the prevalence of mitochondrial mutations in CF patients it would be really interesting to see if the same conclusions would be reached when assessing the prevalence of A1555G mutation in a group children with CF.
**Penetrance and expressivity of A1555G mutation:**

Penetrance is a calculation of the proportion of subjects in a population who carry the disease-causing mutation of interest and those who express the disease phenotype. In this case it is the proportion of individuals that carry the A1555G mutation and are affected by hearing impairment. Variable expressivity is when the phenotypic presentation of the disease varies between different individuals having the same genotype e.g. in the degree/severity of hearing loss, age of onset, and response to different levels of exposure to aminoglycosides. Research has shown that several factors have been identified that affects the penetrance, age of onset and the phenotypic expression of individuals harboring this mutation. As presented earlier, exposure to aminoglycosides was shown to be one of the most significant modifying factors leading to expression of hearing loss even with exposure to a single dose and up to 100% penetrance of this mutation (Bitner-Glindzicz et al., 2009, Pandya, 1993). Individuals with the mutation but no history of exposure to AGs may still present with non-syndromic hearing loss at a median age of 20 years with around 40% exhibiting hearing loss by the age of 30 years and 80% by the age of 65 years (Estivill et al., 1998). Other modifying factors include different mitochondrial haplotypes, major nuclear modifier genes, mtDNA variations and environmental factors. Many other researchers confirmed 100% penetrance in subjects with positive genotype and confirmed exposure to AGs (Veenstra et al., 2007, Pandya et al., 1997, Fischel-Ghodsian et al., 1997, Estivill et al., 1998, Bitner-Glindzicz et al., 2009, Ballana et al., 2006). Even the articles that reported a low penetrance of hearing loss in specific populations such as the Han Chinese pedigrees (average penetrance 5.3%, range 0-17%), it was still reaffirmed that aminoglycosides appeared to be a major modifying factor for the phenotypic expression of the A1555G mutation in these Chinese families. It is also worth noting that these studies were not actually designed to calculate penetrance as they were retrospectively analyzing data from hearing loss groups and their matrilineal relatives and therefore were biased towards subjects who express the disease phenotype (Tang et al., 2007, Guan, 2011, Dai et al., 2006).
**Association between A1555G mutation and ototoxicity but not nephrotoxicity**

Rahman et al. hypothesized that the main reason why this mutation is mainly associated with cochleotoxicity and not vestibulotoxicity or nephrotoxicity is related to the cochlea’s unique endocochlear potential (EP) and function of the stria vascularis. The stria vascularis is the epithelial layer of cells lining the lateral wall of the cochlear duct and is highly metabolically active with cells rich in mitochondria. Its main role is the active energy-dependent transport of ions, mainly potassium, into the endolymph to produce a positively charged EP of +80 mV, which is essential for inner hair cell transduction. AGs are normally at lower concentrations within the endolymph because there are positively charged, as is the endolymph. The presence of the A1555G mutation has a significant effect on the mitochondria rich stria vascularis cells leading to decreased OXPHOS, accumulation of ROS and initiation of apoptosis, which decreases the EP. This in turn aids in the attraction and accumulation of the positively charged AGs inside the endolymph. They are then transported through the apical mechano-transduction channels of the hair cells where they accumulate and have a long half-life lasting up to six months following the last intake of these drugs causing ototoxicity from what appears as ‘normal’ drug levels (Marcotti et al., 2005, Gale et al., 2001). The AGs again attach to the mutation site in the hair cells’ mtDNA and reduce OXPHOS, increase ROC and generate AG-iron complexes, which induce hair cell death. As the EP is unique to the cochlea and not in the vestibular or renal apparatus the effect of the mutation appear to be restricted to enhancing cochleotoxicity (Usami et al., 1997, Rahman et al., 2012).
Figure 1-11: Mechanism that is proposed for enhanced sensitivity of the cochlea to aminoglycosides.

In the cochlea: (A) Normal function of stria vascularis with maintenance of active K+ transport into the endolymph to keep the EP positively charged at +80mV. (B) Main effect is on stria vascularis -> decrease in ATP and inaccurate translation of OXPHOS proteins -> decrease in K+ and EP (norm +80mV) -> increase in AG uptake -> (C) increase entry into hair cells through mechanotransduction channels -> more ROS & generation of iron species (toxic Fe-AG complex) -> apoptosis. Half-life of AG in hair cells is very long (6 mths). AG, aminoglycosides; I–V represents complexes I–V of the mitochondrial OXPHOS system. (From: Rahman et al, 2012)
1.7.4 Drug metabolizing genes and ototoxicity

Pharmacogenetics is the study of the role of inheritance in individual variation in drug response. This individual variation can result in a phenotype spectrum that can range between adverse drug reaction at one end and a lack of therapeutic efficiency at the other end. Pharmacogenetics has quickly evolved into pharmacogenomics due to rapid development in human genomics (Wang and Weinshilboum, 2008, Weinshilboum, 2006, Weinshilboum and Wang, 2005, O’Kane et al., 2003). For many medications, these inter-individual dissimilarities result in part from polymorphisms in genes that encode drug-metabolizing enzymes, drug transporters, and/or drug targets (such as drug receptors or enzymes). Pharmacogenomics is a growing field aimed at revealing the genetic basis of differences in drug efficacy and toxicity through the use of genome-wide or candidate-gene approaches to identify the group of genes that govern an individual’s reaction to drug therapy (Evans and Relling, 2004).

There is substantial inter-individual variation in ototoxicity in individuals receiving similar doses of ototoxic drugs like cisplatin and aminoglycosides. This may be explained by the hypothesis behind pharmacogenomics in that some individuals have polymorphisms in genes encoding drug-metabolizing enzymes that render them more or less susceptible to the ototoxic effect of these drugs.

Ross et al. published in Nature Genetics in 2009 a study on the pharmacogenomics of cisplatin and ototoxicity. This study used a candidate gene approach and tested the top 220 genes associated with the absorption, distribution, metabolism and elimination (ADME) of drugs. They used an Illumina GoldenGate assay to genotype 1,949 SNPs of these drug-metabolizing genes to assess if any of these variants were associated with cisplatin ototoxicity within two study groups of children with cancer in Canada. They used an initial discovery cohort of 54 children treated in a paediatric oncology unit at the BC Children’s hospital in Vancouver and then ran the same assay with a second replication cohort of 112 children recruited through a national surveillance network of adverse drug reactions in Canada. By using this tiered analysis strategy they were aiming to increase power in the discovery cohort as there was less biasing and then used the replication cohort to ensure generalizability of the clinically significant findings and minimize the likelihood of false positives. Variants in two genes were discovered. TPMT gene (thiopurine S-methyltransferase) variants rs12201199, rs1142345 and rs1800460 (confer odds ratio OR: 16.9, 11.0 and 18.0 respectively) and COMT gene (catechol O-methyltransferase) variants rs9332377 and rs4646316 (OR: 5.5 and 15.0 respectively) were found to be significantly (p<0.01) associated with cisplatin ototoxicity. COMT gene is present on Chromosome 22.
and the TPMT gene is on Chromosome 6 (Ross et al., 2009). Similar involvement with aminoglycoside ototoxicity was investigated in this current research.

TPMT and COMT are methyltransferases dependent on the S-adenosylmethionine (SAM) methyl donor substrate in the methionine pathway. The TPMT gene encodes the thiopurine S-methyltransferase enzyme, which is known to have a significant role in the metabolism of thiopurine cytotoxic drugs such as azathioprine, 6-mercaptopurine (6MP) and 6-thioguanine through AdoMet-dependent S-methylation. Variants of this gene that are associated with decrease or loss of function can lead to accumulation of these thiopurine drugs leading to bone marrow toxicity which consequently leads to anaemia, leucopenia, bleeding tendencies and infections due to myelosuppression (Fujita and Sasaki, 2007, Evans, 2004). The COMT gene is known to regulate catecholamines such as epinephrine, norepinephrine and dopamine, which have important functions as hormones and neuromodulators in the brain.

The variants implicated in cisplatin ototoxicity included TPMT rs12201199, rs1142345 (TPMT*3C Tyr240Cys) and rs1800460 (TPMT*3B Ala154Thr) – with carriers of both of the latter two being defined as carriers of the TPMT*3A haplotype, and COMT rs4646316 and rs9332377. These variants were described as loss-of-function variants that decrease the function of normal TPMT and COMT enzyme activity. This reduced function leads to increased levels of S-adenosylmethionine (SAM), which was also previously shown to increase nephrotoxicity by 3-6.2 fold when administered with cisplatin (Ochoa et al., 2009). Cisplatin normally causes cell death in rapidly dividing cancer cells by forming intra- and interstrand DNA cross-links when binding thiol-containing compounds and purines and inducing apoptosis. The authors proposed that TPMT and COMT are involved in reduced inactivation of cisplatin-purine compounds, which consequently enhances cisplatin cross-linkage and therefore cytotoxic effect on inner hair cells. However, cisplatin is not a thiopurine drug and a direct mechanistic pathway linking the TPMT and COMT polymorphisms and cisplatin toxicity was not clearly presented in the Ross et al. study. Other concerns were raised regarding the statistical analysis of some of the data in this study. One of the main concerns was related to the concept that no significance was given to the fact that more than half the ototoxicity cases had concomitant exposure to vincristine, which has also been linked to ototoxicity and therefore should have been considered as a confounding factor (Boddy, 2013). On the other hand, this publication had significant impact as the US Food and Drug Administration (FDA) has recognized this work by implementing a change in the Cisplatin product label in 2011 to include a new safety warning related to the association of TPMT gene variants and risk of cisplatin-induced ototoxicity in children.
The same Canadian group in a new cohort of 155 children with cancer replicated this work. The associations were replicated for genetic variants in TPMT (rs12201199, \( p = 0.0013, \) OR 6.1) and for other variants including the ATP-binding cassette transporter C3 (ABCC3) (rs1051640, \( p = 0.036, \) OR 1.8). They also showed that the prediction of the risk of hearing loss was improved using a novel predictive model including genetic (TPMT, ABCC3 and COMT variants) in addition to clinical variables (patient’s age, vincristine treatment, germ-cell tumour, and craniospinal irradiation) compared to a model with clinical variables alone (Pussegoda et al., 2013). Conversely, a separate group from the USA also replicated the work in 213 children with medulloblastomas and another independent cohort of 41 children with solid-tumours, however they failed to establish any significant association between TPMT or COMT variants and cisplatin-induced hearing loss. They also performed laboratory investigations with TPMT knockout (KO) vs. wild-type mice and found no difference in functional hearing loss or hair cell damage between the two following cisplatin treatments. They also showed that neither TPMT nor COMT variants were associated with cisplatin cytotoxicity in lymphoblastoid cell lines (Yang et al., 2013). The Yang et al. study doesn’t just contradict the two Canadian studies but it stresses the critical need for independent validation studies especially when outcomes are associated with possibilities of major changes in clinical management.

I was interested in whether these enzymes were also associated with aminoglycoside ototoxicity. The authors’ proposed mechanism of action of these TPMT and COMT variants is not fully established and a clear definition of how they induce, or are linked, with cisplatin ototoxicity is not proved. Additionally the fact that many other studies have shown that there are common apoptotic cell death pathways induced by cisplatin, aminoglycoside and even noise-induced hearing loss (Cheng et al., 2005, Rybak, 2007), offers a rationale for investigating the role of TPMT and COMT polymorphisms in aminoglycoside ototoxicity.

**Other possible evidence of involvement of TPMT and COMT with ototoxicity:**

A better understanding of the normal function of these enzymes may be useful in identifying how they are linked with ototoxicity.

Du et al. identified a previously annotated gene on chromosome 7 which had a similar function as COMT (chromosome22q11), which they called COMT2 (Du et al., 2008). COMT2 was shown to be highly expressed in inner ear sensory hair cells (OHCs, IHCs and vestibular hair cells) without detectable expression elsewhere in the nervous system. Mice homozygous for a missense mutation in COMT2 show vestibular impairment, profound
sensorineural deafness and progressive degeneration of the cochlea. They showed that defects in catecholamine modification by COMT are not just implicated in the development of schizophrenia, but identified a role for catecholamines (specifically dopaminergic neurotransmitters) in the function of auditory and vestibular sense organs in mice and humans. Whether or not these dopaminergic neurotransmitters are affected by the apoptotic or necrotic cell death pathways induced by cisplatin and aminoglycosides is the next question that needs to be answered.

Pharmacogenomic studies have shown that TPMT has 21 already described polymorphisms or mutations that may have an effect on its function. Studies have shown that the functional effects are primarily caused by a decrease in the levels of TPMT enzyme protein. Further work (Salavaggione et al. 2005) has shown that the level and quality of enzyme protein of wild type (WT) TPMT was much higher than for different described allozymes like TPMT *3A variants which showed little or no protein. Other common genetic polymorphisms that alter only one or two amino acids have also been observed to be associated with a similar phenomenon (Weinshilboum, 2006). This led to the following question of: what caused the drop in protein levels in these polymorphisms? Several studies have reported that it may be due to decreased production, increased mRNA instability or increased protein degradation. These studies have implicated the accelerated protein degradation to be the major contributor to this outcome (Weinshilboum and Wang, 2004, Tai et al., 1999). This then led to the question of how one or two amino acid mutations within; for example, the 245 amino acids constituting the TPMT protein are ‘recognized’ by the cell leading to its degradation. Wang et al. showed that there are associated molecular chaperones such as heat shock protein 90 (hsp90) and hsp70 and heat shock organizing protein (hop) that are much more highly associated with TPMT allozymes as TPMT*3A than with WT variants. This also agreed with the more generalized observation that complexes involving molecular chaperones and their client proteins participate in a cellular ‘protein quality control’ mechanism that can either lead to proper folding of the client protein or target the misfolded protein for proteasome-mediated degradation (Wang et al., 2005).

Molecular chaperones, specifically hsp70, hsp27 and hsp32 have been shown to have an inhibitory effect with cisplatin and aminoglycoside ototoxicity. Taleb et al. showed that heat shock results in significant inhibition of both cisplatin- and AG-induced hair cell death as it can inhibit JNK- and caspase-dependent apoptosis. They showed that Hsp70 is the most strongly induced Hsp, which was unregulated over 250-fold at the level of mRNA 2 hours after heat shock. Hsp70 overexpression inhibits aminoglycoside-induced hair cell death in vitro (Taleb et al., 2009). Therefore, the relationship between heat shock proteins and TPMT
polymorphisms may offer a possible explanation for a pathway linking these genetic variations with ototoxicity.

1.8 Conclusion:

As seen from the topics introduced above, research in the field of ototoxicity is extensive and varied assessing multiple aspects of this important and preventable cause of inner ear damage. The current research aimed to assess some of these avenues of research in ototoxicity, specifically in children, to improve understanding and possibly make recommendations for better clinical management of this condition if needed.

To summarize, it is established that ototoxic medications, especially aminoglycosides and chemotherapeutic agents such as cisplatin, significantly damage hearing yet there is still a discrepancy in the recommended and commonly used audiological tests used to detect this effect. It would be beneficial to establish whether a test battery including extended high frequency audiometry and DPOAEs would be better at detecting early or milder cases of ototoxicity than the commonly used standard audiometry. It has been established that patients with cystic fibrosis are potentially exposed to high and repeated levels of aminoglycosides to combat pulmonary exacerbations yet the reported prevalence of ototoxicity in this patient groups is highly variable, ranging from 0 to 51% in adults and 0 to 6% in children. Some literature even implies that the prevalence of ototoxicity in this group is much lower than expected suggesting that a possible ‘otoprotective’ function of this genetic disorder may exist.

Several factors have highlighted the need for more investigation into the assessment of ototoxicity in CF patients in the UK. These include: (i) The variability in reported prevalence; (ii) the minimal guidelines provided within the UK CF Trust document on ‘Antibiotic Treatment for cystic fibrosis’ recommending that only ‘An annual pure tone audiogram should be considered for patients receiving frequent courses of an intravenous aminoglycoside’; and (iii) the absence of UK national guidelines for ototoxicity monitoring. It must however be acknowledged that there is excellent research already undertaken in this field in the UK but there is a need to confirm whether the recommended outcomes of this research is actually translated into clinical practice.

Assessment of prevalence; the benefits of use of different audiological tools; and the search for possible factors that increase the risk of ototoxicity in these patients are all areas of research that could be investigated further. The possible risk factors increasing susceptibility to ototoxicity suggests investigating the prevalence of genetic mutations or variations that
may be associated with this increased susceptibility. As discussed above, mitochondrial DNA mutations, specifically A1555G mutation of the 12S rRNA gene have been associated with increased susceptibility to aminoglycoside ototoxicity and yet only one research article investigated this prevalence in patients with CF (Conrad et al., 2008). This article only presented data from adult CF patients, it would be interesting to assess the prevalence of this mutation in a group of children with CF. There was also only one article published that established a significant association between specific drug-metabolizing genes and ototoxicity in children with cancer receiving cisplatin. It would therefore be interesting to investigate whether a similar association is established with AG ototoxicity.

Patients with cancer receiving ototoxic chemotherapeutic drugs such as cisplatin are also an important and interesting group of patients to investigate. Ototoxicity monitoring in this patient group is slightly more established yet there is very limited research investigating the effect the extra disability of hearing loss has on the general well-being and quality of life of these patients. This is an especially important question for children as a permanent hearing loss can potentially affect their speech and language development, social and psychological development and their ability to effectively communicate within their usually highly noisy and distracting environments. As higher numbers of these patients, who also have evidence of auditory monitoring to confirm ototoxicity, can be accessed, a good sample size can be achieved to assess if a significant change in the quality of life of these patients occurs due to the hearing loss. It would be interesting to see if similar outcomes could be extrapolated for other patient groups such as those with CF. It would also be very interesting to establish the current practice in the UK regarding monitoring for ototoxicity in these patient groups to identify if more work is needed to actually translate research evidence into clinical practice.
Aims of the research
Chapter 2: **Aims of the research**

2.1 **Overview**

This research project aimed to identify and investigate the different elements that affect the management of patients at risk of ototoxicity. The work undertaken had three main themes. The first theme aimed to observe the prevalence of ototoxicity in a population of children with CF, who are commonly exposed to ototoxic aminoglycosides, with the added aim of validating and applying the most appropriate tools for the audiological detection of ototoxicity. The second theme aimed to identify possible causative factors that may be responsible for wide-ranging susceptibilities of these children to ototoxicity, with two main areas of investigation: i) analyzing patients’ history and ii) testing for genetic variants commonly associated with increased susceptibility to ototoxicity. The third and final theme aimed to assess the clinical impact of ototoxicity on patients, in addition to assessing the current practice in monitoring ototoxicity from the clinicians’ perspective. The ultimate aim of the whole study was to gain a better understanding of these aspects of ototoxicity in order to make informed, clear recommendations for the implementation of the best and most suitable ototoxicity monitoring service for susceptible patients.

2.2 **Themes of the research project:**

2.2.1 **Theme A: Audiological assessment and assessment tools**

A clinical observational study investigated aminoglycoside ototoxicity in patients with CF. The main aims of this work were:

- To assess the auditory status of children with CF with different levels of exposure to aminoglycosides using an audiological test battery of objective and subjective tests in order to identify the prevalence of ototoxicity in this cohort.
- To compare the performance of the different audiological tests used and identify the most effective audiological tools in early identification of ototoxicity.
The second aim prompted further research into factors that may affect the reliability of DPOAE testing as an objective audiological tool for monitoring ototoxicity. A control study were undertaken to:

a. Investigate the short-term repeatability of DPOAE recordings in school-aged normal hearing children. This aimed to assess the repeatability of DPOAE recordings after removal and re-insertion of the OAE probe.

b. It also aimed to calculate the standard error of measurement (SEM) beyond which a change would be considered as a true change in OHC function due to ototoxicity or any other inner ear disorder.

2.2.2 Theme B: Causation

Aimed at identifying possible causative factors that can be associated with the occurrence of ototoxicity in the population of children with CF included in the audiological observational study above. The two main areas of investigation were:

i) Analysis of the clinical history of the CF patients with the aim of identifying factors such as age or drug dosage that may be significantly associated with occurrence of their ototoxicity.

ii) Genetic analysis of biological samples obtained from these children in order to identify if an association between previously reported mutations, known to increase susceptibility to ototoxicity, and children with ototoxicity in this study population existed. The genetic analysis aimed to:

- To assess the prevalence of the A1555G mutation in the 12S rRNA gene in the mitochondrial DNA in children with CF and assess its association with aminoglycoside ototoxicity. This mutation is the most well documented mutation to be associated with increased susceptibility to aminoglycoside ototoxicity.

- To sequence the 12S rRNA gene in the children identified with the A1555G genotype to identify any other known or new variations within this gene were present.

- To assess the prevalence of variants rs12201199 of TPMT and rs4646316 of COMT genes in CF children and assess if they are associated with aminoglycoside ototoxicity. These two variants were shown to be significantly associated with cisplatin ototoxicity.
2.2.3 Theme C: Impact of ototoxicity and current service provision

Aimed at assessing the impact of ototoxicity on patients in order to assess if more effective ototoxicity monitoring would be justified by the benefits. An investigation into the effect of hearing loss on the quality of life of paediatric cancer survivors receiving ototoxic chemotherapeutic agents was undertaken. The aim was to assess the difference in the reported quality of life of oncology children with and without associated ototoxicity using validated parent-proxy generic and customized quality of life questionnaires.

A further aim of this theme also included assessing current clinical practice in the UK in relation to ototoxicity monitoring. A survey of Oncology, Audiology and CF services in the UK was done to assess the current UK practice regarding auditory monitoring for ototoxicity, in the absence of nationally agreed guidelines, using online questionnaires.
Material & Methods
Chapter 3: **Material and Methods**

### 3.1 Study populations

The main study populations were recruited at Great Ormond Street Hospital (GOSH) to address several aims of this project. They included children with CF from the CF Unit, Respiratory Medicine Department (for Themes A and B) and the children with cancer from the Oncology Department (for Theme C).

Two control groups were recruited for the studies in Theme A investigating the short-term repeatability of DPOAE recordings in normally hearing school-aged children and investigating the effect of changes in ear canal pressure on OAE recordings. For the former study, 60 normal hearing healthy primary school-aged children (7-11 year-olds) were recruited from Abbey Meads community primary school in Swindon. For the later study, eight normal hearing healthy adults were recruited from students and staff members of the Ear Institute.

In addition to the recruited subjects or patients mentioned above, healthcare professionals including audiological professionals, oncology and CF clinicians were included in the final survey study in Theme C, which aimed at evaluating the current provision of ototoxicity monitoring services in the UK.

Ethics approval was obtained from the appropriate ethical committees before commencing any of the studies. These are all listed with each equivalent part of the methodology below.

### 3.2 Materials:

Typically equipment used for audiological assessment in all studies:

- **Otoscopy:**
  - Welch Allen Otoscope (Guymark UK Limited, UK)
- **Tympanometry:**
  - Grason-Stadler GSI TympStar diagnostic tympanometer, version 1 OR
  - Grason-Stadler GSI 33 middle ear analyzer
• Audiometry:
  o Standard:
    ▪ GSI-61 diagnostic audiometer with Telephonics TDH-39 supraural headphones (Guymark UK Limited, UK) OR
    ▪ Kamplex KC50 clinical Audiometer with TDH-39P headphones (Interacoustics, PC Werth, UK)
  o High-frequency:
    ▪ GSI-61 diagnostic audiometer with Sennheiser HDA200 circumaural headphones (Guymark UK Limited, UK)

• DPOAE & TEOAE
  ▪ Otodynamics ILO292: USB DP-Echoport equipment (Otodynamics, Hatfield, UK)

Equipment used in the Genetics Study (Theme B) is listed in section: 3.5.2.2.
3.3 **Methods:**

The methodology used for each of the studies undertaken will be presented under the three corresponding themes highlighted in the aims section. The same layout will also be used for the results/discussions chapters that will follow.

3.4 **Theme A: Audiological assessments and assessment tools**

3.4.1 Clinical observational study investigating aminoglycoside ototoxicity in patients with CF

Ethics approval was granted for this study through the NHS ICH/GOS Research Ethics Committee, REC reference number: 07/Q0508/21

This study aimed to assess the auditory function of children with CF with a variable history of exposure to aminoglycosides:

- To assess the prevalence of ototoxicity in this patient group,
- To recommend the most appropriate audiological test battery to allow for early identification of ototoxicity,
- To assess the possible risk factors that would make the affected children more susceptible to the ototoxic effect of these antibiotics (results of this aim were presented within Theme B)

A cross-sectional study design of this observational study was adopted.

3.4.1.1 Subjects for Theme A:

Children with confirmed CF (positive sweat test or genetics) (De Boeck et al., 2006) aged 4 – 16 years, were recruited from the paediatric CF clinic at Great Ormond Street Hospital (GOSH), London, UK. Inclusion criteria were limited to the child being ≥4 years old; have a confirmed diagnosis of CF irrespective of whether they already received aminoglycosides or not; with a negative history of previous ear infections or surgery; for parent and child to voluntarily consent to join the study and to come for the audiological assessment at the Audiology department, GOSH. There was no requirement to have a specific history of
exposure to aminoglycosides. Children with negative history of exposure were considered as a control group and were assessed to confirm whether CF as a disorder was associated with increased prevalence of hearing loss, compared to normal healthy subjects, or not. Informed consent was obtained from the parents and the child (where appropriate). Full ethical approval was obtained for the study from the hospital ethics committee.

3.4.1.2 Retrieval of patient information in relation to ototoxicity:

This data was obtained through verbal interviews with the children’s parents/carers; through review of annual review reports, discharge letters and clinical notes; and through review of patient records of haematological tests for therapeutic drug monitoring (TDM) of the aminoglycosides administered while the children were admitted to GOSH. History taking was performed and the following information was obtained for each patient through their parent/carer by a verbal interview: number and type (e.g. amikacin, tobramycin and gentamicin) of i.v. AG courses received and duration of exposure; history of hearing problems (including hearing loss, tinnitus or ear infections), balance problems, nasal, sinus or renal problems and intake of other ototoxic medication. The history of having nasal polyps or sinus disease were not exclusion criteria but were asked about as they are conditions that may be associated with or predispose the occurrence of middle ear infections. Measurements of forced expiratory volume in 1 second (FEV$_1$) obtained within the previous year were recorded and confirmation of AG drug type, number of courses, years of exposure and drug trough levels for TDM, were obtained from hospital records. Drug trough levels were obtained from a blood sample drawn within 30 minutes before the intake of the 2nd and 8th doses of AG using a once-daily (pulse dosing) regimen. The UK Cystic Fibrosis Trust recommended measuring a trough level, specifying that a trough/pre-dose tobramycin concentration of < 1 μg/mL should be accepted as per the TOPIC study (Smyth et al., 2005) while the goal trough concentrations for gentamicin was considered to be <0.5–1 μg/mL (Mohamed et al., 2012, Rao et al., 2011) and 4-10 μg/ml for Amikacin (Begg et al., 2001).

3.4.1.3 Audiological Test Battery

Each patient underwent the following hearing tests:

3.4.1.3.1 Otoscopy and tympanometry with acoustic reflexes

Otoscopic examination, tympanometry and stapedial reflex thresholds using Grason-Stadler (GSI-33) immittance equipment (Guymark, UK Ltd) were performed to exclude external and middle ear problems. Normal middle ear function was defined as a type ‘A’ Tympanogram
with the BSA agreed pediatric normative range of -150 to +50 daPa peak pressure and 0.3 to 1.6 cm\(^3\) static compliance (BSA, 1992).

Both ipsilateral and contralateral acoustic reflex thresholds for frequencies 0.5, 1, 2 and 4 kHz were recorded. Patients were retested on a separate occasion if they had evidence of middle ear disease. If there was persistent evidence of middle ear disease as seen by abnormal Otoscopy or tympanometry results, the patient was excluded from further analysis.

Assessment of middle ear function of the CF patients was made to investigate the rate of occurrence of middle ear infections (otitis media) in this group and compare the results with previously published data of presumably ‘normal hearing children’ such as the Avon Longitudinal Study of Parents and Children (ALSPAC) birth cohort, a cohort of children who were not selected for hearing loss (Bitner-Glindzicz et al., 2009).

### 3.4.1.3.2 Standard and extended high frequency pure-tone audiometry (PTA)

Standard PTA frequencies of 0.25-8 kHz were tested at 1-octave steps using a GSI-61 diagnostic audiometer (Guymark, UK Ltd) and Telephonics TDH-39 supra-aural earphones. Regular calibration of the audiometer using the TDH-39 earphones for frequencies (0.125-8 kHz) was performed in accordance with the EN 60645-1:1995 specification and for the EarPhone SPL’s: Reference equivalent threshold sound pressure levels (RETSPL) + 60dB + NPL Correction using the ISO 389-1 specification.

Extended high-frequency (EHF) audiometry was performed to measure hearing thresholds for frequencies ranging from 9-16 kHz at 1/6\(^{th}\) octave test frequencies, using the same GSI-61 audiometer and Sennheiser HDA200 circumaural high frequency earphones. Regular calibration of the Sennheiser HDA200 earphones for high frequencies (8-16 kHz) was performed using the EN 60645-1:1995 specification and for the EarPhone SPL’s: RETSPL + Reference HL level + NPL Correction + LD2559 mic Correction using the ISO 389-5:1998 specification.

Testing was carried out in standard soundproof rooms of the hospital audiology department satisfying the criteria for ambient noise levels of <30dBA during audiometric testing. Care was taken to allow for accurate placement of the headphones so that the diaphragm was in line with the concha then the headband was tightened accordingly. This was done to decrease the test-retest differences to within the clinically acceptable range of ±10dB (Hunter et al., 1996). Thresholds were defined as the quietest sound that the child could hear 50% percent of the time with a minimum of two ascending responses at each frequency using conditioned play audiometry, or standard methods, depending on the child’s age and development.
3.4.1.3.3 Distortion-product otoacoustic emissions (DPOAE) testing

This was measured using commercial Otodynamics ILO292:USB DP-Echoport equipment. Two simultaneous pure-tone signals were presented at 2 different frequencies ($f_1$ and $f_2$, where $f_2 > f_1$) with $f_1:f_2$ ratio of 1.22 and at intensity of 65 and 55 dB SPL respectively. The intensity of the $2f_1 - f_2$ intermodulation distortion product components were measured as a function of $f_2$ frequency at 1/2-octave frequency intervals ranging between 1 and 8 kHz and were presented in the form of DP-grams.

Calibration of the probe was performed regularly using the manufacturer’s recommended procedure and the small cavity provided. The total duration of recording per ear was ~2 minutes. As the recording was performed in a quiet room at the audiology department, the noise floors during the measurements were typically quite low ranging between -20 ± 5 dB SPL over the 1-8 kHz regions. DPOAE responses were considered valid when signal/noise ratio was >6 dB SPL. The children were asked to sit quietly while a small probe, placed in the ear canal, recorded the DPOAEs.

3.4.1.4 Procedure

3.4.1.4.1 Recruitment

CF children were recruited with the aid of the CF nurse specialists and other members of the CF unit. They were approached while they attended the CF 3-monthly or annual review clinics. The CF outpatient clinic is held on a weekly basis and takes place in the Outpatient Department on the first floor of the Royal Homeopathic Hospital on Great Ormond Street on Tuesday afternoons. Patients were also approached while being admitted to Badger Ward at GOSH, usually for a planned course of i.v. antibiotics.

At that initial introduction, the patients and their parents were given the age-appropriate information sheet and consent form and a verbal explanation of the study aims and procedures to be done. The parents were advised to read the information sheets at home and once they are satisfied were instructed to complete the written consent form and to send it back in the enclosed stamped envelope.

On receipt of the consent form, the date of the patient’s next CF outpatient appointment was checked and an extra appointment for audiological assessment was booked either one hour before or after their CF clinic. This avoided patients having to undertake an extra journey to the hospital just for the audiological assessment. With regards to the DNA sample needed for the genetics part of the research (Theme B), the blood samples were collected through
coordination with the outpatients’ phlebotomist responsible for these clinics or with the ward nurse responsible for the child.

3.4.1.4.2 Audiological assessment

On arrival to the audiology department at GOSH, history taking was done followed by a full test battery of audiological assessments as previously detailed in section: 3.4.1.3.

At the end of the assessment, all the test results were explained to the patient and their parent/s and two copies were made of the test results – one for the parent to keep for future reference and the other to add to the patient hospital notes (investigations section).

3.4.1.4.3 Reporting back to the CF Unit

If any patient fulfilled the criteria for ototoxicity through the audiological tests, as stated below, this information was relayed directly to the supervisory consultant (Dr. Ranjan Suri) who would then take the necessary action of reviewing the patient’s current medications and ensure continuous monitoring of their audiological status.

3.4.1.5 Patient Grouping

In accordance with the history of exposure to i.v AGs, each patient was placed into one of three groups: the first is the ‘non-exposure’ group for patients with no previous history of exposure to AGs; the second is the ‘low-exposure’ group for patients with history of exposure to <10 i.v AG courses in their lifetime. The last group is the ‘high-exposure’ group with history of intake of ≥10 i.v AG courses in their lifetime. The cut-off threshold of 10 i.v. AG courses to divide groups into low and high exposure groups was based on previous studies including Mulheran and Degg who showed that the median number of i.v. AG courses received in patients with CF and normal hearing was 9 whereas it was 20 for those with evidence of ototoxicity (Mulheran and Degg, 1997a, Mulheran et al., 2001, Tan et al., 2003). Following the audiological assessment of the patients, they were further grouped into ‘ototoxic’ and ‘non-ototoxic’ groups.

Determination of Occurrence and Severity of Hearing Loss

Hearing loss was assessed using the ASHA criteria for both standard and EHF PTA (Table 1) with severity of hearing loss determined using the British Society of Audiology (BSA) audiometric descriptors (BSA, 2011), which define that pure-tone average thresholds of ≤20 dBHL is considered normal hearing; 21-40 dBHL is mild hearing loss (HL); 41-70 dBHL is
moderate HL, 71-95 dBHL is severe HL and >95 dBHL is profound HL. Therefore, the criteria of ototoxicity used included an increase in audiometric hearing thresholds of >20dBHL in two or more of the high frequencies using either standard or EHF audiometry. A drop in the DPOAE amplitudes determined evidence of ototoxicity using DPOAEs so that the difference between the DPOAE amplitude and the noise floor (also termed signal-to-noise ratio) was ≤6dB at two or more higher f2 frequencies that are at least ½ octave or more apart. Standard audiograms were analysed and assigned numeric grades to describe the degree of acquired hearing loss. Grades were assigned according to the ototoxicity grading systems developed by Brock et al. (Table 1-2) with the grading and thresholds illustrated for clarity in Figure 3-1. In cases of asymmetric hearing loss, the Brock’s hearing loss grade was based on results from the ear with better hearing (Brock et al., 1991).

Figure 3-1: A graphic representation of a typical audiogram with areas shaded to illustrate the different Brock’s grades. (Keeping in mind that each grade includes the specified frequency and all the higher frequencies above it. Note that the results of the better ear were the ones considered when grading.)
3.4.2 A control study investigating the short-term repeatability of DPOAE recordings in school-aged normal hearing children

Ethics approval was granted for this study through the UCL Ethics Committee (Ref.ID: 2476/001)

This control study observed the short-term repeatability of DPOAE recordings in school-aged children using a repeated measures cross-sectional study design. This was to assess whether probe positioning/re-insertion had a significant effect on DPOAE level outcomes and therefore should be considered as a contributing factor for inaccurate test results in study populations of this age group. Calculation of the standard error of measurement, beyond which a change would be considered as a true change in inner ear OHC function due to inner ear disorders such as ototoxicity, was also made to help inform more accurate guidelines for ototoxicity monitoring when using DPOAE measurements.

3.4.2.1 Procedure

3.4.2.1.1 Recruitment and set-up:

Children aged 7 – 11 years attending Swindon, Abbey Meads community primary school were recruited.

The school headmaster was approached in order to get approval to conduct the study on the school premises. All the relevant paperwork was presented and enquiries addressed. A CRB check was undertaken by the school administration for the audiologist testing the children before commencing with the study. The audiologist undertaking the testing and collecting the data was Mrs Bali (an MSc student and audiologist) under my (the candidate) sole supervision.

Copies of the Parent/Patient information sheet, the consent form and an associated pre-assessment questionnaire were given to the school administrators in order to distribute them to the children to take home. Written consent by the parents/guardians was requested. 150 copies were sent off with the children.

The Parent/Patient information sheet included details about: the aim of the study; explanation of tests that will be performed on the day; confirmation that recruitment is voluntary and that the parents/children are free to withdraw at any time without having to provide an explanation; that all data anonymized and stored and assessed in a secure fashion.
Confirmation that the tests will be performed within the school premises within school hours and that a copy of the hearing tests results will be given to each child was also provided in addition to provision of details of the audiologist’s contact details. The pre-assessment questionnaire attached to the consent form asked if the child had any history of known hearing loss, had a recent ear infection within the last three months, any history of ear surgery or having ventilation/Grommet tube insertion in their ears.

To encourage children and parents to consent to the study, ‘Free hearing tests’ fliers were displayed at different locations on the school premises. The flier clearly highlighted that the hearing test was free; tests were quick and non-invasive and that they are to be conducted by a qualified NHS audiologist.

An agreement with the school to conduct the test on 100 children over a mutually agreed period of 3 weeks was made. A small quiet room was made available within the school premises for testing within this period.

Each test was conducted over a period of 10-15 minutes. A simple Hearing test report clearly indicating if the child has passed both tests was issued for the child to give to the parents. In case of failure of one or both tests, the parents were advised to have further detailed assessment of child’s hearing through their GP and local Hospital if they had any concerns.

3.4.2.1.2 Inclusion criteria

These included the following: (1) based on the information provided by the parents/guardians through the pre-assessment questionnaire: - absence of previous history of hearing loss, recent history of ear infections or ear surgery including Grommet’s tube insertion. (2) Normal otoscopic examination to confirm absence of external or middle ear infections, occluding wax, foreign bodies or perforation of the ear drum. (3) Confirmation of normal middle ear function through tympanometry. (4) Collection of the signed written consent form with verbal confirmation of the child that he/she is happy to have their ears tested.

3.4.2.2 Test Battery

3.4.2.2.1 Tympanometry

Tympanometry was performed with a Grason-Stadler GSI 33 middle ear analyzer using a 226 Hz probe tone with a sound pressure level of 85 dB SPL to exclude external and middle ear problems using the same criteria as described in section: 3.4.1.3.1
3.4.2.2  DPOAE repeated recordings with probe removal and replacement

DPOAEs were recorded simultaneously in both ears of all the test subjects as described previously in section: 3.4.1.3.3

Three short-term within subject - within session repeats of the recordings were performed per ear for each child giving a total of six recordings per child. The probe was removed and refitted before each recording.
3.5 **Theme B: Causation**

3.5.1 **Investigation to identify potential risk factors that can be associated with ototoxicity in CF children**

Ethical approval is included within the same study referenced in section 3.4.1.

As section 3.4.1 mainly aimed to assess the audiological status of the chosen population of CF children exposed to aminoglycosides to identify the prevalence of ototoxicity and the most appropriate audiological tools to detect it, this section aimed to analyze the patient and treatment factors that may be associated with ototoxicity. Separating the analysis of this data from the earlier section is to highlight the main theme of the second part of this research, which involved investigating possible causative factors associated with ototoxicity.

**3.5.1.1 Procedure:**

All the factors retrieved from taking the patient history from the parent/carer or from information obtained from clinical records as specified in section 3.4.1.1 were statistically analyzed after grouping the patients into two groups (with and without ototoxicity) according to the outcomes of the audiological assessment of these children (as shown in Theme A). The statistical package SPSS version 17.0 was used where the $p$-value of $<0.05$ was considered to be significant.
3.5.2 Genetic studies investigating susceptibility to aminoglycoside ototoxicity in patients with CF

Ethical approval is included within the same study referenced in section 3.4.1.

The aim of these genetic investigations was to assess whether genetic mutations known to increase susceptibility to ototoxicity could be associated with ototoxicity detected in the study population of CF children exposed to aminoglycosides.

The best known and most frequent susceptibility factor of the A1555G mutation in the 12S rRNA gene (GenBank GI: 251831106) of the mitochondrial DNA (mtDNA) was genotyped to see if this explained some of the variance in ototoxicity between the CF children. Further search for other mutations/variations in the mtDNA 12S rRNA gene was also performed if cases with the A1555G genotype were discovered.

Genotyping of variants in the drug-metabolizing genes TPMT rs12201199 (thiopurine S-methyltransferase) and COMT rs4646316 (catechol-O-methyltransferase), which were previously reported by Ross et al. to be significantly associated with cisplatin ototoxicity in patients with cancer (Ross et al., 2009), was undertaken to assess if they were also significantly associated with aminoglycoside ototoxicity in patients with CF and could therefore be included as a possible causative factor for ototoxicity. A pilot study to gauge the frequency of the TPMT variant in a UK population and assess whether it is linked to hearing loss using the 1958 British cohort was also performed, as these samples were already available.

3.5.2.1 Patient samples:

DNA was obtained from the children with CF either through collection of a blood or saliva sample.

Peripheral venous blood samples were collected in BD vacutainer blood tubes (Becton, Dickson and Company) containing EDTA (red-top tubes) to avoid coagulation. 2-5 ml were collected by the hospital phlebotomist in the outpatients’ clinic or by the ward nurse if the child was an in-patient for administration of i.v. antibiotics. Samples were pipetted into 1.5ml aliquots and stored at -80° C freezers until DNA extraction was performed.

Saliva samples were collected using the DNA Genotek collection kits while the children were in the CF review outpatients’ clinic.
All samples and data were analyzed under the anonymized-linked procedure and allocated codes ‘CF001’ onwards.

3.5.2.2 General Equipment:

Applied Biosystems

7500 Real-Time PCR System

Eppendorff

Centrifuges: 5417R, 5417C, 5804R

Thermal Cycler for PCR: Mastercycler gradient

Jenway

6305 UV/Vis Spectrophotometer

Millipore

Elix and Milli-Q® water purification system

Nanodrop®

ND-1000 spectrophotometer

Sorvall

RC 5C plus centrifuge

UVP

3UV™ Transilluminator; GelDoc-it imaging system

3.5.2.3 General Reagents:

50x TAE (Stock solution diluted to 1x for use)

- 2M Tris
- 1M Glacial acetic acid
- 50mM EDTA pH 8.0
2% Agarose Gel

- 2g Agarose powder
- 100ml 1x TAE
- 3μl Ethidium Bromide

3.5.2.4 Procedures

3.5.2.4.1 DNA extraction

DNA Extraction from the Blood samples:

Qiagen (20) QIAamp blood Midi kit (Midispin protocol) for DNA extraction from whole blood was used according to manufacturer’s instructions. All thirteen steps in the recommended protocol for (http://www.immunoseq.com/wpcontent/uploads/manual/QIAamp_DNA_Blood_Midi_Maxi_Handboo.pdf) were followed to obtain a maximum yield of ~600μl using step 13b of the protocol.

To summarize; 1-2 ml blood per sample were equilibrated to room temperature (15-25°C). 200 μl QIAGEN Protease was pipetted into a 15ml centrifuge tube to which the blood was added and mixed. 2.4ml Buffer AL was then added followed by vigorous shaking for at least 1 min to ensure adequate lysis. The tubes were incubated at 70°C for 10 minutes. 2 ml 100% ethanol was added per sample and mixed by vigorous shaking to ensure efficient binding. Half the solution was transferred onto the QIAamp Midi column in a 15 ml centrifuge tube and centrifuged at 1850 x g (3000 rpm) for 3 minutes. The filtrate was discarded and the remaining solution was again transferred and centrifuged. After discarding the filtrate again, 2 ml Buffer AW1 was added and centrifugation at 4500 x g (5000 rpm) was performed for 1 min. 2 ml of Buffer AW2 was then added followed by centrifugation at the same speed but for 25 minutes. After placing the QIAamp Midi column in a clean 15 ml centrifuge tube, 300 μl Buffer AE was pipetted directly onto the membrane of the QIAamp Midi column, incubated at room temperature for 5 minutes then centrifuged at 4500 x g (5000 rpm) for 2 minutes. To maximize yield, 300 μl more Buffer AE was pipetted and the same steps were followed. Around 500-600 μl were eluted from the column containing the purified DNA.
**DNA Extraction from the Saliva samples:**

Oragene® DNA saliva kits (Ref.no. OG-100) were used to collect 2-4ml of saliva from children with CF. The children were instructed to spit into the special vials up to a specified marked line on the container. Once the specified amount was collected the cap of the container was screwed on which broke a seal on the cap and released Oragene.DNA, which immediately stabilized the saliva solution.

The samples, once mixed with Oragene.DNA are stable at room temperature for years without processing but it is preferable to store them at a -20°C freezer for long-term storage. This is done after transferring the sample into a 15ml centrifuge tube with incubation at 50°C for at least 1 hour in a water bath. The protocol for manual purification for each of the collected samples was followed as per manufacturer’s instructions (http://www.dnagenotek.com/ROW/pdf/PD-PR-006.pdf) and is summarized as follows: -

1/25th volume Oragene.DNA Purifier was added to the sample (i.e. 40µl/1ml of the sample); mixed by vortexing and incubated on ice for 10 minutes. Samples were then spun in a SS34 rotor in a RC 5Cplus Sorvall centrifuge for 20 minutes.

An equal volume of 100% ethanol was added to the supernatant and left to stand for 15 minutes at room temperature to allow the DNA to fully precipitate. DNA was then pelleted by centrifugation at 20°C for 15 minutes at 5,000 rpm. Most of the supernatant was carefully removed without disturbing the DNA pellet, which was then transferred into 1.5ml eppendorff tubes. Centrifugation at 20°C for 10 minutes at 13,600 rpm resulted in a compact pellet of DNA. An ethanol wash step was performed by adding 1ml of 70% Ethanol to the DNA pellet followed by further centrifugation at 20°C for 5 minutes at 13,600 rpm. The supernatant was again discarded carefully and the DNA pellet was left to dry for 3 minutes. Rehydration of the DNA was then done by adding an appropriate volume of 0.1X TE pH8.0 (200µl or 400 µl is added depending on the size of the pellet- larger volume for bigger pellet) and then gently pipetting and placed on a rotor at 4°C with speed set at 0.2-0.3 for 48 hours to allow for gentle mixing and full dissolving of the DNA. After 48hrs: the sample was briefly centrifuged at maximum speed and then gently pipetted 15-20x with a p200 filter tip to ensure the DNA has dissolved. If the DNA was too viscous, more 0.1xTE was added and the sample was returned to the rotor for another 24 hours. Once dissolved fully, the DNA sample was centrifuged at 20°C for 15 minutes at 13,000 rpm, to maximize DNA recovery and ensure complete rehydration and removal of any remaining turbid material. The supernatant containing the DNA was moved to a 1.5ml sterile screw-top tube leaving the
pellet of remaining impurities behind. Quantification of the DNA was then performed using the Nanodrop ND-1000 spectrophotometer.

**Assessing DNA purity and concentration:** Nanodrop ND-1000 spectrophotometer was typically used to assess the concentration and purity of the DNA eluted solutions. Qiagen Buffer AE solution (blood samples) and 0.1X TE solution (saliva samples) were used as the blanking solution (2μl) followed by the same quantity of the sample to be tested. The 260/280 ratio was recorded (should range between 1.7-1.95) in addition to the concentration (measured in ng/μl) as seen in Figure 3-2.

![Figure 3-2: A screen shot of the information displayed by the Nanodrop test](image)

- The single peak at 260 nm wavelengths is an indication of presence of DNA with absence of impurities. The 331.1-ng/μl is the concentration for this sample.

The yield of the samples varied quite significantly to range between 55-486ng/μl (Figure 3-3).
Samples were obtained for all CF patients' blood and saliva samples (n=105). Yield from all samples ranged between 55-486ng/µl.

### 3.5.2.4.2 Agarose gel electrophoresis:

This is a procedure used to separate fragments of DNA of different sizes (bps) by passing an electrical current through the DNA mix placed in an agarose gel. Typically 5-10µl DNA solutions were electrophoresed on a 2% agarose gel containing 0.03% ethidium bromide at approximately 80mA for 45 minutes. The gel was then visualized using the UVP 3UV™ Transilluminator where the gel is exposed to ultraviolet light. This procedure is used following Polymerase Chain Reactions (PCR) or Restriction Fragment Length Polymorphism (RFLP), as discussed below, in order to verify outcomes of these procedures. A 100bp ladder by Promega was used as a marker to help identify the fragment sizes (Figure 3-4).
3.5.2.4.3 Polymerase Chain Reaction (PCR)

<table>
<thead>
<tr>
<th>Variant/Mutation</th>
<th>Forward primer (5’-3’)</th>
<th>Reverse primer (5’-3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1555G</td>
<td>GCTCAGCCTATATACGCCATCTTCAGCAA (30)</td>
<td>TTTCCAGTACACTACCACATGTACGTAC (30)</td>
</tr>
<tr>
<td>12S rRNA</td>
<td>GAACCAACCAACCCCCAAAG (20)</td>
<td>TGAGCAAGAGGTGGAAGT (20)</td>
</tr>
<tr>
<td>(Fragment 1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12S rRNA</td>
<td>TGGCTTTAACATATCTGAACACA (23)</td>
<td>CTCCTAAGTGAATAGTTGG (23)</td>
</tr>
<tr>
<td>(Fragment 2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TPMT rs12201199</td>
<td>CTCAGTTTCCATAGTTGGAG (23)</td>
<td>GCAATGCAGCATGGAGTGG (21)</td>
</tr>
<tr>
<td>COMT rs4646136</td>
<td>CAGCCTAGCCTTACCTCAAAGGCC (24)</td>
<td>GAGTGCAGCTAGACAGCGGGTG (22)</td>
</tr>
</tbody>
</table>

Table 3-1: Primers used for PCR amplification of the DNA segments containing the different target genes or mutations. Primers were purchased from Eurofins and re-suspended at 100μM in ddH₂O.
The Eppendorff Gradient Mastercycler was used for all the PCR reactions. PCR was used for amplification for all the different genomic DNA segments containing the specific variants/mutations of interest within each study. Table 3-1 contains the primers used for each reaction.

**PCR parameters:**

The following is a typical cocktail recipe used to produce a total of 25μl/sample for each of the reactions that are described below:

- 0.2μl dNTPs (25mM)
- 5μl 5X GoTaq Green Buffer
- 1.25μl Forward Primer
- 1.25μl Reverse Primer
- 2μl MgCl2 (2mM)
- 0.125μl GoTaq Polymerase (0.025U)
- 10.175μl Water (Milli-Q dd H2O)
- 5μl Genomic DNA (250ng)

Varying the MgCl2, dNTP, and genomic DNA quantities and the annealing temperature in order to ensure specific amplification of the required amplicons was undertaken to optimize the PCR reactions.

The following is a typical PCR thermal cycler program used:

1. 95°C 5 minutes (initial denaturation)
2. 95°C 1 minute (denaturation)
3. 62.4°C* 30 seconds (annealing)
4. 72°C 30 seconds (extension)
5. **Repeat Steps 2 to 4 for 35 Cycles**
6. 72°C 5 minutes (final extension)
7. 4°C Hold

*Typically this annealing temperature is the only parameter changed for the different reactions to provide the optimal setting for each reaction.

5μl of the samples were then electrophoresed on a 2% Agarose gel and visualized as described in section: 3.5.2.4.2

3.5.2.4.3.1 **PCR for A1555G mutation**

The A1555G 12S rRNA gene mutation in mitochondrial DNA (mtDNA) has been repeatedly reported in the literature to be associated with increased susceptibility to AG ototoxicity and with non-syndromic deafness (Prezant et al., 1993b, Li et al., 2005, Kupka et al., 2002a). To
amplify the segment of the mtDNA containing the A1555G mutation in the \textit{12S rRNA} gene (also called \textit{MTRNR1} gene: GenBank GI: 251831106), forward and reverse primers previously specified by Estivill et al. and Kokotas et al. were used as shown in Table 3-1 (Estivill et al., 1998, Kokotas et al., 2009, Kokotas et al., 2011). The PCR reaction was performed using the same typical recipe and thermal cycle settings as those mentioned above. Successful amplification of the required mtDNA 339-bp segment was confirmed through 2% agarose gel electrophoresis and visualization under UV light.

![Figure 3-6: 2% agarose gel electrophoresis of 10 PCR amplicons for the mtDNA segment containing the 1555A/G variants. Lane 1 contains 100bp ladder maker with the 500 and 300bp bands highlighted with the arrows and the last lane (11) contains the no template control.](image)

3.5.2.4.3.2 \textbf{PCR for mtDNA 12S rRNA gene}

In order to detect further mutations or variations in the \textit{12S rRNA} gene that may be significantly associated with ototoxicity in the study group, PCR amplification of two overlapping fragments of the mtDNA, containing the whole of the \textit{12S rRNA} gene, were prepared using the two sets of primer pairs as shown in Table 3-1.

The first fragment reaction was performed as above but with 500ng genomic DNA, 0.2μM (0.5μl) of each primer and 1.5μl MgCl\textsubscript{2}. The PCR thermal cycle settings were the same as the typical reaction above with the exception of an annealing temperature of 54.3°C. The second fragment PCR reaction used the same components and thermal cycler settings as the first fragment with the exception of an MgCl\textsubscript{2} concentration of 2μl and an annealing temperature at 52.3°C.
The first fragment yielded a product of 707 bp spanning from mtDNA positions 545 to 1251 and the second fragment yielded a product of 616 bp from positions 1028 to 1644. These products were then purified and sequenced as described later in section 3.5.2.4.5.

3.5.2.4.3.3  **PCR for TPMT rs12201199 variant**

Primers TPMT-F and TPMT-R (for sequence see Table 3-1) were designed to generate a PCR fragment of 168 bp of the intron region of the TPMT gene (NCBI Reference Sequence: NM_000367.2) containing the SNP rs12201199 of interest. The amplified PCR fragment was confirmed by 2% agarose gel electrophoresis where visualizing of a single band of the correct size was made. Reactions were performed using the typical 25µl recipe stated above with the exception of using 10µl (500ng) genomic DNA, 2.5µl (1µM) of each primer and 1µl MgCl₂ (water quantities were adjusted accordingly). Thermal cycling was performed as stated above with only the annealing temperature changed to an optimal temperature of 58°C for 30 seconds.

3.5.2.4.3.4  **PCR for COMT variant:**

Primers COMT-F and COMT-R (for sequence see Table 3-1) were used in a typical PCR reaction, as described in above, to generate a 496 bp PCR fragment of the intron region of the COMT gene (NCBI reference sequence: NM_000754.3.) containing the SNP rs4646316 (RefSNP Alleles: C/T). The PCR was performed using the typical recipe and standard reaction as specified above with the only difference being the annealing temperature of 63.8°C.

3.5.2.4.4  **Genotyping by Restriction Fragment Length Polymorphism (RFLP)**

Restriction fragment length polymorphism is a procedure used to aid detection of differences between homologous DNA sequences. A specific restriction enzyme, also called restriction endonuclease, is used to divide the DNA sample into different restriction fragments. This reaction is also called ‘digestion of the DNA fragment’. The digested fragments of varying sizes can then be visualized using agarose gel electrophoresis. RFLP is capable of detecting the different alleles of a single nucleotide polymorphism (SNP) or mutation so that the wild-type (WT) homozygous, heterozygous and mutant homozygous variants can be differentiated from each other.
**RFLP parameters:**

The following is a typical cocktail recipe used to produce a total of 20μl/sample for each of the reactions that are described below:

- 2μl 10X Buffer (Promega or NEB)
- 6.5μl Water
- 1.5μl Restriction endonuclease enzyme
- 10μl DNA (PCR amplicons)

To ensure complete digestion the RFLP mixture was placed in a 37°C water bath overnight.

RFLP was used to identify the A1555G mutations and to identify TPMT and COMT SNPs under investigation as follows:

#### 3.5.2.4.4.1 For A1555G mutation:

RFLP was performed using the restriction enzyme HaeIII as specified by Estivill et al. and Kokotas et al. where HaeIII with the 1555A wild-type produces two fragments of 216 bp and 123 bp, while the patients affected with the A1555G mutation have three fragments of 216 bp, 93 bp and 30 bp owing to the creation of a HaeIII site by the A1555G mutation. (Estivill et al., 1998, Kokotas et al., 2009, Kokotas et al., 2011). Table 3-2 below summarises these expected outcomes.

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Enzyme</th>
<th>Amplified fragment (bp)</th>
<th>Normal sample (bp)</th>
<th>Mutant sample (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1555G</td>
<td>HaeIII</td>
<td>339</td>
<td>216, 123</td>
<td>216, 93, 30</td>
</tr>
</tbody>
</table>

Table 3-2: Summary of the expected outcomes of RFLP using HaeIII restriction enzyme on the 12S rRNA (*MTRNR1*) segment of the mtDNA. (Estivill et al. 1998; Kokotas et al. 2009 and Kokotas et al. 2011).

#### 3.5.2.4.4.2 For TPMT rs12201199 variant:

A restriction mapper website ([http://www.restrictionmapper.org/](http://www.restrictionmapper.org/)) was used to identify the restriction endonuclease (RE) enzyme that can differentiate between the ‘A’ wild-type and the ‘T’ variant SNP of the *TPMT* rs12201199 SNP by producing different digestion fragments with each. Table 3-3 shows the restriction endonuclease *MnLI* that was identified and the cut positions occurring with each SNP variant. The table shows that the sample with
an A-wild type homozygous allele will be digested into four fragments of sizes 116, 12, 27 and 13 bp whereas the T-variant homozygous allele would only be digested into three fragments of 128, 27 and 13 bps.

<table>
<thead>
<tr>
<th>TPMT with MnLI</th>
<th>Sequence</th>
<th>Overhang</th>
<th>Frequency</th>
<th>Cut Positions</th>
<th>Fragments</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘A’ WT SNP</td>
<td>CCTC</td>
<td>3_prime</td>
<td>3</td>
<td>116, 128, 155</td>
<td>116, 12, 27, 13</td>
</tr>
<tr>
<td>‘T’ variant SNP</td>
<td>CCTC</td>
<td>3_prime</td>
<td>2</td>
<td>128, 155</td>
<td>128, 27, 13</td>
</tr>
</tbody>
</table>

Table 3-3: Describing action of the restriction enzyme MnLI used for digestion of the TPMT amplicon and its cutting positions for both ‘A’ or ‘T’ alleles.

Restriction enzyme digestion using MnLI enzyme was performed. A 20µl digest mixture using 10µl of the TPMT PCR product was prepared as described above. The restriction fragments were visualized on the 2% agarose gels and under UV light using the 20 µl Digest solution + 4 µl 6X Xylene Blue loading dye (LD).

3.5.2.4.4.3 For COMT rs4646316 variant

The restriction mapper website (http://www.restrictionmapper.org/) was used again to identify the RE that can differentiate between the ‘C’ wild-type (WT) and the ‘T’ variant SNP of the COMT rs4646316 SNP. The restriction enzyme XcmI was identified as the suitable enzyme to use. Table 3-4 shows the different digestion sites and fragment sizes produced.

<table>
<thead>
<tr>
<th>COMT with XcmI</th>
<th>Sequence</th>
<th>Overhang</th>
<th>Frequency</th>
<th>Cut Positions</th>
<th>Fragments</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘C’ WT SNP</td>
<td>CCANNNNNNNNTGG</td>
<td>3_prime</td>
<td>1</td>
<td>79</td>
<td>417, 79</td>
</tr>
<tr>
<td>‘T’ variant SNP</td>
<td>CCANNNNNNNNTGG</td>
<td>3_prime</td>
<td>2</td>
<td>79, 250</td>
<td>246, 171,79</td>
</tr>
</tbody>
</table>

Table 3-4: Describing action of the restriction enzyme XcmI used for digestion of the COMT amplicon and its cutting positions for both ‘C’ or ‘T’ alleles.

As described above, A 20µl digest mixture using 10µl of the COMT PCR product was made. The restriction fragments were visualized on 2% agarose gels and under UV light using the 20 µl Digest solution + 4 µl 6X Xylene Blue LD.

3.5.2.4.5 Sequencing for mtDNA 12S rRNA gene

In an attempt to identify differences between the cases where the mtDNA A1555G mutation was found, sequencing of the whole 12S rRNA gene was performed. The 12S rRNA gene (GenEmbl AF346971) was amplified, using PCR, in two overlapping fragments using two
sets of primer pairs (Table 3-1) as described previously in Gurtler et al. (Gurtler et al., 2005), yielding a product of 707 base pairs and 616 base pairs for each of the two fragments respectively. The fragments encompassed nucleotides 545–1251 and 1028–1644, respectively. Each fragment was purified using the QIAquick PCR Purification Kit (Qiagen) and subsequently analyzed by direct sequencing at Source Bioscience Sequencing Cambridge UK [sequencing.cambridge@sourcebioscience.com]. Bidirectional sequencing was carried out using the forward and reverse PCR amplification primers presented above. Therefore, each fragment was sequenced twice after independent PCR amplification to detect and confirm sequence changes. The resultant sequence data were compared with the updated consensus Cambridge sequence (GenBank accession number: NC_012920 gi:251831106) (Andrews et al., 1999).

3.5.2.4.6 Real-time PCR for TPMT variant:

Unfortunately, the agarose gel electrophoresis was not sensitive enough to differentiate between the T/T-homozygous and the A/T heterozygote genotypes due to the similarity and small sizes of the produced fragments. Only the A/A-wild type homozygous genotype was differentiated from the other two on the 2% agarose gel. Real time PCR was used instead to identify all three alleles.

Reagents & Equipment used:

Custom TaqMan® SNP Genotyping Assay for TPMT rs12201199 SNP was ordered through Applied Biosystems. The ordered 40x SNP Genotyping Assay contained:

- Sequence-specific forward and reverse primers to amplify the polymorphic sequence of interest.
- Two TaqMan® MGB probes:
  - One probe labelled with VIC® dye detects the Allele 1 sequence
  - One probe labelled with FAM™ dye detects the Allele 2 sequence

These dyes emit fluorescence during the PCR reaction to indicate which allele is being amplified. In this case the VIC dye detected the Wild-type ‘A’ allele and the FAM dye detected the variant ‘T’ allele. If both alleles are amplified, then this sample was a heterozygote ‘A/T’. All TaqMan SNP Genotyping Assays are designed and optimized to work with TaqMan® Universal PCR Master Mix (with or without AmpErase® UNG) using the same thermal cycling conditions. They require only one PCR amplification step and an endpoint reading to obtain results.
The equipment used was **Applied Biosystems 7500 Real-Time PCR System**. This instrument allows real-time analysis of PCR, which is helpful for troubleshooting. Cross-referencing the real-time dye output with the final endpoint plate read can be done to correctly identify each sample SNP.

The total volume of each component needed for each assay was calculated as shown in the Table 3-5 below:

<table>
<thead>
<tr>
<th>Working stock for a 96-well plate</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>TaqMan Universal PCR Master Mix (2x), No AmpErase UNG</td>
<td>5 µl</td>
</tr>
<tr>
<td>20X working stock of SNP Genotyping Assay</td>
<td>0.5 µl</td>
</tr>
<tr>
<td>DNase-free water</td>
<td>0.5 µl</td>
</tr>
<tr>
<td>DNA sample @ 2.5ng/µl</td>
<td>4 µl</td>
</tr>
<tr>
<td>TOTAL</td>
<td>10 µl</td>
</tr>
</tbody>
</table>

Table 3-5: Formula used for preparation of 10 µl/sample reaction mix for Real-time PCR using a 96-well plate.

Amplification cycles protocol to perform the Real-time PCR used the following specified thermal cycling conditions:

<table>
<thead>
<tr>
<th>The Standard Protocol was used</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>AmpliTaq Gold Enzyme Activation</td>
<td>PCR (50 cycles)</td>
</tr>
<tr>
<td>HOLD</td>
<td>Denature</td>
</tr>
<tr>
<td>10 min at 95 °C</td>
<td>15 sec at 92 °C</td>
</tr>
</tbody>
</table>

Table 3-6: The thermal cycles conditions used for Real-time PCR.

After PCR amplification, an endpoint plate read was performed using the Applied Biosystems 7500 Real-Time PCR System. The Sequence Detection System (SDS) Software used the fluorescence measurements made during the plate read to plot fluorescence (Rn) values based on the signals from each well. The plotted fluorescence signals indicated which alleles were in each sample. Manual allele calls or reviewing automatic allele calls was performed then the allele calls were converted to genotypes. The data was also exported into excel sheets in order to save and analyze it further.
3.6 Theme C: Impact of ototoxicity and Current service provision

3.6.1 Investigating the effect of hearing loss on the quality of life of paediatric cancer survivors receiving ototoxic chemotherapeutic agents.

This study was registered as a clinical audit with the Research and Development (R&D) office at Great Ormond Street Hospital (GOSH) for Children

This audit was aiming to assess if the quality of life of a cancer patient deteriorates even further if the patient has the added complication of ototoxicity. It also aimed to assess whether both generic quality of life questionnaires, such as the Health Utility Index 3 (HUI3) questionnaire, or disease-specific quality of life questionnaires, such as the Paediatric Audiology Quality of Life (PAQL) questionnaire, were able to accurately detect this effect or if one type was more sensitive than the other.

As the main aim of this research theme was to highlight the importance of early and accurate identification of ototoxicity, assessing the impact of ototoxicity on the quality of life of patients suffering from it was an important factor in completing this argument.

3.6.1.1 Recruitment

Patients were recruited retrospectively from an assessment of auditory ototoxicity monitoring records of oncology patients assessed through a dedicated ototoxicity clinic at the Audiology department at GOSH during the past three years. The following inclusion and exclusion criteria were used to identify two groups of children: those who received ototoxic chemotherapy but still had normal hearing and those who received ototoxic chemotherapy and showed evidence of ototoxicity.

Inclusion criteria:

Children aged 5-18 years with confirmed diagnosis of cancer and proofs of intake of chemotherapeutic medication such as cisplatin were included in the study. All included subjects had to have records of baseline audiological assessment before the intake of any chemotherapeutic drugs and of post treatment audiological follow up.
**Exclusion criteria:**

Children outside the above mentioned age range were excluded (n=339); other exclusion criteria included: children with recorded cognitive/psychological problems pretreatment (n=3), private non-NHS patients residing outside the UK (n=25), types of chemotherapeutic agents used not included in the record (n=59), absence of a baseline audiological assessment record (n=30) and death of the patient as shown through the hospital database (n=23).

The hospital medical records were examined to extract the following information: The patients’ age, gender, age at diagnosis, cancer diagnosis confirmed by histopathology and the treatment protocol used. The ototoxicity clinic records were used to extract data regarding the baseline and post-treatment pure-tone standard audiometric results and to document records of auditory rehabilitation through amplification.

**Identification of hearing loss**

Patients were divided into two groups according to their post-treatment audiometric assessment into normal hearing and ototoxic hearing loss groups using the BSA audiometric descriptors of hearing loss and evidence of high frequency sloping hearing loss associated with ototoxicity caused by chemotherapeutic drugs. Brock’s grading was also used to describe the severity of ototoxicity with grades as seen in Table 1-2 and Figure 3-1.

**3.6.1.2 Assessment of quality of life using questionnaires:**

Once patients were identified to satisfy the inclusion criteria, parents were sent the parent/patient information sheet, consent forms and a copy of two questionnaires: the Health Utilities Index Mark 3 (HUI3) and the Paediatric Audiology Quality of Life (PAQL) Questionnaires. These were sent either through the mail with a pre-stamped return envelope enclosed or sent through email with a link to the questionnaires being available through the UCL Opinio survey software to allow for online completion of the questionnaire. Miss Abiodun (MSc student) collected this data under my (the candidate) sole supervision.

**The Health Utilities Index Mark 3 (HUI3) questionnaire:**

A parent-proxy version of this questionnaire was sent for completion by parents/carers of the children aged 5 years and above (not suitable for younger children). The questionnaire is a health utility measure of generic health-related quality of life (HRQOL) (Horsman et al., 2003). It consists of a 15-item questionnaire (15Q) with answers set in a multiple-choice
format with four to six choices given for the different questions and usually takes approximately 5–10 minutes to complete (Hawthorne and Richardson, 2001). The answers are arranged so that the first one (level one) is always the ‘normal’ best possible condition (e.g. ‘Able to hear what is said without a hearing aid or cochlear implant’), which then gradually get worse so that the last answer is the ‘most disabled’ worst possible condition (e.g. ‘Unable to hear at all’).

The questionnaire covers eight attributes: vision, hearing, speech, emotion, pain/discomfort, ambulation, dexterity, and cognition (Appendix 9.2 and 9.3). The HUI responses were converted to utilities as defined by Feeny et al. (Feeny et al., 2002) with scores from each subscale aggregated to calculate the final utility score.

The final multiplicative, multi-attribute utility score was based on the variation of responses across the eight attributes and was expressed on an interval scale ranging from –0.36 (representing the ‘most disabled’ health state with the lowest level of function for all attributes i.e. worse than death, which is attributed a score of 0.00) to 1.00 (representing the ‘maximum perfect health’ state with the highest level of function for all attributes).

**The Paediatric Quality of Life (PAQL) Questionnaire:**

This questionnaire is used to assess the Quality of Life (QOL) of children as young as 3 years. Parents/carers provided the answers to the questionnaire, which was divided into three sections (Appendix 9.4). The main first part of the questionnaire consisted of 22 questions’ assessing ‘how concerned or worried parents felt about’ issues covering four subscales: communication and independence (6 items), emotional well-being (7 items), peer comparisons (5 items) and acceptance by peers (4 items). **Communication and Independence** refers to the child’s ability to communicate with his/her peers, family members and teachers and the extent to which they are able to be independent. **Emotional well-being** refers to his/her emotional state on a daily basis and the extent to which negative emotions are under control. **Peer comparisons** and **acceptance by peers** refers to how well the child gets along with his/her peers and how their peers view them.

Each question was given a ranked score ranging from 1-to-5 with the highest score of 5 being attributed to a response of ‘Not at all concerned’ and the lowest score of 1 attributed to a response of ‘Extremely concerned’. Due to the difference in the number of questions included in each subscale, the weighting of subscales differed as follows:
1. Communication and Independence: questions 4, 8, 13, 14, 20 and 22 – with maximum score of 30
2. Emotional Well-being: questions 9, 10, 15, 17, 18, 19 and 21 – with maximum score of 35
3. Peer Comparisons: questions 1, 2, 3, 7 and 16 – with maximum score of 25
4. Acceptance by peers: questions 5, 6, 11 and 12 – with maximum score of 20

The Total Quality of life (QOL) score was calculated from the sum of the four subscales. The highest total score that could be achieved was 110.

The second part of the questionnaire was specifically tailored for those with a hearing loss who do wear hearing aids; here only two questions were presented with responses again in the form of a tick box with three alternative answers (N/A applied to those who do not have a hearing loss or do not wear hearing aids even if a hearing loss is present). The last part of the questionnaire was designed to obtain some demographic information about the child and ascertain the length of hearing aid use.
3.6.2 Survey of Oncology, Audiology and CF services in the UK to assess current practice of auditory monitoring for ototoxicity

Ethics approval was granted for this study through the NHS City Road and Hampstead Research Ethics Committee, REC reference number: 13/LO/0624.

This study aimed to assess the current UK practice in regards to auditory monitoring for ototoxicity from the perspective of both the audiology professionals and clinicians managing the patients exposed to ototoxic medication (in this case oncologists and CF clinicians). This would help identify deficiencies in existing clinical practice, which should be addressed when making recommendations for appropriate UK-wide ototoxicity monitoring guidelines in order to improve clinical practice.

3.6.2.1 Questionnaires’ design and delivery:

Review of the literature revealed that there were no previous survey studies investigating ototoxicity monitoring from the oncology or audiology professionals’ perspective except for a small pilot study of two oncology centers in South Africa (de Andrade, 2009). There were a few studies reporting on ototoxicity monitoring for CF patients as part of surveys investigating aminoglycosides usage and monitoring in CF patients in the UK (Tan et al., 2002), the USA (Van Meter et al., 2009) and in Australia (Phillips and Bell, 2001, Soulsby et al., 2009). Therefore custom questionnaires (Appendices 9.6, 9.7 and 9.8) were specifically designed for each of the three professions (audiology, oncology and CF clinicians) with the MSc students (MDJ and MK) and with feedback from clinicians (Drs Suri, Sirimanna, Rajput and Brock) in order to ensure that it contained appropriate content and detail to suit each of the professional groups.

The questionnaires mainly followed a multiple-choice format with some open-ended questions included to allow respondents to elaborate on some of their answers. Table 3-7 shows a summary of the overall structure of the questionnaires. The surveys were delivered electronically using a web-based survey tool called ‘Opinio’, which is available to UCL staff and graduate students, that provides a framework for authoring and distributing surveys (http://www.ucl.ac.uk/isd/staff/e-learning/core-tools/opinio). This format allowed for ease of access and quick response. The hyperlink to the survey was sent through an invitation email. This message highlighted that answering the survey would only take 5 minutes to complete, which the consulted clinicians stressed was a vital requirement to ensure more cooperation. It also included a brief explanation of the main aims and purpose of the study and provided reassurance that the anonymity of all the respondents would be maintained.
<table>
<thead>
<tr>
<th>Section I</th>
<th>Demographics:</th>
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<tbody>
<tr>
<td></td>
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<th>Aminoglycosides used</th>
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<td></td>
<td>• Proportion of patients receiving ototoxic medication;</td>
<td>• AG type as 1st line treatment;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Cisplatin/Carboplatin as first line treatments;</td>
<td>• Frequency of administration;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Receive head and neck radiotherapy;</td>
<td>• TDM</td>
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<th>Section III</th>
<th>Auditory monitoring for ototoxicity</th>
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<tbody>
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<td></td>
<td>• Verification whether auditory monitoring was performed or not</td>
</tr>
<tr>
<td></td>
<td>• Pre-treatment counselling and baseline assessments</td>
</tr>
<tr>
<td></td>
<td>• Protocols/other measures for when, what, and where audiological assessments are performed</td>
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<th>Section IV</th>
<th>Significance of ototoxicity monitoring</th>
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<tr>
<td></td>
<td>• Criteria for confirming evidence of ototoxicity</td>
</tr>
<tr>
<td></td>
<td>• If and how changes in management are made</td>
</tr>
<tr>
<td></td>
<td>• If other measurements of ototoxicity are undertaken</td>
</tr>
<tr>
<td></td>
<td>• If monitoring is considered important</td>
</tr>
<tr>
<td></td>
<td>• If genetic screening for A1555G was performed (only in CF survey)</td>
</tr>
</tbody>
</table>

Table 3-7: Summary of the structure of the three surveys
3.6.2.2 Survey participants and data collection:

As email was the route of delivery of the survey hyperlinks, the method accessing these email addresses defined who the invited participants were going to be. It was possible to obtain a list of all the UK CF centers from the 2010 CF Trust Registry available on the CF Trust website (www.cftrust.org.uk). There were 48 CF centers, including both adult and paediatric services. The lead clinician of each CF center was identified through the CF Trust website and they were contacted. This made calculation of a response rate possible.

On the other hand, it was not possible to identify the oncology and audiology centers and therefore a different approach was taken. Audiology professionals (including audiologists, clinical scientists, Audiovestibular physicians, and ENT doctors) and oncology professionals (including oncology physicians, specialist nurses and pharmacists) were invited to participate. The British Academy of Audiology (BAA), the British Society of Audiology (BSA), the UK Oncology Nursing Society, the Children’s Cancer & Leukaemia Group and the CHAIN support network were contacted and they agreed to send the invitation email with the survey hyperlinks to members on their mailing lists. The anonymity of the respondents was maintained for all the surveys.

The surveys were available for three months. Follow-up reminder emails were sent. The Opinio survey tool generated data reports that provided information related to the frequency of responses to each of the questions. Integration of all the outcomes of all three surveys was undertaken to identify common trends and obtain a better understanding of current practice.
3.7 **Statistical Analyses of studies included within all three themes:**

Software used in all studies:

- SPSS statistical package version 17- Significance was two-tailed and set at 5% level ($p<0.05$) for all statistical tests.
- MATLAB (only used in ‘Effect of ECP changes on OAE recordings’ study)
- G*Power online software version 3.1 was used for sample size and power calculations.
- Genetic Power Calculation was used in Theme B using online software at [http://pngu.mgh.harvard.edu/~purcell/gpc/](http://pngu.mgh.harvard.edu/~purcell/gpc/) (Purcell et al., 2003).

3.7.1 **Theme A: Audiological assessments and tools - Statistical analysis**

**Clinical observational study described in section 3.4.1:** Descriptive analysis including frequency plots for AG use and plots of mean ± standard deviations (SD) of the audiological assessments were performed and statistically significant differences between non-ototoxic vs. ototoxic groups were analyzed using a repeated measures analysis of variance (ANOVA) with post-hoc pairwise comparisons with Bonferroni adjustment for multiple comparisons.

A priori sample size calculation was performed for this clinical observational study using the G*Power v.3.1 software. Mean and standard deviation (SD) data was based on the work of Stavroulaki et al. (2002). This study used paired samples 2-tailed t-test to compare the baseline audiometric and otoacoustic emissions (TEOAE and DPOAE) recordings for CF patients with those following gentamicin treatment. Significant differences were found between the pre-treatment and post-treatment DPOAE amplitudes at all frequencies above 3 kHz. The outcomes for DPOAEs at $f_2$ frequency 5042 Hz were used as the group parameters to generate the effect size ($d_z$). The mean pre-treatment amplitude (mean group 1) mean was 17.68 dBSPL with SD of 5.47 dBSPL and the mean post-treatment amplitude (mean group 2) was 10.80 dBSPL with SD of 9.54 dBSPL (Stavroulaki et al., 2002). This produced an effect size of 0.63 as shown in Table 3-8. This is considered a moderate-to-large effect size according to Cohen's index of effect size for t-tests where 0.2 is considered a small effect size, 0.5 a medium and 0.8 a large effect size (more detailed explanation of the
meaning of effect size and power is provided below in section 3.7.2). A power estimate of 0.95, and a significance level of 0.05 (two-tailed) were used. According to the calculation below, a total of 36 patients with CF and history of exposure to aminoglycosides were required for this study (Table 3-8).

<table>
<thead>
<tr>
<th>Analysis:</th>
<th>A priori: Compute required sample size</th>
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<tbody>
<tr>
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<td></td>
</tr>
<tr>
<td>Tail(s)</td>
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</tr>
<tr>
<td>Effect size dz</td>
<td>0.6256</td>
</tr>
<tr>
<td>α err prob</td>
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<tr>
<td>Power (1-β err prob)</td>
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<td>Noncentrality parameter δ</td>
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<tr>
<td>Critical t</td>
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</tr>
<tr>
<td>Df</td>
<td>35</td>
</tr>
<tr>
<td>Total sample size</td>
<td>36</td>
</tr>
<tr>
<td>Actual power</td>
<td>0.9544</td>
</tr>
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</table>

Table 3-8: A priori sample size calculation performed to assess the number of subjects needed to detect an effect of ototoxicity.

Control Study assessing repeatability of DPOAE recordings described in section 3.4.2: A one-way repeated measures analysis of variance (ANOVA) was conducted to analyze the variance in the DPOAE amplitudes and signal-to-noise ratios (SNR) between the three repeated recordings at all eleven f2 frequencies tested.

A Pearson’s Interclass Correlation Coefficient (ICC) was also computed for the different f2 frequencies tested between the three repeats. The strongest and weakest correlations were presented as scatter charts with $r^2$ values and trend lines displayed.

Standard error of measurement (SEM) was calculated using ICC values using the following formula $SEM = s\sqrt{(1-ICC)}$ where ‘s’ is the standard deviation (combined SD of the three recordings) across all the measurements. The SEM values were used to calculate the minimum detectable difference (MDD), which can be taken into consideration as an actual change above the measurement error in a subject's score. MDD$_{95\%}$ equals 1.96*a\sqrt{2*SEM}.

3.7.2 Theme B: Causation- Statistical analysis

Study assessing potential risk factors for aminoglycoside described in section 3.5.1: Risk factors were analyzed using non-parametric tests including chi-squared test for categorical data, with $\chi^2$ or Fisher’s exact test used where appropriate; and
Mann Whitney U-test for continuous numerical variables. The null hypothesis that there was no significant difference in distribution across the two groups was rejected when the level of significance of p<0.05 was reached. Further analysis of the risk factors was performed using Binomial Logistic Regression to confirm which factors were significantly associated with ototoxicity. A non-parametric Spearman’s rho correlation test was performed to test the correlation between the average lung function (FEV$_1$ scores) and number of i.v AG courses administered and between the type of AG used and the occurrence of high frequency hearing loss. A statistician from Institute of Child Health (ICH) (Mr. Manolis Bagkeris) provided advice regarding some of the results of statistical analysis presented within this section.

**Genetics studies described in section 3.5.2:** Descriptive analysis and Chi-squared statistical analyses correlating the frequency of the different polymorphisms/mutations with the audiometric analysis were carried out using the SPSS version 17 statistical software packages. A Test for deviation from Hardy-Weinberg equilibrium and association analysis was performed to assess the level of significance of association between the frequencies of the genotypes/alleles of the TPMT rs12201199 and COMT rs4646316 variants and the occurrence of ototoxicity in the CF children. A power calculation was performed for both gene variants using the online genetic power calculator at http://pngu.mgh.harvard.edu/~purcell/gpc/ (Purcell et al., 2003). The case-control for discrete traits option was used. The data from Table 2 of the Ross et al. 2009 article was used to derive reference information for the number of cases and genotypic relative risk Aa and AA values. The high risk allele frequency (A) data was derived from the population genetics 1000-Genome Study allele frequencies information presented in the e!Ensembl website at http://www.ensembl.org/Homo_sapiens. Power is the probability of correctly rejecting the null hypothesis when the null hypothesis is truly false (i.e. the probability of not making a Type II error, which is also known as a false negative rate (β). Therefore power is also referred to as 1-β. A power value of 80% or 0.8 is considered acceptable but studies may aim to achieve a higher probability of finding a true difference by choosing a value of 95% or 0.95 instead. Power usually increases as the sample size or the effect size increase. The effect size is a measure of the size of difference or association recorded when comparing independent variables. It is equivalent to the amount of change that occurs in order to successfully differentiate between groups. It is also known as the expression variance. Common measures of effect size include the r or R-value calculated in correlation/regression analysis, the Cohen’s d or dz calculated in t-tests, partial eta-squared (η$^2$) calculated in ANOVA tests and phi (θ) calculated in chi-squared goodness of fit tests. Cohen provided rules of thumb for interpreting these effect sizes. According to Cohen's index of effect size
for the goodness of fit test a small effect size is equivalent to 0.10, a medium effect size is equivalent to 0.30, and a large effect size is equivalent to 0.50 (Walker, 2008).

3.7.3 Theme C: Impact and current service provision - Statistical analysis

Study on the affect of ototoxicity on quality of life described in section 3.6.1: Descriptive analysis of the age, gender, type of tumour, chemotherapeutic drugs administered, Brock’s ototoxicity grading and presence of other disabilities/not was presented. The HRQL and total QOL scores and scores of the different subscales were compared between the two groups of cancer patients, those with and those without hearing loss. As the data was not normally distributed, a Mann Whitney U test was performed to assess whether the differences between the groups were statistically significant or not. Correlation analysis between the outcomes of the two questionnaires was also performed.

Survey study on current practice described in section 3.6.2: Descriptive analysis and graphical representation of the outcomes of the questionnaires was presented in clear formats to highlight the results and integrate the responses from all three professional groups.
Results Chapters
Chapter 4:  **Results & Discussion of Theme A:**
**Audiological assessment and assessment tools**

This part of the research assessed the prevalence of ototoxicity in a group of children with CF exposed to aminoglycosides and validated a sensitive audiological test battery that allows for reliable detection of early/mild ototoxicity.

The rationale for this theme is a follow up of previous reports that the prevalence of ototoxicity in CF patients is quite variable (McRorie et al., 1989, Mulheran et al., 2001, Mulheran et al., 2006, Mulrennan et al., 2009, O'Donnell et al., 2010, Pedersen et al., 1987, Prayle et al., 2010, Scheenstra et al., 2010, Smyth and Bhatt, 2012) and specifically low in children (Cheng et al., 2009, Crifo et al., 1980).

### 4.1 Clinical observational study investigating AG ototoxicity in children with CF

#### 4.1.1.1 Study groups and demographics:

The study population of interest was the children with CF who were under the care of the CF unit, department of Respiratory Medicine at Great Ormond Street Hospital (GOSH). 76 children with CF aged 4-16 years old (median: 11.4; range: 4.3 - 16.4 years) were recruited. The main inclusion criteria, other than having CF, were mainly that they are willing to be assessed and did not have previous history of ear infections or surgery with more details included in section 3.4.1.1. Six (8%) patients were excluded from the analysis; five had middle ear disease, with negative tympanometric pressures $\leq -100$daPa, and one was unable to produce reliable audiometric test results, leaving 70 children whose results were analyzed.

The patients were divided into 3 groups based on the number of courses of i.v AG they had received during their lifetime: **Non-exposure group** - those who had never received IV AG (n=7); **low-exposure group** - those who had received <10 courses of IV AG (n=38); **high-exposure group** - those who had received $\geq 10$ courses of IV AG (n=25). Patients in the high exposure group had received between 10 to 40 courses of IV AG (median: 20; range: 10-40). Figure 4-1 shows the distribution of number of i.v AG courses across all 70 children.
4.1.1.2 Results of Standard and EHF audiometry and DPOAE testing:

As ototoxicity is known to cause high frequency sensorineural hearing loss, the first aim of the audiological assessment was to exclude external and middle ear pathologies. Otoscopy and tympanometry with acoustic reflex testing was performed for all the children in order to achieve this aim and exclude causes of conductive hearing loss (not related to ototoxicity).

Standard audiometry (0.25-8.0 kHz at one-octave intervals) and extended high-frequency (EHF) audiometry (9.0-16.0 kHz at 1/6th octave intervals) were then used to assess the children’s hearing status. A recording of their DPOAEs followed this.

Reviewing the distribution of audiometric threshold levels recorded at each of several different frequencies showed a clear dichotomy in the results. Figure 4-2 demonstrates this bimodal/double-peaked distribution shown over six of the twelve frequencies tested. This figure also shows that as the frequencies increase the number of children having higher thresholds increases, which is in line with the evidence that ototoxicity first affects the higher
frequencies at the basal turn of the cochlea, and then gradually extends apically damaging the lower frequencies. Inspection of the audiograms revealed a bimodal/ double-peaked distribution, with one group of children having thresholds of ≤20 dBHL, indicating normal hearing, and the other group having thresholds >20 dBHL extending up to 95 dBHL in the high frequencies.

The BSA has defined audiometric descriptors (BSA, 2011) and the ASHA criteria of ototoxicity (ASHA, 1994a), including an increase in thresholds of ≥20dB HL at one or more frequencies, to differentiate between normal and abnormal hearing. Using <20 dBHL cut-off criteria for normality, as defined by these bodies, the children were grouped into ‘non-ototoxic’ and ‘ototoxic groups’.

Figure 4-3 shows the mean ±SD thresholds of the right and left ears of these two groups which confirms the presence of these two distinct groups with the non-ototoxic group of children showing normal, even exceptionally good, hearing and the ototoxic group showing decrease in hearing thresholds mainly at frequencies at and above 8 kHz.
Figure 4-2: Frequency plots of the number of cases (children) displaying the different threshold intensities at a range of frequencies recorded using standard and EHF pure-tone audiometry. Note a clear bimodal /double-peaked distribution is shown at the lower frequencies and as the frequency increases the number of cases exceeding the normal hearing thresholds (≤20 dBHL) increase. X-axis shows the threshold intensity range at each of the specified frequencies; Y-axis shows the number of cases.
Standard PTA:

On applying these criteria for ototoxicity to the standard audiometric frequencies only (frequency range 0.25 to 8 kHz), 13 of the 70 children showed evidence of ototoxicity, i.e. 18.6 % with 95% confidence interval (95% CI: 9.5% to 27.7%). This included three children showing unilateral hearing loss confined to the right ears only. All of the children in the non-exposure group (7/70) fell into the non-ototoxic group. Despite this representing a small number of children, absence of ototoxicity in all the non-exposure children confirmed that CF as a disorder was not commonly associated with hearing loss and that other causes were most likely to be associated with appearance of hearing loss in these patients, taken here to be ototoxicity. Therefore, standard PTA identified 13/63 (20.6%) children who had previously received i.v AGs and satisfied the criteria of ototoxicity. In 5 cases evidence for ototoxicity was only found at the highest standard frequency of 8kHz. The remaining eight children showed frank threshold elevations in ≥2 consecutive standard PTA frequencies. In contrast to the BSA/ASHA criteria, Brock’s grading uses an increase in thresholds of ≥40 dBHL in the better ear at different frequencies to grade ototoxicity (criteria shown in Table 1-2). When Brock’s grading was applied using this data, 5/13 of these children scored grade 0 (absent ototoxicity) and 6 scored grade 1 (mild ototoxicity) (Table 4-1). Figure 4-4 shows
the audiological results of four children with ototoxicity, a variable level of AG exposure and the corresponding Brock’s grading as examples to demonstrate the non-linear relationship between these factors. In this figure, examples 4-4A and 4-4B show audiometric outcomes of two children that had similar exposure to >10 i.v. AG courses and similar normal standard PTA and Brock’s grading but with a significant difference in the EHF PTA results where the second child has a moderate sloping SNHL. Example 4-4C has a history of high exposure (26 i.v. AG courses) and a moderate-to-severe EHF PTA SNHL. However, within the standard PTA the hearing loss is confined to the 8 kHz frequency point and therefore has a Brock grading of 1. Thus if only standard PTA and Brock’s grading were used a significant underestimation of the extent of ototoxicity affecting this child would occur. Example 4-4D is of a child that had a history of low exposure of only 3 courses yet significant sloping SNHL in both standard and EHF PTA frequencies and a Brock’s grade 4 rating. Thus

Figure 4-4 demonstrates when accepted criteria are applied to the standard audiograms of these children there is some ambiguity about ototoxic vs. non-ototoxic group of children.
**Extended high-frequency (EHF) PTA**

The High frequency audiometry is an extension of the standard PTA, which adds information from PTA 9 kHz to PTA 16 kHz at 1/6th octave intervals. Frequencies 18 and 20 kHz were also tested but it was very clear early on that responses to these two frequencies were very unreliable and so they were excluded from the analysis.

The EHF PTA data indicated that 15 of the 70 children developed ototoxicity i.e. 21.4 % (95% CI: 11.8% to 31.0%). Again all the non-exposure group of children did not show evidence of ototoxicity. The three children who showed unilateral hearing loss with standard PTA continued to display the same pattern of unilateral loss. EHF audiometry identified 15/63 of the AG exposed children as having ototoxicity (23.8%). That included two extra children with early signs of ototoxicity at frequencies ≥9 kHz, not identified by standard PTA. In relation to the AG exposure history, 11 of the 15 children (73%) with ototoxicity identified by EHF audiometry belonged to the ‘high exposure’ group (≥10 courses of i.v AG) and only 4 children (27%) with ototoxicity belonged to the low-exposure group, each receiving between 3 to 8 courses of i.v AG, (median: 17; range: 3-40 across all ototoxic group). Therefore 44% (11/25 children) of the ‘high exposure’ group had ototoxicity (Figure 4-5). Their hearing loss ranged between 25 and 85 dBHL (mean ±SD: 57.5±25.7dBHL) across all the EHF audiometry frequencies.

An important observation was that 14 children in the high-exposure group (56%) were in the non-ototoxic group. Many demonstrated exceptionally good hearing, where thresholds were in the range of 0 to -10/-20 dBHL rather than in the 0 to 20 dBHL range which is still within the normal hearing range, across all frequencies despite having a similar history of high exposure to i.v AG (median: 17.5; range: 10-38 courses). This again highlighted that high or cumulative AG exposure was not always sufficient to cause ototoxicity and that other factors must be associated with making some children more susceptible to it’s effect.

As with standard PTA, three children showed unilateral signs of ototoxicity, with the hearing loss occurring only in their right ears at the EHF audiometric frequencies. In addition, eight of these children reported absence of any hearing problems, while four complained of both tinnitus and hearing loss; two reported hearing impairment only and one only complained of tinnitus (Table 4-1).

The threshold data from these two audiometrically identified groups (non-ototoxic vs. ototoxic) were analyzed using a repeated measures analysis of variance (ANOVA) for the effect of ear (right vs. left) and the audiometric frequencies (0.25–16 kHz). The overall
between-subjects effect of the ototoxic grouping (ototoxic vs. non-ototoxic) was significant ($F_{1, 68} = 277.5$, $p<0.001$, partial $\eta^2 = 0.810$). The non-ototoxic group threshold means for the right and left ears respectively were 3.85 and 3.52 dBHL (95% CI: 1.78 to 5.93 and 0.83 to 6.22 respectively) and were 47.78 and 46.65 dBHL (95% CI: 43.33 to 52.22 and 37.88 to 49.41 respectively) for the ototoxic group. The audiometric thresholds did not differ significantly between the groups at the lower frequencies ($\leq 4$ kHz) but differed significantly from one another at $p<0.05$ for frequencies 8 to 16 kHz with related $t$-tests when Bonferroni adjustment was made for the number of comparisons.

Figure 4-5 shows the distribution of children in each of these two groups (as defined by both standard and EHF audiometry) in relation to their level of lifetime exposure to i.v AGs. None of the seven (0%) children with absent history of exposure to IV AGs had any signs of ototoxicity confirming, as stated earlier, that CF as a disease is not commonly associated with hearing loss. This increased to 4/38 (11%) of the low exposure (<10 IV AGs) group and then showed even more dramatic increase in the high exposure ($\geq$10 IV AGs) group with 11/25 (44%) children showing evidence of ototoxicity.
Figure 4-4: Audiometric data (Standard and EHF) of four children with Brock's grading and level of AG exposure stated as examples to demonstrate the non-linear relationship between these factors.

(A) Normal hearing non-ototoxic child with Brock's grade 0 yet history of intake of 14 i.v AG courses;

(B) Ototoxicity only in EHF PTA, normal Standard PTA and Brock's grade 0 with history of intake of 15 i.v AG courses;

(C) Ototoxicity clear in EHF PTA, only at 8kHz in standard PTA, Brock's grade 1 with history of intake of 26 i.v AG courses;

(D) Frank ototoxicity with Brock's grade 4 with history of intake of only 3 i.v AG courses.

The grey shaded areas represent the normal hearing range.
Figure 4-5: The distribution of identified non-ototoxic vs. ototoxic group of children using both standard and EHF audiometric data presented according to their lifetime exposure to i.v AG courses.
Most of the ototoxic group of children were within the high exposure group (≥10 courses) with 44% of this group showing evidence of ototoxicity.
**Results of DPOAE recordings:**

Only 65/70 children underwent DPOAE testing, as it was not possible to test five patients. These five children included 3 of the 15 children identified with ototoxicity using EHF audiometry. The main reason for not performing the test was the presence of infections such as MRSA, where it is a contraindication to perform this test to avoid cross-contamination through the probe, or it was due to lack of availability of the equipment on the day.

Unlike PTA there are no generally agreed criteria for determining pathological DPOAEs, other than their complete absence. DPOAEs are considered to show evidence of a functional deficit when the DPOAE amplitude levels (dBSPL) are below the normal range at any two or more adjacent f2 test frequencies with consequent decrease in the signal-to-noise ratio (SNR) (Robinette, 2003, Dille et al., 2007, Durrant et al., 2009, Lonsbury-Martin and Martin, 2003, Lonsbury-Martin et al., 1993b, Mulheran and Degg, 1997a). For the purposes of this study a small SNR was used. There is general consensus that SNR can be used to assess the presence of a DPOAE but no clear consensus as to what this limiting SNR should be. Cut-off SNR values of both <3dB and <6dB were explored in the analysis. When cut-off SNR levels of <6dB was used 12/65 (18.5%; 95% CI: 7.8% to 26.0%) children showed evidence of ototoxicity and when SNR levels of <3dB cut-off was used this decreased to 7/65 (10.8%; 95% CI: 3.3% to 18.4%).

Figure 4-6 shows the mean DPOAE amplitudes of right and left ears of each of the two groups (non-ototoxic vs. ototoxic as determined by the audiometric assessments) displayed against the corresponding noise floor levels which were shown to be at low levels so that a low SNR score meant that the DP amplitude level was abnormally low too. Figure 4-7 shows the mean corresponding SNR scores. The mean SNR scores for both ears were clearly poorer in the audiometric ototoxic group compared to the non-ototoxic group especially at f2 frequencies 4-8 kHz. In 7/15 children with ototoxicity identified by EHF audiometry there was an associated drop in DPOAE amplitudes, confirming ototoxicity using both cut-off SNR values. The decrease in the left ear mean DPOAE amplitudes in the ototoxic patients was more prominent, occurring across all the f2 frequencies tested (0.8-8 kHz) whereas in the right it was limited to the higher f2 frequencies of 3-8 kHz. Statistical analysis of the difference in DPOAE amplitudes between the two groups was performed using a repeated measures analysis of variance (ANOVA) for the effect of ear (right vs. left) and the f2 frequencies (0.8-8 kHz). The overall between-subjects effect of the ototoxic grouping (ototoxic vs. non-ototoxic as defined by the PTA criteria) was significant (F₁,₆₃= 21.7, p<0.001, partial η²= 0.262). There was a significant difference between the ears within the groups (F₁,₆₃= 7.62, p= 0.008, partial η²= 0.111). The non-ototoxic group DPOAE means for
the right and left ears respectively were 7.47 and 7.70 dBSPL (95% CI: 6.19 to 8.75 and 6.10 to 9.30 dBSPL) and were 2.06 and -3.32 dBSPL (95% CI: -1.30 to 5.42 and -7.51 to 0.88 dBSPL) for the ototoxic group. The f2 frequencies also differed significantly between the groups in the right ears at f2 frequencies 4–8 kHz & left ears at f2 frequencies 1.3–6.3 kHz (p<0.05) with related t-tests when Bonferroni adjustment was made for the number of comparisons.

Table 4-1 displayed the characteristics of the fifteen children that were included in the ototoxic group and presented the differences in the audiological outcomes when each of the three audiological assessment tools was used. It highlighted that EHF audiometry was the assessment tool that was able to clearly detect ototoxicity for all 15 children; and that 7/15 also showed evidence of ototoxicity using DPOAEs (low amplitude levels and SNR scores), 3/15 where not tested using DPOAEs and 5/15 demonstrated within normal DPOAE results. If the five children that demonstrated normal DPOAEs were compared with standard PTA outcomes it shows that 1/5 also had normal thresholds and another 2/5 showed hearing loss only at 8 kHz. This shows that there is a reasonable agreement between audiometric and DPOAE testing with EHF PTA showing the most loss.

Figure 4-6: DP-Gram showing DPOAE mean amplitudes for right and left ears of the two audiometrically defined groups (ototoxic vs. non-ototoxic) and the corresponding noise floor recordings. The grey areas in the background show the normal range of DPOAE and Noise floor recordings. ANOVA test showed a statistically significant difference in distribution of DPOAE Amplitudes across the two categories in RT ears at Frequencies 4 – 8 kHz & LT ears at frequencies 1.25 – 6.3 kHz (p<0.05). (RT, right; LT, left; NF, noise floor)
Figure 4-7: Showing the SNR scores of the audiometric non-ototoxic vs. ototoxic group for the Rt and Lt ears respectively. The SNR scores were significantly poorer in the ototoxic group at f2 frequencies 4-8 kHz.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yrs.)</th>
<th>Gender</th>
<th>Auditory symptoms</th>
<th>No. IV AGs</th>
<th>Ear Affected</th>
<th>Assessment tools for ototoxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>CF035</td>
<td>15.9</td>
<td>M</td>
<td>HL</td>
<td>3</td>
<td>RT</td>
<td>+                  0            ++         ++</td>
</tr>
<tr>
<td>CF062</td>
<td>14.6</td>
<td>F</td>
<td>HL &amp; T</td>
<td>3</td>
<td>Both</td>
<td>++                 3            ++         ++</td>
</tr>
<tr>
<td>CF067</td>
<td>15.1</td>
<td>F</td>
<td>None</td>
<td>4</td>
<td>Both</td>
<td>-ve                0            ++         -ve</td>
</tr>
<tr>
<td>CF046</td>
<td>12</td>
<td>F</td>
<td>None</td>
<td>8</td>
<td>RT</td>
<td>++                 0            ++         -ve</td>
</tr>
<tr>
<td>CF032</td>
<td>5.5</td>
<td>F</td>
<td>None</td>
<td>14</td>
<td>RT</td>
<td>+                  0            ++         -ve</td>
</tr>
<tr>
<td>CF096</td>
<td>10.8</td>
<td>F</td>
<td>HL &amp; T</td>
<td>14</td>
<td>Both</td>
<td>++                 2            ++         ++</td>
</tr>
<tr>
<td>CF011</td>
<td>14</td>
<td>M</td>
<td>None</td>
<td>15</td>
<td>Both</td>
<td>-ve                0            ++         ++</td>
</tr>
<tr>
<td>CF003</td>
<td>16.4</td>
<td>F</td>
<td>None</td>
<td>17</td>
<td>Both</td>
<td>++                 1            ++         -ve</td>
</tr>
<tr>
<td>CF014</td>
<td>12.5</td>
<td>M</td>
<td>HL</td>
<td>18</td>
<td>Both</td>
<td>+                  1            ++         Not tested</td>
</tr>
<tr>
<td>CF066</td>
<td>13.8</td>
<td>F</td>
<td>HL &amp; T</td>
<td>24</td>
<td>Both</td>
<td>++                 4            ++         Not tested</td>
</tr>
<tr>
<td>CF026</td>
<td>16.2</td>
<td>F</td>
<td>None</td>
<td>26</td>
<td>Both</td>
<td>+                  1            ++         -ve</td>
</tr>
<tr>
<td>CF022</td>
<td>12.9</td>
<td>F</td>
<td>None</td>
<td>30</td>
<td>Both</td>
<td>++                 1            ++         ++</td>
</tr>
<tr>
<td>CF006</td>
<td>14.4</td>
<td>M</td>
<td>HL &amp; T</td>
<td>32</td>
<td>Both</td>
<td>++                 2            ++         Not tested</td>
</tr>
<tr>
<td>CF004</td>
<td>11.8</td>
<td>F</td>
<td>T</td>
<td>37</td>
<td>Both</td>
<td>+                  1            ++         ++</td>
</tr>
<tr>
<td>CF034</td>
<td>10.6</td>
<td>F</td>
<td>None</td>
<td>40</td>
<td>Both</td>
<td>++                 1            ++         ++</td>
</tr>
</tbody>
</table>

Table 4-1: Characteristics and audiological results of the fifteen children with ototoxicity with comparison of diagnosis based on different assessment tools.

HL, hearing loss; T, tinnitus; freq, frequency; RT, Right ear. Hearing loss is bilateral unless otherwise stated. For assessments: ‘+’ indicates ototoxicity recorded at one frequency only (8kHz at standard PTA); ‘++’ indicated ototoxicity recorded at ≥ 2 consecutive frequencies and ‘-ve’ indicate normal non-ototoxic response. Brock’s hearing loss grading (0 to 4): 0=No ototoxicity, 4=severe ototoxicity.
4.1.1.3 Relationship between the three audiological assessment tools:

In the previous section, the differentiation of patients into an ototoxic and a non-ototoxic group was dependent on the outcomes of the audiometric assessments (both standard and EHF PTA) and then the outcomes of the DPOAEs within this two pre-determined groups was assessed. In this section, a statistical analysis of the relationship between the three audiological assessment tools is presented. The aim was to determine whether or not all tools were able to identify ototoxicity in the CF children and to identify how well the outcomes of each of the tools correlated with each other. An appropriate statistical test to use in order to assess the reliability of different assessment tools is the ‘Kappa statistic’. A reliability analysis using the Kappa statistic was performed to determine consistency among methods (Landis and Koch, 1977). Cohen’s kappa coefficient is a statistical measure of agreement for binary outcomes that can be used to address agreement of the three different methods in identifying a patient’s hearing loss. A 2x2 contingency table was used to analyze the categorical data obtained from two methods at a time to calculate the Kappa statistic. An example of these tables is shown in Table 4-2, Table 4-3 and Table 4-4. The interrater reliability comparing the Standard and EHF PTA method was found to be Kappa = 0.911 (p <0.001), 95% CI (0.77, 1.0). This has indicated that a significant agreement above that expected by chance between the two methods was present. As EHF PTA indicated more children had ototoxicity, this method was used as a ‘gold standard’ to compare with the DPOAE tool using the two SNR cut-off criteria. The interrater reliability comparing EHF PTA and DPOAE with cut-off SNR value of <6dB was found to be Kappa = 0.707 (p <0.001), 95% CI (0.57, 0.95) (Table 4-3) which increased to Kappa = 0.754 (p <0.001), 95% CI (0.58, 0.96) when the cut-off SNR value of <3dB was used. This has also indicated that a significant agreement above that expected by chance between the two methods was present yet to a slightly lesser extent than that between the two audiometric tests.

<table>
<thead>
<tr>
<th>EHF PTA</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non ototoxic</td>
</tr>
<tr>
<td><strong>Standard PTA</strong></td>
<td></td>
</tr>
<tr>
<td>Non ototoxic</td>
<td>55</td>
</tr>
<tr>
<td>Ototoxic</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>55</td>
</tr>
</tbody>
</table>

Table 4-2: A 2x2 contingency table of outcomes using Standard PTA vs. EHF Audiometry. The interrater reliability comparing Standard and EHF PTA method was found to be Kappa = 0.911(p <0.001), 95% CI (0.77, 1.0). There is significant agreement above that expected by chance between the two methods.
Table 4-3: A 2x2 contingency table of outcomes using DPOAEs vs. EHF Audiometry. The interrater reliability comparing DPOAEs with 6dB SNR and EHF PTA method was found to be \( \text{Kappa} = 0.707 \) (\( p < 0.001 \)), 95% CI (0.57, 0.95). There is significant agreement above that expected by chance between the two methods.

Table 4-4: A 2x2 contingency table of outcomes using DPOAEs vs. Standard Audiometry. The interrater reliability comparing DPOAEs with 6dB SNR and EHF PTA method was found to be \( \text{Kappa} = 0.488 \) (\( p < 0.001 \)), 95% CI (0.03, 0.56). There is significant agreement above that expected by chance between the two methods.

Receiver operating characteristics (ROC) analysis was also performed using the EHF PTA data as the gold standard to determine the ototoxicity detection rate (sensitivity) and the non-ototoxicity detection rate (specificity) of the other two methods compared to it. The Area under the curve (AUC) corresponding to Standard PTA, DPOAE with SNR cut-off at <6dB and of <3dB were 0.909, 0.824 and 0.827 respectively, with sensitivity levels of 81.8%, 70.4% and 65.4% and specificity levels of 100%, 95.5% and 100% respectively. It is worth noting that, ‘Sensitivity’ defines the percentage of cases accurately detected to have ototoxicity by the different audiological assessment tools compared to those detected by EHF audiometry. Whereas, ‘Specificity’ defines the percentage of cases that were accurately confirmed to NOT have ototoxicity (i.e. normal hearing) by the different audiological assessment tools compared to those detected by EHF audiometry. Therefore, this analysis confirmed that despite all methods having significant agreement above that expected by chance, the ototoxic-induced DPOAE reduced amplitudes and SNR scores occurred at lower rates than those shown with behavioral threshold changes especially when compared with those at the EHF range. This was especially more apparent with the relatively low levels of sensitivity of the DPOAE test (especially when cut-off <3dB (65.4%) was used). Yet it is also worth considering that the two and three patients that showed evidence of ototoxicity with DPOAEs and not with EHF audiometry (Table 4-3) or standard audiometry (Table 4-4) respectively may be actually early subclinical evidence of functional damage of the OHCs not yet detected through audiometry (even EHFs).
A correlation test was then performed between the high frequency PTA and DPOAE at frequencies where there was a statistically significant difference between groups (Table 4-5). This showed a significant correlation between PTA thresholds and DPOAE amplitudes. The correlation was negative because patients with increased audiometric thresholds tended to have smaller DPOAEs amplitude levels. Many frequency pairs showed a highly statistical significance at \( p<0.001 \). The strongest negative correlation was seen between the DPOAEs results at 5 kHz f2 frequency and all the PTA results, for example, with the mean 8-12.5 kHz averaged result \( (r= -0.729, \, df=129, \, p<0.001) \). These correlation calculations were corrected from biasing due to multiple comparisons using the bootstrap method as shown in more detail in Table 4-6. Therefore, as PTA thresholds increased, in this case due to ototoxicity, the DPOAE amplitudes decreased. This confirmed that even though the sensitivity of DPOAE recordings to audiometric loss were shown to be lower than expected using the Kappa statistic or the ROC analysis, when specifically looking at the higher frequency recordings, they were actually highly correlated with the higher PTA frequency recordings.

An analysis of audiological data in relation to different patient and treatment related factors that were assessed for an association with occurrence of ototoxicity are presented in section: 5.1 as part of Theme B of this research which is focused on investigating ‘causation’ of ototoxicity.

<table>
<thead>
<tr>
<th>Mean PTA 8 - 12.5kHz</th>
<th>DP 3.2 kHz</th>
<th>Pearson Correlation</th>
<th>Sig.</th>
<th>DP 4 kHz</th>
<th>Pearson Correlation</th>
<th>Sig.</th>
<th>DP 5kHz</th>
<th>Pearson Correlation</th>
<th>Sig.</th>
<th>DP 6.3 kHz</th>
<th>Pearson Correlation</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTA: 4kHz</td>
<td>-.463**</td>
<td>&lt;.001</td>
<td></td>
<td>-.637**</td>
<td>&lt;.001</td>
<td></td>
<td>-.729**</td>
<td>&lt;.001</td>
<td></td>
<td>-.527**</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>PTA: 8kHz</td>
<td>-.402**</td>
<td>&lt;.001</td>
<td></td>
<td>-.588**</td>
<td>&lt;.001</td>
<td></td>
<td>-.592**</td>
<td>&lt;.001</td>
<td></td>
<td>-.328**</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>PTA: 9kHz</td>
<td>-.381**</td>
<td>&lt;.001</td>
<td></td>
<td>-.573**</td>
<td>&lt;.001</td>
<td></td>
<td>-.630**</td>
<td>&lt;.001</td>
<td></td>
<td>-.455**</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>PTA: 10kHz</td>
<td>-.443**</td>
<td>&lt;.001</td>
<td></td>
<td>-.631**</td>
<td>&lt;.001</td>
<td></td>
<td>-.721**</td>
<td>&lt;.001</td>
<td></td>
<td>-.511**</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>PTA: 11.8kHz</td>
<td>-.463**</td>
<td>&lt;.001</td>
<td></td>
<td>-.638**</td>
<td>&lt;.001</td>
<td></td>
<td>-.730**</td>
<td>&lt;.001</td>
<td></td>
<td>-.544**</td>
<td>&lt;.001</td>
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<td>PTA: 12.5kHz</td>
<td>-.465**</td>
<td>&lt;.001</td>
<td></td>
<td>-.627**</td>
<td>&lt;.001</td>
<td></td>
<td>-.735**</td>
<td>&lt;.001</td>
<td></td>
<td>-.518**</td>
<td>&lt;.001</td>
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<tr>
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<td>-.490**</td>
<td>&lt;.001</td>
<td></td>
<td>-.629**</td>
<td>&lt;.001</td>
<td></td>
<td>-.723**</td>
<td>&lt;.001</td>
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<td>-.531**</td>
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<tr>
<td>PTA: 16kHz</td>
<td>-.482**</td>
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<td></td>
<td>-.605**</td>
<td>&lt;.001</td>
<td></td>
<td>-.725**</td>
<td>&lt;.001</td>
<td></td>
<td>-.570**</td>
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<tr>
<td></td>
<td>-.468**</td>
<td>&lt;.001</td>
<td></td>
<td>-.624**</td>
<td>&lt;.001</td>
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<td>-.709**</td>
<td>&lt;.001</td>
<td></td>
<td>-.528**</td>
<td>&lt;.001</td>
<td></td>
</tr>
</tbody>
</table>

** Correlation is significant at the 0.01 level (2-tailed)
* Correlation is significant at the 0.05 level (2-tailed).

Table 4-5: Correlation between the audiometric thresholds and DPOAE amplitudes at frequency recordings that showed significant changes in outcomes indicating ototoxicity.
<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
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<tr>
<td><strong>PTA_AV8_12.5</strong></td>
<td>Pearson Correlation</td>
<td>-.463***</td>
<td>-.637**</td>
<td>-.729**</td>
</tr>
<tr>
<td></td>
<td>Sig. (2-tailed)</td>
<td>.000</td>
<td>.000</td>
<td>.000</td>
</tr>
<tr>
<td><strong>N</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>131</td>
<td>131</td>
<td>131</td>
</tr>
<tr>
<td><strong>Bootstrap</strong></td>
<td>Bias</td>
<td>.013</td>
<td>.010</td>
<td>.006</td>
</tr>
<tr>
<td><strong>Std. Error</strong></td>
<td></td>
<td>.110</td>
<td>.072</td>
<td>.065</td>
</tr>
<tr>
<td><strong>BCa 95% Confidence</strong></td>
<td>Lower</td>
<td>-.645</td>
<td>-.773</td>
<td>-.832</td>
</tr>
<tr>
<td></td>
<td>Upper</td>
<td>-.185</td>
<td>-.396</td>
<td>-.541</td>
</tr>
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<td>-.402**</td>
<td>-.588**</td>
<td>-.592**</td>
</tr>
<tr>
<td></td>
<td>Sig. (2-tailed)</td>
<td>.000</td>
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<td>.000</td>
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<td></td>
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<tr>
<td><strong>Bootstrap</strong></td>
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<td>.011</td>
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<td><strong>Std. Error</strong></td>
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<td><strong>BCa 95% Confidence</strong></td>
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<td></td>
<td>Upper</td>
<td>-.105</td>
<td>-.387</td>
<td>-.405</td>
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<tr>
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<td>-.381**</td>
<td>-.573**</td>
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<td></td>
<td>Sig. (2-tailed)</td>
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<td></td>
<td>131</td>
<td>131</td>
<td>131</td>
</tr>
<tr>
<td><strong>Bootstrap</strong></td>
<td>Bias</td>
<td>.012</td>
<td>.008</td>
<td>.004</td>
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<td><strong>Std. Error</strong></td>
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<td><strong>BCa 95% Confidence</strong></td>
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<td>-.570</td>
<td>-.729</td>
<td>-.784</td>
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<td></td>
<td>Upper</td>
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<td>-.443**</td>
<td>-.631**</td>
<td>-.721**</td>
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<tr>
<td></td>
<td>Sig. (2-tailed)</td>
<td>.000</td>
<td>.000</td>
<td>.000</td>
</tr>
<tr>
<td><strong>N</strong></td>
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<tr>
<td><strong>Bootstrap</strong></td>
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<td>.012</td>
<td>.010</td>
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<tr>
<td><strong>Std. Error</strong></td>
<td></td>
<td>.104</td>
<td>.071</td>
<td>.066</td>
</tr>
<tr>
<td><strong>BCa 95% Confidence</strong></td>
<td>Lower</td>
<td>-.608</td>
<td>-.761</td>
<td>-.838</td>
</tr>
<tr>
<td></td>
<td>Upper</td>
<td>-.195</td>
<td>-.386</td>
<td>-.531</td>
</tr>
<tr>
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<td>-.638**</td>
<td>-.730**</td>
</tr>
<tr>
<td></td>
<td>Sig. (2-tailed)</td>
<td>.000</td>
<td>.000</td>
<td>.000</td>
</tr>
<tr>
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<td><strong>Bootstrap</strong></td>
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<tr>
<td><strong>Std. Error</strong></td>
<td></td>
<td>.110</td>
<td>.073</td>
<td>.066</td>
</tr>
<tr>
<td><strong>BCa 95% Confidence</strong></td>
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<td>-.762</td>
<td>-.837</td>
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<tr>
<td></td>
<td>Upper</td>
<td>-.190</td>
<td>-.407</td>
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<td>-0.627**</td>
<td>-0.735**</td>
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<tr>
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<td>BCa 95% Confidence Interval</td>
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<td>-0.629**</td>
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</tr>
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<td>.000</td>
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<td>131</td>
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<tr>
<td>Bootstrap$^c$</td>
<td>Bias</td>
<td>0.011</td>
<td>0.009</td>
<td>0.005</td>
</tr>
<tr>
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<td>0.106</td>
<td>0.072</td>
<td>0.064</td>
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<tr>
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<td>.000</td>
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<tr>
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</tr>
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<tr>
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<td>0.061</td>
<td>0.080</td>
</tr>
<tr>
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<td>-0.729</td>
<td>-0.829</td>
</tr>
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<td>-0.418</td>
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<tr>
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<tr>
<td>Bootstrap$^c$</td>
<td>Bias</td>
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</tr>
<tr>
<td>Std. Error</td>
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<td>BCa 95% Confidence Interval</td>
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<td>-0.814</td>
</tr>
<tr>
<td>Upper</td>
<td>-0.184</td>
<td>-0.429</td>
<td>-0.549</td>
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**. Correlation is significant at the 0.01 level (2-tailed).

c. Unless otherwise noted, bootstrap results are based on 131 bootstrap samples, BCa 95% CI= Bias-corrected and accelerated (BCa)

Table 4-6: Detailed Bivariate Correlation analysis with bootstrap corrections between the higher PTA and DPOAE frequencies that previously showed significant differences between the two groups (non-ototoxic vs. ototoxic group of children)
4.1.2 Discussion of the audiological observational study of CF children:

4.1.2.1 Summary of the main findings

This study has shown a high prevalence (23.4%) of ototoxicity in children with CF who have previously received i.v AG courses (median: 5; range: 1-40 courses). This prevalence was significantly higher than the previously reported 0-6% prevalence in CF children (Crifo et al., 1980) and closer to that reported in adult CF patients (Mulheran et al., 2001, Mulherin et al., 1991, O'Donnell et al., 2010). This was particularly the case for those who have received at least 10 courses (high-exposure group) where 11/15 children showed evidence of ototoxicity. These children received an average of 18.2 courses (median: 16.0; range: 10-40) indicating that even at this young age repeated cumulative high exposure was common (Table 4-1). This is contrary to previous reports that CF children are less prone to ototoxicity because of the low exposure to aminoglycosides (Mulheran et al., 2001). However a clear dichotomy was seen in both the low and high exposure groups where some children showed evidence of ototoxicity while others showed normal, sometimes even exceptionally good, hearing.

Just under half (44% -11/25) of CF children in the high-exposure group had evidence of ototoxicity. Thus it may be concluded that repeated exposure to i.v AGs represents a significant risk factor for ototoxicity in some children with CF and probably has a cumulative effect. This is in agreement with previous studies such as Cheng et al. who investigated the prevalence of SNHL in 50 children with CF and reported that 3% in those who received ≤10 AG courses had evidence of ototoxicity versus 43% in those who received >10 courses (Mulheran et al., 2001, Mulheran and Degg, 1997a, Smyth and Tan, 2006, Tan et al., 2003, Cheng et al., 2009). In contrast, the remaining 4/15 children with ototoxicity received only three to eight i.v AG courses during their lifetime. It is possible that these individuals may be at increased risk of ototoxicity by being carriers of mutations known to confer increased susceptibility to AG ototoxicity such as the A1555G mutation in the mitochondrial 12S rRNA gene (Bitner-Glindzicz et al., 2009, Fischel-Ghodsian et al., 2004, Guan, 2011, Li et al., 2005, Conrad et al., 2008). These possible ‘risk factors’ and others were specifically investigated further in this patient cohort and the results were reported in section: 5.1 (Theme B) results. The lack of occurrence of ototoxicity in the other children receiving similar numbers of AG courses, especially the high-exposure group (14/25), is worthy of attention and again highlights that other factors may be involved in increased susceptibility to ototoxicity in addition to AG exposure.
4.1.2.2 Complaints of auditory symptoms:

Another finding of this study is that more than half the children with ototoxicity did not complain of any hearing problems. Symptom presentation is therefore not a substitute for actively monitoring hearing through audiological testing. Children, in general, do not complain of hearing loss until the speech frequencies (mainly 0.5-6 kHz) become affected. At this point other serious consequences, including delayed speech and language development, will occur with associated adverse effects on social, educational and psychological wellbeing (Pimperton and Kennedy, 2012, Theunissen et al., 2013). Results of the survey study in section: 6.2 showed that patients’ complaints of hearing loss, tinnitus or dizziness were the most commonly used criteria for referral for audiological assessment by clinicians. This needs to be avoided and an appropriate ototoxicity monitoring protocol should be implemented instead. Results of further investigation into the effect that hearing loss due to ototoxicity has on the quality-of-life of children are presented within Theme C of this research in section: 6.1 of chapter six.

4.1.2.3 Assessment of standard PTA vs. EHF PTA and DPOAEs as monitoring tools for ototoxicity

Overall, EHF audiometry was shown to be most sensitive in detecting ototoxicity in children with CF, and superior to the more commonly used standard PTA. DPOAEs showed a lower than expected degree of sensitivity with respect to EHF PTA yet an equal degree of specificity in identifying ototoxicity compared to EHF audiometry.

When compared with standard PTA, EHF audiometry detected two additional children with mild or early ototoxicity and showed a significant drop in hearing thresholds (25-85 dBHL) across several adjacent frequencies, whereas with standard PTA hearing loss was confined to only the highest (8 kHz) frequency in 33.3% (5/15) of the subjects identified with ototoxicity. Threshold changes that occur only at a single frequency are subject to much greater variability in interpretation. Consequently when Brock’s hearing loss grading was employed to the standard PTA results, as much as 73% of these children were given a low Brock’s grade of 0 or 1 equating to absent or mild hearing impairment respectively, even though increased EHF audiometric thresholds provided evidence that a large proportion of the basal turn of the cochlea had already been damaged.

DPOAE results showed a prevalence of loss of 18.5% and 10.8% respectively when SNR cut-off scores of <6dB and <3dB were used. A significant drop in DPOAE amplitudes at higher f2 frequencies in 7/15 children diagnosed with ototoxicity by the EHF audiometry
was recorded whereas DPOAE was not performed in another 3/15 of these children. Despite the kappa scores showing that this tool had a significantly higher agreement with EHF PTA above chance, results using this method were shown to be less sensitive than audiometry, especially EHF PTA. DPOAE sensitivity of 70.4% and specificity of 95.5% with the SNR cut-off of <6dB was obtained when compared to EHF PTA. This was similar to the hit rate of 78% reported by Reavis et al. who also indicated that DPOAEs were less sensitive to ototoxic injury compared with behavioral audiometry especially the 1/6th octave EHF PTA (Reavis et al., 2008). However, they also highlighted that the timing of DPOAE changes relative to behavioral threshold changes differed between patients. Roughly equal proportion (33-34%) of patients had DPOAE changes preceding, occurring during and lagging behind behavioral changes. They could not explain the reason for this difference on examining several independent variables but did state that DPOAE sensitivity increased when patients had better measurable pre-exposure hearing and larger magnitudes of post-exposure hearing change. As the current study had a cross-sectional design, further investigation into the relationship between DPOAE and behavioral thresholds was not possible but this explanation could offer a possible explanation for the patients identified to have ototoxicity by DPOAEs and not by the audiometric tests (Table 4-3 and Table 4-4). Here DPOAE changes preceding behavioral threshold changes could explain these results. This also supports that a recommendation should be made to use the outcomes of both the EHF PTA and DPOAEs as a ‘test battery’ and consider that to be a gold standard instead of EHF PTA alone and definitely instead of standard PTA alone if the main aim of monitoring is early identification of ototoxicity. If this was the case and if ototoxicity was identified using results of both/either of the EHF PTA or DPOAE showed evidence of ototoxicity 14/65 (21.5%) of this cohort that underwent both tests would have been identified versus 12/65 (18.5%) with EHF PTA alone or 10/65 (15.4%) with standard PTA alone (Table 4-3 and Table 4-4). However, as the results of the survey of current ototoxicity monitoring in the UK presented in section 6.2 demonstrated that a significant percentage of clinicians don’t monitor for ototoxicity at all and others provide a very variable service, considerations to providing the minimum level of assessment with just standard audiometry may be an important starting point to establish. More details of the implications of this survey are presented later.

To support this recommendation, analysis of the outcomes of the higher frequencies, where early evidence of ototoxicity is expected to occur, showed a highly significant negative correlation between the DPOAE amplitudes and the audiometry thresholds (Table 4-5). Thus indicating that both tools produce similar outcomes at the more important higher frequencies. This was in agreement with Arnold et al. and Reavis et al. who reported that DPOAEs at the
4-8 kHz range were significantly correlated with the EHF PTA results at the 8-20 kHz range indicating that high-frequency hearing influences DPOAEs recorded at significantly lower frequencies even when the corresponding PTA thresholds at these 4-8 kHz were still within the normal thresholds (Arnold et al., 1999, Reavis et al., 2008). This may be because the emissions are sensitive to subtle changes in outer hair cells not yet detected by pure-tone thresholds in this region or because alterations in the basal cochlea affect the generation of lower-frequency DPOAEs originating from more apical cochlear regions. As such, DPOAEs should be considered to be good markers for early detection of high-frequency hearing loss caused by ototoxicity. The strong correlation between high frequency DPOAE amplitudes and PTA high-frequencies shown in Table 4-5 supports this conclusion yet the cross-sectional design of the current study do not aid in clearly demonstrating this point.

A relative drawback to DPOAE testing was that it could not be measured in the presence of middle ear pathology, or in a child who is crying, vocalizing, or moving. Five children who were recruited in this current study had middle ear disease and DPOAEs could not be validly measured in them. On the other hand, DPOAE measurement is objective, non-invasive and does not require the patient’s active participation and therefore has the advantage of being very effective in monitoring ototoxicity in young children or any difficult to test patients who may not consistently provide reliable, ear-specific pure-tone threshold responses (Beattie et al., 2003, Knight et al., 2007). This fact again supported the conclusion that EHF audiometry and DPOAE testing complemented each other and provided a test battery that would be more superior than standard audiometry alone in detecting early/mild ototoxicity in children of this age group where crying and vocalizing in no longer an issue. Standard audiometry is usually used as the only audiological tool for ototoxicity monitoring despite repeated evidence showing that it is less sensitive than the other two tools used as a test battery (Al-Malky et al., 2011, Knight et al., 2007, Konrad-Martin et al., 2010, Lonsbury-Martin and Martin, 1990, Reavis et al., 2011, Stavroulaki et al., 2002).

The question of how reliable EHF audiometry is in children and what DPOAE testing can add to monitoring for ototoxicity may be posed. Investigations into the test-retest reliability of EHF audiometry in children have been limited (Margolis et al., 1993, Reuter et al., 1998, Schmuziger et al., 2004, Dreschler et al., 1989) but an important study by Beahan et al. was recently published specifically evaluating this issue in relation to criteria for ototoxicity (Beahan et al., 2012). They tested 125 children aged between 4–13 years with normal hearing in the 0.25–4 kHz range and divided them up into three age groups (4-6; 7-9 and 10-13 years). They investigated the test-retest reliability of EHF PTA at frequencies 8-16 kHz. Their results demonstrated that a slight age effect was evident by reporting that normal
variability in thresholds (within ±10 dB) occurred in 89.9%, 93% and 97% of the three age groups respectively. It was also reported that a significant deterioration in hearing thresholds across test-retest conditions in relation to the ASHA (1994) ototoxicity criteria in the three age groups demonstrated false-positive rates of 24.6%, 11% and 7.6% respectively. They therefore concluded that EHF PTA demonstrated high test-retest reliability in all but the 4-6 year-old groups and recommended that testing should be supplemented with an ‘objective’ test of auditory function, such as DPOAEs, to confirm the diagnosis of ototoxicity with serial monitoring of hearing. This again endorsed the conclusion that these two tests are complementary to each other and should be used as a test battery.

Overall, EHF audiometry and DPOAE as a complementary (subjective and objective) test battery were shown to be sensitive in diagnosing ototoxicity in children with CF, and superior to the commonly used standard PTA alone. As DPOAE was shown to be a valuable objective tool in detecting or monitoring ototoxicity further effort was invested in trying to improve this technique. Results of this study and previous research have shown that DPOAE maybe affected by middle ear problems and probe fitting, and do not have universally accepted criteria for diagnosing ototoxicity as criteria for DPOAE strength is not standardized (Knight et al., 2007, Beattie et al., 2003). Further investigation into factors, such as repeatability of recordings or impact of differences in probe placement on OAE recordings was undertaken within a control study whose results are presented in section 4.2.

4.1.2.4 Effect of ototoxicity on patients and the importance of accurate criteria for ototoxicity

The importance of setting the appropriate criteria for determining ototoxicity was highlighted by using Brock’s grading as an example of the significant difference in outcomes that can be obtained when different criteria are used (Figure 4-4). When Brock’s grading was applied using the standard audiometry data, 5/13 of these children scored grade 0 (absent ototoxicity) and 6 scored grade 1 (mild ototoxicity) (Table 4-1). Brock’s grading is only one example of numeric grading systems that are at risk of under-estimating the degree of drug-induced hearing loss. Knight et al. compared the ASHA criteria (Table 1-1) with the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE - Table 1-5) used in assessment of chemotherapeutic ototoxicity (Knight et al., 2005). They concluded that the commonly used reporting of toxicity data (CTCAE) had under-reported ototoxicity, did not correlate with the ASHA criteria outcomes and minimized the significance of hearing loss in children as it did not specifically consider high frequency hearing loss. The problems of clinical interpretation of the PTA data may be addressed by use of EHF, which detected a
much greater loss across a number of adjacent consecutive frequencies in this cohort (Figure 4-3) or through the use of more accurate criteria for ototoxicity. The recently proposed International Society of Pediatric Oncology Boston Ototoxicity Scale (SIOP Boston Scale: Table 1-4) appears to be a good scale to use, as there is a drive to have a consensus from all different professionals to use it, with the aim to have internationally common criteria for ototoxicity (Brock et al., 2012a). This scale is easy to interpret and takes into account mild levels of hearing loss at the high frequencies allowing for early identification of ototoxicity. It is also dependent on absolute thresholds so that if baseline assessment were not performed for any reason (the child was very ill/not cooperative) later assessments can be made independently.

Hearing loss in childhood has major implications on speech and language, social and psychological development in patients and may significantly affect quality of life. Therefore it is recommended to perform serial auditory monitoring using EHF audiometry and DPOAEs in all children with CF especially those who receive frequent courses of i.v AG for early detection of signs of ototoxicity. Early detection allows the managing clinicians to make informed decisions about the different antibiotics regimens they are using with each patient, weighing their risk/benefits in relation to their recorded side effects. Appropriate management might allow for more conservative use of these antibiotics and initiate early auditory rehabilitation to decrease the impact of hearing loss on quality of life.

4.1.3 Conclusions:

This study showed that ototoxicity due to exposure to aminoglycosides in children with cystic fibrosis was more common than previously reported. It affected one in five (23.4%) of the children tested and this prevalence went up to around two in five (44%) when only the high exposure children were assessed. This prevalence was closer to the previously reported prevalence in adults. It was also shown that despite their young age, many children were exposed to a large number of repeated cumulative aminoglycoside courses with some recorded to have received up to 40 courses before the age of 16 years. Many of the children were admitted to hospital for prophylactic intake of i.v antibiotics every 2-3 months in order to combat chronic pseudomonas chest infections. This confirms that the misconception that CF children are not at risk of ototoxicity because of limited exposure to AGs is not true. It was also shown that even when audiological assessment confirmed the existence of ototoxicity most of the children affected did not complain of any auditory symptoms therefore waiting for the child to complain before assessing them would not detect early or mild ototoxicity. It could also be concluded that in order to protect their quality of life, which would be affected by ototoxicity and the possible consequent problems such as
deterioration of speech and language abilities, regular active monitoring of their hearing status is needed. This would allow clinicians to make informed decisions regarding treatments regimens with the aim to protect hearing from progression of ototoxicity.

On comparing the different audiological assessment tools it was concluded that a test battery including EHF audiometry and DPOAE testing would be more sensitive for ototoxicity monitoring than the commonly used standard audiometry. However, the lower than expected sensitivity of DPOAEs as an independent monitoring tool compared to EHF audiometry prompted further investigations into possible factors that may account for this outcome. These are presented below in sections 4.2 and Error! Reference source not found.. A major finding was the dichotomy in audiological outcomes of the CF children. Some children with both low and high exposure had exceptionally good hearing whereas others had substantial hearing loss. This is clear evidence that multiple factors are involved in the causation of ototoxicity, which needs to be investigated further.
Assessment of DPOAE testing as an audiological tool - rationale behind the control studies:

- **Significance of DPOAE as a monitoring tool for ototoxicity in children:**

  Review of the literature has confirmed that DPOAE measurements have an important yet underutilized role in the monitoring of ototoxicity (Biro et al., 2006, Cevette et al., 2000, Konrad-Martin et al., 2012). The results reported above and other studies have shown that it is a quick non-invasive objective test that can be well recorded in children. It provides information regarding the integrity of OHCs and preservation of the non-linearity of the inner ear. As the OHCs are one of the first cells to be damaged by ototoxic drugs, this would be the ideal test to use for early detection of ototoxicity. However, this test was not found to be as sensitive as EHF PTA – but maybe this could be improved by research.

- **Pilot study on DPOAEs in school-aged children vs. adults:**

  Identification of some gaps in the literature regarding research in DPOAE measurements in the school-aged group of children prompted investigation of this aspect as a possible explanation for the recorded lower sensitivity when using this tool with the CF children exposed to aminoglycosides. There had been extensive research regarding DPOAE testing in neonates and also in adults. They showed that there was a variation in outcomes of each of these subject groups, even within normal hearing subjects, yet there was limited research in the school-aged children’s group (Gorga et al., 2000b, Lonsbury-Martin et al., 1993a, Lonsbury-Martin et al., 1993b, Zhao and Stephens, 1999). A pilot study where repeated DPOAE testing for two normal-hearing children aged 6 years and 8 years and two normal-hearing adults aged >18 years over a period of four weeks was undertaken. Testing was repeated on average ten times/day at different times of the day and at different positions (i.e. sitting up or lying down). Preliminary analysis of these results showed that there was significantly more variability in the DPOAE results of the children compared to the adults despite all the results still being considered within the normal range of >6dB SNR scores. The DPOAE amplitudes and noise levels varied more with the children. On trying to identify the possible causes for this in line with previous research it was decided that the two main factors that may have caused this variability were differences in the positioning and placement of the OAE probe into the ear canal and possible changes in the children’s middle
ear pressure due to the higher probability of nasal congestion or susceptibility to otitis media with effusion in children.

- **Generation of research questions:**

  On the basis of this small pilot study it was decided to undertake a control study. This study was to assess the effect of probe re-insertion on the repeatability of DPOAE recordings in school-aged children. The results of this study is presented below.
4.2 **A control study assessing the short-term test-retest repeatability of DPOAE recordings in normal hearing school-aged children**

The aim of this study was to assess the short-term repeatability of DPOAE recordings in school-aged children after removal and re-insertion of the OAE probe. The aim was also to calculate the minimum detectable difference (MDD), which is the ‘band of error’ beyond which a change is considered to be a true sign of abnormality.

4.2.1.1 **Study group:**

150 patient information sheets and consent forms were sent home with the children from Swindon, Abbey Meads community primary school to obtain parental approval to test the children. 83 consent forms were returned. Assessments were undertaken in a dedicated quiet room in the school. Otoscopy and tympanometry were performed to confirm normal external and middle ear function. 23 (28%) children were excluded as they had occluding earwax or Type B or C tympanograms. Therefore a total of 60 children were included in the study.

The ages of the 60 children ranged between 7 and 12 years (mean ±SD: 10.1 ±5.3) with 37 boys and 23 girls. Figure 4-8 shows the distribution of children according to age. All children had normal otoscopy and tympanometry with mean ±SD: -17.9 ±24.0 daPa and 0.78 ±0.49 ml peak pressure and compliance respectively.

![Figure 4-8: Distribution of the recruited children according to age (years)](image-url)
DPOAE results:

DPOAE recordings were obtained from both right and left ears (120 recordings), which were repeated three times. DPOAE recordings at eleven 1/4-octave f2 frequencies ranging between 0.8 and 8 kHz were made. Figure 4-9 shows the mean ± standard error (SE) DPOAE amplitudes for all f2 frequencies for the three recordings. All three recordings were very similar with mean DPOAE amplitudes ranging between 5.2 and 13.1 dB SPL (±6.2 to 9.2 SD) across all f2 frequencies with the exception of responses at 8 kHz, which were the only responses with negative mean amplitudes of -14.5 (±11.5) dB SPL. Responses peaked at frequencies 1.6 and 5 kHz. The equivalent noise floor, as seen in Figure 4-10 showed gradual decrease in mean amplitudes as frequencies increased with mean levels highest at 0.8 kHz at 1.1 dB SPL and lowest at frequency 8 kHz at -16.4 dB SPL. This higher noise floor at the lower frequencies contributed to diminished ability to distinguish between the DPOAE responses from the background noise and hence a lower SNR. This affects the reliability of the recordings at f2 frequencies <1.3 kHz (Figure 4-11). Reliability improves, as defined by the larger SNR, at f2 frequencies ≥1.3 kHz due to the decreased noise floor levels and associated increase in DPOAE amplitudes. A minimal SNR score of 3dB, i.e. a DPOAE amplitude of ≥3 dB above the mean noise floor + 2 SDs, is considered as a true 2f1-f2 DPOAE response. A repeated measures analysis of variance (ANOVA) was performed to assess if the difference between the three recordings was significant or not. Results showed no significant within-subject differences between the three recordings across all 11 f2 frequencies (F2, 118 = 2.609, p=0.078, partial η²= 0.042). Pairwise comparison of combinations of each pair of repeats showed absence of significance in any of the combinations (p>0.05). This absence of significant differences was also confirmed by Post Hoc tests with Bonferroni correction (p>0.05).
Figure 4-9: Mean ±SE DPOAE amplitudes for each f2 frequency for each of the repeated three recordings. The three recordings were similar at all f2 frequencies, which were all positive values except for the 8kHz frequency recordings.

In an attempt to explain the reason for the significantly low 2f₁-f₂ DPOAE amplitudes at 8 kHz, as seen in Figure 4-9 and Figure 4-10 compared to all the other responses, it was discovered that the average level of the actual output of L1/L2 primary tones was significantly lower than the target L1/L2: 65/55 dB SPL. From a random sample of 60 recordings obtained from 10 of the recruited children, the average L1/L2 values at 8 kHz were actually 52/40 dB SPL ± SD: 5/7 dB SPL and at 6 kHz were 58/45 dB SPL ± SD: 8/5 dB SPL. This was not the case for the other f2 frequencies where the target L1/L2: 65/55 dB SPL were achieved in most cases with a small degree of variance. This, in addition to the theory of standing waves in the ear canal cancelling DPOAE responses at the higher frequencies, may explain the low amplitudes at 8 kHz and to a lesser extent at 6 kHz. This information was fed back to the manufacturing company (Otodynamics Ltd.), which then took action and modified the recording algorithm for responses at frequencies >5 kHz by introducing correction factors to overcome this problem (see Appendix 9.1 for report from Otodynamics).
Figure 4-10: Mean ±SD of the three repeated recordings of DPOAE amplitudes and its associated noise floor recordings. The grey background shows the average range of DPOAE amplitudes and noise floor of adult patients. The mean values were so similar that they overlapped.

Figure 4-11: Mean ±SD of the signal-to-noise ratios (SNR) of the 3 repeated DPOAE recordings as a function of the f2 frequencies. The recordings are shown to be strongly repeatable leading to overlap of the results.
4.2.1.2 Statistical analysis of DPOAE repeatability and Calculation of the SEM:

An interclass correlation coefficient (ICC) analysis was made to assess the degree of correlation between the three repeated recordings at each of the DPOAE f2 frequencies recorded. A strong ICC was obtained for the averaged measures across all tested frequencies (ICC = .885, 95% CI = .838 - .919) with a strong reliability statistic in the form of Cronbach’s Alpha of .948 as shown in Table 4-7 and Table 4-9. When the ANOVA statistic was performed to assess the significance of these correlations and ensure that there is a true within subjects – between items significant effect, the outcomes were highly significant (F = 165.3; df= 32,118; p<0.001) as shown in Table 4-8. Table 4-10 shows a strong correlation between the repeats at all f2 frequencies (p<0.01) especially at f2 frequencies 1.3 to 6.4 kHz (ICC: 0.791 to 0.908). The scatter plots demonstrate the weaker correlation obtained at DPOAE f2 frequency 0.8 kHz (Figure 4-12) with positive regression shown at R²=0.30 and the strongest correlation obtained at DPOAE f2 frequency 5 kHz (Figure 4-13) with positive regression at R²=0.77.

<table>
<thead>
<tr>
<th>Reliability Statistics</th>
<th>Cronbach's Alpha</th>
<th>Based on Standardized Items</th>
<th>N of Items</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cronbach's Alpha</td>
<td>.948</td>
<td>.957</td>
<td>33</td>
</tr>
</tbody>
</table>

Table 4-7: Reliability statistics showing a strong correlation between all 33 tested items (11 DPOAE f2 frequencies, 3 Repeats)

<table>
<thead>
<tr>
<th>ANOVA</th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between People</td>
<td>85643.290</td>
<td>118</td>
<td>725.791</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within People</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between Items</td>
<td>197953.042</td>
<td>32</td>
<td>6186.033</td>
<td>165.270</td>
<td>.000</td>
</tr>
<tr>
<td>Residual</td>
<td>141334.968</td>
<td>3776</td>
<td>37.430</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>339288.010</td>
<td>3808</td>
<td>89.099</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>424931.299</td>
<td>3926</td>
<td>108.235</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Grand Mean = 6.779
Table 4-8: ANOVA statistic assessing the significance of the ICC calculations performed.

<table>
<thead>
<tr>
<th>Intraclass Correlation Coefficient (ICC)</th>
<th>Intraclass Correlation</th>
<th>95% Confidence Interval</th>
<th>F Test with True Value 0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single Measures</td>
<td>.190b</td>
<td>.135 - .257</td>
<td>19.391 (df1 = 118, df2 = 3776, Sig = .000)</td>
</tr>
<tr>
<td>Average Measures</td>
<td>.885c</td>
<td>.838 - .919</td>
<td>19.391 (df1 = 118, df2 = 3776, Sig = .000)</td>
</tr>
</tbody>
</table>

Two-way mixed effects model where people effects are random and measures effects are fixed.

a. The estimator is the same, whether the interaction effect is present or not.
b. Type A intraclass correlation coefficients using an absolute agreement definition.
c. This estimate is computed assuming the interaction effect is absent, because it is not estimable otherwise.

Table 4-9: ICC calculations showing the strength of the overall correlations between the repeated recordings of the DPOAE f2 frequencies as single measures (as shown below) and with all the measures averaged together.

<table>
<thead>
<tr>
<th>DPOAE F2 Frequency</th>
<th>Repeats</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.8 kHz</td>
<td>R1</td>
<td>1</td>
<td>.548*</td>
<td>.401*</td>
</tr>
<tr>
<td></td>
<td>R2</td>
<td>.548*</td>
<td>1</td>
<td>.270*</td>
</tr>
<tr>
<td></td>
<td>R3</td>
<td>.401*</td>
<td>.270*</td>
<td>1</td>
</tr>
<tr>
<td>1 kHz</td>
<td>R1</td>
<td>1</td>
<td>.646*</td>
<td>.746*</td>
</tr>
<tr>
<td></td>
<td>R2</td>
<td>.646*</td>
<td>1</td>
<td>.799*</td>
</tr>
<tr>
<td></td>
<td>R3</td>
<td>.746*</td>
<td>.799*</td>
<td>1</td>
</tr>
<tr>
<td>1.3 kHz</td>
<td>R1</td>
<td>1</td>
<td>.807*</td>
<td>.900*</td>
</tr>
<tr>
<td></td>
<td>R2</td>
<td>.807*</td>
<td>1</td>
<td>.791*</td>
</tr>
<tr>
<td></td>
<td>R3</td>
<td>.900*</td>
<td>.791*</td>
<td>1</td>
</tr>
<tr>
<td>1.6 kHz</td>
<td>R1</td>
<td>1</td>
<td>.845*</td>
<td>.896*</td>
</tr>
<tr>
<td></td>
<td>R2</td>
<td>.845*</td>
<td>1</td>
<td>.836*</td>
</tr>
<tr>
<td></td>
<td>R3</td>
<td>.896*</td>
<td>.836*</td>
<td>1</td>
</tr>
<tr>
<td>2 kHz</td>
<td>R1</td>
<td>1</td>
<td>.888*</td>
<td>.890*</td>
</tr>
<tr>
<td></td>
<td>R2</td>
<td>.888*</td>
<td>1</td>
<td>.868*</td>
</tr>
<tr>
<td></td>
<td>R3</td>
<td>.890*</td>
<td>.868*</td>
<td>1</td>
</tr>
<tr>
<td>2.5 kHz</td>
<td>R1</td>
<td>1</td>
<td>.877*</td>
<td>.932*</td>
</tr>
<tr>
<td></td>
<td>R2</td>
<td>.877*</td>
<td>1</td>
<td>.845*</td>
</tr>
<tr>
<td></td>
<td>R3</td>
<td>.932*</td>
<td>.845*</td>
<td>1</td>
</tr>
<tr>
<td>3.2 kHz</td>
<td>R1</td>
<td>1</td>
<td>.876*</td>
<td>.899*</td>
</tr>
<tr>
<td></td>
<td>R2</td>
<td>.876*</td>
<td>1</td>
<td>.901*</td>
</tr>
<tr>
<td></td>
<td>R3</td>
<td>.899*</td>
<td>.901*</td>
<td>1</td>
</tr>
<tr>
<td>4 kHz</td>
<td>R1</td>
<td>1</td>
<td>.771*</td>
<td>.874*</td>
</tr>
<tr>
<td></td>
<td>R2</td>
<td>.771*</td>
<td>1</td>
<td>.812*</td>
</tr>
<tr>
<td></td>
<td>R3</td>
<td>.874*</td>
<td>.812*</td>
<td>1</td>
</tr>
<tr>
<td>5 kHz</td>
<td>R1</td>
<td>1</td>
<td>.878*</td>
<td>.928*</td>
</tr>
<tr>
<td></td>
<td>R2</td>
<td>.878*</td>
<td>1</td>
<td>.911*</td>
</tr>
<tr>
<td></td>
<td>R3</td>
<td>.928*</td>
<td>.911*</td>
<td>1</td>
</tr>
<tr>
<td>6.4 kHz</td>
<td>R1</td>
<td>1</td>
<td>.880*</td>
<td>.908*</td>
</tr>
<tr>
<td></td>
<td>R2</td>
<td>.880*</td>
<td>1</td>
<td>.889*</td>
</tr>
<tr>
<td></td>
<td>R3</td>
<td>.908*</td>
<td>.889*</td>
<td>1</td>
</tr>
<tr>
<td>8 kHz</td>
<td>R1</td>
<td>1</td>
<td>.681*</td>
<td>.699*</td>
</tr>
<tr>
<td></td>
<td>R2</td>
<td>.681*</td>
<td>1</td>
<td>.683*</td>
</tr>
<tr>
<td></td>
<td>R3</td>
<td>.699*</td>
<td>.683*</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 4-10: Outcome of interclass correlation coefficient (ICC) analysis assessing the correlation between the three repeated DPOAE recordings as a function of each f2 frequency tested. Corrections for biasing from multiple correlations was performed using the simple Bootstrap method.

** Correlation is significant at the $p<0.01$ level (2-tailed).
Figure 4-12: Scatter plot with trend line showing a positive weaker correlation ($R^2=0.30$) between the 1st and 2nd repeat DPOAE recordings at f2 frequency 0.8 kHz.

Figure 4-13: Scatter plot with trend line showing a positive strong correlation ($R^2=0.77$) between the 1st and 2nd repeat DPOAE recordings at f2 frequency 5 kHz.

The final useful calculation that was performed was to assess the standard error of measurement (SEM) and the minimum detectable difference (MDD). The SEM was used to
assess test-retest reliability and was computed using the formula: SEM = s*√(1–ICC) where ‘s’ is the combined standard deviation (SD) of all the three repeated recordings at each DPOAE f2 frequency and the ‘ICC’ is the interclass correlation coefficient also known as the reliability coefficient (r) for the test. The SEM results were then used to calculate MDD, which is considered as the minimum difference above which an actual change exceeds the measurement error in the score and is therefore considered abnormal i.e. it is the ‘band of error’ beyond which a change is a true sign of abnormality. The $MDD_{95\%}$ is equivalent to $1.96*\sqrt{2}*SEM$ which is within ±2 standard errors (SEs) of the MDD calculation and therefore has a confidence interval of 95%. Table 4-11 shows the outcome of these calculations for all the DPOAE f2 frequencies tested.

These values allow us to assess the limits above which a change can be attributed to deterioration in cochlear function and therefore would be vital for the use of DPOAEs as a monitoring tool for ototoxicity. The SEM scores averaged across the strongly correlated mid-frequencies of 1.6 to 6 kHz equaled 2.7 dB SPL with an averaged $MDD_{95\%}$ of 7.5 dB SPL. This data suggests that there is a 95% probability that a drop in DPOAE amplitudes of >7.5 dB SPL in these frequencies signifies change in the OHC non-linear cochlear function secondary to exposure to ototoxic medications, or any source of inner ear insult, in these patients. The $MDD_{95\%}$ average for the lower and highest f2 frequencies, of 0.8 – 1.3 kHz and 8 kHz, is higher, giving values of 11.1 dB SPL and 18.0 dB SPL respectively.

<table>
<thead>
<tr>
<th>DPOAE f2 frequencies (kHz)</th>
<th>0.8</th>
<th>1.0</th>
<th>1.3</th>
<th>1.6</th>
<th>2.0</th>
<th>2.5</th>
<th>3.0</th>
<th>4.0</th>
<th>5.0</th>
<th>6.0</th>
<th>8.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICC</td>
<td>0.3</td>
<td>0.7</td>
<td>0.8</td>
<td>0.8</td>
<td>0.9</td>
<td>0.9</td>
<td>0.9</td>
<td>0.8</td>
<td>0.9</td>
<td>0.9</td>
<td>0.7</td>
</tr>
<tr>
<td>SEM</td>
<td>4.5</td>
<td>4.0</td>
<td>3.5</td>
<td>2.6</td>
<td>2.4</td>
<td>2.6</td>
<td>2.3</td>
<td>3.2</td>
<td>2.6</td>
<td>3.2</td>
<td>6.5</td>
</tr>
<tr>
<td>$MDD_{95%}$</td>
<td>12.5</td>
<td>11.0</td>
<td>9.7</td>
<td>7.2</td>
<td>6.6</td>
<td>7.2</td>
<td>6.3</td>
<td>9.0</td>
<td>7.2</td>
<td>8.9</td>
<td>18.0</td>
</tr>
</tbody>
</table>

Table 4-11: Showing the calculated Interclass correlation coefficient (ICC), Standard error of measurement (SEM) and Minimum detectable difference at 95% probability ($MDD_{95\%}$) for the repeated measurements at each of the DPOAE f2 frequencies recorded.
4.2.2 Discussion of DPOAE test-retest repeatability control study:

This study aimed to investigate the short-term test-retest reliability of DPOAE recordings in primary school-aged children, taking into account the effect of reinsertion of the probe into the ears. This would enable assessment of the normal variability of DPOAEs so that these variations can be accounted for when they are used to monitor cochlear function in children receiving potentially ototoxic medications or offer an explanation for their lower sensitivity in the clinical CF study.

The DPOAE recordings, obtained in this study from 60 normal hearing children (120 ears), were shown to be highly repeatable even when the probe was removed and reinserted in each child’s ear. This was confirmed by absence of a significant within-subject difference between the three recordings at all f2 frequencies using a repeated measures ANOVA test (F2,118 = 2.609, p=0.078, partial η2 = 0.042) and through the strong correlation between the repeated recordings shown in Table 4-10. The single most significant variable influencing the reliability of DPOAE measurements was the f2 frequency. This was evidenced by the SNR scores (Figure 4-11) where the minimum 3dB SNR score required to confirm the presence of a true 2f1-f2 DPOAE response above a mean noise floor +2SDs was not reached at the lower f2 frequencies <1.3 kHz or at the highest frequency of 8 kHz. SNR scores at ≥1.3 to 6.4 kHz were well above these pass criteria and were repeatable. Decreased SNR scores in the lower f2 frequencies were mainly attributed to higher noise floor levels and relatively lower DPOAE amplitudes in comparison with the higher f2 frequencies (Figure 4-10). This was in agreement with Roede et al., Zhao & Stephens, and Beattie et al., who reported that environmental, instrumentation and internal-subject noise affected the DPOAE reliability mainly at the lower frequencies (Roede et al., 1993, Zhao and Stephens, 1999, Beattie et al., 2003). Other researchers such as Gorga et al. hypothesized that the forward and reverse middle ear transmission and cochlear nonlinear function contributed to the significant effect of environmental noise on lower frequency DPOAEs (Gorga et al., 1994).

On the other hand the decreased SNR scores at 8 kHz were mainly attributed to the less than target stimulus levels actually delivered to the ears (target L1/L2: 65/55 whereas average stimulus levels of only 52/40 dB SPL where actually recorded). Kepper et al. also reported lower DPOAE reliability at 8 kHz and confirmed that higher primary-tone level DPOAE combinations improved reliability of DPOAEs amplitudes (Kepper et al., 2010). This same issue was reported by other researchers who attributed the increased variability in stimulus levels at 8kHz to difficulties with calibration at f2 frequencies >6kHz (Franklin et al., 1992, Roede et al., 1993). The theory that the presence of standing waves in the ear canal leads to
systematic errors in estimating eardrum sound pressure levels by the probe microphone at the higher frequencies was also proposed (Siegel and Hirohata, 1994).

One of the main drivers to performing this study was due to the fact that there is very limited published data on test-retest reliability of DPOAEs in the paediatric population (Gorga et al., 2000b, O'Rourke et al., 2002, Sockalingam et al., 2007) in comparison to neonatal and adult populations, which are both potentially very different. The neonates were shown to have very robust DPOAEs attributed to healthy undamaged cochlear function but also explained by higher amplification of the DPOAE response within the smaller ear canal space between the DPOAE probe and the tympanic membrane. Conversely, they were also shown to have significantly high noise levels at the lower frequencies (<1.0 kHz) which were mainly caused by internal noises from breathing and crying (Gorga et al., 2000b). DPOAEs in adults were shown to be repeatable but with lower amplitudes due to more cochlear damage from noise exposure and ageing. Lower frequency noise was still high but was mainly attributed to environmental noise (Gorga et al., 2000a, Gorga et al., 1993, Keppler et al., 2010, Franklin et al., 1992, Roede et al., 1993). The results of the current study were an intermediate outcome compared to these two population groups in that the responses were robust and repeatable as in the neonates but will less internal noise affect as in the adult population.

Results showed that testing the school-aged group of children in a quiet room rather than in a soundproof booth did not have a significant effect on the measurements. This was in agreement with other studies (Gorga et al., 2000b, Cone-Wesson et al., 2000) that showed that the external test environment did not have a significant effect of DPOAE recordings even when testing babies in the perinatal period as long as the test environment was relatively quiet. They showed that the main source of increased noise levels with consequent decrease in SNRs was the baby’s own internal sounds like heavy breathing, crying, or nasal congestion. As these internal noises decrease, as the child gets older, this effect decreases. This confirms that children can have their auditory status monitored through DPOAE testing for evidence of ototoxicity during a hospital visit, on the ward or even in the patient’s home as long as the room is a relatively quiet.

The calculations of SEM and MDD95% were very useful in assessing the test-retest reliability of DPOAE recordings in normal-hearing children of this age group. Ideally a test should produce the same results when the same subject is tested under the same conditions on two separate occasions. Unfortunately, in reality this goal is not met and clinical tests show less-than-perfect reliability in test-retest conditions. The SEM is a reliability estimate that is capable of determining a true DPOAE from a single measurement so that if a change on repeated monitoring recordings is found clinicians can be confident that this is due to a
change in the auditory system and not simply due to measurement error (Demorest and Walden, 1984). The SEM at the lower frequencies (<1.3 kHz) averaged 4.0 dB and was averaged 2.7 dB at the higher frequencies (1.6-6 kHz). This was similar to results obtained in 50 young adult women tested by Beattie et al. who reported SEMs of ~4.6 dB at 550 Hz and ~2.5 dB at the higher frequencies (1 to 4 kHz) which was also similar to other studies (Beattie 2003), (Beattie and Bleech, 2000, Zhao and Stephens, 1999, Franklin et al., 1992, Beattie et al., 2003). The larger SEM in the lower frequencies was attributed to a more significant effect of changes in the position of the probe tip on the level of background noise in the ear canal (Zhao and Stephens, 1999) and to the middle ear changes in fluid and air pressure having more effect on the lower frequencies (Roede et al., 1993). Beattie et al. (2003) assessed SEMs for immediate repeated recordings (on same day with no probe replacement), for very-short term (on same day but after a 10-20 minute break and replacement of probe) and for short-term recordings (5-10 days following the first recording) and showed that the major factor contributing to variability was the probe removal and reinserion. Therefore careful probe insertion and fitting may contribute to decreasing variability. Reavis et al. conducted a similar study where potential occurrences for adjacent frequency shifts in normal ears of eight subjects were calculated and showed that a 4dB shift in amplitudes was only observed in 5% of the recordings (21/409). They concluded that DPOAE change criteria of ≥4dB level reduction or loss of response due to drop in the DPOAE amplitudes to < -10dB at two or more adjacent f2 frequencies would be considered as a true change of outcome with a 5% false positive rate to be expected (Reavis et al., 2008).

The SEM was used to determine the confidence interval for a child’s true DPOAE to establish whether the DPOAE has significantly deviated from a cut-off or normal level or not. The 95% confidence intervals (MDD$_{95\%}$) averaged for the lower frequencies (<1.3kHz) were 11.1 dB SPL and 7.5 dB SPL for the higher frequencies (1.6 – 6 kHz). This was in agreement with Beattie et al. (2003) which calculated similar MDD$_{95\%}$ of ~10 dB at 550 Hz and ~5 dB for 1-4 kHz suggesting that differences between DPOAE recordings should exceed 14 dB and 7 dB for the lower and higher frequencies respectively to be statistically significant at the 0.05 level of confidence. Values exceeding these limits following exposure to ototoxic medications are likely to represent an actual decrease in cochlear function rather than due to a measurement error.

4.2.3 Conclusion:

Review of the literature had shown that research related to DPOAE testing in ears of children during the primary school age was limited. As this was the prevalent age group of the children with CF that were included in the main study, this control study was undertaken to
exclude possible confounding factors that may have affected the outcomes of the main study group of children with CF.

Results showed that DPOAE recordings could be highly repeatable in the ears of children within this age group. There was a strong correlation between repeated recordings across all test frequencies even after probe removal and reinserion was performed. It therefore suggested that this was probably not a contributing factor to changes in sensitivity of the DPOAE when testing the children with CF as long as good probe insertion was maintained. It established that on repeated DPOAE recordings, while regularly monitoring for ototoxicity, a minimal change of $>11.1$ and $>7.5$ dB SPL for the lower and higher frequencies respectively is required to establish a true shift in auditory function with 95% confidence, and not to attribute a change to an error of measurement. These thresholds were similar to recommendations of previous researchers. It also confirmed that accurate repeatable recordings could be made even when testing in a quiet room (as opposed to a soundproof room). This makes this tool suitable for use in a clinic room or a wardroom if the patient is too ill to go to an audiology department. DPOAE testing is a quick, non-invasive, repeatable and accurate measurement of inner ear OHC function. The outcomes of this study have confirmed that a recommendation to use it as serial monitoring tool to detect evidence of ototoxicity in children can be made with confidence.
Chapter 5: **Results and Discussion of Theme B: Causation**

The aim of this theme was to investigate factors that may be associated with ototoxicity in CF children. The clear dichotomy seen in the hearing status of the children with CF and exposure to aminoglycosides suggested that there might be risk factors that make some children more susceptible to ototoxicity than others. The results of two studies are presented and discussed. The first one assessed factors in the CF children’s clinical / drug history and investigations that may be significant risk factors associated with ototoxicity. The second study was a genetic study, which initially investigated the prevalence of the mtDNA A1555G mutation of the 12S rRNA gene. This mutation is the commonest mutation reported to increase susceptibility to aminoglycoside ototoxicity and non-syndromic hearing loss (Bottger, 2010, Gallo-Teran et al., 2004, Shohat et al., 1999). It also investigated the association between certain variants of drug-metabolizing genes and aminoglycoside ototoxicity as they were previously shown to be significantly associated with cisplatin ototoxicity (Ross et al., 2009).
5.1 Potential risk factors associated with AG ototoxicity in CF children

5.1.1.1 Study group:

The study group consisted of the same 70 children with CF that were audiologically assessed for Theme A. Data obtained from the children’s clinical and drug history and from outcomes of investigations were statistically analyzed to investigate the relationship between them and ototoxicity. This data was obtained through verbal interviews with the children’s parents/carers; through review of annual review reports, discharge letters and clinical notes; and through review of patient records of haematological tests for therapeutic drug monitoring (TDM) of the aminoglycosides administered while the children were admitted to GOSH for intake of i.v. antibiotic courses.

5.1.1.2 Analysis of potential risk factors:

Table 5-1 shows the factors that were tested for association with the occurrence of ototoxicity in this study group. As the data for these risk factors was not normally distributed, non-parametric Chi-square ($\chi^2$) test and Mann Whitney U-test were employed to compare the distribution of the categorical and numerical possible risk factors respectively, across the two categories of ‘ototoxicity absent’ and ‘ototoxicity present’.

Results of the analysis showed that the ototoxic group had: a significantly higher age; lower mean FEV₁ lung function; exposure to a greater number of i.v AG courses; exposure to a greater number of amikacin and tobramycin courses; and that they were also significantly associated with intake of vancomycin. However, gender, ethnicity, other AG types (gentamicin) and plasma AG trough levels did not show a significant difference between the groups (Table 5-1). A Pearson’s correlation test showed that there was a moderate negative correlation between lung function, recorded as the FEV₁ % predicted score, and the number of IV AG courses received ($p<0.01; r = -0.505; r^2 = 0.218$) (Figure 5-1).
Figure 5-1: Comparison between the number of courses of IV AG received by each of the children with CF and average FEV₁ % predicted score.
The bold line is the negative regression line and the dotted lines are the 95% confidence intervals. A significant (p < 0.01) negative Pearson’s correlation was obtained between the number of administered IV AG courses and average lung function with R² = 0.218

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>Ototoxicity</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Absent (n = 55)</td>
<td>Present (n = 15)</td>
</tr>
<tr>
<td><strong>Age &amp; Gender</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median age years (range)</td>
<td>10.1 (3.1-16.2)</td>
<td>13.0 (5.5-16.4)</td>
</tr>
<tr>
<td>Female sex [n (%)]</td>
<td>31 (56%)</td>
<td>11 (73%)</td>
</tr>
<tr>
<td>Male sex [n (%)]</td>
<td>24 (44%)</td>
<td>4 (27%)</td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian [n (%)]</td>
<td>50 (91%)</td>
<td>13 (87%)</td>
</tr>
<tr>
<td>Other race [n (%)]</td>
<td>5 (9%)</td>
<td>2 (13%)</td>
</tr>
<tr>
<td><strong>Lung Function</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median FEV₁ % predicted score (range)</td>
<td>79.1 (69.1)</td>
<td>54.5 (55.1)</td>
</tr>
<tr>
<td><strong>Aminoglycoside risk factors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median no. IV AG courses (range)</td>
<td>4 (0-31)</td>
<td>16 (3-40)</td>
</tr>
<tr>
<td>Median yrs. of intake of IV AGs (range)</td>
<td>5 (0-16)</td>
<td>7 (1-15)</td>
</tr>
<tr>
<td>Median no. of Amikacin courses (range)</td>
<td>2 (0-21)</td>
<td>10 (2-26)</td>
</tr>
<tr>
<td>Median no. of Tobramycin courses (range)</td>
<td>0 (0-11)</td>
<td>1.5 (0-18)</td>
</tr>
<tr>
<td>Median no. of Gentamicin courses (range)</td>
<td>0 (0-11)</td>
<td>0 (0-24)</td>
</tr>
<tr>
<td><strong>Other Ototoxic Agents</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. Receiving Vancomycin courses (n (%))</td>
<td>3 (5%)</td>
<td>4 (26%)</td>
</tr>
<tr>
<td>No. Receiving Cisplatin courses (n (%))</td>
<td>1 (2%)</td>
<td>2 (13%)</td>
</tr>
</tbody>
</table>

Table 5-1: Assessment of potential risk factors associated with ototoxicity.

* indicates the categorical variables with patient numbers and percentage within each group presented and chi-squared test used for analysis using χ² or Fischer’s exact p-values as appropriate. **indicates the...
numerical variables with median and range values presented and the Mann Whitney U-test used for analysis. Significant data ($p < 0.05$) is shown in bold.

External review of these results led to recommendations for further statistical analysis. Specifically for multivariable linear mixed models to be applied for each of the testing methods. Multivariable linear mixed models were applied for each of the testing methods, Standard PTA, EHF PTA and DPOAE to quantify the association of these different possible risk factors on the response levels at different frequencies. This allowed modeling the trajectories of hearing level (dB) change according to frequency and adjusting for the possible risk factors, taking into account the repeated measures at different frequencies from different ears within person (Appendix 9.13).

The motivation for this analysis was the possibility that adding all the frequency responses within each of the three audiological tools into a multivariable linear mixed model would take advantage of the richness of the data collected rather than just depending on the binary analysis of identifying ‘non-ototoxic’ versus ‘ototoxic’ groups. The binary analysis from each of these tools as previously presented in the Kappa analysis in Table 4-2, Table 4-3 and Table 4-4 or in the chi-square analysis in Table 5-1. The criteria for otoxicity was applied to each frequency tested by the three tools rather than to the overall outcome of each of the tests. However, this analysis provided some outcomes that are contradictory to those previously presented and appears to be quite circular as the same individual frequencies within each audiological tool were the same frequencies used to define ototoxicity. In this analysis only the 8kHz frequency outcomes from standard audiometry showed a statistically significant coefficient change, which was expected from previous analysis, yet for the EHF audiometry only the 11.8 and 12.0 kHz frequency were significant. This was not expected as data presented in Figure 4-1 and Figure 4-2, which showed the clear difference in thresholds across all EHF audiometry responses at all 9 to 16 kHz frequencies, and from the ANOVA statistical analysis which showed: ‘The audiometric thresholds did not differ significantly between the groups at the lower frequencies ($\leq 4$ kHz) but differed significantly from one another at $p < 0.05$ for frequencies 8 to 16 kHz with related t-tests when Bonferroni adjustment was made for the number of comparisons.’ (Section: 4.1.1.2). The analysis of the DPOAE responses was even more divergent from previous analysis as it showed that all f2 frequencies (except for 8kHz) tested showed significant coefficient changes that were much larger than the standard or the EHF audiometry data (i.e. coefficient values range of 3.79 - 18.59 for DPOAEs vs. 0.72 - 3.74 for EHF PTA). This was different from the DPOAE amplitude and SNR data presented in Figure 4-6 and Figure 4-7 respectively and in the kappa analysis presented in Table 4-3 and Table 4-4. These results had shown that even though the DPOAE results were generally in agreement with the audiometric data, the
sensitivity of the EHF audiometry to detection of ototoxicity was higher than with DPOAE. This current analysis may shed more light on the possibility that DPOAEs may be more sensitive to inner ear ototoxic damage than previously presented. However, using the audiometric data, as the gold standard for audiometry was still considered a valid approach to analysis. For these reasons the table detailing this multivariate analysis has been removed from this section and added as an appendix item (Appendix 9.13) for reference.

5.1.1.3 Significance of the type of Aminoglycoside used:

The three AGs administered were Amikacin, Tobramycin and Gentamicin and these are typically prescribed as a once daily dose over an average of 14 days. Serum levels were monitored before the 2nd and 8th doses with pre-dose trough levels. These were always administered in combination with other i.v antibiotics such as ceftazidime, meropenem or piperazobactam. Table 5-1 shows that in this study group, there was a statistically significant difference (p<0.001) between the ototoxic versus the non-ototoxic group of children mainly in regards to Amikacin and to a lesser extent to Tobramycin (p=0.038) where both drugs were more associated with the ototoxic group. Pearson’s correlation analysis was performed between the number of Amikacin courses taken and an audiometric high-frequency pure-tone average (8-12.5 kHz). It showed a significant mild negative correlation (p<0.05; r= -0.247; r²= 0.280) between the two, where increase in intake in the number of courses was associated with hearing loss and deterioration in audiometric thresholds with the r² value indicating that increased intake is responsible for 28% of the occurrence of high frequency hearing loss. There was no significant correlation found when the same analysis was performed for Tobramycin intake (p=0.730; r= -0.042; r²= 0.038) or Gentamicin intake (p=0.373; r= -0.109; r²= 0.034). Assessment of AG trough levels used for TDM, as an indication of exceeding therapeutic blood levels of the AG used when higher than normal levels are recorded, did not show a significant difference between the ototoxic and non-ototoxic groups (p >0.05), suggesting that ototoxicity can occur despite adherence to therapeutic levels.
5.1.2 Discussion of potential risk factors associated with AG ototoxicity in CF children

5.1.2.1 Age, gender and ethnicity

An analysis of possible risk factors predictive of ototoxicity in this study, showed a significant difference between the ages of the non-ototoxic and the ototoxic groups. It is worth noting that 14 out of 15 ototoxic group of children (Table 4-1) were over 10 years of age (median: 13.8; range: 5.5 - 16.4 years), suggesting that they were more likely to have received a larger number of courses of i.v AG and therefore age is probably more of an indicator of amount of AG exposure than an actual risk factor to ototoxicity. Ethnicity differences were not of significance between the two groups, probably because our study subjects were mainly Caucasians, and neither were there gender differences (Table 5-1). Previous studies have shown a higher prevalence of ototoxicity in Hispanic (O'Donnell et al., 2010) and Asian (Knight et al., 2005) patients with CF compared to Caucasians.

Children with greater disease severity (lower FEV₁ indicating poorer lung function) had a higher risk of ototoxicity \( (p<0.01) \). There was also a negative correlation between FEV₁ and the number of courses of i.v AG received \( (p<0.01; r=-0.505; r^2=0.218) \), as expected (Figure 5-1). Children with lower lung function would be more likely to have a greater number of i.v AG courses and hence at an increased risk of ototoxicity indicating, again, that poor lung function could be another possible confounding factor. Concomitant use of other potentially ototoxic medications was also assessed as risk factors for ototoxicity and Vancomycin was found to be significantly associated with ototoxicity in this group. O'Donnell et al. considered risk factors for AG ototoxicity in adult CF patients and also reported a borderline significance for concomitant use of Vancomycin (O'Donnell et al., 2010).

5.1.2.2 Type of aminoglycoside taken

The majority of patients in this study had received i.v Amikacin, compared to Tobramycin or Gentamicin. This was in line with the prescribing protocol at Great Ormond Street Hospital, which used Amikacin as the first-line treatment for \textit{pseudomonas aeruginosa} infections since the 1990s when gentamicin-resistant strains emerged. Tobramycin was also commonly prescribed, but after/alternating with amikacin courses. Results of the survey study of current ototoxicity monitoring practice in the UK presented in section 6.2, have shown that this is not common practice in the UK as 97% of the responding CF clinicians indicated that Tobramycin was their first line of treatment (Table 6-9). A mild significant negative correlation between Amikacin and poor high-frequency hearing \( (p<0.05; r=-0.247; r^2= \)
0.280) was found but was mostly absent for the other two AG types. Despite this finding, the preferential use of Amikacin did not allow for an accurate comparison of ototoxicity between different AGs and therefore the suggestion that Amikacin is more ototoxic than the other AGs studied is made with caution. However, this has been investigated previously by Matz et al. who reported a prevalence for ototoxicity of 18% for Gentamicin and 12.9%, 11.5% and 2% for Amikacin, Tobramycin and Netilmicin, respectively (Matz, 1986) and others, have reported auditory toxicity occurring in 4.4, 10.8, and 23.5% of 187 patients given Netilmicin, Tobramycin, and Amikacin, respectively (Gatell et al., 1987).

Assessment of AG trough levels is used as an indication of exceeded therapeutic blood levels of AG. It did not show a significant difference between the ototoxic and non-ototoxic groups (p >0.05), suggesting that ototoxicity can occur despite adherence to therapeutic levels. O’Donnell et al. showed ototoxicity was predicted by trough serum concentrations >10 mg/L for Amikacin or >2 mg/L for Gentamicin and Tobramycin (O’Donnell et al., 2010). However, Tan et al. highlighted that tests for therapeutic drug monitoring should be interpreted with caution, as they are limited in sensitivity (Tan et al., 2003).

5.1.2.3 Cumulative repeated AG courses

This study has shown that one of the most significant risk for ototoxicity (p<0.001) in children with CF was history of high exposure to i.v AGs, particularly those who have received at least 10 courses (Table 5-1). This was also confirmed in the multivariate analysis where high exposure showed the highest unit increase in thresholds mainly in EHF PTA but also in standard PTA results (Table 5-9-1). This was in agreement with Mulheran et al. who assessed 70 CF patients and 91 controls and reported ototoxicity in 12 CF patients including 1 paediatric case. The median number of AG courses in the hearing impaired group was 20 versus only 9 in the normal hearing group (Mulheran et al., 2001). Several other studies have also reported on the effects of repeated AG exposure on the auditory status of CF patients (Mulherin et al., 1991, Crifo et al., 1980, Pedersen et al., 1987, Wood et al., 1996). This suggests that repeated doses have a cumulative effect and that a child that has many doses of AG but still hasn’t presented with ototoxicity may still develop hearing loss at a later stage and therefore must be monitored. However, the fact that there were children in the ototoxic group with history of exposure to a small number of AG courses and others with history of high exposure yet completely normal hearing, as demonstrated in Figure 4-4, excludes that a simple linear relationship exists between these two factors suggesting that other factors are possibly involved, including genetic predisposition, as suggested by others (Mulheran et al., 2001, Smyth, 2010).
5.1.2.4 Information regarding antibiotic intake

The most significant limitation of this study was accuracy of information about previous drug history and details regarding intake of AGs (i.e. number of courses in the child’s lifetime and the years over which these courses were taken). The first source of information was obtained through the history-taking interview with the child’s parent/carer before audiological assessment was undertaken. It was clear at a very early stage that this was a variable source of information as some parents provided very accurate information and even kept written records of all of their child’s medications while others were very overwhelmed by their child’s condition and only vaguely knew what medications they were receiving. The second source of information was examining the patients’ hospital notes where records of medications when children are admitted to the hospital for i.v AGs were kept and review of the results of the haematological tests where the dates for trough level measurements of the AGs used for TDM were kept. Even though these provided a more accurate source of information they were not comprehensive. Some children were admitted to their local hospitals for these treatments or were administered the i.v. treatments at home if they had a patent venous line through a portacath. Local hospitals and the GPs were consistently requested to provide this information to the GOSH CF unit as this was the main center of care for these children, but this information was not always fed back. Finally, every Annual Review letter written by the CF clinician was examined in order to extract medication information provided for that past year. Crosschecking with any data available within databases that only members of the CF unit staff could access was also done.

This highlighted a significant clinical problem in the accuracy and accessibility of this information not only for future ototoxicity monitoring but also for monitoring of any other side effects of these medications, assessment of benefit, comparison of outcome between treatment regimens or even identification of drug resistance. CF unit staff members confirmed that this is a problem especially as this is information that is submitted to the CF Trust database in order to produce the UK CF Registry annual data reports published online (https://www.cysticfibrosis.org.uk/about-cf/publications/cf-registry-reports.aspx). The difficulty encountered in trying to retrieve this information actually highlighted the need to include clear and accurate treatment records as an essential component of any ototoxicity monitoring service being developed whether on a local departmental, national or international scale. Regarding its affect on the current study, this was a limiting factor, as the accuracy of the drug history was not guaranteed 100% but the crosschecking of the information managed to keep the information as accurate as possible.
5.1.3 Conclusions:

Analysis of the data related to ototoxicity in the CF children assessed has shown that repeated cumulative exposure to aminoglycosides is the most significant risk factor associated with the occurrence of ototoxicity. The older age of the child and the poorer lung function were also significantly associated with ototoxicity but this may be because this is the group with increased exposure to more i.v. AG courses. As shown in the data in the earlier audiological assessment study, higher audiometric frequencies (≥8 kHz) were more significantly associated with differentiating between non-ototoxicity and ototoxicity, as were the higher DPOAE f2 frequencies. It could not be categorically confirmed that Amikacin was more ototoxic than other AGs due to the prescription bias in this group of children because of the treatment protocol of GOSH, however it was the AG drug type that was most consistently associated with ototoxicity.

This study has suggested that there are several possible ‘risk factors’ that may increase the susceptibility of CF children to ototoxicity confirming that audiological monitoring and good record keeping of these factors is very important if the aim is to try to prevent ototoxicity and avoid deterioration of the children’s quality of life. The lack of a clear linear relationship between ototoxicity and any of the factors assessed, even high exposure, mandates that further investigation into other causes that affect individual susceptibility to ototoxicity, such as genetic causes, may help elucidate the mechanisms involved.
5.1.4 Investigating susceptibility to AG ototoxicity in CF children through Genetic studies.

Results from the audiological clinical study reported earlier in section 4.1, Figure 4-3 showed a clear dichotomy in the recorded hearing of the children, with some showing evidence of hearing loss following the high frequency sloping SNHL representation of ototoxicity while others maintained exceptionally good hearing. Here, despite higher cumulative exposure to repeated intravenous courses of AGs being identified as a significant risk factor to development of ototoxicity it could not account for hearing impairment in all cases. It was not clear why some children manifest significant hearing impairment following minimal exposure to AGs while others sustained normal hearing even after multiple courses of intravenous AGs. This supported the aim to investigate the role of genetic variation in increasing susceptibility to aminoglycosides and a possible explanation for this dichotomy.

As presented in section 1.7, genetic susceptibility to AG ototoxicity is well established especially in relation to mutations in the mitochondrial DNA such as the A1555G mutation in the 12S rRNA gene. The frequency of this mutation varies by ethnicity but two large UK-based population studies report a prevalence of the A1555G mutation of 0.19% and of 0.26% respectively (Bitner-Glindzicz et al., 2009, Rahman et al., 2012). A higher prevalence of 2 to 5% was reported in Caucasians with sensorineural deafness (Hutchin et al., 2001, Kupka et al., 2002b, Tekin et al., 2003). A study of a population of adult cystic fibrosis patients showed a prevalence of 1.3%, which was considered to be higher than expected (Conrad et al., 2008).

5.1.4.1 Study group:

The parents/carers of the children with CF recruited in the audiological observational study also provided consent for their children to provide either a blood or saliva sample for DNA extraction to test for genetic variations that may be associated with increased susceptibility to ototoxicity. All 70 children who underwent audiological assessment were recruited but three of the parents/children refused to give consent to this aspect of the research or it was not possible to obtain a sample from the patient. Therefore, a total of 67 DNA samples were collected. Two of the missing samples were from children in the ‘Non-ototoxic’ group and one was from the ‘Ototoxic’ group. This meant that there were 53 children with normal hearing and 14 children with evidence of ototoxicity included in the genetic study.

The first aim of this study was to assess the prevalence of the mtDNA A1555G mutation in the 12S rRNA gene in CF children as this is the most commonly reported gene mutation to be associated with increased susceptibility to aminoglycoside ototoxicity (Bai et al., 2008, ...
Bottger, 2010, Casano et al., 1998, Estivill et al., 1998, Gallo-Teran et al., 2004, Gallo-Teran et al., 2003, Scrimshaw et al., 1999, Shohat et al., 1999). The second aim was to assess if the two variants in the TPMT and COMT genes (rs12201199 and rs4646316 respectively) were significantly associated with occurrence of aminoglycoside ototoxicity in the CF children. These variants were previously shown to be significantly associated with cisplatin ototoxicity in children with cancer (Ross et al., 2009). The overall aim of the genetic study was to assess whether the presence of any of these variants in subjects might explain the dichotomy in outcome versus dose observed in the audiological study.

5.2 Analysis of mtDNA A1555G and other variations in the 12S rRNA gene in CF patients

5.2.1 Outcomes of genotyping for the A1555G mutation:

Polymerase chain reaction (PCR) was used to amplify a section of the 12S rRNA gene containing the site of the A1555G mutation using primers as described in Table 3-1. Restriction fragment length polymerase (RFLP) was then used to digest the amplified fragment into different sized fragments according to whether the A1555G mutation was present or not. The HaeIII restriction endonuclease enzyme was used to genotype the samples for the A1555G mutation using this PCR-RFLP technique. The A1555G genotype was identified in 2/67 subjects and both appeared to be homoplasmic for this mutation. Figure 5-2 shows a representative UV image of the 2% agarose gel electrophoresis of six digested samples including the two samples showing A1555G mutation where three fragments of sizes 216 bp, 93 bp and 30 bp are visualized instead of the two fragments of sizes 216 and 123 bp which were seen with the wild-type m.1555A variant. Table 5-2 is a 2x2 contingency table showing the distribution of children with and without ototoxicity according to the A1555G genotype where there was only one child with the A1555G mutation in each of the two groups. Fisher’s exact test showed a non-significant difference between ototoxicity and the A1555G mutation ($\chi^2 (1, n=67) =3.84, p = 0.333$). The overall prevalence of the A1555G mutation was equal to 3% of this study cohort. This was a surprising outcome of this study as this prevalence was higher than the expected prevalence reported in population studies. A 3% prevalence with 2/67 cases is much higher than the reported ~0.2% frequency of this mutation in the UK and other populations (Bitner-Glindzicz et al., 2009, Kokotas et al., 2009). It is equivalent to a 15-fold increase in prevalence. In order to try to confirm if there is a higher prevalence of A1555G mutations in patients with CF, more DNA samples were collected from this cohort. In total 105 samples
were collected with the same patient information, consent and assessments performed for the additional subjects as for the original recruited children with the exception of 19 children who only provided the DNA sample but didn’t have the hearing assessments undertaken by the time the analysis of the data was performed. These assessments were later undertaken as part of the regular ototoxicity-monitoring clinic that was established as a result of this current research (see Appendix 9.12. for detailed ototoxicity monitoring protocol established for the CF and Audiology units at GOSH). Interestingly, PCR-RFLP revealed absence of any more A1555G mutations in any of the added cases making the prevalence 2/105 (1.9%), which was still found to be a non-significant difference between determination AG ototoxicity and occurrence of A1555G mutation ($\chi^2 (1, n=105) =3.84, p = 0.395$) as shown in Table 5-3.

Figure 5-2: RFLP analysis of the 12S RNA A1555G mutation using HaeIII restriction enzyme. Well M, 100bp DNA ladder marker. Well 1, undigested sample (339bp). Lanes 2-7, HaeIII digested samples. Wild type (WT) samples show 2 fragments at 216 bp & 123 bp (WT – wells 2, 5-7). The A1555G (Mut.) affected samples show 3 fragments at 216 bp, 93 bp and 30 bp (Mut. – wells 3-4); Well 8, non-template control.
### A1555G mutation vs. ototoxicity

<table>
<thead>
<tr>
<th></th>
<th>Non-ototoxic (n=53)</th>
<th>Ototoxic (n=14)</th>
<th>Total</th>
<th>OR (95% CI)</th>
<th>p-valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absent A1555G</td>
<td>52 (98%)</td>
<td>13 (93%)</td>
<td>65 (97%)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Present A1555G</td>
<td>1 (2%)</td>
<td>1 (7%)</td>
<td>2 (3%)</td>
<td>4.818 (0.28-83.035)</td>
<td>0.333</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>53 (100%)</td>
<td>14 (100%)</td>
<td>67 (100%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a Fisher’s exact test 2-sided significance was used as 2 cells had cell count <5

Table 5-2: Contingency table showing a record of patient numbers with and without the A1555G mutation in the two groups (ototoxicity was defined by the audiometric data). \chi^2- test shows non-significant difference between presence of A1555G mutation and occurrence of ototoxicity. Note the normal hearing case with the A1555G mutation (not reported previously in literature). *Fisher’s exact test (two tailed, \(p< 0.05\)).

### A1555G mutation vs. ototoxicity

<table>
<thead>
<tr>
<th></th>
<th>Non-ototoxic (n=67)</th>
<th>Ototoxic (n=19)</th>
<th>Hearing tests not performed yet (n=19)</th>
<th>Total</th>
<th>(p)-valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absent A1555G</td>
<td>66 (98.5%)</td>
<td>18 (95%)</td>
<td>19 (100%)</td>
<td>103 (98%)</td>
<td></td>
</tr>
<tr>
<td>Present A1555G</td>
<td>1 (1.5%)</td>
<td>1 (5%)</td>
<td>0 (0%)</td>
<td>2 (2%)</td>
<td>0.395</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>67 (100%)</td>
<td>19 (100%)</td>
<td>19 (100%)</td>
<td>105 (100%)</td>
<td></td>
</tr>
</tbody>
</table>

*a Fisher’s exact test 2-sided significance was used as 2 cells had cell count <5

Table 5-3: Contingency table showing a record of patient numbers with and without the A1555G mutation in the groups defined by the audiometric outcomes when more samples were genotyped. \chi^2- test showed no significant difference between presence of A1555G mutation and occurrence of ototoxicity. *Fisher’s exact test (two tailed, \(p< 0.05\)).

#### 5.2.2 Further analysis of the two children with the A1555G genotype:

Sanger sequencing was used to confirm a positive result in the A1555G genotype. The audiological data and sequencing chromatograms for the two individuals with the A1555G
mutation are presented in Figure 5-3. Child A displayed bilateral severe high frequency hearing loss with diminished DPOAEs typical of AG ototoxicity. Surprisingly, child B showed well preserved hearing as demonstrated by thresholds across all frequencies and by DPOAE measurements despite repeated intravenous aminoglycoside use in the presence of the A1555G mutation. This finding was surprising because it has been repeatedly reported that exposure to AGs is one of the most significant modifying factors associated with increasing the penetrance of the A1555G mutation up to 100% (Bitner-Glindzicz et al., 2009, Pandya, 1993). The clinical characteristics of both children A and B are presented in Table 5-4. Due to the unexpected nature of this observation a second, independent DNA sample was obtained from this patient to confirm the presence of the A1555G mutation. Functional evidence from several earlier studies suggests that the pathogenic mechanism of A1555G may be via an altered secondary structure of the 12S rRNA, which facilitates an interaction with aminoglycoside antibiotics. If so, it is possible that further mutations in the 12S rRNA molecule could modify the secondary structure to ameliorate this pathogenicity (Ballana et al., 2006). To determine whether child B, or even child A, had such a mutation the complete 12S rRNA gene was sequenced for both children. The results confirmed the presence of the A1555G mutation in child A and B in addition to the identification of two other known polymorphisms of 750A>G and 1438A>G, which were also present in both subjects.
Figure 5-3: Outcomes of audiological and genetic assessments for both child A and child B. Aⅰ, Bⅰ shows standard and EHF pure tone audiometry. Child A Aⅰ shows a severe high-frequency sloping sensorineural hearing loss whereas child B, Bⅰ shows normal hearing across all frequencies (0.25 – 16 kHz). Aⅱ and Bⅱ show DPOAE results of each child. Child A, Aⅱ displays an abnormal DP-gram with low DPOAE amplitudes and signal/noise ratio <6 dBSPL representing poor cochlear function whereas child B, Bⅱ results are within normal DP-gram with high DPOAE amplitudes and a signal/noise ratio ≥6 dBSPL representing normal cochlear function. Aⅲ and Bⅲ show the sequencing chromatograms of the section of the mtDNA 12S rRNA gene that contains the A1555G mutation in both children, highlighted by a grey bar.
<table>
<thead>
<tr>
<th>Patient</th>
<th>Ototoxic</th>
<th>Non-ototoxic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Child A</td>
<td>14.1y</td>
<td>11.5y</td>
</tr>
<tr>
<td>Child B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>Female</td>
<td>Female</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>Caucasian</td>
<td>Caucasian</td>
</tr>
<tr>
<td>CFTR Genotype</td>
<td>ΔF508/1717-1G&gt;T</td>
<td>ΔF508/ΔF508</td>
</tr>
<tr>
<td>Mean FEV&lt;sub&gt;1&lt;/sub&gt; % predicted (Lung Function)</td>
<td>72%</td>
<td>104%</td>
</tr>
<tr>
<td>12S rRNA genotype</td>
<td>750A&gt;G</td>
<td>750A&gt;G</td>
</tr>
<tr>
<td></td>
<td>1438A&gt;G</td>
<td>1438A&gt;G</td>
</tr>
<tr>
<td></td>
<td>A1555G</td>
<td>A1555G</td>
</tr>
<tr>
<td>Hearing status</td>
<td>Severe HF-SNHL</td>
<td>Normal</td>
</tr>
<tr>
<td>Family history of maternal HL</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Vestibular toxicity</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>IV AG courses (n, type)</td>
<td>3 (Amikacin)</td>
<td>3 (Tobramycin)</td>
</tr>
<tr>
<td>Years during which IV courses were given</td>
<td>1 y</td>
<td>3 y</td>
</tr>
<tr>
<td>Nebulized AG (type, duration)</td>
<td>Gentamycin (3m)</td>
<td>TOBI (3y)</td>
</tr>
<tr>
<td>Other IV/ototoxic Rx</td>
<td>Vancomycin/Azithromycin/Ceftazidime/Flucloxacillin</td>
<td>Vancomycin/Ciprofloxacin/Flucloxacillin/Azithromycin</td>
</tr>
<tr>
<td>Family history of HL</td>
<td>Negative</td>
<td>Negative</td>
</tr>
</tbody>
</table>

*CFTR*, cystic fibrosis transmembrane conductance regulator gene; *IV*, intravenous; *AG*, aminoglycoside; *FEV<sub>1</sub>%, forced expiratory volume in one second (percentage); *y*, years; *m*, months; *HF-SNHL*, high-frequency sloping sensorineural hearing loss; *TOBI*, nebulized tobramycin; *HL*, hearing loss; *Rx*, treatment.

Table 5-4: Characteristic features of the two children with CF who had A1555G mutation and history of exposure to AGs.

Due to the significance of this finding, another sample was collected from the child and both the genetic and auditory testing was repeated and findings confirmed. The child’s parents were informed of the outcomes of this genetic test and a more detailed history was obtained to confirm the absence of hearing loss in any of the family members especially the matrilineral relatives. The family was offered the chance to have genetic counseling and the managing clinician confirmed that this child should not be prescribed any AGs in the future.
except where it was essential and recommended that regular audiological monitoring of this child is maintained. The same actions were also taken for Child A but additionally this child was referred to Audiology where she was fitted with bilateral hearing aids and offered rehabilitation and counseling regarding her hearing loss. Child A reported that she had initially refused to wear hearing aids but changed her mind later because she was struggling to hear in school and found it very difficult to get any part-time jobs because of her hearing loss. She currently wears her hearing aids and is much happier.
5.2.3 Discussion of outcomes from investigating the A1555G mutation and sequencing the 12S rRNA gene:

The most commonly reported mutation, as a cause of increased susceptibility to aminoglycoside ototoxicity, is the A1555G mutation in the 12S rRNA gene in the mitochondrial DNA. Results of the current study identified two children with the A1555G mutation in 67 CF children (Table 5-2). As this was equivalent to a 3% prevalence, which is a 15-fold increase in prevalence when compared to the reported population prevalence of 0.2% (Bitner-Glindzicz et al., 2009, Rahman et al., 2012, Vandebona et al., 2009) it was decided to test more children from this cohort to confirm if this mutation was more common in children with CF than in the general population. A total of 105 samples were tested but no more mutations were uncovered (Table 5-3). This sample size is considered a small sample group in genetics studies as a ‘chance’ occurrence of two individuals can give an overestimation of the prevalence of this mutation. This indicated that a significantly larger cohort might need to be examined before such a conclusion could be made. A chi-squared analysis was performed to assess if the difference between the observed and expected frequencies of the presence of the A1555G mutation and occurrence of ototoxicity was significant or not. The results showed that it was not significant ($\chi^2$ (1, n=105) =3.84, p=0.395) (Table 5-3). However, Conrad et al. investigated the frequency of mtDNA 12S rRNA variants in 157 North American adult CF patients (Conrad et al., 2008). As in this current study, they also highlighted that the frequency of the A1555G mutation (1.27%) was higher than that observed in non-CF general populations where it was detected at a frequency of 1/385-520 (0.19-0.26%) in different studies. Conrad et al. identified five CF patients with homoplasmic variations in this gene with two of these patients having the A1555G mutation. These two patients presented with moderate-to-severe SNHL after exposure to only one and four courses of tobramycin respectively. The other three patients each displayed one form of polymorphism of 827A>G, 961T>G and 1494C>T with AG-induced auditory dysfunction reported as unknown, none (normal hearing) and mild hearing loss respectively. They didn’t observe any correlation between the CFTR genotypes and the novel 12S rRNA variants that they identified (Conrad et al., 2008). The Conrad et al. study was the only study reporting the prevalence of 12S rRNA mutations in patients with CF in addition to this current study. If the data of both the current study and Conrad’s study were combined, a total of 4/262 patients with CF would be reported to have the A1555G mutation. This would still equate to a higher prevalence of 1.5% compared to the reported average population prevalence of 0.2%. This again highlights the importance of conducting larger scale investigations with this patient group especially since most of the patients with CF will need to take these antibiotics sooner.
or later in their lifetime and so identifying subjects that are more susceptible to their side effects would be beneficial.

However, the aim of this current study was not to perform an association study between the A1555G mutation and AG ototoxicity as this has already been established and the sample size was too small to detect such an effect. As stated before, previous population studies had shown an prevalence of 0.2%, which means that it is expected to only find around two cases per 1000 subjects tested. Therefore the sample size included within this current study would never have fulfilled this aim. The main aim of this study was to assess if this mutation could explain part of the causation of ototoxicity in this patient cohort and see if it could partially explain the dichotomy in hearing status observed. The small number of children carrying this mutation (only two children) could not support that this mutation was a strong cause of ototoxicity occurring in this cohort especially that one of these children was within the non-ototoxic group having normal hearing. However, the other child within the ototoxic group is one of the few children (4/15 children with ototoxicity) that were within the low-exposure group. She started complaining of tinnitus and hearing loss even after exposure to the first course of amikacin, which progressed to severe-profound SNHL after exposure to only three courses. This is an agreement with previous research, which has confirmed that the presence of this mutation does not just increase susceptibility to AG ototoxicity but also increases the occurrence of ototoxicity even after intake of one dose of AG (Pandya, 1993).

The most significant finding of this study was the identification of a child with the A1555G mutation with normal hearing despite having documented evidence of AG exposure (Table 5-4). It has been repeatedly documented that AG exposure is a major modifying factor and has been perceived to make the A1555G mutation 100% penetrant in the presence of AGs (Bae et al., 2012, Estivill et al., 1998, Li et al., 2005, Liu et al., 2006). A possible explanation of this finding of normal hearing despite the positive genotype and exposure to AGs may be due to low dosage intake of the treatment leading to diminished cochlear load of AGs preventing them from reaching pathogenic levels. This however cannot be the cause as it is well documented that a single therapeutic dose of AG is capable of causing ototoxicity in subjects with the A1555G mutation (Pandya, 1993). Child B reported here has documented evidence from the hospital records confirming that she has received three i.v courses of tobramycin (14 days of 10mg/kg body weight/day/i.v course) over a 3-year period in addition to repeated intake of nebulized tobramycin (TOBI). Delayed post-treatment onset of hearing loss may not be the explanation either because the child was assessed several years after the intake of the last AG course. If testing was confined to performing standard audiometry, as in common practice, it may have been hypothesized that this child had early
effects of ototoxicity restricted to the higher frequencies and not detected by standard PTA. However EHF audiometry and DPOAEs were performed, as they were shown by the earlier study (section 4.1) and by other previous studies to be better markers of early ototoxicity (Al-Malky et al., 2011, Knight et al., 2007, Stavroulaki et al., 2002). This child had normal audiometric thresholds up to 16kHz and normal DPOAE amplitudes (Figure 5-3) again excluding undiagnosed hearing loss.

The A1555G mutation is considered the most common base change in the 12S rRNA that is associated with AG ototoxicity (Fischel-Ghodsian et al., 2004, Guan, 2011, Prezant et al., 1993a). It occurs in a conserved region of the gene producing a secondary structure (Ballana et al., 2006) which closely resembles the bacterial E. coli 16S rRNA gene and creates an AG-binding site in the human 12S rRNA structure which subsequently results in decreased mitochondrial ribosomal function and build up of reactive oxygen species (ROS) ensuing initiation of apoptosis (Cheng et al., 2005, Roland, 2004). Another possible explanation for hearing preservation in this child may be that this child has another rare mutation(s) in the 12S rRNA gene, which negates the A1555G pathogenic mechanism by changing this secondary structure. However, as presented earlier, DNA sequencing of the gene has only uncovered two other known nucleotide variations (750A>G and 1438A>G) which were also present in Child A and were not reported to have significant effects on the penetrance and expressivity of hearing impairment with the A1555G mutation (Bae et al., 2012, Li et al., 2004, Lu et al., 2010b, Zhao et al., 2004). Should this point be investigated further, sequencing of the mtDNA rRNA gene and other reported nuclear modifier genes such as TRMU, MTO1 or p53 would need to be explored (Guan et al., 2006, Li et al., 2004, Li et al., 2002). More tentatively, it has been suggested by Mulheran et al. that the CF condition may provide some form of ‘protection’ of the ear from AG ototoxicity even in the presence of the A1555G mutation (Mulheran et al., 2001). The CFTR protein is expressed in outer hair cells (OHC) where it has been shown to interact with prestin, the OHC motor protein, and so it is not implausible that mutations in the CFTR gene might in some way vary the hair cell’s susceptibility to aminoglycosides (Homma et al., 2010). However, there is no tangible evidence to support this theory especially given that many studies report high rates of ototoxicity in CF patients, as exhibited by Child A here.

The most plausible suggestion to explain this A1555G non-penetrance case has been reached from a detailed review of the relevant literature. Here, the true penetrance of this mutation with the added modifying factor of AG exposure is questioned based on the existence of a recruitment bias in previous studies. A large number of studies document the frequency of A1555G in cohorts of families or unrelated individuals with a previous/existing diagnosis of
hearing loss (Abe et al., 1998, Abreu-Silva et al., 2006, Bae et al., 2012, Bai et al., 2008, Gallo-Teran et al., 2002, Guo et al., 2008, Kokotas et al., 2009, Malik et al., 2003, Ou et al., 2011, Tekin et al., 2003, Zu-Jian et al., 2009). This recruitment method would not permit detection of non-penetrance as the phenotype is already exhibited. Many of the pedigree-based studies also involve a retrospective characterisation of the matrilineal family members of a proband diagnosed with AG-induced ototoxicity. Previous exposure of these family members to AGs is usually dependent on self-report. This may compromise researchers’ confidence in confirming this exposure in the absence of a robust source of information especially when people generally take a wide variety of ‘antibiotics’ during their lifetime without always exactly knowing which ‘type of antibiotic’ they are prescribed. In this scenario it is possible that non-penetrance may have been under reported. With this in mind, the report here of a single case of non-penetrance may be a very rare observation or it could represent a more frequent phenomenon, which has been overlooked due to a recruitment bias towards hearing impaired individuals. It is unlikely that the A1555G interaction with AGs is not highly penetrant, especially given that even the studies that reported low penetrance showed that all probands/matrilineal relatives that had a history of exposure to AGs were hearing-impaired (Dai et al., 2006, Tang et al., 2007). However, this case has highlighted the lack of studies capable of establishing the true penetrance of this effect. In addition, it has been reported that there are issues of publication bias where it is quite difficult to publish negative findings compared to positive observations. Case reports are also increasingly difficult to publish which makes evidence of the existence of these cases of non-penetrance extremely difficult to add to the literature. This problem was highlighted by Boddy when commenting on the recently published literature related to the genetics of cisplatin ototoxicity (Boddy, 2013) as well as through personal experience when trying to publish this case report.

In conclusion, providing accurate information regarding the true penetrance of this interaction is highly significant because clinical centres are starting to advocate screening patients for the A1555G mutation based on recommendations of population studies even if it hasn’t been deemed to be cost-effective yet (Bitner-Glindzicz et al., 2009, Vandebona et al., 2009, Veenstra et al., 2007). This is particularly significant since avoidance of first-line aminoglycoside therapy based on pharmacogenetic testing for the A1555G genotype can cause an increased risk of morbidity or even mortality in some conditions. A large well-characterised study with accurately documented AG exposure is urgently required.
5.3 **Investigating the association between variants in the TPMT & COMT genes and AG ototoxicity:**

Ross et al. had undertaken a study where association analyses of 1,946 SNPs from 220 drug-metabolism genes with increased susceptibility to cisplatin ototoxicity in cancer children treated with this drug were performed. They identified some variants of both the TPMT and COMT genes to be highly statistically significantly associated with cisplatin ototoxicity. This was the first relatively large-scale study in which this link between drug-metabolizing genes and ototoxicity was investigated. The aim of this part of the research was to assess whether a similar association was present with these identified SNPs and aminoglycoside ototoxicity in the study CF population.

5.3.1 **Genotyping for the rs4646316 COMT SNP:**

The DNA was extracted from the blood and saliva samples in accordance with the manufacturer’s instructions as described in the methods section 3.5.2.4.1. Amplification of the gene fragment of interest was done using Polymerase Chain Reaction (PCR) by using primers specific for each reaction as specified in Table 3-1. A representative gel showing the PCR amplified DNA fragment containing the rs4646316 SNP of the COMT gene was 457 bp in size and was shown in Figure 5-4. Genotyping was performed using the Restriction Fragment Length Polymerase (RFLP) technique where the PCR amplified fragment is digested further into smaller fragments using an enzyme. The number and size of the digested fragments varies according to the genotype of the SNP of interest. RFLP was carried out for all the amplified samples where the restriction endonuclease enzyme XcmI was used to digest the samples. The three genotypes produced are shown in Figure 5-5. The wild-type (W/T) C/C genotype was digested into two fragments of sizes of 378 and 79 bp; the heterozygous C/T genotype was digested into four fragments of 378, 207, 171 and 79 bp; whereas the variant homozygous genotype T/T was digested into three fragments of 207, 171 and 79 bp.
**5.3.2 Genotyping for the rs12201199 TPMT SNP:**

For the rs12201199 TPMT SNP, the same PCR-RFLP procedure was used first. The PCR amplified DNA fragment was 168 bp in size. This was digested using the *MnII* enzyme in RFLP, which would cut the W/T genotype A/A at positions 116, 128 and 155 and would cut the variant genotype T/T at positions 128 and 155. Analysis by agarose gel electrophoreses produced four fragments of sizes 116, 12, 27 and 13 bp with the W/T genotype A/A. It produced only three fragments of sizes 128, 27 and 13 bp with the variant T/T genotype. These small fragments could not be distinguished with clarity so the analysis was dependent
on the 116 and 128 bp fragments alone. This procedure was not able to clearly discriminate between the three genotypes due to the similar small band sizes, which are not easily visualized using agarose gel electrophoresis (fragments 116 and 128 bp had only 12 bp difference in size). This was shown in Figure 5-6 where it was clear that it was not easy to differentiate between the genotypes even when a λDNA Hind III marker (M2 marker in the figure) was used because it had a 125 bp fragment to help differentiate between the A/A and T/T genotypes. Therefore, it was decided to send the fourteen samples, which were suspected to be either A/T or T/T, for Sanger sequencing to confirm exact genotypes, together with a few wild type samples as a control. Representative chromatograms obtained for the three different genotypes are shown in Figure 5-7. These outcomes were matched with the UV electrophoresis image of the same samples displayed in Figure 5-6. As the PCR-RFLP technique was shown to be unclear and the cost of sequencing all the samples was going to be too high, it was subsequently decided that Real-time PCR using TaqMan® SNP Allelic Discrimination Assays was to be used as it was the most cost-effective and accurate technique to use in order to confirm the correct allele calls for all the samples. All these techniques are described in the methods section 3.5.2.4. Through the Real-time PCR Taqman genotyping assay technique only one heterozygous A/T genotype was identified in the ototoxic group of children (as defined by the audiometric tests) and no homozygous variant T/T genotypes were identified. For the non-ototoxic group, five children had the A/T genotype while one child had the T/T genotype Table 5-5. This data was presented and further analyzed in section 5.3.3 below.
The fragments obtained were small and very similar in size making it difficult to identify the different genotypes. The small fragments 12, 27 and 13 bp were all blurred and vaguely seen at the lower end of the gels. Only the relatively larger 116bp and 128 bp fragments could be seen clearly. The genotypes (A/A, A/T and T/T) marked on this picture were identified through the DNA sequencing as shown in figure 5-6 and then matched with the same samples on this UV image. The heterozygous A/T and homozygous variant T/T were impossible to differentiate through this technique.

M1, 1kb Ladder marker; M2, λDNA Hind III marker used because it had a 125bp-band fragment that could differentiate between the 116bp A/A digested fragment and the 128bp T/T digested fragment.

Figure 5-7: DNA sequencing chromatogram of TPMT rs12201199 SNP.

Showing 3 sequencing chromatograms with the Heterozygous A/T (top), Wild-type Homozygous A/A (middle) and Variant Homozygous T/T (bottom) TPMT rs12201199 SNP respectively. Only 14 samples were sent for sequencing and only 9 of these were correctly identified through this technique (the other samples were too noisy). The identified samples were matched to the UV electrophoresis image displayed in figure 5-5.
5.3.3 Statistical analysis of the results:

The outcomes for both gene variants were then correlated with evidence of ototoxicity in the sample using Chi-squared ($\chi^2$) statistical analysis as shown in Table 5-5. The mutant T/T genotype in rs12201199 *TPMT* SNP was only present in one (1.9%) normal hearing child and the mutant T/T rs4646316 *COMT* SNP was only present in two (3.8%) normal hearing children and one (7.1%) child with ototoxicity. Chi-square analysis showed that both variants were not statistically significantly associated with aminoglycoside ototoxicity in this cohort of children with CF ($p=0.691$ and $0.442$ respectively). More detailed analysis was presented below.

<table>
<thead>
<tr>
<th>Genotype / allele</th>
<th>CF Patients</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-Ototoxic (n=53)</td>
<td>Ototoxic (n=14)</td>
</tr>
<tr>
<td><strong>TPMT rs12201199</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/A</td>
<td>47 (88.7%)</td>
<td>13 (92.9%)</td>
</tr>
<tr>
<td>A/T</td>
<td>5 (9.4%)</td>
<td>1 (7.1%)</td>
</tr>
<tr>
<td>T/T</td>
<td>1 (1.9%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>A</td>
<td>99 (93.4%)</td>
<td>27 (96.4%)</td>
</tr>
<tr>
<td>T</td>
<td>7 (6.6%)</td>
<td>1 (3.6%)</td>
</tr>
<tr>
<td><strong>COMT rs4646316</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C/C</td>
<td>32 (60.4%)</td>
<td>11 (78.6%)</td>
</tr>
<tr>
<td>C/T</td>
<td>19 (35.8%)</td>
<td>2 (14.3%)</td>
</tr>
<tr>
<td>T/T</td>
<td>2 (3.8%)</td>
<td>1 (7.1%)</td>
</tr>
<tr>
<td>C</td>
<td>83 (78.3%)</td>
<td>24 (85.7%)</td>
</tr>
<tr>
<td>T</td>
<td>23 (21.7%)</td>
<td>4 (14.3%)</td>
</tr>
</tbody>
</table>

Table 5-5: Chi-square analysis showing lack of association between SNPs in TPMT & COMT and ototoxicity.

$^a$ determined using Fisher Exact Probability test as the expected cell count was <5 in some cells ($p<$0.05)

5.3.3.1 Modeling under different genetic models of inheritance

In order to further analyze the significance of association between the variant genotype and allele (in this case ‘T/T’ and ‘T’ respectively, for both the TPMT and COMT SNPs of interest) and cases with ototoxicity, further statistical analysis using the tests for deviation from Hardy-Weinberg equilibrium and association analysis for alleles and genotypes was performed. The output for the tests for deviation from Hardy-Weinberg equilibrium showed non-significant $p$-values ($p>0.05$) for both controls (i.e. non-ototoxic) and cases (i.e. ototoxic) for both SNPs. This indicated that there was no deviation of the genotype frequencies from those expected under the Hardy–Weinberg equilibrium. Case-control
association analysis for alleles and genotypes under different genetic models of inheritance was performed using a $\chi^2$-test or Fisher’s exact test if the expected cell count was <5. The $\chi^2$-test tests the null hypothesis, which is based on the assumption that there is no statistically significant difference between the observed and expected results and that any difference is due to chance. Therefore, if there is no statistical difference obtained because the $\chi^2$ value is larger than the critical value corresponding to the 0.05 (5%) probability then the difference is due to chance, and so the null hypothesis is accepted. The results for the association analysis were displayed in Table 5-6 for TPMT rs12201199 and in Table 5-7 for COMT rs4646316. Here the theory of the null hypothesis is that there is no significant relationship between each of these SNPs and the AG ototoxicity phenotype. As all the $\chi^2$ results showed that there is no significant difference ($p$>0.05) then the null hypothesis was accepted. Therefore this data did not show an association like the one seen with the cisplatin ototoxicity previously reported. The risk probability associated with the presence of either or both COMT and TPMT variant SNPs were also shown to be non-significant. This was displayed in Table 5-8 in addition to the presentation of the different levels of sensitivity and specificity of the observed results.

<table>
<thead>
<tr>
<th>SNP</th>
<th>TPMT rs12201199</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NON-OTOTOXIC (controls n=53)</td>
</tr>
<tr>
<td>A/A</td>
<td>47 (88.7%)</td>
</tr>
<tr>
<td>A/T</td>
<td>5 (9.4%)</td>
</tr>
<tr>
<td>T/T</td>
<td>1 (1.9%)</td>
</tr>
<tr>
<td></td>
<td>(a) i- Full genotype table for a general genetic model</td>
</tr>
<tr>
<td>A</td>
<td>99 (93.4%)</td>
</tr>
<tr>
<td>T</td>
<td>7 (6.6%)</td>
</tr>
<tr>
<td></td>
<td>(a) ii-Allele frequency</td>
</tr>
<tr>
<td>A</td>
<td>47</td>
</tr>
<tr>
<td>T</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>(b) Dominant model: allele T increases risk</td>
</tr>
<tr>
<td>A/A+A/T</td>
<td>52</td>
</tr>
<tr>
<td>T/T</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>(c) Recessive model: two copies of allele T required</td>
</tr>
<tr>
<td>A (2a+b)</td>
<td>99</td>
</tr>
<tr>
<td>T (b+2c)</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>(d) Multiplicative model: r-fold increased risk for AT, $r^2$ increased risk for TT. Analysed by allele, not by genotype</td>
</tr>
<tr>
<td>Risk allele A</td>
<td>0.421</td>
</tr>
<tr>
<td>Risk allele T</td>
<td>5.49</td>
</tr>
</tbody>
</table>

Table 5-6: TPMT association analysis for alleles and genotypes under different genetic models.
<table>
<thead>
<tr>
<th>SNP</th>
<th>COMT rs4646316</th>
<th>Gene</th>
<th>SNP</th>
<th>Genotyp e</th>
<th>Non-Ototoxic (n=53)</th>
<th>Ototoxic (n=14)</th>
<th>OR (95% CI)</th>
<th>P value</th>
<th>Sens</th>
<th>Spec</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPMT</td>
<td>rs12201199</td>
<td>T/</td>
<td>6 (11.3%)</td>
<td>1 (7.1%)</td>
<td>0.08</td>
<td>0.63</td>
<td>8.3%</td>
<td>88.9%</td>
<td>14.3%</td>
<td>81.4%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>A/A</td>
<td>47 (88.7%)</td>
<td>13 (92.9%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COMT</td>
<td>rs4646316</td>
<td>T/</td>
<td>21 (39.6%)</td>
<td>3 (21.4%)</td>
<td>0.31</td>
<td>0.19</td>
<td>16.7%</td>
<td>61.1%</td>
<td>8.7%</td>
<td>76.7%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>C/C</td>
<td>32 (60.4%)</td>
<td>11 (78.6%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Unique carriers of either*</td>
<td>27 (46.3%)</td>
<td>4 (25.0%)</td>
<td>0.39</td>
<td>0.21</td>
<td>25.0%</td>
<td>53.7%</td>
<td>10.7%</td>
<td>76.3%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Non-carriers</td>
<td>29 (53.7%)</td>
<td>9 (75.0%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5-8: Combined effect of TPMT and COMT genotypes on AG-induced ototoxicity.

---

(a) i- Full genotype table for a general genetic model

<table>
<thead>
<tr>
<th>Genotyp e</th>
<th>Non-Ototoxic (n=53)</th>
<th>Ototoxic (n=14)</th>
<th>OR (95% CI)</th>
<th>χ²</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C/C</td>
<td>32 (60.4%)</td>
<td>11 (78.6%)</td>
<td>1</td>
<td>3.157</td>
<td>0.287</td>
</tr>
<tr>
<td>C/T</td>
<td>19 (35.8%)</td>
<td>2 (14.3%)</td>
<td>0.174</td>
<td>(0.021-1.464)</td>
<td>0.191</td>
</tr>
<tr>
<td>T/T</td>
<td>2 (3.8%)</td>
<td>1 (7.1%)</td>
<td>0.174</td>
<td>(0.021-1.464)</td>
<td>0.191</td>
</tr>
</tbody>
</table>

(a) ii- Allele frequency

<table>
<thead>
<tr>
<th>Allele</th>
<th>Non-Ototoxic (n=53)</th>
<th>Ototoxic (n=14)</th>
<th>OR (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>83 (78.3%)</td>
<td>24 (85.7%)</td>
<td>0.914</td>
<td>(0.762-1.095)</td>
</tr>
<tr>
<td>T</td>
<td>23 (21.7%)</td>
<td>4 (14.3%)</td>
<td>1.519</td>
<td>(0.572-4.034)</td>
</tr>
</tbody>
</table>

(b) Dominant model: allele T increases risk

<table>
<thead>
<tr>
<th>Genotyp e</th>
<th>Non-Ototoxic (n=53)</th>
<th>Ototoxic (n=14)</th>
<th>OR (95% CI)</th>
<th>χ²</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C/C</td>
<td>32</td>
<td>11</td>
<td>1</td>
<td>2.135</td>
<td>0.191</td>
</tr>
<tr>
<td>C/T+T/T</td>
<td>21</td>
<td>3</td>
<td>0.314</td>
<td>(0.063-1.578)</td>
<td>0.96</td>
</tr>
</tbody>
</table>

(c) Recessive model: two copies of allele T required

<table>
<thead>
<tr>
<th>Genotyp e</th>
<th>Non-Ototoxic (n=53)</th>
<th>Ototoxic (n=14)</th>
<th>OR (95% CI)</th>
<th>χ²</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C/C+C/T</td>
<td>51</td>
<td>13</td>
<td>1</td>
<td>2.364</td>
<td>0.485</td>
</tr>
<tr>
<td>T/T</td>
<td>2</td>
<td>1</td>
<td>2.364</td>
<td>(0.197-28.424)</td>
<td>0.485</td>
</tr>
</tbody>
</table>

(d) Multiplicative model: r-fold increased risk for AT, r² increased risk for TT. Analysed by allele, not by genotype

<table>
<thead>
<tr>
<th>Genotyp e</th>
<th>Non-Ototoxic (n=53)</th>
<th>Ototoxic (n=14)</th>
<th>OR (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C (2a+b)</td>
<td>83</td>
<td>24</td>
<td>0.528</td>
<td>(0.145-1.927)</td>
</tr>
<tr>
<td>T (b+2c)</td>
<td>23</td>
<td>4</td>
<td>0.528</td>
<td>(0.145-1.927)</td>
</tr>
</tbody>
</table>

(e) Additive model: r-fold increased risk for AT, 2r increased risk for TT. Genotypes analysed by Armitage's test for trend

| Risk allele C | 1.351 | 0.92 | 0.337 |
| Risk allele T | 0.791 | 0.92 | 0.337 |

Table 5-7: COMT rs4646316 association analysis for alleles and genotypes under different genetic models.

*Number of individuals (percentage of the group) †Determined using Fisher's Exact Test (2-sided) ‡Combination of unique carriers of either the TPMT risk genotype (rs12201199 T/_) or the COMT risk genotype (rs4646316 T/_.) Sens, sensitivity; Spec, specificity; PPV, positive predictive value; NPV, negative predictive value.
It was also considered that the lack of significant association with AG ototoxicity might be due to the sample size not being large enough to achieve enough power to reject the null hypothesis with confidence. To confirm if this was the case, a power calculation was performed for both gene variants using the online genetic power calculator at [http://pngu.mgh.harvard.edu/~purcell/gpc/](http://pngu.mgh.harvard.edu/~purcell/gpc/) (Purcell et al., 2003). The case-control for discrete traits option was used. The data from Table 2 of the Ross et al. article was used to derive reference information for the expected genotypic relative risk Aa and AA values. The high risk allele frequency (A) data was derived from the population genetics 1000-Genome Study allele frequencies information presented in the e!Ensembl website at [http://www.ensembl.org/Homo_sapiens](http://www.ensembl.org/Homo_sapiens). For the rs12201199 TPMT SNP the calculation suggested that a sample size of 302 cases with ototoxicity would be needed to have 80% power to detect an allelic association based on an allele frequency of 0.151, prevalence of 0.21% and a 3.6 fold relative risk under an additive model. As for the rs4646316 COMT SNP the calculation suggested that a sample size of 42 cases with ototoxicity would be needed to have 80% power to detect an allelic association based on an allele frequency of 0.764, prevalence of 0.21% and a 2 fold relative risk under an additive model. Therefore, these calculations confirm that the sample size of the current study was not large enough to assess the significance of this association with confidence and so should be considered as a pilot study for this effect.

### 5.3.3.2 Genetic association with TPMT and hearing in the British 1958 birth cohort

The rs12201199 TPMT SNP was also genotyped in a sample of 351 individuals from the British 1958 birth cohort study group. This is a cohort of subjects born in 1958 for which samples were collected for a large-scale population study investigating many aspects of health of a representative sample of the British population. Therefore subjects were not selected for hearing impairment but audiological assessment was undertaken as part of an extensive test battery. Within this population study of 6000 individuals the top 1000 and bottom 1000 (16.67%) people were chosen according to their hearing scores (4kHz thresholds) to represent ‘good’ and ‘poor’ hearing for this 44-45 years age group. These samples were available in the lab and were used here to assess if a similar genotype representation was seen in a group of these subjects representing ‘Good’ and ‘Poor’ hearing as with the CF children in the study population (with and without ototoxicity). A sample of subjects representing ‘Good Hearing’ (n=175; 87 females and 88 males) and others representing ‘Poor Hearing’ (n=176; 88 females and 88 males) were tested to confirm if there was an association between the variant T/T genotype and poor hearing. Table 5-9
shows that again there was no significant difference in the genotype or allele frequencies between the two groups. As shown with the CF children, there were no association between \textit{TPMT} variants and poor hearing ($p=0.298$). Figure 5-8 shows the allele frequencies of both the 1958 cohort and CF study group data confirming the similarity in the genotype distributions between the two groups.

<table>
<thead>
<tr>
<th>Genotype/allele</th>
<th>With GH (n=175), n (%)</th>
<th>With PH (n=176), n (%)</th>
<th>OR (95% CI)</th>
<th>p value$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/A</td>
<td>150 (85.7%)</td>
<td>144 (81.8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/T</td>
<td>23 (13.1%)</td>
<td>29 (16.5%)</td>
<td>1.313 (0.726-2.377)</td>
<td>0.367</td>
</tr>
<tr>
<td>T/T</td>
<td>2 (1.1%)</td>
<td>3 (1.1%)</td>
<td>0.640 (0.105-3.886)</td>
<td>0.625</td>
</tr>
<tr>
<td>A</td>
<td>323 (92.3%)</td>
<td>317 (90.1%)</td>
<td>0.757 (0.448-1.280)</td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>27 (7.7%)</td>
<td>35 (9.9%)</td>
<td>1.321 (0.781-2.234)</td>
<td>0.298</td>
</tr>
</tbody>
</table>

Table 5-9: Genotypes for the British 1958 birth cohort samples. These were used to assess the prevalence of rs12201199 TPMT variants in a sample of the general population of normal hearing and poor hearing subjects. GH, Good Hearing and PH, Poor Hearing.

Figure 5-8: Comparison between the TPMT rs12201199 allele frequencies of the 1958 cohort study population representing good and poor hearing and the study CF group with and without ototoxicity (as defined by standard & EHF PTA outcomes). There was no statistically significant difference between the groups ($p<0.05$).
5.3.4 Overview for outcomes of genetic analysis of the children with ototoxicity

Table 5-10 displays the genetic characteristics of the fourteen children with evidence of ototoxicity. The table shows that their ages ranged between 5.5 and 16.4 years; that they were all Caucasian, with the exception of one child; that 9/14 were affected by the ΔF508/ΔF508 CFTR mutation, which is the commonest type of CFTR mutation recorded. Only Child A (CF062) was genotyped positive for the A1555G mutation. Only one child (CF035) had an A/T genotype for the TPMT SNP and only two (CF046 and CF011) had the C/T and one (CF026) had the T/T genotypes for the COMT SNP. None of these children had a positive family history for hearing loss. Overall there were no characteristic factors from the patients’ assessed data that separated Child A from the rest of the children that did not have the mutation, other than she had the less common ΔF508/1717-1G>T CFTR gene mutation.

<table>
<thead>
<tr>
<th>Pt. code</th>
<th>Age</th>
<th>Ethnicity</th>
<th>CFTR mutation</th>
<th>TPMT genotype</th>
<th>COMT genotype</th>
<th>A1555G mutation</th>
<th>Family H of HL</th>
</tr>
</thead>
<tbody>
<tr>
<td>CF035</td>
<td>15.9</td>
<td>Caucasian</td>
<td>ΔF508/ΔF508</td>
<td>A/T</td>
<td>C/C</td>
<td>- ve</td>
<td>- ve</td>
</tr>
<tr>
<td>CF062</td>
<td>14.6</td>
<td>Caucasian</td>
<td>ΔF508/1717-1G&gt;T</td>
<td>A/A</td>
<td>C/C</td>
<td>+ ve</td>
<td>- ve</td>
</tr>
<tr>
<td>CF067</td>
<td>15.1</td>
<td>Caucasian</td>
<td>ΔF508/ΔF508</td>
<td>A/A</td>
<td>C/C</td>
<td>- ve</td>
<td>- ve</td>
</tr>
<tr>
<td>CF046</td>
<td>12.0</td>
<td>Caucasian</td>
<td>ΔF508/ΔF508</td>
<td>A/A</td>
<td>C/T</td>
<td>- ve</td>
<td>- ve</td>
</tr>
<tr>
<td>CF032</td>
<td>5.5</td>
<td>Caucasian</td>
<td>ΔF508/ΔF508</td>
<td>A/A</td>
<td>C/C</td>
<td>- ve</td>
<td>- ve</td>
</tr>
<tr>
<td>CF011</td>
<td>14.0</td>
<td>Caucasian</td>
<td>ΔF508/ΔF508/1507</td>
<td>A/A</td>
<td>C/T</td>
<td>- ve</td>
<td>- ve</td>
</tr>
<tr>
<td>CF003</td>
<td>16.4</td>
<td>Caucasian</td>
<td>ΔF508/1154 ins TC</td>
<td>A/A</td>
<td>C/C</td>
<td>- ve</td>
<td>- ve</td>
</tr>
<tr>
<td>CF014</td>
<td>12.5</td>
<td>Caucasian</td>
<td>ΔF508/ΔF508</td>
<td>A/A</td>
<td>C/C</td>
<td>- ve</td>
<td>- ve</td>
</tr>
<tr>
<td>CF066</td>
<td>13.8</td>
<td>Caucasian</td>
<td>ΔF508/G542X</td>
<td>A/A</td>
<td>C/C</td>
<td>- ve</td>
<td>- ve</td>
</tr>
<tr>
<td>CF026</td>
<td>16.2</td>
<td>Caucasian</td>
<td>ΔF508/ΔF508</td>
<td>A/A</td>
<td>T/T</td>
<td>- ve</td>
<td>- ve</td>
</tr>
<tr>
<td>CF022</td>
<td>12.0</td>
<td>Caucasian</td>
<td>ΔF508/ΔF508</td>
<td>A/A</td>
<td>C/C</td>
<td>- ve</td>
<td>- ve</td>
</tr>
<tr>
<td>CF006</td>
<td>14.4</td>
<td>Caucasian</td>
<td>ΔF508/G551D</td>
<td>A/A</td>
<td>C/C</td>
<td>- ve</td>
<td>- ve</td>
</tr>
<tr>
<td>CF004</td>
<td>11.8</td>
<td>Indian</td>
<td>ΔF508/ΔF508</td>
<td>A/A</td>
<td>C/C</td>
<td>- ve</td>
<td>- ve</td>
</tr>
<tr>
<td>CF034</td>
<td>10.6</td>
<td>Caucasian</td>
<td>ΔF508/ΔF508</td>
<td>A/A</td>
<td>C/C</td>
<td>- ve</td>
<td>- ve</td>
</tr>
</tbody>
</table>

CFTR, cystic fibrosis transmembrane conductance regulator; TPMT, thiopurine S-methyltransferase; COMT, Catechol-O-methyltransferase; Family H of HL, family history of hearing loss.

Table 5-10: Genetic characteristics of the children with ototoxicity.
5.3.5 Discussion of the investigation of association between TPMT & COMT variants and AG-ototoxicity:

Cisplatin and aminoglycoside ototoxicity were shown to follow a similar apoptotic cell death pathway leading to cochlear toxicity (Hutchin and Cortopassi, 1994, Ravi et al., 1995, Rybak et al., 2007) and consequently typically lead to a similar phenotypic profile of permanent bilateral symmetrical high frequency sloping SNHL. Ross et al. proposed that variants in two genes, thiopurine S-methyltransferase (TPMT) and catechol O-methyltransferase (COMT), were significantly associated with patients having evidence of cisplatin ototoxicity (Ross et al., 2009). They investigated 1,949 SNPs in 220 drug-metabolizing genes identified to have key roles in absorption, distribution, metabolism and elimination (ADME) of medical treatments. They had two study groups, a discovery group of 54 children exposed to cisplatin from the same oncology center and an independent replication group of 112 children from several paediatric oncology units across Canada in order to ensure increased power and generalizability of any significant findings they discover. They showed that SNPs rs12201199; rs1142345 and rs1800460 of the TPMT gene and rs4646316 and rs9332377 of the COMT gene were significantly associated with oncology children exhibiting cisplatin ototoxicity (p<0.01). They were all reported to be ‘loss-of-function’ variants of these genes leading to decrease in their enzyme activity and hypothesized to consequently increase the toxic effect of cisplatin. As the TPMT rs12201199 was in Linkage disequilibrium (LD) with the other two SNPs (LD D’=1.0 for both) and the COMT rs4646316 was in LD with the rs9332377 SNP (LD D’=0.94) it was decided for the current study to only investigate these two SNPs for association with AG ototoxicity in CF children. Results of the Ross et al. study had shown that the risk allele for the TPMT rs12201199 was present in 9 (27.3%) and 16 (21.9%) of the children showing cisplatin ototoxicity in the discovery and replication cohorts and was not present in the controls of the discovery cohort in only 1 (2.4%) of the controls of the replication cohort. This was a significant even after Bonferroni adjustment for multiple testing in the combined analysis of both cohorts (Fisher exact allelic test p=0.00022, Bonferroni-corrected p=0.032). The COMT rs4646316 variant allele (T) was actually seen more in the controls with 5 (25%) and 2 (5.6%) of the controls in the discovery and replication cohorts presenting with it and in only 1 (1.4%) of the ototoxic cases in the replication cohort only. This indicated that this allele conferred a protective effect in this case (Fisher exact allelic test p=0.00055, Bonferroni-corrected p=0.076) (Ross et al., 2009). Results of the current study showed absence of any significant association between either SNPs of both genes and AG ototoxicity in the CF children (p >0.05) (Table 5-5, Table 5-6, Table 5-7 and Table 5-8) where the variant risk alleles (T) of both gene SNPs occurred more commonly in the control children. The COMT rs4646316 variant did not follow a similar
trend of protection as conferred in the Ross et al. study. Therefore, trend in allele frequency was in the same direction as the Ross et al. study with the TPMT rs12201199 SNP but was in the opposite direction for the COMT rs4646316 SNP. Samples from the British 1958 cohort study were used as samples representative of the general UK population with ‘Good’ or ‘Poor’ hearing to assess if similar genotype frequencies were obtained compared to those from the study population of CF children. Case-control association analysis between the TPMT rs12201199 and ‘poor hearing’ in samples from this population was also shown to be not statistically significant (Table 5-9) and of a similar genotype frequency distribution as the CF children (Figure 5-8). A similar association analysis using the British 1958 cohort study samples could not be done for the COMT rs4646316 variants. This was because there was not enough DNA in the available samples to identify the different variants when using RFLP whereas with the TPMT, Real time PCR using Taqman Allelic Discrimination assays was used which required much smaller quantities of DNA.

These results confirmed that AG ototoxicity did not show a significant association with these two gene variants in this CF cohort and therefore did not explain the dichotomous in the hearing status of these children. This was contrary to the findings previously reported to show a highly significant relationship with cisplatin ototoxicity. Another possible explanation for the lack of significant association with AG ototoxicity that was considered was that the sample size was not large enough to achieve enough power to reject the null hypothesis with confidence. The genetic power calculation that was performed suggested that a sample size cohort of 302 and 42 cases with AG ototoxicity would be needed to have 80% power to detect an allelic association for TPMT rs12201199 and COMT rs4646316 SNPs respectively. This was therefore considered as the main limitation of this study. Therefore, a recommendation to consider this cohort, as a pilot study group should be made and consideration to recruit the calculated sample size if this research question is to be pursued.

It is also worth noting that this current investigation was undertaken during the period of 2010-2012 following the initial Ross et al., 2009 article. In 2013 a number of articles were published with the main aim of replicating the significant outcomes of this original paper (Carleton et al., 2014, Yang et al., 2013, Pussegoda et al., 2013). Pussegoda et al. (who belong to the same Canadian group of investigators from the Ross et al., 2009 article) replicated the genetic findings in an independent group of 155 paediatric oncology patients exposed to cisplatin. They investigated the association between genetic variants in TPMT (rs12201199, rs1800460, rs1142345), and COMT (rs9332377, rs4646316), in addition to other variants, including the ATP-binding cassette transporter C3 (ABCC3) (rs1051640) and
cisplatin-induced ototoxicity. They were able to replicate these associations for genetic variants in *TPMT* (rs12201199, *p* = 0.0013, odds ratio (OR) 6.1) and *ABCC3* (rs1051640, *p* = 0.036, OR 1.8) in support of the original article. They also presented evidence that a predictive model that combined genetic variants in *TPMT*, *ABCC3*, and *COMT* with clinical variables (patient age, concomitant vincristine treatment, germ-cell tumour, and craniospinal irradiation) significantly improved the prediction of ototoxicity development as compared with a predictive model using clinical risk factors alone (Pussegoda et al., 2013). On the other hand, a separate research group from St. Jude Children’s Research Hospital, Memphis, Tennessee, USA, replicated this work in 213 children with medulloblastomas and could not find any significant association between *TPMT* or *COMT* variants and cisplatin-induced hearing loss. Instead, they confirmed that a significant connection with ototoxicity was only established for younger age (*p* = 0.013) and additional exposure to craniospinal irradiation (*p* = 0.001). They also replicated the study with 41 other children with solid-tumours (neuroblastoma and osteosarcoma) to avoid confounding factors in the children with medulloblastomas such as being treated with craniospinal irradiation and with amifostine, an otoprotectant against cisplatin ototoxicity. They still did not establish a significant association between *TPMT* and *COMT* variants with cisplatin-induced hearing loss. Experimental *in-vivo* and *in-vitro* laboratory studies were also undertaken using TPMT knock-out (KO) mice and lymphoblastoid cell lines, which also established a lack of association (Yang et al., 2013). Therefore the outcomes of the Yang et al. study for cisplatin ototoxicity showed similar outcomes as with the current study but for AG ototoxicity where no significant association was found between the *TPMT* or *COMT* variants and AG ototoxicity.

Publication of this conflicting data is important as it re-establishes the importance of replicating outcomes of such significant pharmacogenetic association studies. The US Food and Drug Administration (FDA) had changed the cisplatin label to indicate the association of *TPMT* with ototoxicity based on the outcomes of the original 2009 paper. This big impact was probably due to the fact that this article was published in the high-impact journal *Nature Genetics* and because the data presented a highly significant pharmacogenetic association with cisplatin-induced hearing loss. This has major implications on the clinical management of cancer patients needing cisplatin, where pre-treatment genotyping of patients in search of the *TPMT* and *COMT* variants would be needed if cisplatin treatment individualization were to be implemented. If cisplatin is the 1st line of treatment for an individual’s type of tumour, risk assessment of this avoidance treatment needs to be considered on top of cost-effectiveness of this genetic testing and the need for genetic counseling and other monitoring
services. These factors have large health and financial implications and therefore outcomes of any study have to be considered with care and caution.

However, there is still a rationale that variants of drug-metabolizing genes could be significantly associated with AG ototoxicity. It is still a valid research hypothesis and should be investigated further for AG ototoxicity. The outcomes of the current study did not support this but maybe by initially replicating Ross et al.’s methodology of assessing this association with variants of the most common 220 drug-metabolizing genes significant outcomes may be achieved with other genes. This is especially pertinent as the actual mechanistic pathway connecting variants of the two TPMT and COMT genes and cisplatin ototoxicity was not really established. Ross et al. had proposed a convoluted hypothesis that the TPMT/COMT variants are associated with deficiency in the functional ability of these genes, which could result in excess intracellular S-adenosylmethionine (SAM). Both TPMT and COMT are methyltransferases that are consistently dependent on SAM as a methyl-donor substrate in the methionine pathways occurring within many cells. Ochoa et al. had shown that administration of SAM and cisplatin together is associated with 3-6.2-fold increase in cisplatin toxicity as substantiated by significantly increased renal dysfunction. This was an unexpected outcome as this study was initiated based on the concept that SAM also had antioxidant properties by acting as a precursor of Glutathione. This is a major cellular antioxidant that may have protective effects on cisplatin nephrotoxicity and ototoxicity without interfering with its chemotherapeutic function. The administration of SAM alone was not cytotoxic, and cisplatin alone resulted in a moderate increase in toxicity (Ochoa et al., 2009). Based on these finding, Ross et al. concluded that cisplatin-induced ototoxicity could be related to increased levels of SAM through reduced TPMT or COMT activity to explain the link between these gene variants and ototoxicity. However, Milek et al. showed that direct measurement of SAM in red blood cells from healthy individuals showed no significant difference between individuals with wild-type TPMT (*1/*1, n = 115) and those carrying TPMT loss-of-function variants (*1/*3; n = 44; p = 0.69), thus defying the notion that TPMT status can substantively influence SAM homeostasis. The direct correlation between COMT and SAM in vivo continues to be unclear (Milek et al., 2012). A study by Babu et al. investigated the antioxidant effect of SAM on gentamicin-induced nephrotoxicity in rats confirming the common pathways involved in toxicity caused by cisplatin and aminoglycosides. They showed similar outcomes as the study with cisplatin where renal dysfunction was demonstrated through increased urea and creatinine and histopathological damage of the proximal tubules seen in the rats given gentamicin + SAM yet this effect was non-significant when compared to the saline alone or gentamicin alone conditions (Babu et al., 2013). Therefore if this line of investigation is to continue in the future with AG
ototoxicity, investigation of the mechanistic pathway of any association should take priority too.

5.3.6 Conclusions from the genetic studies:

It is established that genetic variations may offer a possible explanation for substantial interindividual variation in ototoxicity in individuals receiving similar doses of potentially ototoxic medications. Therefore, following this line of investigation was an essential aspect in the search for ‘causation’ of ototoxicity and to offer a possible explanation for the dichotomy in hearing observed in the CF study population. Specifically, we investigated whether some prevalences of ototoxicity after low doses of AG may be explained by a known genetic susceptibility. The 12S rRNA gene of the mitochondrial DNA was genotyped for the A1555G mutation in the CF children. This is the most commonly reported mtDNA mutation known to cause increased susceptibility to aminoglycoside-induced ototoxicity.

Two out of 105 (1.9%) children had the A1555G mutation. Even though it was not an aim of the current study to investigate the prevalence of this mutation in CF patients, it was noted that the prevalence of occurrence in this study cohort was higher than the previously reported ~0.2% in UK populations. However, if this aim were to be pursued, a larger cohort study would be needed to confirm if the prevalence of this mutation is truly higher than expected in CF patients. It is worth noting that the only previous study of A1555G in a similar sample size of CF cases also found a raised frequency. In the case of child A - the A1555G was associated with early onset severe-profound SNHL in one of the few ototoxic group of children that belonged to the low AG-exposure group, consistent with the well established effect of this mutation. In this child the presence of this mutation provided an explanation as to why she had severe-profound SNHL following intake of only three AG courses whereas other children had exceptionally good hearing even after intake of 30-40 AG courses. This finding, in the context of what is already established in the literature, supports the recommendation to screen patient groups that are known to require the intake of these medications (like this CF patient group) at an early stage before they start developing infections and need these antibiotics. This will enable the clinicians to make informed decisions regarding the drug regimens they will use for each patient and be able to provide proper counselling and management.

The unexpected and significant finding was that one of the two children with the mutation was seen to have completely normal hearing despite confirmed intake of i.v and nebulized AGs. Exposure to AGs has been consistently identified as a major modifying factor for this mutation leading to increased penetrance to 100%. From a retrospective review of the literature many of these studies utilized hearing loss cohorts and therefore were not capable
of establishing penetrance. This finding was published as a case report as this was a rare outcome and in order to highlight the need for further studies to confirm the true penetrance of this mutation with exposure to AGs (Al-Malky et al., 2014)(Appendix 9.14). Genotyping for the A1555G mutation in CF patients is not common practice in the UK. As aminoglycosides are commonly used as 1st line treatments of pseudomonas chest exacerbations, genetic testing for this mutation raises many issues including dealing with patient/parent anxiety, the need to provide genetic counselling and auditory monitoring services, and weighing up the benefits of protecting hearing versus possible increased morbidity due to cessation of this treatment. Therefore it is recommended that further larger scale investigations should be done before genotyping for this or any other mutations with consequent stoppage of AG prescription in CF patients becomes common practice.

The research by Ross et al. was the first to identify a correlation between variants in two of the known most common 220 drug-metabolizing genes and cisplatin ototoxicity. A similar link between these variants and AG ototoxicity could not be established in the current study. There was also no significant correlation seen between the TPMT rs12201199 variant and a representative sample of the British population using samples from the 1958 British Cohort study. Yang et al. later showed that this association with cisplatin ototoxicity could not be replicated. As both cisplatin and aminoglycosides were shown to follow similar pathways of entry into the inner ear and initiation of apoptosis of its cells (Forge and Schacht, 2000, Koegel, 1985, Lautermann et al., 2004, Ravi et al., 1995, Roland, 2004, Rybak and Ramkumar, 2007, Schacht et al., 2012), it was hypothesized that a similar correlation with the identified variants of the TPMT and COMT drug-metabolizing genes could be established with aminoglycoside ototoxicity. The small sample size should be considered as a limiting factor within this current study, as it may be a possible cause for this absence of significant relationships. The genetic power calculation showed that a sample size of 302 and 42 cases with ototoxicity would be needed to establish an association with 80% power between AG ototoxicity and TPMT rs12201199 and COMT rs4646316 respectively. Conversely, even though this similar link could not be established, the concept that variations in drug-metabolizing genes could offer a possible explanation for the dichotomy in hearing thresholds of children exposed to AGs could still be valid. These genes affect the way the body deals with the absorption, dissemination, metabolism, and elimination (ADME) of drugs and are the basis for pharmacogenomics. If variants of these genes change the rate this ADME occurs this will lead to consequent changes in the drug serum levels and the amounts that pass the blood-labyrinth barrier and therefore may account for increased individual susceptibility to ototoxicity. This current pilot study failed to find a correlation with the TPMT and COMT variants but it may still be worth replicating the Ross et al. study and
searching for a correlation between AG ototoxicity and variants of all the 220-commonest drug-metabolizing genes, as variants other than those of *TPMT* and *COMT* may be associated with it. In this case establishing the mechanistic pathway through which this occurs should also be defined.
Chapter 6: **Results & Discussion of Theme C: Impact of ototoxicity and current service provision**

In this current research, results of the audiological assessment of the CF children exposed to AGs has shown that ototoxicity occurs in around one-fifth of the children exposed, which increases even further to 44% in children exposed to cumulative high doses (Figure 4-5). Previous research has shown that cisplatin leads to ototoxicity in 60-100% of children (Knight et al., 2005, Brock et al., 1991, Rybak et al., 2009). The main aims of this final theme of the work were firstly to assess the impact that the added disability of ototoxic hearing loss has on patients that are already unwell. Evidence that this would significantly affect their quality of life would support the need to try and prevent the occurrence of ototoxicity by early identification through monitoring and change in treatment regimens if possible. The second aim was to survey current practice regarding monitoring for ototoxicity in the UK from the perspective of the audiological professionals and clinicians managing patients vulnerable to ototoxicity. This would allow for a more informed assessment of what is currently available and identify what is needed in order to improve this service.
6.1 Investigating the effect of hearing loss on the quality of life of paediatric cancer survivors receiving ototoxic chemotherapeutic agents.

6.1.1.1 Initial retrospective assessment of the study group:

This study aimed to assess the effect an added disability caused by hearing loss has on the quality of life of children with cancer. Records of patients that were monitored for ototoxicity in a dedicated ototoxicity clinic for oncology patients at GOSH were reviewed. Within this clinic children were routinely monitored for ototoxicity with baseline and regular post-treatment assessments undertaken. Data for 219 patients that were assessed over the period from 04/2005 to 12/2012 were retrieved. The mean age of the patients that were reviewed was 9.7 years (±SD=3.1 years) having a mean age at cancer diagnosis of 5.5 years (±SD=2.9 years). 82 of the 219 (37%) patients were females. On assessing their audiological data, 114 patients (52%) had normal hearing with standard audiometric thresholds of ≤20dB across all 1-8 kHz frequencies with recordable DPOAEs and normal type ‘A’ tympanograms, whereas 105 (48%) demonstrated evidence of hearing loss. Only 21 (20%) of the hearing loss patients had conductive/mixed hearing loss whereas the majority (80%) had sensorineural high frequency sloping hearing loss commonly seen in cases of ototoxicity (36% mild-moderate, 40% severe and 4% profound high frequency sloping SNHL). 70/105 (67%) of the patients with hearing loss wore amplification, which in most cases was in the form of hearing aids except for two children who were fitted with cochlear implants. Most of the hearing loss patients were reported to have progressively worsening SNHL, which was evidenced through repeated assessments (mean number of assessments 3.6 ±SD=2.8; range 1-16) (Table 6-1). The parents of all these children were contacted by phone or through email, the study was explained to them and a copy of the information sheet, consent form and questionnaires were sent to them either through the post or electronically through an email with the link to the questionnaires using the UCL Opinio survey tool.
<table>
<thead>
<tr>
<th>Descriptive Data</th>
<th>Number of Patients (n=219)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total PTAs Performed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-4 PTAs</td>
<td>160</td>
<td>73</td>
</tr>
<tr>
<td>5-8 PTAs</td>
<td>38</td>
<td>17</td>
</tr>
<tr>
<td>9-12 PTAs</td>
<td>13</td>
<td>6</td>
</tr>
<tr>
<td>13-16 PTAs</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>OAE results</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact OAEs</td>
<td>118</td>
<td>54</td>
</tr>
<tr>
<td>Absent OAEs</td>
<td>74</td>
<td>34</td>
</tr>
<tr>
<td>Tympanometry results</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal Type 'A'</td>
<td>152</td>
<td>69</td>
</tr>
<tr>
<td>Flat Type 'B'</td>
<td>31</td>
<td>14</td>
</tr>
<tr>
<td>Negative Type 'C'</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td>Hearing Status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal Hearing</td>
<td>114</td>
<td>52</td>
</tr>
<tr>
<td>Conductive HL</td>
<td>21</td>
<td>10</td>
</tr>
<tr>
<td>Mild-Moderate HF Sloping HL</td>
<td>38</td>
<td>17</td>
</tr>
<tr>
<td>Severe HF Sloping HL</td>
<td>42</td>
<td>19</td>
</tr>
<tr>
<td>Profound HF Sloping HL</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Progression of HL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stable</td>
<td>67</td>
<td>31</td>
</tr>
<tr>
<td>Progressive</td>
<td>79</td>
<td>36</td>
</tr>
</tbody>
</table>

Table 6-1: Description of the audiological data retrieved from the records of oncology patients (n=219) assessed in the ototoxicity clinic. Some data was missing for patients in each category hence the discrepancy in the reported numbers.

### 6.1.1.2 Responding participants:

Completed questionnaires were received from 41 parents of children with ototoxic hearing impairment and 37 parents of children with normal hearing (total of 78 responses, 36% response rate). The mean age of the children was 9.8 years (±2.8 years) with a mean age at cancer diagnosis of 5.9 years (±2.3 years). 35/78 (45%) of the children were females. Table 6-2 summarizes the types and distribution of the cancers affecting the children highlighting that a wide range of cancers require the intake of ototoxic chemotherapy or radiotherapy with the most common being the solid tumours (47.5%). All children were exposed to at least one type ototoxic agent with many exposed to more (e.g. both cisplatin and carboplatin) with details shown in Table 6-3. The children were divided into those with normal hearing (n=37) and those with audiological evidence of ototoxicity (n=41) and the responses to the questionnaires were analyzed and compared between the two groups. Of the 41 children with ototoxicity 26 (63%) had mild to moderate high-frequency (HF) sloping SNHL while 11 (27%) and 2 (5%) had severe and profound HF sloping SNHL respectively. Thirty (73%) of the study children with ototoxicity were wearing hearing aids and six (15%) had other...
disabilities, including partial sightedness, which was comparable with the normal hearing group (14%).

<table>
<thead>
<tr>
<th>Leukaemias /Lymphomas</th>
<th>Number of children (n=78)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute Lymphoblastic Leukaemia (ALL)</td>
<td>2</td>
<td>2.6</td>
</tr>
<tr>
<td>Acute Myeloid Leukaemia (AML)</td>
<td>4</td>
<td>5.1</td>
</tr>
<tr>
<td>Anaplastic Large Cell Lymphoma (ALCL)</td>
<td>1</td>
<td>1.3</td>
</tr>
<tr>
<td>B Cell Lymphoma</td>
<td>1</td>
<td>1.3</td>
</tr>
<tr>
<td>Burkitts Lymphoma</td>
<td>2</td>
<td>2.6</td>
</tr>
<tr>
<td>Follicular Lymphoma</td>
<td>1</td>
<td>1.3</td>
</tr>
<tr>
<td>Hodgkin’s Lymphoma</td>
<td>3</td>
<td>3.8</td>
</tr>
<tr>
<td>Non Hodgkin’s</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td><strong>Solid Brain Tumours</strong></td>
<td><strong>27</strong></td>
<td><strong>34.6%</strong></td>
</tr>
<tr>
<td>Astrocytoma</td>
<td>4</td>
<td>5.1</td>
</tr>
<tr>
<td>Ependymoma</td>
<td>2</td>
<td>2.6</td>
</tr>
<tr>
<td>Glioma/Optic Glioma</td>
<td>5</td>
<td>6.4</td>
</tr>
<tr>
<td>Medulloblastoma</td>
<td>15</td>
<td>19.2</td>
</tr>
<tr>
<td>Pineal Tumour</td>
<td>1</td>
<td>1.3</td>
</tr>
<tr>
<td><strong>Solid Tumours</strong></td>
<td><strong>37</strong></td>
<td><strong>47.5%</strong></td>
</tr>
<tr>
<td>Adrenal Carcinoma</td>
<td>1</td>
<td>1.3</td>
</tr>
<tr>
<td>Choriocarcinoma</td>
<td>1</td>
<td>1.3</td>
</tr>
<tr>
<td>Ewing’s Sarcoma</td>
<td>2</td>
<td>2.6</td>
</tr>
<tr>
<td>Germ Cell Tumour</td>
<td>4</td>
<td>5.1</td>
</tr>
<tr>
<td>Hepatoblastoma</td>
<td>4</td>
<td>5.1</td>
</tr>
<tr>
<td>Hepatoma</td>
<td>1</td>
<td>1.3</td>
</tr>
<tr>
<td>Immature Teratoma</td>
<td>1</td>
<td>1.3</td>
</tr>
<tr>
<td>Neuroblastoma</td>
<td>7</td>
<td>9.0</td>
</tr>
<tr>
<td>Neurofibromatosis</td>
<td>1</td>
<td>1.3</td>
</tr>
<tr>
<td>Osteosarcoma</td>
<td>4</td>
<td>5.1</td>
</tr>
<tr>
<td>Retinoblastoma</td>
<td>4</td>
<td>5.1</td>
</tr>
<tr>
<td>Rhabdoid Tumour</td>
<td>1</td>
<td>1.3</td>
</tr>
<tr>
<td>Wilm’s Tumour</td>
<td>6</td>
<td>7.7</td>
</tr>
</tbody>
</table>

Table 6-2: Summary of types and distribution of tumours affecting the children. The commonest tumour was Medulloblastoma (19.2%) whereas the commonest group of tumours was the solid tumours (47.5%).
<table>
<thead>
<tr>
<th></th>
<th>Number Of Children (n=78)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-5y</td>
<td>6</td>
<td>7.7</td>
</tr>
<tr>
<td>6-10y</td>
<td>42</td>
<td>53.8</td>
</tr>
<tr>
<td>11-15y</td>
<td>30</td>
<td>38.5</td>
</tr>
<tr>
<td><strong>Age At Diagnosis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-5y</td>
<td>45</td>
<td>57.7</td>
</tr>
<tr>
<td>6-10y</td>
<td>29</td>
<td>37.2</td>
</tr>
<tr>
<td>11-15y</td>
<td>4</td>
<td>5.1</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>43</td>
<td>55.1</td>
</tr>
<tr>
<td>Female</td>
<td>35</td>
<td>44.9</td>
</tr>
<tr>
<td><strong>Ototoxic Chemotherapy received</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single type</td>
<td>32</td>
<td>41.0</td>
</tr>
<tr>
<td>Multiple types</td>
<td>46</td>
<td>59.0</td>
</tr>
<tr>
<td><strong>Hearing Status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>37</td>
<td>47.4</td>
</tr>
<tr>
<td>Mixed HL</td>
<td>2</td>
<td>2.6</td>
</tr>
<tr>
<td>Mild-Moderate HF Sloping SNHL</td>
<td>26</td>
<td>33.3</td>
</tr>
<tr>
<td>Moderate-Severe HF Sloping SNHL</td>
<td>11</td>
<td>14.1</td>
</tr>
<tr>
<td>Moderate-Profound HF Sloping SNHL</td>
<td>2</td>
<td>2.6</td>
</tr>
<tr>
<td><strong>Brock's Grading</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>45</td>
<td>57.7</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>6.4</td>
</tr>
<tr>
<td>2*</td>
<td>5</td>
<td>6.4</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>12.8</td>
</tr>
<tr>
<td>4</td>
<td>13</td>
<td>16.7</td>
</tr>
<tr>
<td><strong>Hearing Aids fitted</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>20</td>
<td>25.4</td>
</tr>
<tr>
<td>No</td>
<td>58</td>
<td>74.4</td>
</tr>
<tr>
<td><strong>Other Disabilities</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>67</td>
<td>85.9</td>
</tr>
<tr>
<td>Yes</td>
<td>11</td>
<td>14.1</td>
</tr>
</tbody>
</table>

Table 6-3: Summary of descriptive and audiological data of all recruited children. Brock's grade 2* is the minimal grade prompting clinicians to consider changing the treatment regimen e.g. switch to less ototoxic chemotherapeutics.
6.1.1.3 Outcomes of the responses from the questionnaires:

Health Utilities Index Mark 3 (HUI3) Questionnaire:

The HUI3 is a standardized set of generic health profiles and preference-based systems designed for the purpose of producing utility scores to report on health-related quality of life (HRQL) and measure health status. It was chosen because it is one of the most commonly used and validated measures of HRQL. The 15-Q parent-proxy version of the HUI3 questionnaires was used. The eight attributes in the HUI3 classification (vision, hearing, speech, ambulation, dexterity, emotion, cognition and pain) are scored using single and multi-attribute utility functions so that single-attribute scores of morbidity are defined on a scale such that the best level has a score of 1.00 and the worst score of 0.00 and the multi-attribute utility functions convert detailed health state descriptors into preference measures of overall HRQL. This again is defined such that the score for perfect health=1.00 and the score for death=0.00 with the HUI3 allowing for negative scores of HRQL representing health states considered worst than death with the lowest score being -0.36 (Horsman et al., 2003).

When comparing the frequency and distribution of suboptimal levels of function in the eight different attributes of the HUI3 questionnaire between the normal hearing group of cancer patients and those with ototoxicity, two attributes showed a significant difference (Table 6-4). The suboptimal levels of function were defined as the less than normal function indicated by scores below level 1 for each attribute (see Appendix 9.3). The two attributes that were significantly different between the two groups were the ‘hearing’ and ‘cognition’ attributes ($p= <0.0001$ and 0.046 respectively). The remaining six of the eight attributes were not statistically significantly different between the two groups of children although suboptimal levels were present in a higher percentage of the ototoxic group children compared to those in the normal hearing group across all attributes (Table 6-4).
Normal Hearing Group (n=37), n (%) | Ototoxic Group (n=41), n (%) | p-value*  
--- | --- | ---  
Vision | 3 (8.1%) | 8 (19.5%) | 0.199  
Hearing | 1 (2.7%) | 33 (80.5%) | <0.001  
Speech | 11 (29.7%) | 19 (46.3%) | 0.132  
Ambulation | 15 (40.5%) | 18 (43.9%) | 0.764  
Dexterity | 9 (24.3%) | 14 (34.1%) | 0.342  
Emotion | 13 (35.1%) | 17 (41.5%) | 0.566  
Cognition | 17 (45.9%) | 28 (68.3%) | **0.046**  
Pain | 13 (35.1%) | 18 (43.9%) | 0.429  

Suboptimal levels of function are defined as below level 1 scores indicating less than normal function for each attribute.  
*Calculated using chi-square test (Fischer's exact test when cells have <5) comparing all children with normal hearing with those with ototoxicity (p<0.05).

Table 6-4: Number of children in each group with suboptimal levels of function in each attribute of the HUI3.

Figure 6-1: Shows mean with 95% CI error bars for the HUI3 single attribute responses for each of the two normal hearing and ototoxic oncology groups. The Hearing and Cognition attributes were significantly worse in the ototoxic group (* p<0.05).
A comparison of the single-attribute response scores in the two groups again showed a significantly lower mean (error bars=95% CI) score in the ototoxic group for the Hearing and Cognition attributes (Figure 6-1).

Figure 6-2 and Table 6-5 show the distribution of the multi-attribute utility scores of the children with normal hearing and those with evidence of ototoxicity. There was a significant difference in the median and range values between the two groups with only one outlier in the normal hearing group. The difference was shown to be statistically significant using the non-parametric Mann Whitney U test (Mann-Whitney U=419.0, p=0.001). This indicates an overall recorded reduction in the health-related quality of life (HRQL) of children with cancer when hearing loss is an added disability.

![Boxplot showing the multi-attribute utility scores of HUI3 for both the normal hearing and ototoxicity group of cancer children.](image)

Figure 6-2: A Boxplot showing the multi-attribute utility scores of HUI3 for both the normal hearing and ototoxicity group of cancer children.

The central line inside the box represents the median score and the lower and upper edges of the box represent the 25th and 75th percentile scores.
Normal Hearing Group | Ototoxicity Group | p-value
---|---|---
N | 37 | 41 |
Mean | 0.734 | 0.539 |
Median | 0.849 | 0.598 | 0.001<sup>a</sup> |
Minimum | -0.155 | -0.264 |
Maximum | 1.000 | 0.931 |
Range | 1.155 | 1.195 |
Std. Deviation | 0.297 | 0.276 |

Table 6-5: Descriptive data of the multi-attribute HUI3 utilities scores for both groups (including the outlier).

<sup>a</sup> Calculated using the Mann-Whitney U test (p<0.05).

**Paediatric Audiology Quality of Life Questionnaire (PAQL):**

The PAQL 22-item questionnaire, which was developed by Edwards et al. (Edwards et al., 2012) to specifically assess the aspects of Quality of Life (QoL) affected by childhood deafness while still being appropriate for hearing children, was the second parent-proxy questionnaire used in this study. The scores of the four PAQL subscales and total QoL score (i.e. the sum of all four subscale scores) were compared for children with cancer who had normal hearing and those showing evidence of ototoxic hearing loss using the non-parametric independent samples Mann-Whitney U-test. The results shown in the boxplots in Figure 6-3 and in the analysis in Table 6-6 demonstrated that a highly statistically significant difference was present between the two groups across all four subscales and in the overall total QoL score at p<0.001, therefore rejecting the null hypothesis that the distribution of any of these scales is the same across the two patient categories. All the scores of the ototoxic group were poorer than those of the normal hearing group. The highest mean difference between the two groups was seen in the ‘communications and independence’ subscale, which was also shown to be the most important subscale in differentiating between the two groups. This was because it was responsible for 89% of the variance between them when factor analysis was performed to identify the significance of each of the subscales (% of variance of 6%, 3% and 2% was shown for the other three subscales respectively). Despite the presence of a wider range of scores in all subscales of the ototoxic group, there were significantly more extreme scores in the subscale scores of the normal hearing group as shown in Figure 6-3 as the outliers.
Figure 6-3: A Boxplot showing the distribution of scores for all four subscales of the PAQL for each of the two groups of children. Outliers were only seen in the Normal Hearing group.
<table>
<thead>
<tr>
<th></th>
<th>Communication &amp; independence</th>
<th>Emotional wellbeing</th>
<th>Peer comparisons</th>
<th>Acceptance</th>
<th>Total QoL scores</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Normal Hearing Group</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>37</td>
<td>37</td>
<td>37</td>
<td>37</td>
<td>37</td>
</tr>
<tr>
<td>Mean</td>
<td>27.70</td>
<td>31.81</td>
<td>22.54</td>
<td>18.57</td>
<td>100.62</td>
</tr>
<tr>
<td>Median</td>
<td>30.00</td>
<td>34.00</td>
<td>24.00</td>
<td>20.00</td>
<td>107.00</td>
</tr>
<tr>
<td>Minimum</td>
<td>16.00</td>
<td>19.00</td>
<td>9.00</td>
<td>11.00</td>
<td>63.00</td>
</tr>
<tr>
<td>Maximum</td>
<td>30.00</td>
<td>35.00</td>
<td>25.00</td>
<td>20.00</td>
<td>110.00</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>3.79</td>
<td>4.36</td>
<td>3.72</td>
<td>2.39</td>
<td>13.11</td>
</tr>
<tr>
<td>Variance</td>
<td>14.38</td>
<td>19.05</td>
<td>13.81</td>
<td>5.70</td>
<td>171.96</td>
</tr>
<tr>
<td><strong>Ototoxic Group</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>41</td>
<td>41</td>
<td>41</td>
<td>41</td>
<td>41</td>
</tr>
<tr>
<td>Mean</td>
<td>22.51</td>
<td>27.05</td>
<td>18.24</td>
<td>15.49</td>
<td>83.29</td>
</tr>
<tr>
<td>Median</td>
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<td>30.00</td>
<td>18.00</td>
<td>16.00</td>
<td>81.00</td>
</tr>
<tr>
<td>Minimum</td>
<td>11.00</td>
<td>12.00</td>
<td>9.00</td>
<td>6.00</td>
<td>42.00</td>
</tr>
<tr>
<td>Maximum</td>
<td>30.00</td>
<td>35.00</td>
<td>25.00</td>
<td>20.00</td>
<td>110.00</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>4.97</td>
<td>6.39</td>
<td>4.07</td>
<td>3.64</td>
<td>17.76</td>
</tr>
<tr>
<td>Variance</td>
<td>24.66</td>
<td>40.80</td>
<td>16.59</td>
<td>13.26</td>
<td>315.31</td>
</tr>
<tr>
<td>Mean Difference</td>
<td>5.19</td>
<td>4.76</td>
<td>4.30</td>
<td>3.08</td>
<td>17.33</td>
</tr>
</tbody>
</table>

**Independent-Samples Mann-Whitney U Test**

<table>
<thead>
<tr>
<th></th>
<th>Mann-Whitney U</th>
<th>Z-score</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>290.00</td>
<td>-4.78</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>396.50</td>
<td>-3.68</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>292.50</td>
<td>-4.70</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>359.50</td>
<td>-4.10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>305.50</td>
<td>-4.55</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 6-6: The distribution and statistical analysis of the four subscales and total scores of the PAQL between the two normal hearing and ototoxic groups of cancer children. Independent-samples Mann-Whitney test U was used. There was a highly significant difference between the groups in all categories.

### 6.1.2 Discussion of the effects of ototoxicity of the quality of life on children:

Cancer is considered to be one of the most life threatening diseases of the century. The UK World Cancer Research Fund (WCRF) reports a prevalence of around 12.7 million cancer cases per year worldwide, which is estimated to rise to 21 million by the year 2030, with the UK reported to have the 22nd highest overall rate of cancer in the world ([www.wcrf-uk.org](http://www.wcrf-uk.org)). With the marked advances in the research, healthcare, pharmaceuticals, technology and many fields related to the diagnosis and management of cancer, the survival rate from this disease has significantly improved with statistical reports such as those from the National Cancer Institute in the US stating that >80% of patients diagnosed at childhood or adolescence survive their initial disease ([http://seer.cancer.gov/faststats](http://seer.cancer.gov/faststats)). This in turn has meant, as with CF patients, that more emphasis is placed on trying to preserve the patients’ quality of life as opposed to just keeping them alive. Chemotherapy is an extremely important method of treatment for a wide variety of tumours. However it can lead to
ototoxicity as a side effect, with the platinum-based group of drugs such as cisplatin and high-dose carboplatin, being the most well documented ototoxic chemotherapeutics (Schell et al., 1989, Parsons et al., 1998, Punnett et al., 2004, Bertolini et al., 2004, Kushner et al., 2006). The hearing loss is especially detrimental in children as it also affects speech and language development, educational progression, social interaction and communication, psychological wellbeing and overall quality of life.

In this study records of 219 children with cancer who were exposed to potentially ototoxic chemotherapeutic agents were reviewed with the aim of recruiting a group of them with evidence of ototoxicity and a matching group with normal hearing in order to assess the effect this added hearing disability has on Quality of Life measures. The 15Q parent-proxy version of the HUI3 and the 22Q PAQL questionnaire were administered. 78 families responded.

Retrospective assessment of the data of the children with cancer showed that they had a wide range of tumour types all requiring the administration of at least one type of potentially ototoxic chemotherapeutic agents e.g. cisplatin, in addition to exposure to head and neck radiotherapy. Radiotherapy is also potentially ototoxic and is reported to have a synergistic effect when combined with chemotherapy (Kortmann et al., 2000, Miettinen et al., 1997, Schell et al., 1989, Walker et al., 1989). Platinum agents are known to be effective against a variety of childhood malignancies including certain brain tumours, such medulloblastomas; solid tumours, such as neuroblastomas and germ cell tumours (Doz et al., 1993, Gaynon, 1994, Ettinger et al., 1994), which were all seen in this study cohort. This data showed that 48% of the children demonstrated evidence of ototoxicity when post-exposure audiological assessments were performed and that 80% of those had high-frequency sloping SNHLs characteristic of ototoxicity. This prevalence of ototoxicity is similar to several other studies reporting prevalences ranging from 34-62% (Kortmann et al., 2000, Kushner et al., 2006, Laverdiere et al., 2005, Packer et al., 1994), which significantly increased to 80-100% when associated with high risk factors such as young age, cumulative dosage, brain tumours and concomitant use of CNS radiation. A large proportion of the children showed evidence of progressive worsening of their hearing status on repeated testing emphasizing the need for repeated audiological monitoring of these patients (Table 6-3). At GOSH change in management is made if Brock grade 2 levels of hearing loss are reached. This may be the reason why only 48% of the children had ototoxicity despite having all the high risk factors just mentioned which would potentially increase this prevalence to up to 100%. The progressive worsening of hearing that was seen in these children has also been reported by Bertolini et al. who showed that 5% of the 120 children exposed to cisplatin and/or
Carboplatin had at least Brock’s grade 2 hearing loss before the end of treatment, which rose to 44% after more than 2 years of long-term follow-up. Al-Khatib et al. also showed a 33% worsening in the audiograms of cisplatin-exposed patients with long-term follow-up for periods ranging from 1.5 to 6.6 years (median of 3.4 years). They, as well as several others, also recommended long-term audiological surveillance even after cessation of treatment (Bertolini et al., 2004, Al-Khatib et al., 2010, Einarsson et al., 2010, Sivaprakasam et al., 2011).

The 78 recruited children were aged between 5-15 years with 54% of them lying within the 6-10 years age category. There was a good match between the group with normal hearing (n=37) and the group with ototoxicity (n=41) in terms of age, gender, tumor type, chemotherapy exposure and recorded additional disabilities. 63% of the group with ototoxicity had mild-moderate HF sloping SNHL and 73% were wearing hearing aids. Despite this match, scores of the single attributes of ‘hearing’ and ‘cognition’ and the overall multi-attribute utility score of HRQL of the HUI3 questionnaire all showed a significant difference between the two groups with children in the ototoxicity group having HRQL utility scores as low as -0.264, which is considered a level worse than death (Table 6-4, Table 6-5, and Figure 6-2). In addition to that, all the PAQL subscales and the overall QoL scores of the group with ototoxicity were also significantly lower than the group with normal hearing (Figure 6-3 and Table 6-6). Therefore, results of both questionnaires demonstrated that the additional hearing loss disability had the significant effect of reducing the overall health-related quality of life (HRQL) and overall quality of life (QoL) of children who are already facing the extensive range of difficulties associated with being a cancer patient.

There is limited data in the literature investigating the impact that hearing loss has on the QoL of cancer survivors therefore the findings of this current study are significant. These limited studies are presented below. There is more evidence in the literature on the significant affect of permanent childhood hearing loss (PCHI) on quality of life. This is also related to the outcomes of the current study as the ototoxicity in these children would be considered a cause of PCHI.

On comparing the health status and HRQL preference-based outcomes, using the HUI3, of 7-9 years old children with bilateral permanent childhood hearing impairment (PCHI) with their normal hearing peers, Petrou et al. found that they scored significantly lower on 6 of the 8 single-attribute utility scores of the HUI3: vision, hearing, speech, ambulation, dexterity and cognition with significantly lower multi-attribute utility scores as well (Petrou et al., 2007). Wake et al. used the 28-item parent-proxy Child Health Questionnaire measure of HRQL to assess the difference between a cohort of 7-8 year-old children with a varying
range of severities of congenital hearing loss that were fitted with hearing aids or cochlear implants and normal hearing children of the same age cohort. They found that the hearing loss cohort scored significantly poorer in 6 of the scales (Behaviour, Mental health, Role/Social-Physical, Parent Impact-time, Parent Impact-Emotional and Family Activities) in addition to poorer overall Psychosocial Summary scores when compared with their normal hearing peers (Wake et al., 2004). Edwards et al. had devised the PAQL to assess the quality of life to deaf children wearing cochlear implants and specifically assess the added impact of having additional needs, such as learning or physical difficulties. They showed that the children with added disabilities had poorer scores in three of the four subscales (Communication and independence, Emotional well-being and Acceptance by peers) yet also showed that both groups of children benefited significantly from having cochlear implants which were shown to improve their overall QoL (Edwards et al., 2012). This PAQL was a good tool to use in the current study because it is a disease-specific quality of life measure and as expected was able to demonstrate the significant impact of ototoxicity on all four subscales.

Regarding the limited literature related to the effect of hearing loss on QoL in cancer survivors, Gurney et al. undertook a survey of 8-17 years-old long-term neuroblastoma (NB) survivors. The parent-proxy and self-reported Pediatric Quality of Life Inventory 4.0 was used which showed that the NB survivors with hearing loss had twice the risk of having academic learning difficulties, such as reading and math skills, and psychosocial problems compared to their normal-hearing counterparts (Gurney et al., 2007). Laverdiere et al. who had also assessed the long-term complications of NB survivors (7.06 years median follow-up from diagnosis) showed that 62% of the children complained of hearing loss. Cisplatin exposure increased the risk for hearing loss (OR 9.74, 95% CI: 0.9-101.6) (Laverdiere et al., 2005). Barr et al. assessed the HRQL of children with Wilm's tumour and those with NB using the parent-proxy HUI Mark 2 and Mark 3 questionnaires. Children with Wilm's tumours generally have a better prognosis and less exposure to platinum compounds. 84% of the eligible 93 families responded and scores were worse in the NB survivors for the single attributes for ‘hearing’ and ‘speech’ utility scores and for the overall multi-attribute HRQL scores when compared to the Wilm's tumour survivors (Barr et al., 2000). These findings are all in agreement with the outcomes in the current study. The worsening in the hearing attribute is expected and confirms that the questions representing this attribute are able to detect the effect of the mild to moderate hearing loss seen in the bigger proportion of the children in the ototoxic group. However, the worsening in the cognition and overall multi-attribute utility scores confirm the findings of the previous research discussed here which shows that the effect of hearing loss extends to affect the children’s cognitive function,
educational abilities and overall psychosocial development. This supports the recommendation to effectively manage the hearing loss caused by ototoxicity through early detection and rehabilitation.

In recognizing the significant effect of long-term (≥ 2 years post treatment) complications of management of paediatric malignancies, a multidisciplinary panel of experts developed “The Children's Oncology Group Long-Term Follow-Up Guidelines for Survivors of Childhood, Adolescent, and Young Adult Cancers”. These guidelines were intended to increase awareness of these late complications and advocate standardized follow-up care. A series of complimentary patient educational material (“Health Links”) were also developed. Hearing loss was recognized as one of these complications recognized to affect the academic, speech and language, social and emotional well-being of children and adolescent cancer survivors. The Guidelines, and related Health Links, can all be downloaded at www.survivorshipguidelines.org. (Eshelman et al., 2004, Grewal et al., 2010, Landier et al., 2004).

6.1.2.1 Limitations of the study:

Limitations of this study included the achieved response rate. Responses were obtained from 78/219 of the potential candidates contacted, which represents a response rate of 36%. Despite this being a common problem in questionnaire studies especially when targeting significantly unwell subjects, the lower response rate may bias the outcomes as the 64% non-respondents may have done so for several reasons. This may include families where ototoxicity had less of an impact on the QoL of their children. However the similar response rate of the non-ototoxic group and ototoxic group (37 vs. 41 responses) helps overcome this to a certain extent. Another limitation of the study was that parent-proxy questionnaires were used and so the children themselves were not directly reporting the effect of their disease on their QoL. The effect perceived by the parents might be different from that perceived by the children themselves. A recommendation for future work may include repeating this study with the actual patient forms of the questionnaires and contact the older children and adults surviving cancer. It would also be interesting to confirm whether the same effect on the QoL would be recorded for the children with CF with ototoxicity. One of the reasons for choosing the children with cancer for this was because they already had a dedicated ototoxicity monitoring service at GOSH and therefore at retrospective review of records of disease management and audiological assessment was possible. It was also possible to have an adequate sample size to have enough power to detect a significant effect if it existed. As the children with CF did not have a similar service this was not possible. CF is a chronic disorder where the child is unwell from birth or early in childhood then
throughout their lives. Parents are used to their children developing repeated complications and know that their children’s life is under threat all the time. In addition, there is also a significant impact on their educational and psychosocial development due to the need for repeated hospitalization and therefore the impact of ototoxicity on the QoL may be more significant. It would be interesting to compare the outcomes of these QoL measures for this group with the children with cancer where the disease is usually more acute and parents have a shorter time to deal with the psychological and physical stress of discovering that their previously healthy child is having a life-threatening condition. A recommendation for future research would be to repeat this study with CF children and compare the results with the cancer children in order to put in place individualized rehabilitation plans that are aimed at addressing the specific needs of each patient group if they were found to be different.

6.1.3 Conclusion:

In conclusion, this study has added to the limited literature assessing the effect of the hearing loss disability on the quality of life of children and adolescents surviving cancer. The use of generic HRQL (HUI3) questionnaires helps compare this effect with a wide variety of conditions and confirm its impact on health status. The use of the dedicated disease specific questionnaire such as the PAQL specifically assesses the effect of childhood deafness on the quality of life (QoL) of children and so highlights this impact in more detail compared to the generic tools. Both questionnaires were shown to effectively detect a significant difference between the two groups of non-ototoxic vs. ototoxic cancer survivors. This study has shown that ototoxicity had a significant effect on the hearing and cognition attributes of the HUI3 questionnaire in addition to a general worsening of the overall multi-attribute scores. It also showed that all four subscales of the PAQL questionnaire were significantly worse. These subscales included Communication and independence, Emotional well-being and Peer comparisons and Acceptance indicating that the handicap caused by the hearing loss extended much further than the hearing disability itself. These finding supported previous research showing that hearing loss exacerbates the deficits in the developmental, academic, psychosocial, emotional and physical well-being of cancer survivors with documented long-term effects recognized. Therefore this study augments the recommendations previously made for increased awareness and better standardized and effective management of this disability in these children in order to improve their quality of life.
6.2 **Survey of UK Oncology, Audiology and CF services to assess current practice of auditory monitoring for ototoxicity**

The previous study showed that hearing loss due to ototoxicity had a significant effect on the quality of life of children who are already unwell such as those with cancer or CF. This highlights the importance of early detection and monitoring for ototoxicity with the aim to prevent or avoid further deterioration of hearing. Providing the clinicians with the ability to make informed decisions regarding change in treatment management can do this. This study aimed to assess current UK practice in relation to monitoring for ototoxicity from the perspective of oncology and CF clinicians and those of the audiologists. This would help identify areas of weakness that can aid in devising an informed plan for improvement of service provision.

The UCL Opinion online survey tool was used to collect data for questionnaires specifically designed to suit each of the three target populations in term of their area of expertise and knowledge. The target professionals were those working in oncology, as they manage patients with cancer who are exposed to ototoxic chemotherapy; those working in CF units as they manage CF patients exposed to ototoxic antibiotics; and those working in audiology as they are the professionals providing the auditory monitoring to both patient groups and any others exposed to ototoxicity. The hyperlink to each applicable survey was sent to the related clinicians through the mailing lists of the relevant professional bodies as described in the methods section 3.6.2. The questionnaires aimed to document current practice in ototoxicity monitoring and address questions like: 1) Are clinicians aware of the need to monitor hearing? 2) Are they following guidelines for referral, testing and correct identification of ototoxicity? 3) Is there uniformity in this service provision? 4) Do individual clinics have a pipeline for monitoring and options for change in management and rehabilitation? If they do, are these criteria adhered to? The responses to all the questions were presented below.

**6.2.1.1 Characteristic features of respondents:**

Responses were received from 56 oncology, 133 audiology and 33 CF unit clinical staff. As it was possible to obtain a list of all 48 CF units through the CF Trust 2010 registry (www.cftrust.org.uk) the lead clinician from each unit was identified and was sent the invitation to complete the questionnaire. Therefore a response rate of 33/48 (69%) could be calculated. Due to the significantly larger number of oncology and audiology professionals
all over the UK and the portal used to reach them, it was not possible to approach them in the same way, making it impossible to calculate a response rate or to confirm exactly which hospitals were involved.

Responses were received from members from all three professions representing all regions of the UK as seen in Figure 6-4. The clinical roles are represented in Table 6-7 for the oncology and audiology surveys; all the CF respondents represented the clinical leads of the UK CF services, as these were the only ones approached. Audiology professionals (including audiologists and audiological scientists) represented 90.2% of the respondents while doctors (including Audiovestibular physicians and ENT surgeons) accounted for only 8.3%. On the other hand, for the oncology survey, doctors (oncologists) constituted 53.6% whereas nurses constituted 41.1% of the 56 respondents. Twenty-eight (84.9%) of the CF centers cared for over 100 CF patients at any given time whereas only five (15.2%) stated that their patient cohort ranged from 50-100 patients. The categorization of the type of services delivered was also shown in Table 6-7 and shows a good representation of paediatric and adults services within each profession.

Figure 6-4: Regional distribution of respondents from the three professions.
Audiology (N=133), n (%) of respondents

<table>
<thead>
<tr>
<th>Clinical roles</th>
<th>Audiology (N=133), n (%) of respondents</th>
<th>Oncology (N=56), n (%) of respondents</th>
<th>CF units (N=33), n (%) of respondents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Audiological professional</td>
<td>120 (90.3%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Doctor</td>
<td>11 (8.3%)(^a)</td>
<td>30 (53.6%)(^b)</td>
<td>33 (100%)(^c)</td>
</tr>
<tr>
<td>Nurse</td>
<td>1 (0.8%)</td>
<td>23 (41.1%)</td>
<td>-</td>
</tr>
<tr>
<td>Other</td>
<td>3 (2.3%)(^d)</td>
<td>3 (5.4%)(^e)</td>
<td>-</td>
</tr>
</tbody>
</table>

Program type\(^1\)

<table>
<thead>
<tr>
<th>Program type</th>
<th>Paediatric</th>
<th>Adult</th>
<th>Other specialist centre</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>90 (67.7%)</td>
<td>105 (79.0%)</td>
<td>6 (4.5%)</td>
</tr>
<tr>
<td></td>
<td>33 (58.9%)</td>
<td>28 (50.0%)</td>
<td>2 (3.6%)</td>
</tr>
<tr>
<td></td>
<td>24 (72.7%)</td>
<td>14 (42.4%)</td>
<td>-</td>
</tr>
</tbody>
</table>

\(^a\),\(^b\),\(^c\) Doctors referred to: \(^a\) AVM & ENT; \(^b\) Oncologists; \(^c\) CF Respiratory consultants respectively

\(^d\),\(^e\) Others included: \(^d\) neonatal consultant, student audiologist & oncologist; \(^e\) radiographer, pharmacist & shared care doctor

Program type\(^1\): Respondents were asked to tick ‘all options that applied’ therefore percentages are not intended to equal 100%.

\(^1\) Table 6-7: Clinical and Program characteristics of respondents.

6.2.1.2 Confirmation of ototoxic drug intake:

Several questions were added to the oncology and CF services surveys in order to achieve a better understanding of the clinicians’ awareness of their patients’ exposure to ototoxic medications, confirm the type of ototoxic drugs patients are exposed to and to assess if monitoring of therapeutic drug levels constitutes part of their regular practice. Table 6-8 shows the oncology respondents’ answers to these questions. Most of the respondents reported that up to 75% of their patients are exposed to potentially ototoxic chemotherapeutics with the highest percentage (39.3%) reporting that 26-50% of their patients are exposed. This confirms that ototoxicity is a complication that can affect these patients and therefore something that the clinicians should be aware of. Cisplatin and carboplatin (or drug protocols including these drugs) were identified as first line treatment options by 94.6% and 78.6% of respondents respectively. Exposure to head and neck radiotherapy, known to exacerbate the ototoxic effect of chemotherapy, was also reported by 58.9% of the respondents. It was interesting to note that 44 (78.5%) of the oncology respondents were either not sure or confirmed that therapeutic drug monitoring (TDM) was not carried out with chemotherapeutics. TDM is performed to avoid sub-therapeutic or toxic levels being reached. This is not the case for aminoglycosides, which are monitored by all the responding CF clinicians (Table 6-9). Therefore TDM for chemotherapy may not be part of general practice. Tobramycin was reported (97.0%) as the first choice aminoglycoside used to combat pulmonary exacerbations in CF patients and is commonly administered using extended interval (once or twice daily) dosing.
<table>
<thead>
<tr>
<th>% Patients exposed to ototoxic chemotherapy</th>
<th>Oncology (N=56), n (%) of respondents</th>
</tr>
</thead>
<tbody>
<tr>
<td>76-100%</td>
<td>1 (1.8%)</td>
</tr>
<tr>
<td>51-75%</td>
<td>13 (23.2%)</td>
</tr>
<tr>
<td>26-50%</td>
<td>22 (39.3%)</td>
</tr>
<tr>
<td>0-25%</td>
<td>20 (35.7%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cisplatin/Cisplatin-based protocol as 1st line treatment?</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>53 (94.6%)</td>
<td>3 (5.4%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Carboplatin/Carboplatin-based protocol as 1st line treatment?</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>44 (78.6%)</td>
<td>12 (21.4%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Patients receive Head &amp; Neck Radiotherapy?</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>33 (58.9%)</td>
<td>23 (41.1%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>How are therapeutic drug levels monitored?</th>
<th>Measure a peak and a trough level</th>
<th>Measure trough level only</th>
<th>Measure a single level at a specified time</th>
<th>I'm not sure</th>
<th>They're not measured!</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 (3.6%)</td>
<td>10 (17.9%)</td>
<td>1 (1.8%)</td>
<td>25 (44.6%)</td>
<td>19 (33.9%)</td>
</tr>
</tbody>
</table>

| **Table 6-8**: Oncology respondents’ estimation of their patients’ exposure and monitoring to ototoxic chemotherapy and radiotherapy. |

<table>
<thead>
<tr>
<th>Which iv AG is used as 1st line treatment of pulmonary exacerbations</th>
<th>CF units (N=32)*, n (%) of respondents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tobramycin</td>
<td>32 (97.0%)</td>
</tr>
<tr>
<td>Amikacin</td>
<td>2 (6.1%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Frequency of administration?</th>
<th>Once daily</th>
<th>Twice daily</th>
<th>Three times daily</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>21 (65.6%)</td>
<td>10 (31.3%)</td>
<td>1 (3.1%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>How are therapeutic drug levels monitored?</th>
<th>Measure a peak and a trough level</th>
<th>Measure trough level only</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12 (37.5%)</td>
<td>19 (59.4%)</td>
<td>1 (3.1%)</td>
</tr>
</tbody>
</table>

* One respondent consistently didn’t answer these questions hence N=32

* The respondent specified that a peak and trough measure is taken with dose 3 then a peak measurement is made on day 10.

**Table 6-9**: CF clinician respondents’ estimation of their patients’ exposure and monitoring to ototoxic aminoglycosides.
6.2.1.3 Ototoxicity monitoring:

All three surveys included a direct question asking if patients received monitoring of auditory function for ototoxicity. As shown in Figure 6-5, 63.9% of the audiology (n=85); 69.6% of the oncology (n=39); and 69.7% of the CF clinicians (n=23) said that they did monitor hearing for signs of ototoxicity. The audiology and oncology surveys also asked whether they believe that ototoxicity monitoring is a priority. Interestingly, 68.4% (n=91) of the audiology respondents and 80.4% (n=45) of the oncology respondents confirmed that they do consider it to be a priority, which are more responses than those that actually perform monitoring. This indicates that even some of the respondents who are not involved in monitoring ototoxicity in their patients still acknowledge its importance. On the other hand, a significant minority of professional groups, 31.6% (n=42) of audiology and 19.6% (n=11) of oncology respondents felt that it was not a priority for their patients. When asked whether other forms of monitoring for toxicities was carried out, 61.6% of the audiology respondents said that none were carried out and of the other 26% that chose the ‘Other monitoring’ option 18/26 (69%) stated that they either ‘didn’t know’ or were ‘not sure’ whether any other monitoring was performed or not (Figure 6-6). On the other hand, 83.9% of the oncology respondents confirmed that monitoring for nephrotoxicity was carried out and the 60.6% who chose the ‘other monitoring’ option mainly stated that monitoring for cardiac toxicities and other adverse effects on the CTCAE register were monitored. Both professionals indicated that limited monitoring for vestibulotoxicity was carried out (≤10% in both groups).

When the services that do undertake monitoring were asked whether their patients were assessed within a dedicated ototoxicity monitoring clinic 81.2% of audiology (n=69) and 64.1% of the oncology (n=25) respondents confirmed that testing was not performed in a dedicated ototoxicity clinic, which would create difficulties in tracking patients’ monitoring within appropriate timelines, performing more specialized testing such as high frequency audiometry or even being able to properly audit this service in order to make recommendations for improvements. When asked where the auditory assessments are undertaken, all three professional groups confirmed that they were overwhelmingly conducted at their local audiology departments [97.7% audiology (n=83); 94.9% oncology (n=37); and 82.0% CF respondents (n=18)]. Only 17.7% of the audiology (n=15) and 2.6% of the oncology respondents (n=1) reported that testing could be conducted at the patients’ bedside on the hospital ward and even less are undertaken in a community setting. The CF clinicians didn’t report this similar small proportion and stated that the remaining 14% and 5% of the testing was performed in the local ENT departments or specialist Audiovestibular
clinics respectively. This indicates limited access of the commonly unwell cancer or CF patients who are not able to come to outpatients’ audiology clinics.

Only 4 respondents from the CF survey did not answer this question, all the rest gave an answer (Yes/No).

Figure 6-5: Distribution of responses of the three professions confirming whether they do or do not monitor their patients’ hearing for signs of ototoxicity.
Figure 6-6: Percentage of respondents confirming if other forms of monitoring for toxicities is carried out.

**Criteria for referral for monitoring:**

An important set of questions was related to assessing the criteria for referral of patients for auditory monitoring. Figure 6-7 and Figure 6-8 show the distribution of responses. The CF responses were presented separately as different response choices were offered in their survey compared to the audiology and oncology respondents. ‘Patients receiving repeated doses of ototoxic medication’ was the most frequently selected referral criteria by both audiology (55.3%) and oncology (66.7%) respondents; whereas the CF service respondents indicated that patients complaining of hearing loss, tinnitus or dizziness were the most frequently selected criteria for referral (90.9%, 72.7% and 68.2% respectively). Only referring patients when they start complaining of a hearing or a balance problem was also a common referral criterion for the audiology and oncology respondents (32.9% and 25.6% respectively). A very limited number of any of the three professions indicated that all their patients are monitored. CF service respondents (31.8%) also indicated that patients with poor renal function or those with positive family history or known genetic susceptibility to ototoxicity were referred.

A follow up question was given in the audiology and oncology surveys asking them ‘if formal referral criteria didn’t exist, what in their clinical judgment would be the criteria for
referral for monitoring?’ Figure 6-9 summarizes the comments of the audiology (n=38) and oncology (n=9) respondents who commented on this question. Interestingly the highest percentage (36%) confirmed that a baseline testing at diagnosis then repeated testing after every 2-3 cycles/courses, whereas some stated that only children should be referred and several audiologists confirmed that they didn’t know what criteria the oncologists used.

**Responses to: What are the criteria used for referring patients for auditory monitoring?**

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Percentage (%) of respondents</th>
<th>Audiology</th>
<th>Oncology</th>
</tr>
</thead>
<tbody>
<tr>
<td>All oncology patients referred</td>
<td>20</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td>Only paediatric patients</td>
<td>30</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>Patients reporting hearing or balance problems</td>
<td>40</td>
<td>30</td>
<td>50</td>
</tr>
<tr>
<td>Patients receiving repeated doses ototoxic medication</td>
<td>60</td>
<td>50</td>
<td>70</td>
</tr>
<tr>
<td>Patients receiving specific medication (please specify)</td>
<td>50</td>
<td>40</td>
<td>60</td>
</tr>
</tbody>
</table>

Respondents were asked to tick ‘all options that applied’ therefore percentages are not intended to equal 100%.

Figure 6-7: Response of Audiology (n=85) and Oncology (n=39) respondents to: “What are the criteria used for referring patients for auditory monitoring”.

210
Figure 6-8: Response of CF respondents (n=23) to: “What are the criteria used for referring patients for auditory monitoring?”

C/O, Complaining of; iv AGs, intravenous aminoglycosides. Respondents were asked to tick ‘all options that applied’ therefore percentages are not intended to equal 100%.

Figure 6-9: Professional opinions from audiologists and oncologists in regards to when patients should be referred for monitoring in the case of absence of referral criteria.
**Auditory monitoring for ototoxicity:**

Table 6-10 shows results of several questions covered in this section. On assessing whether there is a protocol defining which tests to use for ototoxicity monitoring, 52.9% of the audiology (n=45) and 25.6% of the oncology (n=10) respondents stated that there is no protocol and that tests are determined on a patient-by-patient basis, with another 17.7% and 12.8% of each of the two professionals respectively stating that they don’t know. Surprisingly 61.5% of the oncology (n=24) respondents believed that there is a protocol compared to 29.4% of the audiologists (n=25).

A significant 70.6% of the audiology respondents stated that they didn’t know whether patients received counseling prior to receiving ototoxic treatment or not and indicated a lack of awareness or involvement in this aspect of ototoxicity monitoring. Another 24.7% of the audiology respondents stated that patients did receive counseling but many of them confirmed that the oncologists/managing physicians provided this. Only 3 audiologists said that they provided information in relation to the side effects of ototoxic medications and how this should be managed, with another few indicating that patients were provided with leaflets or written information by the oncologists. On the other hand, 79.5% of the oncology respondents confirmed that counseling is provided but the comments (n=28) provided suggested this is often in the form of just informing patients that ototoxicity is a potential side effect. Several comments included ‘side effects are mentioned at the time of consent’; ‘routine part of consent for chemotherapy’; ‘patients are warned of it only’; while some gave a bit more detail such as ‘We tell all patients that the treatment will cause transient tinnitus and some degree of hearing loss’ or ‘told may reduce hearing and will be repeatedly tested’.

In regards to confirmation if baseline testing is conducted prior to the start of ototoxic medications, 50.6% of the audiologists (n=43) and 76.9% % of the oncologists (n=30) stated that it was performed however the remaining proportions of both groups confirmed that it was either only done some of the time or not at all.

Again, there was a large variation in opinions as to how frequently the monitoring was performed. Some said within the course of treatment, others said when patients report symptoms such as hearing loss or tinnitus but the highest percentage of audiologists (40%) chose the ‘Other’ option where they gave comments like ‘As and when requested by the referring clinician’ or ‘at the referring consultant’s discretion’ or ‘don’t know’. These comments indicated the possible lack of knowledge and the seemingly ad hoc basis by which clinicians refer patients from the audiologists’ perspective. The same applied when the question as to how long ototoxicity monitoring continues following cessation of treatment
was posed. A large variation between audiologists’ responses ranged between ‘it doesn’t continue after cessation of treatment’ to ‘up to 6-12 months later’. However the ‘Other’ option was again chosen by the highest proportion (40%) that specified that they were either ‘not sure’; ‘variable with no consistency’ or ‘as requested by the oncologist’.

The same question regarding how often the monitoring was performed was also posed in the oncology and CF surveys. Contrary to the audiologists’ responses the majority of oncology (69.2%) said that monitoring was performed within or after each course of treatment. This included nine respondents (23.1%) stating that they would only be tested when they start complaining of hearing loss or tinnitus and another nine (23.1%) choosing the ‘other’ option and specifying that monitoring is performed as per guidelines/protocol and others specifying a frequency of every two cisplatin-containing regimens. The highest proportion of CF clinicians (52.4%) stated that auditory assessment was only performed when patients start complaining of hearing loss, another 28.6% said monitoring was done after every 10-12 courses and another 19.1% specified that it was done annually. A smaller proportion of oncology respondents (15.4%) compared to the audiologists reported that monitoring does not continue after cessation of treatment. Most of them reported that monitoring does continue but responses varied in regards to the duration, ranging between 3-6 months, 12 months, 5 years or even 10 years post-treatment. A significant proportion of respondents that provided comments (n=17) confirmed that surveillance continues mainly when hearing loss/tinnitus was present during treatment with only one respondent saying ‘not sure’.
There was a general agreement between all three professionals that standard pure-tone audiometry (0.25-8 kHz) was the most commonly used audiological test used for ototoxicity monitoring (Table 6-11) followed by tympanometry to exclude middle ear disease. All professionals confirmed that when certain tests were performed at baseline testing they were repeated at the follow-up monitoring. However it is worth noting that the percentage of responses made by the oncology respondents for each of the audiological test procedures is very small, which may be an indication of their limited knowledge of these tests and identification of which ones are used (Table 6-11). Unfortunately, extended high frequency
audiometry (EHFA) and DPOAEs, which were shown to be more effective at early detection of ototoxicity, were not as commonly used as hoped (EHFA: in 17.7%, 12.8% and 22.7%; DPOAEs: in 23.5%, 2.6% and 13.6% of each of the three professions). A follow up question asking respondents to specify the highest frequency tested confirmed that frequencies up to and including 8kHz were the commonest as reported by 27/43 audiology respondents and 4/9 oncology respondents. Two audiologists stated that they did have the facilities for EHFA but was deemed unnecessary, as changes in these higher frequencies would not prompt changes in management. A total of 15/43 and only 1/9 audiology and oncology respondents respectively said that they test higher frequencies ranging up to 10 to 20 kHz (1/6th octave intervals). Four respondents indicated that they didn’t know the extent of frequencies tested.

<table>
<thead>
<tr>
<th></th>
<th>Audiology (N=85), n (%)</th>
<th>Oncology (n=39), n (%)</th>
<th>CF clinicians (N=22), n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTA, including VRA/play audiometry (250Hz-8kHz)</td>
<td>64 (75.3%)</td>
<td>14 (35.9%)</td>
<td>19 (86.4%)</td>
</tr>
<tr>
<td>EHFA; including VRA/play audiometry (above 8kHz)</td>
<td>15 (17.7%)</td>
<td>5 (12.8%)</td>
<td>5 (22.7%)</td>
</tr>
<tr>
<td>TEOAEs</td>
<td>21 (24.7%)</td>
<td>1 (2.6%)</td>
<td>3 (13.6%)</td>
</tr>
<tr>
<td>DPOAEs</td>
<td>20 (23.5%)</td>
<td>1 (2.6%)</td>
<td>3 (13.6%)</td>
</tr>
<tr>
<td>Tympanometry</td>
<td>46 (54.1%)</td>
<td>11 (28.2%)</td>
<td>1 (4.5%)</td>
</tr>
<tr>
<td>ART</td>
<td>8 (9.4%)</td>
<td>3 (7.7%)</td>
<td>N/A</td>
</tr>
<tr>
<td>ABR; neurological</td>
<td>1 (1.2%)</td>
<td>1 (2.6%)</td>
<td>N/A</td>
</tr>
<tr>
<td>ABR; threshold</td>
<td>8 (9.4%)</td>
<td>1 (2.6%)</td>
<td>N/A</td>
</tr>
<tr>
<td>Speech audiometry</td>
<td>5 (5.9%)</td>
<td>5 (12.8%)</td>
<td>N/A</td>
</tr>
</tbody>
</table>

VRA, Visual Reinforcement Audiometry; ABR, Auditory Brainstem Response; ART, Acoustic reflex thresholds; N/A, not applicable as this choice wasn’t offered on the CF survey. Respondents were asked to tic 'all options that applied' therefore percentages are not intended to equal 100%.

Table 6-11: Distribution of responses to “What audiological testing is conducted for ototoxicity monitoring?”

**Patient management:**

The final section of the surveys aimed to assess how evidence of ototoxicity, shown through auditory monitoring, affects the medical management of patients. When asked if there is a protocol used to identify when changes in auditory status become clinically significant, 43.5% of the oncology (n=17) respondents confirmed that a published protocol was used but just under half of them (48.7%) where either not sure or said there was no protocol and that clinical judgment was used. Contrary to that, the highest proportion 83.4% of the audiology (n=71) respondents were either not sure or used clinical judgment, with a much smaller percentage (21.1%) confirming there is a departmental or published protocol used (Figure 6-10). The follow up question asked respondents to specify what audiological results may
lead to change in medical management. A summary of the comments added by the audiology and oncology respondents is shown in Figure 6-11. Many audiologists stated that the decision to change management was not up to them especially because they are non-medical. Many comments included comments such as: ‘not for me to say. I'm not a clinician’; ‘Not known. Decisions not made by technician’ and ‘decision of oncologist not audiologist’. More audiologists quoted specific threshold shifts, as seen in Figure 6-11, than the oncology respondents. Mainly audiologists mentioned ASHA criteria whereas oncologists mainly mentioned Brock’s criteria. Several audiologists wrote detailed comments such as: ‘Any significant change (considered as 20dB alteration at single frequency, or 10dB at contiguous frequencies) should be reported by Audiology to Oncologists as potentially important. Clearly whether action is taken will depend upon treatment aims (palliative/curative). Regarding adults: Audiological results should be discussed with patient before making recommendations’.

*Responses to: Is there a protocol used to identify when changes in auditory status become clinically significant?*

![Graph showing responses to the question: Is there a protocol used to identify when changes in auditory status become clinically significant?](image)

Figure 6-10: Distribution of responses to the question: “Is there a protocol used to identify when changes in auditory status become clinically significant?”

Figure 6-12 shows the responses of the oncologists as to what changes would be made. A majority of 79.9% (n=31) confirmed that they would change the ototoxic treatment. All the CF clinicians (100%, n=22) confirmed that results of auditory monitoring confirming occurrence of ototoxicity changes their antibiotic management. Figure 6-13 shows what
actions they would take. The highest proportion stated that they would stop or avoid using aminoglycosides when ototoxicity occurs, with others specifying that they would reduce dosage or number of courses by using alternative antibiotics such as colistin or folomycin. Others said they would avoid intravenous AGs but replace them with nebulized AGs such as TOBI. They were also asked if they undertake genetic screening of all their patients for the mitochondrial DNA mutation A1555G in the 12S rRNA gene, which is known to increase susceptibility to aminoglycoside ototoxicity. Only 19.2% CF clinicians (n=5) said they did screen their patients whereas the majority 80.8% (n=21) confirmed that they didn’t.

Responses to: What changes in audiological results should prompt consideration or an actual change in medical management?

![Responses to: What changes in audiological results should prompt consideration or an actual change in medical management?](image)

Figure 6-11: Distribution of respondents’ comments to the question: "What changes in audiological results should prompt consideration or an actual change in medical management?"
**Responses to: What changes would be made if monitoring shows evidence of ototoxicity?**

**Oncology (n=39)**

- Stop ototoxic treatment, 3
- Reduction in dosage, 23
- Change of ototoxic treatment, 31
- No change as treatment is of curative intent, 1
- Don't know, 2

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**Responses to: What changes do you implement to your medical management if auditory monitoring shows evidence of ototoxicity?**

**Number of CF clinician responses**

- Stop/avoid using AGs: 14
- Reduce the AG dose: 8
- Use an alternative e.g colistin: 6
- Change to nebulised AG (TOBI): 2
- More frequent monitoring: 2
- Individualised plan: 0

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Figure 6-12: Distribution of oncology respondents’ response to question: ‘What changes would be made if auditory monitoring shows evidence of ototoxicity?’

Figure 6-13: Distribution of CF clinicians’ comments to the question: “What changes do you implement to your medical management if auditory monitoring shows evidence of ototoxicity?”
### 6.2.2 Discussion of survey study of current UK service provision:

The 1994 ASHA guidelines for audiological management of individuals receiving cochleotoxic drug therapy emphasized that a basic program of audiological monitoring for ototoxicity requires the following components: “*(a)* specific criteria for identification of toxicity, *(b)* timely identification of at-risk patients, *(c)* pre-treatment counseling regarding potential cochleotoxic effects, *(d)* valid baseline measures (pre-treatment or early in treatment), *(e)* monitoring evaluations at sufficient intervals to document progression of hearing loss or fluctuation in sensitivity, and *(f)* follow-up evaluations to determine post-treatment effects” (ASHA, 1994a). Similar UK guidelines do not yet exist. On the contrary, to emphasise the relatively limited interest in ototoxicity monitoring in clinical practice the only two recommendations made within a 102-page document by the UK CF trust on ‘Antibiotic treatment in cystic fibrosis’ were “*(a)* consideration should be given to an annual pure tone audiogram in patients receiving frequent courses of an aminoglycoside and *(b)* that the use of an aminoglycoside should be restricted to alternate courses of intravenous antibiotics, where the patient’s clinical condition permits in order to reduce cochlear and vestibular toxicity” (UK CF Trust, 2009).

The current study underwent a survey of oncology, CF and audiology services in the UK in order to assess how often ototoxicity monitoring is performed and how effectively the ASHA components above are implemented. Review of the literature had shown that no previous surveys of oncology and audiology services had been published with the exception of one pilot study of two centres in South Africa (de Andrade, 2009). However, there were two previous UK surveys of CF centres (Tan et al., 2002, Smyth and Campbell, 2013, Smyth and Campbell, 2014) and another four from the USA and Australia (Phillips and Bell, 2001, Soulsby et al., 2009, Van Meter et al., 2009, Prescott, 2011, Prescott, 2014) that briefly assessed ototoxicity monitoring as part of overall surveys investigating current practice in aminoglycoside usage and monitoring in CF patients in each of these countries.

There was a relatively good response from 133 Audiology, 56 Oncology and 33 CF respondents representing all regions of the UK (Figure 6-4) and representing both paediatric and adults services (Table 6-7). Both oncology and CF respondents acknowledged that their patients receive potentially ototoxic treatments such as cisplatin, carboplatin and head & neck radiotherapy in the case of oncology patients and aminoglycoside antibiotics, mainly extended interval dosing of tobramycin, in the case of CF patients (Table 6-8 and Table 6-9). A once daily tobramycin regimen was also confirmed as the first line treatment in most of the UK CF centres by the survey study on prescribing practice of i.v. AGs in UK CF centres.
This survey also showed that many centres have stopped gentamicin prescription (Smyth and Campbell, 2014). Tobramycin was also confirmed as the most commonly used regimen in adult US CF programs where it is also dosed at 10 mg/kg/d every 24 hours using an infusion over 30-60 mins (Prescott, 2014).

The most encouraging piece of information obtained from all three surveys was the positive responses from 63.9% of the audiology, 69.6% of the oncology and 69.7% of the CF respondents confirming that their patients do receive auditory monitoring for ototoxicity (Figure 6-5). This was a significant improvement over the responses from the Tan et al. survey which showed that only 3/23 (13%) of the responding UK CF centres routinely assessed hearing function using only standard pure-tone audiometry. Van Meter et al. reported that 22/51 (43%) of the US CF Foundation-accredited care centres and affiliated programs (CFFACCs) administering once-daily tobramycin therapy routinely performed audiometric evaluations and Prescott (2014) confirmed that around one-half of the 68 US CF adult centres responding to his survey used audiometry to monitor ototoxicity even though all of them monitored for nephrotoxicity using serum creatinine measurements. Similarly, Phillips and Bell reported in their 1999 survey that only 4/26 (15%) of the Australian CF centres monitored their patients for otoxicity/vestibulotoxicity which was reported to have improved to 17/27 (63%) eight years later in the repeated survey by Soulsby et al. (Phillips and Bell, 2001, Soulsby et al., 2009, Tan et al., 2002, Van Meter et al., 2009, Prescott, 2014). In the current study, monitoring for other toxicities, especially nephrotoxicity, was common as it was reported by 83.9% of the oncology respondents. This was in line with previous studies that reported that renal function was measured in 18/23 (78%) of UK CF centers (Tan et al., 2002), in 51/51 (100%) of US CFFACCs (Van Meter et al., 2009), and in 26/27 (96%) of the Australian CF centers (Soulsby et al., 2009).

Conversely, all three professional groups reported variability in practice in relation to all aspects of this monitoring service, which highlighted the main problem facing its adequate provision in light of absence of agreed UK guidelines. In relation to the ‘timely identification and referral of at-risk patients’; patients receiving repeated doses of ototoxic medication and patients complaining of hearing or balance problems were the two most common criteria used for referring patients for auditory monitoring (Figure 6-7 and Figure 6-8). The distribution of answers shown in Figure 6-9, of oncology and audiology respondents as to what should be the referral criteria in their professional opinion highlights the wide variability in these opinions with a few stating that only paediatric patients should be referred, some audiologists confirming that they didn’t know how oncologists decide, and only 27% stating that all patients exposed to ototoxic chemo/radiotherapy should be referred.
One of the main purposes of auditory monitoring for ototoxicity is early detection of minor changes to cochlear function in order to inform the managing clinicians to implement changes in the drug regimens, if possible, aiming to prevent further hearing damage (Durrant et al., 2009). Referring patients after exposure to repeated courses or after cochlear damage has extended from the higher frequencies to the lower frequencies associated with speech understanding and communication, defies the main aim of monitoring. In this case, monitoring becomes a tool for management of side effects of the treatment and not for early prevention of hearing damage.

Only around half of the audiologists confirmed that baseline testing is conducted before the start of medication with the remaining proportion stating that baseline recording is either done some of the time or not at all (Table 6-10). It is imperative to keep in mind that ototoxic medications are not administered in isolation from other damaging effects to the ear. Exposure to noise, infections, and trauma, ageing or even to other ototoxic drugs or environmental chemicals causes synergistic damage to the inner ear (Laurell and Borg, 1986, Fechter et al., 2007, Rybak, 1992). Therefore baseline recording in addition to good history taking to identify these risk factors is essential not just to confirm that evidence of hearing loss from post-treatment assessments are attributed to the ototoxic drug exposure but to help in further protection of the patients’ hearing by identifying how often monitoring should occur or even whether use of this ototoxic drug should be avoided from the start. This also brings us to the important topic of pre-treatment counseling. Even though it was expected, it was disturbing to record that 75.3% of the audiology respondents either didn’t know or didn’t believe that patients received counseling prior to receipt of potentially ototoxic medications. This is an indication that audiology professionals are not involved in this process even though their specialist training and knowledge best places them to be able to have a very active role in that aspect. This can be through taking the relevant history; providing patients/their parents with the correct information regarding the prevalence, warning signs, symptoms of ototoxicity from the specific drugs they will be exposed to; and advising them on what to do if unavoidable permanent hearing loss does occur to maintain the most effective hearing and communication possible through rehabilitation (hearing aids/assistive listening devices/cochlear implants) and good communication tactics. This lack of involvement in patient counseling or lack of ownership of the whole ototoxicity monitoring process by the audiology respondents may possibly be attributed to the fact that most of the audiology professionals in the UK are not medically qualified (90.3% of the respondents identified themselves as audiological professionals i.e. audiologists or audiological scientists with only 8.3% AVM or ENT doctors). As they mainly work in consultant-led services, they probably do not believe that patient management is part of their role. This was evident from
some of the comments made such as ‘not for me to say. I'm not a clinician’ or ‘Not known. Decisions not made by technicians’. This, in addition to other discrepancies between audiologists’ and clinicians’ responses, displays a unilateral relationship between them where the audiologists receive the referrals, they perform the tests as requested and they send the results back with no sense of partnership or ownership of the process. The American Academy of Audiology has published a position statement and clinical guideline on ototoxicity monitoring in 2009 to specifically focus attention on the fact that audiologists are best placed to take the lead in developing ototoxicity monitoring programs as their professional training equips them to achieve their two main objectives of preventing or minimizing hearing loss, through early identification, and helping patients maintain the most effective hearing and communication possible should hearing loss occur (Durrant et al., 2009). The majority of oncologists (79.5%) confirmed that they provide counseling to their patients but many of their comments showed this was limited to mentioning hearing loss/tinnitus as possible side effects as part of the consent process. Again this highlights that audiologists can offer so much more to this part of the program.

There were mixed levels of agreement between audiologists and oncology or CF clinicians regarding the frequency and timing of audiological monitoring before, during and post-treatment; whether there was a protocol to specify which audiological tests to perform or whether it was determined on a patient-by-patient basis, or what criteria for ototoxicity are used to advocate change in management (Table 6-10 & Figure 6-10). The lack of generally agreed protocols and guidelines again increases the uncertainty of all the professionals involved and weakens their ability to make best use of the outcomes of monitoring. It also highlights that patients may be at risk of receiving inappropriate and variable monitoring depending mainly on their local clinicians’ awareness of the significance of ototoxicity and its impact of their quality of life and on their relationship with the local audiology service.

A significant proportion of the audiology and CF respondents confirmed that standard PTA was the most commonly used audiological test used in monitoring followed by tympanometry. Most of the respondents indicated that there is no set protocol of audiological tests for ototoxicity monitoring and that testing is not differentiated from a routing test battery of basic audiological assessments. This misses opportunities for early detection and prevention of hearing loss.

Additionally, the very limited percentage of responses made by the oncology respondents to any of the audiological test procedures (the highest percentage being 35.9% for standard PTA with much lower responses for the other options) (Table 6-11) may be an indication of lack of knowledge of which audiological tests are actually performed for their patients. This
is a very significant outcome as it is common practice that audiologists normally perform assessments and then just adds the test results (e.g. a copy of the audiogram, tympanograms, DP-gram or speech test) to the patients’ clinical notes with no reporting/explanation of these results or recommendations for action. If oncologists don’t know what audiological tests are performed how are they interpreting the output of these tests in the clinical notes and how are they changing their management accordingly? It is also clear from the audiologists’ comments to several questions that they do not normally have direct communication with the clinicians. As these were the responses of the 39 oncology respondents that confirmed that ototoxicity monitoring is performed in their service and as this is a very small percentage of overall oncology workforce, which is reported in the Royal College of Radiologists’ (RCR) 2011 census to be 1,118 clinicians (Radiologists, 2011), the question that may be posed could be: Is that a real indication of very limited interest in this area of patient management?

The use of EHFA and DPOAEs were sparsely reported. These tests have been repeatedly shown to detect ototoxic cochlear damage earlier than standard audiometry (Al-Malky et al., 2011, Beahan et al., 2012, Fausti et al., 1992a, Fausti et al., 1993, Fausti et al., 2003, Knight et al., 2007, Stavroulaki et al., 2002). These procedures were also shown to be less effected by low frequency environmental noise and therefore more efficacious for bedside testing in quiet hospital rooms/community settings especially when using portable equipment, which is now widely commercially available (Gordon et al., 2005, Gorga et al., 2000b, Konrad-Martin, 2005a). Therefore these tests can be very effective in monitoring and early detection of auditory changes in ill patients who cannot be repeatedly transported to audiology departments especially if they were based in different hospitals or are too far from patients’ homes. Responses from the three surveys indicated that most patients are tested in the local audiology departments with only 17.7% of audiology, 2.6% of oncology and none of the CF respondents reporting that bedside testing is performed. DPOAEs have the added advantage of being a very quick objective test and therefore do not require patient cooperation. This allows testing for quite unwell children and adults. As shown by the DPOAE repeatability study (section 4.2) deterioration in DPOAE amplitudes of > 7.5 dB SPL, which was also reported by Beattie et al., indicates the criteria that should be used to identify evidence of cochlear damage when using this test as an ototoxicity monitoring tool (Beattie et al., 2003).

EHFA may not be recordable in all patients such as elderly patients, where presbyacusis characteristically affects higher frequencies more (Wiley et al., 1998), and such as young children (<7 years old) due to lower test-retest reliability (Beahan et al., 2012). Therefore, from the outcomes of this survey and through review of the related literature, I am recommending the use of a screening protocol consisting of a test battery of EHF audiometry sensitive range of ototoxicity (SRO) measurements and DPOAEs as an accessible, sensitive
and cost-effective monitor or sensitive early detection of ototoxicity especially in unwell patients.

The respondents also showed variability in the reported results criteria for ototoxicity with the oncologists specifying that they follow published criteria quoting Grade 2 Brock’s criteria and the audiologists quoting the ASHA criteria (Figure 6-10 and Figure 6-11). Both oncology and CF respondents indicated that they would change their management if evidence of ototoxicity were recorded (Figure 6-12 and Figure 6-13). It is important for all parties involved to use the same ototoxicity grading criteria in order to maintain consistent adherence to guideline and management of patients. Brock’s Grade 2 hearing loss is equivalent to a >40dB hearing loss at 4 and 8kHz which is already a significant hearing loss especially in children. It is known that even mild to moderate hearing losses in children will affect their educational progress and speech and language development (Grewal et al., 2010, Park et al., 2013). The recommended modification to the Brock’s scale such as those made by Chang and Chinosornvatana or the International Society of Pediatric Oncology Boston (SIOP) Boston Ototoxicity Criteria are more appropriate criteria to use for early detection of ototoxicity because they take into account mild (>20 dB) levels of hearing loss (Brock et al., 2012a, Chang and Chinosornvatana, 2010). A clearly agreed grading system and grade at which change in management is considered has to be defined by both the audiologists and managing clinicians.

6.2.2.1 Limitations of the study:

The main limitation of this study was the method through which professionals were approached. Lead clinicians of each of the 48 CF centers identified through the ‘CF Trust registry reports’ allowed for the calculation of a center response rate of 33/48 (69%), which was excellent at confirming an adequate representation of UK services, but it limited responses to only the lead clinician of each center. Previous literature and clinical experience confirms that other team members such as specialist oncology/CF nurses and pharmacists undertake key roles in recording drug regimens, identifying planned treatments and arranging baseline and monitoring assessments (Durrant et al., 2009). The audiology and oncology professionals were contacted through mailing lists of relevant professional bodies such the BAA, the Children’s Cancer and Leukaemia group or the UK Oncology Nursing Society and through personal communications. This guaranteed that wide ranges of professionals in the two fields (consultants, registrars, nurses, pharmacists and audiologists/scientists) were approached. Even though this allowed for recruitment of a wider range of healthcare professionals, it did not allow for a calculation of a response rate or for identification of how well each profession is represented to confirm whether the
results are an accurate representation of current UK practice or not. The audiology response of 133 was relatively high but it was not possible to assess why only 56 oncology professionals responded even though the survey could have been potentially sent to hundreds of people. It was not possible to estimate exactly how many professionals received the email, how many deleted the message without even considering to read it, how many wanted to complete the survey but were too busy or didn’t because they don’t perform monitoring or whether this was a true reflection of the significant lack of interest of oncology professionals in ototoxicity monitoring. It was only after the survey was complete that a document of a 2011 UK clinical oncology workforce census conducted by the Royal College of Radiologists (RCR) was discovered. It reported that the overall workforce included 1,118 clinicians (consultants, specialist registrars and other grades) within 59 UK centers (Radiologists, 2011). A repeat of the survey targeting each center may allow for calculation of a response rate and estimation of how well UK services are represented. Davis et al. estimated that there is an excess of 3000 professionals working in the audiology services (Davis et al., 2007) however there is no official workforce census to date. A BAA Audiology 2013 census is the first census to be undertaken to assess the audiology workforce in the UK. An online questionnaire was sent to audiology professionals through the BAA mailing list over the month of October 2013. However, the outcomes of this census have not been published to-date.

The other limitation of the study was the inability to match the audiology services to the corresponding oncology and CF services, in order to directly compare responses of both ends of each service, because the anonymity of the respondents was maintained. It was also not possible to confirm whether more than one respondent from the same hospital had completed the questionnaire. However, the data presented in Figure 6-4 confirms that the three professional groups represented most of the UK regions.

6.2.3 Conclusions:

Hearing loss due to ototoxic medications is potentially preventable but only if all healthcare professionals involved in managing exposed patients play an active role in preventing it through a robust audiological monitoring service. This study aimed to survey healthcare professionals to assess the current UK provision of ototoxicity monitoring services. Due to the lack of clear UK-wide guidelines for the audiological monitoring of ototoxicity, there were significant variations in all aspects of the monitoring services provided and in the perceptions of the audiology professionals compared to the oncology and CF clinicians. The high response confirming that monitoring is performed was encouraging but there was a wide variation in the criteria for referral; the audiological tests used and the frequency of
monitoring; the grading criteria to confirm ototoxicity; and the options for change in management once evidence of ototoxicity is provided. The two main aims of ototoxicity monitoring services is early identification of initial signs of ototoxicity and then adequate management by informing the managing clinicians to modify treatment to prevent further damage or by maintaining communication abilities through audiological rehabilitation in case permanent damage could not be avoided. As recommended in the AAA position statement for ototoxicity monitoring (Durrant et al., 2009), audiologists are best placed to take the lead in initiating and implementing adequate ototoxicity monitoring services but this will only be possible through establishing strong working links with the clinicians involved and through establishing a UK-wide program where clear roles, responsibilities and patient care pathways are defined.
Summary & General Discussion
Chapter 7: **Summary and General Discussion**

7.1 **Recap of research aims:**

This research had the overall aim of furthering the understanding of different aspects of ototoxicity in children to enable recommendations for development of better but realistic guidelines for monitoring and management of ototoxicity and for making recommendations for future research in this field.

The research undertaken was presented within three main themes.

**Theme A** focused on audiological assessment for detection of ototoxicity. It aimed to assess the prevalence of ototoxicity in children with CF exposed to AGs and to evaluating the performance of the different audiological tools in order to define the best test battery that can detect early signs of ototoxicity. A further control study was done to gauge the impact of certain factors on DPOAE testing and which were considered to have an affect on its ability to be used as an effective auditory monitoring tool. It analyzed the effect of probe removal and re-insertion on the repeatability of recordings in ears of school-aged children.

**Theme B** focused on causation. Results of the audiological assessments of AG exposed children with CF showed a dichotomy in the hearing test results of these children with some showing completely normal hearing and others showing evidence of ototoxicity. This dichotomy could partially but not completely be explained by the cumulative exposure to repeated courses of AGs. Within this theme potential risk factors within the children’s history were examined for significant associations with the occurrence of ototoxicity. In addition, genetic investigations were performed to assess if certain genetic mutations were associated with increased susceptibility to ototoxicity in some of the children within this patient cohort and therefore offer a possible explanation for the observed dichotomy. Even though the sample size investigated was not large enough to provide significant outcomes with enough power, it provided a good pilot study to raise specific research questions. For example, if this association was established should consideration be made as to whether or not it would be useful to advocate screening to identify these mutations before patients are exposed to AGs? Should more investigations into other genetic candidate or modifying genes be pursued? Is there a need for more investigation into the penetrance of A1555G
mutations when patients are exposed to AGs? Do drug-metabolizing genes have a role in increasing susceptibility to AG ototoxicity?

**Theme C** focused on the clinical impact of ototoxic hearing loss on patients. It aimed at assessing the impact of hearing loss on the quality of life of paediatric cancer survivors compared to their corresponding normal hearing peers. Significant outcomes would highlight the importance of early detection, management and ultimately prevention of ototoxicity in these children. The second aim within this theme was to assess the current service provision for auditory monitoring for ototoxicity in the UK from the perspective of both the audiology professionals and the oncology and CF clinicians. The overall aim here was to assess how much of the published recommendations and guidelines are actually applied clinically and whether the absence of agreed national UK guidelines has an impact on these services.

### 7.2 Summary of findings

**Studies within Theme A** discussed several aspects of auditory assessment of ototoxicity. The auditory function was examined in the children with CF who were one of the population groups of interest in this research. The fact that the results showed that 21.4% of the tested subjects had evidence of ototoxicity, is a very significant finding as this equates to a ratio of 1:4/5 children with CF exposed to AGs. This is a much higher prevalence than previously reported in the literature for paediatric CF patients and is closer to the reported prevalence for adults (Mulheran et al., 2001, Mulherin et al., 1991, Mulrennan et al., 2009, O’Donnell et al., 2010, Prayle et al., 2010, Smyth and Bhatt, 2012, Tan and Bunn, 2000). This stresses the importance of monitoring for ototoxicity in this patient group, which was not the case at GOSH and was shown to be quite varied and inconsistent in services all over the UK, as shown from the results of the survey study in Theme C. It was also observed that a clear dichotomy existed in the hearing status of these children, where some had completely normal hearing while others had high frequency SNHL attributed to ototoxicity. The study also emphasized the benefits of using both EHF PTA and DPOAEs as a complementary test battery for ototoxicity monitoring as opposed to the commonly used standard PTA especially when testing children. EHF PTA was shown to identify the highest number of children with evidence of ototoxicity and therefore was considered as the gold standard for testing. The DPOAEs have the significant advantage of being objective, quick and non-invasive but in the current study did not show a drop in amplitudes in a number of children where EHF PTA thresholds had increased. This could be attributed to the fact that the DPOAE recordings made only covered frequencies up to 8 kHz and not the extended higher frequencies assessed through EHF PTA. However, two control studies were undertaken with normal hearing
individuals to assess other possible factors that may affect the accuracy of DPOAE recordings and its use as an effective auditory monitoring tool.

These control study showed that DPOAE recording in the ears of school-aged children is robust and repeatable even when performed in a quiet non-sound proof room. It was shown that removal and re-insertion of the OAE probe does not significantly affect the repeatability of DPOAE recordings as long as it’s inserted carefully. It also showed that a minimal change of >11.1 and >7.5 dB SPL for the lower and higher frequencies respectively on repeated DPOAE recordings is required when monitoring for ototoxicity to establish a true shift in auditory function and which can not be attributed with 95% confidence to an error of measurement. These findings helped to confirm that DPOAE testing could be effectively used as an auditory monitoring tool for detection of ototoxicity especially when used for serial recordings. Here, the baseline pre-exposure DPOAE amplitude recordings would be used as the control measurement for each subject and a drop in amplitudes >11.1 and >7.5 dB SPL for the lower and higher frequencies respectively at post-exposure recordings would be an indication of OHC damage within the inner ear. DPOAE have the advantage of being a quick, non-invasive and accurate object measure of inner ear function.

Theme B studies that focused on causation of ototoxicity and assessed possible risk factors that can be used to explain the observed dichotomy in hearing outcomes of the children with CF. The first study showed that high exposure to ototoxic medications like AGs is the most significant, but not exclusive, risk factor for ototoxicity and highlighted the non-linear relationships between age, exposure, lung function and possible genetic predispositions to ototoxicity. It also stressed that waiting for children to start complaining of hearing problems is not an appropriate referral criteria for auditory monitoring and that a monitoring protocol should be put in place commencing with baseline recordings before the start of treatment.

The second study within this theme assessed the role of some genetic variants / mutations in increasing susceptibility to ototoxicity in the CF children exposed to aminoglycosides. These findings will be discussed further in the general discussion below.

Theme C studies focused on assessment of the clinical impact of hearing loss due to ototoxicity and on evaluating the current UK practice in auditory monitoring for ototoxicity. The first study showed that both the generic HUI3 HRQL and disease-specific PAQL (specific for assessing paediatric deafness) parent-proxy questionnaires proved that there was a significant deterioration in the HRQL and QoL of the cancer children with ototoxic hearing loss compared to those with normal hearing. Scores of the single attributes of ‘hearing’ and ‘cognition’ and the overall multi-attribute utility score of HRQL of the HUI3 questionnaire
all showed a significant difference between the two groups in addition to all the PAQL subscales and the overall QoL scores. It highlighted the impact hearing loss has on children’s speech and language development, social and psychological well-being and educational progress, which again stresses on the need for a strong ototoxicity monitoring protocol for patients involved.

The second survey study within this theme assessed the current status of auditory monitoring service provision in the UK. It highlighted the variability in many aspects of provision of this service within and between the three professional groups targeted in this survey. It showed that there was evidence of limited awareness and/or agreement of responsibilities of team members so that audiologists’ roles were underutilized for counseling and rehabilitation and clinicians had limited understanding of the criteria for ototoxicity and test battery used to diagnose it. There were no generally agreed criteria for when, how often and what audiological tools should be used to monitor for ototoxicity. There were no agreed criteria for what audiological outcomes should be used to advocate change in management and no provision to monitoring patients outside the outpatients’ audiology department even if the child was too unwell to reach it. Recommendations included the development of UK-wide clinical guidelines and professional education programmes, as advocated by the WHO (1994), to increase the profile and standardisation of ototoxicity monitoring in clinical practice.

Overall, all the studies within the three themes confirmed findings of previous research studies that stressed that ototoxicity was a significant cause of hearing loss especially in the more vulnerable patient groups such as children with CF or cancer that have to be repeatedly exposed to ototoxic medications. Theme A studies confirmed that a test battery including EHF PTA and DPOAE testing, which is preceded by tympanometric assessment of middle ear function, would constitute an effective auditory monitoring battery especially when pre-and-post exposure serial recordings could be regularly performed. The importance of using agreed specific criteria for identification of ototoxicity and defining at what stage a change in medical management should be implemented was stressed. The newly developed SIOP Boston grading system was recommended as a good grading tool for the degree of ototoxicity as there is more international agreement to use it, the lower audiometric threshold shifts of >20dB are considered and it is simple and easy to follow by all professionals involved. It was also concluded that more work was still needed to implement all the recommendations of this current and previous research regarding the auditory monitoring and management of ototoxicity in these different patient groups.
7.3  **General Discussion & Recommendations for future work and practical applications**

7.3.1 **Significance of monitoring for ototoxicity in susceptible patient populations**

All three themes of this research have directly or indirectly stressed the importance of monitoring for ototoxicity especially in vulnerable patient groups such as children exposed to repeated and well-established permanently ototoxic agents as cisplatin and aminoglycosides. The survey study in Theme C established that services in the UK are still far from achieving this goal and the best way forward is for national guidelines to be agreed between all parties.

Presentation of the results of the clinical observational study to the whole CF team at GOSH highlighting that 1 in 4 of their patients have evidence of ototoxicity in addition to the efforts made to bring the CF unit and Audiology services together and the enthusiastic support of the two consultants involved has now allowed the establishment of an ototoxicity monitoring service for the CF children at GOSH. This service has just recently been set up and hopefully will be reviewed and improved once it becomes embedded more as an integral part of the management of CF patients. Appendices 9.9, 9.10, 9.11 and 9.12 include the protocols, pathways and paperwork that I prepared as part of this work in order to help set up this service. The need for increased awareness of the use of appropriate auditory monitoring tools, criteria for referral and for defining ototoxicity, assessment times, clearly defined roles, good effective lines of communication between professionals, and individualized patient management plans are all essential components needed for a monitoring service to be successful.

7.3.2 **Recommendations for achieving an ideal ototoxicity monitoring protocol:**

Based on the outcomes and clinical experience acquired from all the studies involved in this research and on the review of the related literature the following points are the ones that I believe would aid in developing an effective ototoxicity monitoring protocol:

1. Clear awareness by all clinicians of common / established ototoxic drugs. This study only focused on aminoglycosides and cisplatin but there are many others involved including other antibiotics such as vancomycin, other chemotherapeutic agents such
as vincristine, chelating agents used in thalassaemia patients such as desferoxamine, antimalarials such as quinine and diuretics such as furosemide.

2. Clear identification of patient groups that are at a higher risk of repeated exposure to ototoxic medications. This includes patients with cancer, CF, TB, renal disorders, and infectious diseases in intensive care units etc.

3. Clear identification of key contacts within each team – this confirms that there are clearly identified personnel within the relevant departments (whether consultant, specialist nurse or audiologist) who can be contacted in relation to any queries in relation to the monitoring program as a whole or in relation to a specific patient.

4. Awareness of the clinicians involved in managing these patient groups of the need to start monitoring the hearing of these patients before or just after patients are first exposed to these medications. Baseline auditory recordings for chemotherapeutics should be done before or within 48 hours of the first dose and for aminoglycosides before or within the first 72 hours of the first dose as recommended by ASHA (ASHA, 1994a).

5. Accurate recording of drug history – dates of intake of courses; type, dose and mode of administration of any medication. This would allow for clear identification of ototoxic medications, confirm cumulative exposure and identify any other medications that may interact or have a synergistic effect with these ototoxic drugs.

6. Providing patients with information – e.g. through a pamphlet on the related drug, its effect on the ear and ototoxicity monitoring service provided.

7. Audiologists’ role and importance of leadership role in this service. Audiological professionals have to actively initiate this partnership with clinicians, providing them with clear detailed information on the service they can provide and providing evidence-based information regarding all aspects of the monitoring protocol.

8. Counseling: role of clinician and audiologist. Counseling does not just involve informing the patient that one of the side effects of this drug includes hearing loss as part of the consent procedure. It involved this plus clarifying points like letting the patient know the early warning signs such as tinnitus or difficulty in hearing in background noise, know that the only way to identify early damage is by regular auditory monitoring which starts as baseline testing before commencement of treatment and explaining what steps will be taken if ototoxicity is identified. These steps could include making changes in the medication if possible, more regular auditory monitoring, and referral back to audiology for management of any significant permanent hearing loss through amplification on other methods of rehabilitation. It has to be clear who and when provides this information and that
both clinicians and audiology professionals provide similar non-contradictory information.

9. Good communication links between clinicians and audiologists: clinicians should get clear reports of the outcomes of monitoring and know who to contact if more clarification is required, audiologists should also know the key members of the clinical team (consultant, specialist nurse, registrar).

10. Setup of dedicated ototoxicity clinics. This allows for the use of a specific ototoxicity monitoring test battery that is different from that used in the standard ENT clinics. Here tympanometry, EHF audiometry and DPOAE testing will be performed for all patients. A dedicated clinic will also allow clear identification for these patients as they would be allocated specific identifier codes that can allow retrieval of all their repeated monitoring data and for easier auditing of this service and putting in place measures to improve the service if needed.

11. Having a clear and agreed patient care pathway that both clinicians and audiologists are aware of.

12. Referral points: Baseline testing, then if there is limited capacity for monitoring during or at the end of each course of medication- regular annual monitoring as long as the patient is not complaining of any hearing loss or tinnitus and has normal audiological test results. Monitoring to increase to during and after every course if any evidence of ototoxicity is discovered. Post-treatment monitoring after cessation of treatment for late onset hearing loss (over 3 and 6 months then 1, 2 and 5 year intervals)

13. Criteria for identification of ototoxicity: there need to be clear aims for the monitoring service: a) early identification should prompt closer monitoring; b) a set cut-off point should be agreed where change in management should be advocated. The use of one agreed grading classification such as the SIOP Boston ototoxicity scale where if a specific grade on this scale were reached the clinician would initiate change in treatment.

14. All professionals involved should be aware of risk factors that can increase susceptibility to ototoxicity such as extreme age, renal impairment, previous exposure to this ototoxic medication, concomitant exposure to other ototoxic drugs, noise exposure and genetic predisposition to ensure that closer monitoring or avoidance of this medication should be done.

15. Clinicians should decide on different options for change in management that could be possible for their patient cohorts. They should be clear on whether they can change the medication, decrease dosage, change dosing regimen, change route of
administration (e.g. from systemic to topical routes such as nebulized) or even completely replace it with another non-ototoxic alternative drug if possible.

16. Clear pathway of referral to audiology rehabilitation services for fitting of hearing aids or cochlear implants etc. if ototoxic SNHL has occurred.

17. Facilitating access to monitoring for very ill unwell patients that have difficulty reaching audiology departments.

18. Professional education and training: training healthcare personnel during their basic and continuing professional education training regarding types and rational use of ototoxic drugs, importance of monitoring and ability to interpret audiological assessments will help raise awareness and improve management. The outcomes of the survey study in Theme C had highlighted the need for this training for all professionals involved. This has been advocated for a long time even by the WHO (WHO, 1994). Audiologists and Otolaryngologists could provide the leadership in introducing and supporting these training programs. Professional education can also be advocated through publication of well-designed high quality research in peer-reviewed journals on this topic.

7.3.3 Role of risk factors and genetic susceptibility in increasing the prevalence of ototoxicity

As technology improves, the availability, ease and value of performing genetic tests shows increases. The challenge is to identify the genetic variances or mutations that are significantly associated with increased susceptibility to specific ototoxic medications or those that have a significant role in modifying the phenotypic presentation of hearing loss. In this research a significant correlation between the two drug-metabolizing gene variants (rs12201199 TPMT and rs4646316 COMT) and AG ototoxicity could not be found. Previous research had shown a significant correlation with these variants and cisplatin ototoxicity through association analysis (Ross et al., 2009, Pussegoda et al., 2013) but this correlation was then shown to be non-significant when this work was replicated by another research group (Yang et al., 2013). However the basic concept behind the Ross et al. study of trying to identify gene variations in the top 220 key genes involved in the adsorption, distribution, metabolism and elimination (ADME) of drugs that are significantly associated with increased prevalence in ototoxicity is a valid and logical approach to discovering these links. The ability to genotype for thousands of SNPs in these genes using as single assay such as the Illumina GoldenGate assay that Ross et al. used is an excellent example of how this research can be easily applied to different medications, in this case AGs instead of cisplatin, as long as we have the appropriate patient cohorts to test. The availability of even
more advanced techniques such as next generation genome sequencing, and improvements in fields such as pharmacogenomics, where evaluation of different mechanisms of actions of drugs on cells is revealed through gene expression (Roukos, 2010), makes the goal towards personalized medicine, better understanding of genotype-phenotype relationships and developments of biomarkers for disease seem more possible. Therefore, based on the negative outcomes of the current study the recommendation for future work is either to completely abandon this hypothesis altogether or to take a step back and not try to establish a link with the TPMT and COMT variants but to replicate the Ross et al. assay of all 1,949 SNPs in the 220 drug-metabolizing genes. Here a genetic association study with ototoxicity from AGs but in a much larger cohort of children with CF with any of the other drug-metabolizing genes would be undertaken in search for significant associations. This should be undertaken in addition to focusing on establishing the pathway for this effect if one was discovered.

As for the A1555G 12S rRNA mutation two children of the 67 children with CF were discovered to carry the mutation. This was not enough to explain the causation for the dichotomy in hearing observed in this study group but did offer an explanation for the significant hearing loss seen after exposure to only three i.v. AG courses in one of these two children. This finding was in line with previous literature that showed that this mutation increases susceptibility to ototoxicity even after exposure to the first dose of AG. The two main outcomes of this study were the discovery that one of these children had normal hearing despite documented evidence of exposure to AGs. The other was the suspicion that this mutation is more prevalent in patients with cystic fibrosis as prevalence here represented a 15-fold increase when compared with the commonly reported 0.2% prevalence in UK and other populations. The same suspicion was also raised by Conrad et al. when they identified 2/157 (1.3%) cases of adults with CF. This was a driver to test more patients. A total of 105 samples were collected but only the two A1555G children that were discovered in the first sample collection were confirmed to carry this mutation. However, this study did not aim to perform an association study or to assess prevalence. If this were the aim it would be recommended to conduct a wide scale multi-center study in order to identify the true prevalence of this mutation in CF patients. The latest CF Trust registry report has stated that there are 10,078 people with CF registered in the UK (UK CF Trust, 2013). A large-scale multi-center UK study sequencing the mtDNA 12S rRNA gene will help identify the true prevalence of the A1555G mutation and identify other new or known mutations/variants within the 12S rRNA and tRNA mitochondrial genes that are associated with AG ototoxicity. Patients with CF would be a very good population to investigate AG ototoxicity as
documented evidence of intake of i.v and nebulized aminoglycosides is already collated making a genotype-phenotype association study more feasible.

The discovery that one of the two children carrying the A1555G mutation had normal hearing, which was confirmed twice using a full audiological test battery, has raised two issues. The first was that it questioned the true penetrance of this mutation, which was reported by many researchers to be 100% when patients are exposed to AGs as it is considered to be a major modifying factor in increasing expression of this mutation. The other issue is that it supported a recommendation to screen all CF patients for this mutation before they are exposed to aminoglycosides. This was because this normal hearing may still be considered as a temporary condition and that late onset hearing loss can still occur months or years after cessation of treatment. Therefore early identification may help prevent the occurrence of hearing loss soon after exposure or later on after cessation of treatment. Bitner-Glindzicz et al. and Chen et al. reported that 18/9371 (0.19%) and 6/865 (0.6%) respectively of the newborns and 19/7350 (0.26%) of the adult 44-45 year olds tested by Rahman et al. that genotyped positively for the A1555G mutation all had within normal hearing thresholds confirming that the mutation alone is not enough for the hearing loss phenotype to be expressed and that AG exposure plays a major role in increasing this expression. They all recommended that it would be both practical and cost-effective to complement auditory monitoring for ototoxicity with genetic screening for this mutation for prevention of hearing loss by avoiding intake of AGs if possible or at least closer, more prolonged monitoring of these patients for early detection and discovery of any late-onset hearing loss that may affect these patients following the exposure to AGs (Rahman et al., 2012, Chen et al., 2011, Bitner-Glindzicz et al., 2009).

Investigating genetic susceptibility to AG ototoxicity is an important area of research aiming at understanding individual variances, explaining the dichotomy in outcomes, identifying patients at higher risk and ultimately devising preventative and curative measures. The A1555G mutation, along with other mtDNA variants, is established as a genetic cause of increased susceptibility to AG ototoxicity in addition to non-syndromic deafness. However investigating the effect of this mutation on different populations and designing studies where the patient inclusion criteria are not defined by the phenotypic presentation of hearing impairment may help establish a better understanding of the true penetrance of this mutation and possible ‘protective’ effects of disorders such as CF on the prevalence of this condition. CF patients are repeatedly exposed to aminoglycosides and therefore they could benefit from pharmacogenetic testing for the A1555G mutation but this should not be implemented until a strong evidence base for proof of protection from ototoxicity, assessment of effect of
avoidance of first-line treatments on morbidity and cost-effectiveness is established. Conversely, it should be noted that the pharmacoeconomic analysis made by Veenstra et al. assessing the potential impact of pharmacogenomics testing on clinical, patient and economic outcomes in CF patients (Veenstra et al., 2007) were based on lower prevalence rates of the A1555G mutation (Bitner-Glindzicz et al., 2009, Rahman et al., 2012) than are currently presented in this study and therefore genetic screening may be more cost effective than previously reported.

7.3.4 Developing an audiological screening protocol using EHF screening PTA and portable DPOAE

Results of studies within the three themes of this research and of those of multiple previous studies have confirmed that in order to implement an effective ototoxicity monitoring service; repeated audiological testing using a comprehensive test battery, clear criteria for grading for ototoxicity, options for change in management and introduction of auditory rehabilitation and good multi-disciplinary communication are all required. The Theme C survey has confirmed that patients are almost always only tested in audiology departments. Many of the patients needing ototoxicity monitoring are usually very ill patients, especially when they are children or elderly. They would probably have very limited ability to move to the outpatients audiology departments, even if they were hospitalized, and would also have limited ability to cooperate effectively to respond to prolonged audiological objective tests. In addition to this, many of the audiology departments are not based in the same buildings/location housing the cancer or CF centers e.g. the London University College London Hospital (UCLH) does not have a dedicated audiology department and therefore patients needing this service are referred to the Royal National Nose, Throat & Ear Hospital (RNTNE) which is another hospital within this trust but is about twenty minutes away from its site. Ill patients would need to be transported by ambulance on a wheelchair or trolley to the other hospital. All this means that effective repeated auditory monitoring for ototoxicity might not be practically feasible for many of the patients that most need it.

The recommendation is to develop a quick validated audiological test battery that can be used in a hospital (clinic/ward) or home setting that can be used for repeated testing of these patients for early signs of ototoxicity and only when these are detected would the patient need to be referred for more comprehensive monitoring within the audiology department.

I have already performed a pilot study with 32 CF children and 32 cancer children to validate the outcomes of a screening test battery using a 25dB five-frequency (8, 10, 12.5,14 and 16 kHz) sweep using a portable high frequency audiometer and a high frequency DPOAE (2-8
kHz f2 frequency) using a portable hand-help Otodynamics Otoport device. Results were validated against the available comprehensive audiological tests performed at the audiology department within soundproof booths. Analysis of the results has shown the screening test battery to have a similar level of sensitivity and specificity to the comprehensive test battery and is even more superior than standard audiometry results alone at identifying children with ototoxicity. Further work is required to confirm these findings and recommend the use of this protocol.

Other research groups have also made similar recommendations. Fausti et al. have developed a new automated portable screening high frequency audiometer, which they called the OtoID (Jacobs et al., 2012). This machine does not have FDA approval yet but experimental results have shown that it can be used effectively of monitor ototoxicity of adult patients in the veterans’ hospital or even from the comfort of their own home. The same research group have also published extensively on the sensitive range of ototoxicity (SRO) where an individualized, one-octave wide sensitive frequency range separated by 1/6th-octave intervals from the highest limit of hearing would be used to detect early signs of ototoxicity (Fausti et al., 2003, Konrad-Martin et al., 2010). Therefore screening auditory monitoring may offer a good solution for increasing access to regular repeated auditory monitoring and should be more utilized in the future.

7.3.5 Investigating a possible otoprotective role of the CFTR gene mutation against ototoxicity

Mulheran el al. had suggested that the less than expected prevalence of ototoxicity in CF patients exposed to repeated courses of AGs might be caused by a possible otoprotective role of the CFTR mutation in the inner ear (Mulheran et al., 2001). The relatively large numbers of children in the audiological study of CF children (Theme A) of this research within the high-exposure group that had exceptionally good hearing 14/25 (Figure 4-5) supports this hypothesis. Further investigation is needed to establish the effect of the CFTR mutation on the inner ear and assess whether this affects the mechanism of entry or accumulation of AGs in the inner ear cells or in some way affects the apoptotic cell death pathways initiated by these drugs. Homma et al. have shown the CFTR interacts with prestin (SLC26A5) in the lateral wall of the OHCs and that both the mRNA and protein of CFTR are present in the OHCs with CFTR localizing in both the apical and lateral membranes. As the OHCs at the basal turn of the cochlea are one of the first cells affected by ototoxic drugs like aminoglycosides and cisplatin, it is important to investigate what other proteins it affects in these cells and whether it is involved in the mechano-electrical transduction channels at the apical ends of the OHCs as this is the route of entry of the ototoxic drugs. A possible study
could be through ‘assessing the expression of the CFTR gene in OHCs and inner ear hair cells (IHCs) in a mouse model’ or through ‘comparing the uptake of AGs by the OHCs or IHCs in a CFTR Knock-out mouse vs. a mouse with a normal CFTR gene’.

7.3.6 Other recommendations for future work include:

- Assessing the sensitivity of DP-gram amplitude recordings vs. DP input/output function vs. high-frequency DPOAE testing as monitoring tools for early detection of ototoxicity
- Investigating the difference in prevalence of ototoxicity following exposure to different types of AGs
- Investigating the prevalence, quality of monitoring and rehabilitation of aminoglycoside vestibular ototoxicity
- Investigating the effectiveness of different otoprotection agents as D-methionine, glutathione, N-acetylcysteine, amifostine, ebselen, sodium thiosulphate, aspirin, oxidizing agents in otoprotection against AG ototoxicity

7.4 Conclusion:

The work presented in this thesis has covered several different aspects of research into ototoxicity. Ototoxicity is a very important preventable cause of inner ear damage. Some of the research questions raised here have been addressed by previous research. Others still need more investigation. One important aspect that was quite clear to me from studying aspects of audiological monitoring and causation versus studying clinical impact and current practice is that there seems to be a wide gap between the extensive body of published research in this field and the actual implementation and integration of the outcomes of this research into clinical practice to provide direct benefit to patients. Despite the great benefit that clinicians provide to their patients there is an increased need for translational research that can transfer the established scientific outcomes into measures of patient management. There is a need for national and international guidelines for ototoxicity monitoring and a need for audiology professionals to have a stronger role in increasing awareness, implementing and supporting clinicians in dealing with this very important preventable cause of hearing loss.
Chapter 8: Bibliography


LANDIER, W., BHATIA, S., ESHELAN, D. A., FORTE, K. J., SWEENEY, T., HESTER, A. L., DARLING, J., ARMSTRONG, F. D., BLATT, J., CONSTINE, L. S., FREEMAN, C. R.,


PETROU, S., MCCANN, D., LAW, C. M., WATKIN, P. M., WORSFOLD, S. & KENNEDY, C. R. 2007. Health status and health-related quality of life preference-based outcomes of children who are aged 7 to 9 years and have bilateral permanent childhood hearing impairment. Pediatrics, 120, 1044-52.


Appendices:
Chapter 9: **Appendices:**

9.1 **Appendix 1: High frequency measurements made by Otodynamics to generate correction factors for the high frequency DPOAE recordings.**

High frequency correction for DP stimulus levels

On the DPOAE stimulus level issue:

The frequency response of a number of our probes (n=14) in an Occluded ear simulator (IEC 60318-4 B&K 4157) was measured using a HP Spectrum Analyzer.

The results were used to construct a correction table for frequencies above 5kHz.
The correction table is used by V6 to allow the appropriate extra drive to the speaker.
Recorded level on V6/Ez Screen may still be lower than the target due to standing waves in the ear canal.
9.2 Appendix 2: HUI3 Questionnaire

The Health Utilities Index Mark 3 (HUI3)

Instructions: This set of questions asks about your child’s day-to-day health. You may feel that some of these questions do not apply to you, but it is important that we ask the same questions of everyone. Please read each question and consider your answers carefully. For each question, please select one answer that best describes your child’s usual level of ability or disability. Please indicate the selected answer by marking (X) the box beside the answer.

A few of the questions are similar to others; please excuse the apparent overlap, and answer each question independently. Thank you.

1. Which one of the following best describes your child’s usual ability to see well enough to see pictures in a book?
   □ Able to see well enough without glasses or contact lenses
   □ Able to see well enough with glasses or contact lenses
   □ Unable to see well enough even with glasses or contact lenses
   □ Unable to see at all

2. Which one of the following best describes your child’s usual ability to see well enough to recognize you across the room?
   □ Able to see well enough without glasses or contact lenses
   □ Able to see well enough with glasses or contact lenses
   □ Unable to see well enough even with glasses or contact lenses
   □ Unable to see at all

3. Which one of the following best describes your child’s usual ability to hear what is said in a group conversation with at least three other people?
   □ Able to hear what is said without a hearing aid or cochlear implant
   □ Able to hear what is said with a hearing aid or cochlear implant
   □ Unable to hear what is said, even with a hearing aid or cochlear implant
   □ Unable to hear what is said, but don’t wear a hearing aid or cochlear implant
   □ Unable to hear at all

4. Which one of the following best describes your child’s usual ability to hear what is said in a conversation with one other person in a quiet room?
   □ Able to hear what is said without a hearing aid or cochlear implant
   □ Able to hear what is said with a hearing aid or cochlear implant
   □ Unable to hear what is said, even with a hearing aid or cochlear implant
   □ Unable to hear what is said, but don’t wear a hearing aid or cochlear implant
   □ Unable to hear at all

5. Which one of the following best describes your child’s usual ability to be understood when speaking the same language with strangers?
   □ Able to be understood completely
   □ Able to be understood partially
   □ Unable to be understood
   □ Unable to speak at all

6. Which one of the following best describes your child’s usual ability to be understood when speaking with people who know him/her well?
   □ Able to be understood completely
□ Able to be understood partially
□ Unable to be understood
□ Unable to speak at all

7. Which one of the following best describes how your child usually feels?
□ Happy and interested in life
□ Somewhat happy
□ Somewhat unhappy
□ Very unhappy
□ So unhappy that life is not worthwhile

8. Which one of the following best describes your child’s usual level of pain and discomfort?
□ Free of pain and discomfort
□ Mild to moderate pain that prevents no activities
□ Moderate pain that prevents a few activities
□ Moderate pain that prevents some activities
□ Severe pain that prevents most activities

9. Which one of the following best describes your usual child’s ability to get around?
□ Able to walk, bend, lift, jump and run normally for age
□ Walks, bends, lifts, jumps or runs with some limitations but does not require Help
□ Requires mechanical equipment (such as canes, crutches, braces or wheelchair) to walk or get around independently
□ Requires the help of another person to walk or get around and requires mechanical equipment as well
□ Unable to control or use arms and legs

10. Which one of the following best describes your child’s usual ability to use his/her hands and fingers? Note: Special tools refer to hooks for buttoning clothes, gripping devices for opening jars or lifting small items, and other devices to compensate for limitations of hands or fingers
□ Full use of two hands and ten fingers
□ Limitations in the use of hands or fingers, but do not require special tools or help of another person
□ Limitations in the use of hands or fingers, independent with the use of special tools (do not require the help of another person)
□ Limitations in use of hands or fingers, require the help of another person for some tasks (not independent even with use of special tools)
□ Limitations in use of hands or fingers, require the help of another person for most tasks (not independent even with use of special tools)
□ Limitations in use of hands or fingers require the help of another person for all tasks (not independent even with use of special tools)

11. Which one of the following best describes your child’s usual ability to remember things?
□ Able to remember most things
□ Somewhat forgetful
□ Very forgetful
□ Unable to remember anything at all

12. Which one of the following best describes your child’s usual ability to think and solve day-to-day problems?
□ Able to think clearly and solve day-to-day problems normally for age
□ Have a little difficulty when trying to think and solve day-to-day problems
□ Have some difficulty when trying to think and solve day-to-day problems
□ Have great difficulty when trying to think and solve day-to-day problems
□ Unable to think or solve day-to-day problems
## 9.3 Appendix 3: HUI Mark 3 (HUI3) Classification System.


<table>
<thead>
<tr>
<th>ATTRIBUTE</th>
<th>LEVEL</th>
<th>DESCRIPTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>VISION</td>
<td>1</td>
<td>Able to see well enough to read ordinary newsprint and recognize a friend on the other side of the street, without glasses or contact lenses.</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Able to see well enough to read ordinary newsprint and recognize a friend on the other side of the street, but with glasses.</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Able to read ordinary newsprint with or without glasses but unable to recognize a friend on the other side of the street, even with glasses.</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Able to recognize a friend on the other side of the street with or without glasses but unable to read ordinary newsprint, even with glasses.</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Unable to read ordinary newsprint and unable to recognize a friend on the other side of the street, even with glasses.</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Unable to see at all.</td>
</tr>
<tr>
<td>HEARING</td>
<td>1</td>
<td>Able to hear what is said in a group conversation with at least three other people, without a hearing aid.</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Able to hear what is said in a conversation with one other person in a quiet room without a hearing aid, but requires a hearing aid to hear what is said in a group conversation with at least three other people.</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Able to hear what is said in a conversation with one other person in a quiet room with a hearing aid, and able to hear what is said in a group conversation with at least three other people, with a hearing aid.</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Able to hear what is said in a conversation with one other person in a quiet room, without a hearing aid, but unable to hear what is said in a group conversation with at least three other people even with a hearing aid.</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Able to hear what is said in a conversation with one other person in a quiet room with a hearing aid, but unable to hear what is said in a group conversation with at least three other people even with a hearing aid.</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Unable to hear at all.</td>
</tr>
<tr>
<td>SPEECH</td>
<td>1</td>
<td>Able to be understood completely when speaking with strangers or friends.</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Able to be understood partially when speaking with strangers but able to be understood completely when speaking with people who know me well.</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Able to be understood partially when speaking with strangers or people who know me well.</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Unable to be understood when speaking with strangers but able to be understood partially by people who know me well.</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Unable to be understood when speaking to other people (or unable to speak at all).</td>
</tr>
<tr>
<td>AMBULATION</td>
<td>1</td>
<td>Able to walk around the neighbourhood without difficulty, and without walking equipment.</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Able to walk around the neighbourhood with difficulty; but does not require walking equipment or the help of another person.</td>
</tr>
</tbody>
</table>
Able to walk around the neighbourhood with walking equipment, but without the help of another person.

Able to walk only short distances with walking equipment, and requires a wheelchair to get around the neighbourhood.

Unable to walk alone, even with walking equipment. Able to walk short distances with the help of another person, and requires a wheelchair to get around the neighbourhood.

Cannot walk at all.

**DEXTERITY**

1. Full use of two hands and ten fingers.
2. Limitations in the use of hands or fingers, but does not require special tools or help of another person.
3. Limitations in the use of hands or fingers, is independent with use of special tools (does not require the help of another person).
4. Limitations in the use of hands or fingers, requires the help of another person for some tasks (not independent even with use of special tools).
5. Limitations in use of hands or fingers, requires the help of another person for most tasks (not independent even with use of special tools).
6. Limitations in use of hands or fingers, requires the help of another person for all tasks (not independent even with use of special tools).

**EMOTION**

1. Happy and interested in life.
2. Somewhat happy.
3. Somewhat unhappy.
4. Very unhappy.
5. So unhappy that life is not worthwhile.

**COGNITION**

1. Able to remember most things, think clearly and solve day-to-day problems.
2. Able to remember most things, but have a little difficulty when trying to think and solve day-to-day problems.
3. Somewhat forgetful, but able to think clearly and solve day-to-day problems.
4. Somewhat forgetful, and have a little difficulty when trying to think or solve day-to-day problems.
5. Very forgetful, and have great difficulty when trying to think or solve day-to-day problems.
6. Unable to remember anything at all, and unable to think or solve day-to-day problems.

**PAIN**

1. Free of pain and discomfort.
2. Mild to moderate pain that prevents no activities.
3. Moderate pain that prevents a few activities.
4. Moderate to severe pain that prevents some activities.
5. Severe pain that prevents most activities.
9.4 Appendix 4: Paediatric Quality of Life Questionnaire

Please rate how concerned or worried you have felt about your child in the following areas, over the past month. Please put a tick in the appropriate box for each item, and don’t leave out any items.

<table>
<thead>
<tr>
<th>How concerned or worried have you felt about:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. How well your child is able to cope academically</td>
</tr>
<tr>
<td>2. Your child’s ability to join in play-time activities at school or nursery</td>
</tr>
<tr>
<td>3. How your child gets along with children of the same age</td>
</tr>
<tr>
<td>4. How well your child is able to communicate with family members</td>
</tr>
<tr>
<td>5. The extent to which your child is teased or bullied by peers</td>
</tr>
<tr>
<td>6. The way your child looks; his/her appearance</td>
</tr>
<tr>
<td>7. How much your child is able to join in indoor activities and games at home</td>
</tr>
<tr>
<td>8. How well your child is able to communicate with other relatives</td>
</tr>
<tr>
<td>9. The extent to which your child gets frustrated or angry</td>
</tr>
<tr>
<td>10. How anxious your child has felt about going to school or nursery</td>
</tr>
<tr>
<td>11. The extent to which your child argues or fights with peers</td>
</tr>
<tr>
<td>12. Your child’s ability to make new friends and keep the friendship</td>
</tr>
</tbody>
</table>
How concerned or worried have you felt about:

<table>
<thead>
<tr>
<th>Question</th>
<th>Not at all Concerned</th>
<th>A little concerned</th>
<th>Moderately concerned</th>
<th>Very concerned</th>
<th>Extremely concerned</th>
</tr>
</thead>
<tbody>
<tr>
<td>13. How well your child is able to cope with visiting a friend, without you there</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14. Your child’s ability to communicate with peers in school or nursery</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15. How sad or miserable your child has felt</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16. Your child’s ability to join in sports and physical activities</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17. How tearful or easily upset your child has been</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18. Your child’s ability to cope with everyday life</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19. The extent to which your child has felt over-anxious or panicky</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20. How well your child is able to communicate with peers outside school</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21. The extent to which your child is easily frightened</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22. The extent to which your child is able to be independent, do things for him/herself (as appropriate for their age)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Overall, do you think your child’s quality of life has improved as a result of having hearing aids?  Yes [ ] No [ ] N/A [ ]

If yes, to what extent has it improved?

A little [ ] Moderately [ ] Very much [ ]
Finally:
Please could you now provide the following information?

Your child’s age …………………

Does your child attend school?  □  nursery?  □

How long has your child had hearing aids?  ………………Years ………… Months

Your child’s gender  Male □  Female □

Your child’s ethnic background  ………………………………………………………

Does your child have any disabilities or learning difficulties in addition to their deafness?

Yes □  No □

If yes, please describe:  ………………………………………………………
9.5 Appendix 5: PAQL Items

PAQL items: (Edwards et al., 2012)

- **A.1. Communication and independence**

  - How well your child is able to communicate with family members
  - How well your child is able to communicate with other relatives
  - How well your child is able to cope with visiting a friend, without you there
  - Your child's ability to communicate with peers in school or nursery
  - How well your child is able to communicate with peers outside school
  - The extent to which your child is able to be independent (as appropriate for their age)

- **A.2. Emotional well-being**

  - The extent to which your child gets frustrated or angry
  - How anxious your child has felt about going to school or nursery
  - How sad or miserable your child has felt
  - How tearful or easily upset your child has been
  - Your child's ability to cope with everyday life
  - The extent to which your child has felt over-anxious or panicky
  - The extent to which your child is easily frightened

- **A.3. Peer comparisons**

  - How well your child is able to cope academically
  - Your child's ability to join in playtime activities at school or nursery
  - How well you child gets along with children of the same age
  - How much your child is able to join in indoor activities and games at home

- **A.4. Acceptance by peers**

  - The extent to which your child is teased or bullied by peers
  - The way your child looks; his/her appearance
  - The extent to which your child argues or fights with peers
  - Your child's ability to make friends and keep the friendship
9.6 Appendix 6: Online survey questionnaire for UK Audiologists

Ototoxicity monitoring of auditory function in chemotherapy patients: audiology survey

We are conducting a survey to understand current auditory ototoxicity monitoring practice in the UK for patients with cancer.

If you see patients who receive potentially ototoxic treatment, we appreciate you completing our survey.

It does not matter if your service currently conducts ototoxicity monitoring or not. Thank you for taking your time to complete this survey. Your responses are invaluable in helping us complete this research.

Demographics

Q1: Please state your clinical role:

- Doctor (ENT, AVM)
- Audiology professional
- Other, please state:

If you have chosen "other", please specify:

Q2: What services do you deliver? (tick all that apply)

- Paediatric
- Adult
- Other specialist centre, please state

If you have chosen "other", please specify:

Q3: Where is your service located?

- Scotland
- England - South West
- England - Midlands
- Wales
- England - South East
- England - North West
- Northern Ireland
- England - Greater London
- England - North East
Ototoxicity monitoring of auditory function

Q4: Do your patients receive ototoxicity monitoring that includes audiological testing?

☐ Yes  ☐ No

Ototoxicity monitoring of auditory function continued

Note: If you have answered chosen item [2] in question 4, skip the following question

Q5: What are the criteria for referring patients for audiological testing? (tick all that apply)

☐ All oncology patients are referred
☐ Only paediatric patients
☐ Only adult patients
☐ Patients reporting hearing or balance problems
☐ Patients receiving repeated doses of ototoxic medication
☐ Other
☐ Patients receiving specific medication (please specify):

If you have chosen "other", please specify:


Note: If you have answered chosen item [2] in question 4, skip the following question

Q6: If no formal referral criteria exist, when in your clinical judgement should patients receive audiological testing:


Note: If you have answered chosen item [2] in question 4, skip the following question

Q7: Do your patients access a dedicated clinic for audiological testing?

☐ Yes, specifically for patients receiving chemotherapy
☐ Yes, for patients receiving a range of ototoxic medications
☐ No

Note: If you have answered chosen item [2] in question 4, skip the following question

Q8: Where is audiological testing conducted (please tick all that apply)?

☐ In the patient’s home
☐ In the audiology department
☐ In a community setting e.g. health centre, GP surgery
☐ At bedside / on ward
☐ Other (please specify):
If you have chosen "other", please specify:

Note: if you have answered chosen item [2] in question 4, skip the following question

Q9: Do patients receive counselling prior to potentially ototoxic treatments?
  ○ I don't know ○ No ○ Yes, please give details:

Note: if you have answered chosen item [2] in question 4, skip the following question

Q10: Is there a protocol for which audiological tests are conducted for ototoxicity monitoring?
  ○ Yes
  ○ No, testing required is determined on a patient-by-patient basis
  ○ I don't know

Note: if you have answered chosen item [2] in question 4, skip the following question

Q11: Are baseline audiological tests conducted prior to starting chemotherapy?
  ○ Yes ○ No ○ Sometimes

Note: if you have answered chosen item [2] in question 4, skip the following question

Q12: How often is audiological testing conducted?
  ○ Within a course of treatment e.g. after a certain number of cycles
  ○ After each course of treatment
  ○ After 2 or more courses of treatment
  ○ When patient reports symptoms e.g. hearing loss, tinnitus
  ○ Other

If you have chosen "other", please specify:
Q13: How long does ototoxicity monitoring continue?

- [ ] It does not continue after cessation of treatment
- [ ] Up to 6-12 months following cessation of treatment
- [ ] Up to 3-6 months following cessation of treatment
- [ ] Other (please state):

If you have chosen “other”, please specify:

---

Q14: What audiological testing is conducted?

<table>
<thead>
<tr>
<th>Test Type</th>
<th>At baseline</th>
<th>At follow up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tympanometry</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acoustic Reflex Thresholds</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visual reinforcement audiometry (VRA) / play audiometry (500Hz-4kHz)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pure tone audiometry, including VRA / play audiometry (250Hz-6kHz)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High frequency audiometry, including VRA / play audiometry (above 8kHz)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transient evoked otoacoustic emissions (TEOAEs)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distortion product otoacoustic emissions (DPOAEs)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ABR - neurological</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ABR - threshold</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Speech audiometry</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Q15: If known, please state the upper frequency range tested i.e. by high frequency audiometry or DPOAE:

[Blank]

Q16: Is a protocol used to identify when changes in auditory status become clinically significant? (tick all that apply):

☐ Yes, not used
☐ Yes, a departmental protocol is used
☑ Yes, a published protocol e.g. Brock's scale, is used (please specify):

If you have chosen "other", please specify:

[Blank]

Q17: In your clinical opinion, what audiological results should lead to consideration of a change in medical management (including level on published protocol if appropriate):

[Blank]

Other

Q18: Do your patients receive (please tick all that apply):

☐ Nephrotoxicity monitoring
☐ Ototoxicity monitoring
☐ Torspecy monitoring - other (please specify):

If you have chosen "other", please specify:

[Blank]

Q19: Do you think that ototoxicity monitoring of auditory function is a priority for your oncology patients?

☐ Yes
☐ No
Appendix 7: Online survey questionnaire for UK Oncologists

Ototoxicity monitoring of auditory function in chemotherapy patients: oncology survey

We are conducting a survey to understand current auditory ototoxicity monitoring practice in the UK for patients with cancer.

If you see patients who receive potentially ototoxic treatment, we appreciate you completing our survey.

It does not matter if your service currently conducts ototoxicity monitoring or not. Thank you for taking your time to complete this survey. Your responses are invaluable in helping us complete this research.

Demographics

Q1: Please state your clinical role:

☐ Doctor (oncology) ☐ Nurse ☐ Pharmacist ☐ Other, please state

If you have chosen "other", please specify:

[Blank space]

Q2: What services do you deliver? (tick all that apply)

☐ Paediatric
☐ Adult
☐ Other specialist centre e.g. Head and Neck, please state

If you have chosen "other", please specify:

[Blank space]

Q3: Where is your service located?

☐ Scotland ☐ Wales ☐ Northern Ireland
☐ England - Midlands ☐ England - North West ☐ England - North East
Chemotherapeutic treatment

Q4: What proportion of your patients receive chemotherapeutic treatment known to be potentially ototoxic?

☐ 76-100%  ☐ 51-75%  ☐ 26-50%  ☐ 0-25%

Q5: Is Cisplatin / a Cisplatin based protocol a first choice treatment for any groups of your patients?

☐ Yes  ☐ No  ☐ If so, for which types of cancer?

If you have chosen “other”, please specify:

Q6: Is Carboplatin / a Carboplatin based protocol a first choice treatment for any groups of your patients?

☐ Yes  ☐ No  ☐ If so, for which types of cancer?

If you have chosen “other”, please specify:

Q7: Do your patients receive Head and Neck radiotherapy?

☐ Yes  ☐ No

Q8: How are therapeutic drug levels monitored?

☐ Measure a peak and a trough drug level  ☐ Measure trough drug level only
☐ Measure a single level at a random time  ☐ Measure a single level at a specified time
☐ I am not sure  ☐ Other - please specify:

If you have chosen “other”, please specify:

Ototoxicity monitoring of auditory function

Q9: Do your patients receive ototoxicity monitoring that includes audiological testing?

☐ Yes  ☐ No
Ototoxicity monitoring of auditory function continued

Note: If you have answered 'Yes' in question 9, skip the following question.

Q10: What are your criteria for referring patients for audiological testing? (Tick all that apply)

☐ All oncology patients are referred
☐ Only paediatric patients
☐ Only adult patients
☐ Patients reporting hearing or balance problems
☐ Patients receiving repeated doses of ototoxic medication
☐ Patients receiving specific medication (please specify):

If you have chosen 'other', please specify:

Note: If you have answered 'Yes' in question 9, skip the following question.

Q11: If no formal referral criteria exist, please specify when in your clinical judgement you would refer patients for audiological testing:

Note: If you have answered 'Yes' in question 9, skip the following question.

Q12: Do your patients access a dedicated clinic for audiological testing?

☐ Yes, specifically for patients receiving chemotherapy
☐ Yes, for patients receiving a range of ototoxic medications
☐ No

Note: If you have answered 'Yes' in question 9, skip the following question.

Q13: Where is audiological testing conducted (please tick all that apply)?

☐ In the patient's home
☐ In the audiology department
☐ Other (please specify):

☐ In a community setting e.g. health centre, GP surgery
☐ At bedside / on ward
If you have chosen "other", please specify:

Note: if you have answered "other" in question 9, skip the following question

Q14: Do patients receive counselling prior to potentially ototoxic treatment?

☐ I don't know  ☐ No  ☐ Yes, please give details:

Note: if you have answered "other" in question 9, skip the following question

Q15: Is there a protocol for which audiological tests are conducted for ototoxicity monitoring?

☐ Yes  ☐ No, testing required is determined on a patient-by-patient basis  ☐ I don't know

Note: if you have answered "other" in question 9, skip the following question

Q16: Are baseline audiological tests conducted prior to starting chemotherapy?

☐ Yes  ☐ No  ☐ Sometimes

Note: if you have answered "other" in question 9, skip the following question

Q17: How often is audiological testing conducted?

☐ Within a course of treatment e.g. after a certain number of cycles
☐ After each course of treatment
☐ After 2 or more courses of treatment
☐ When patient reports symptoms e.g. hearing loss, tinnitus
☐ Other

If you have chosen "other", please specify:
Note: if you have answered item [2] in question 9, skip the following question

Q18: How long does ototoxicity monitoring continue?

- It does not continue after cessation of treatment
- Up to 6-12 months following cessation of treatment
- Other (please state):

If you have chosen "other", please specify:

Note: if you have answered item [2] in question 9, skip the following question

Q19: What audiological testing is conducted?

<table>
<thead>
<tr>
<th>Test Type</th>
<th>At baseline</th>
<th>At follow up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tympanometry</td>
<td></td>
<td></td>
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<tr>
<td>Acoustic Reflex Thresholds</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pure tone audiometry, including VRA / play audiometry (250Hz-8kHz)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High frequency audiometry, including VRA / play audiometry (above 8kHz)</td>
<td></td>
<td></td>
</tr>
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<td></td>
</tr>
<tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>Speech audiometry</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I'm not sure what audiological tests are conducted</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Q20: If known, please state the upper frequency range tested i.e. by high frequency audiometry or DPOAE:

[Blank Box]

Q21: Is a protocol used to identify when changes in auditory status become clinically significant? (tick all that apply):

- [ ] I'm not sure
- [ ] No, clinical judgement is used
- [ ] Yes, a departmental protocol is used
- [ ] Yes, a published protocol e.g. Berch's scale, is used (please specify):

If you have chosen "other", please specify:

[Blank Box]

Q22: Please specify what radiological results may lead to a change in medical management (including level on published protocol if appropriate):

[Blank Box]

Q23: What changes would be made:

- [ ] Reduction in dosage
- [ ] Change of treatment
- [ ] Other (please specify):

If you have chosen "other", please specify:

[Blank Box]
Other

Q24: Do your patients receive (please tick all that apply):

- ☐ Neutropenia monitoring
- ☐ None
- ☐ Ventilatory monitoring
- ☐ Toxicity monitoring - other (please specify)

If you have chosen "other", please specify:


Q25: Do you think that ototoxicity monitoring of auditory function is a priority for your oncology patients?

- ☐ Yes
- ☐ No
9.8 Appendix 8: Online survey questionnaire for UK CF Clinicians

Survey of ototoxicity monitoring in CF centres in the UK

INTRODUCTION

Dear CF centre director,

We are conducting a survey of ototoxicity monitoring in all the cystic fibrosis specialist centres in the UK. Your input is very much appreciated and will be invaluable in further research into ototoxicity in CF.

The survey should only take about 5 minutes of your time and is anonymous. If you have any questions please contact us on mikicic@hotmail.com or g.al-malky@ucl.ac.uk at UCL or Ranjan.Suri@gosh.nhs.uk at GOSH.

To complete the survey please use the following navigation buttons online.

Thank you very much for taking the time to complete this survey.
Survey of ototoxicity monitoring in CF centres in the UK

AMINOGLYCOSEIDES USED

4. Which is your first line intravenous aminoglycoside used to treat pulmonary exacerbations?
   - A. Tobramycin
   - B. Gentamicin
   - C. Amikacin
   - D. Other - please state

5. What is the frequency of administration?
   - A. Once a day
   - B. Twice a day
   - C. Three times a day
   - D. Other - Please state

6. How do you monitor for therapeutic drug levels?
   - A. Measure a trough level only
   - B. Measure a peak level only
   - C. Measure a peak and trough level
   - D. Measure a single level at another specified time
   - E. Measure a level at a random time
   - F. Other – please specify

TOXICITY MONITORING

7. Do you monitor hearing or for ototoxicity (hearing loss)? If the answer is No please go to Question 13.
   - A. Yes
   - B. No

8. What criteria do you use for monitoring ototoxicity? (Please tick all criteria used in your unit)
   - A. All patients on intravenous aminoglycosides
   - B. Patients receiving multiple doses of intravenous aminoglycosides
   - C. Patients who complain of hearing loss
   - D. Patients who complain of tinnitus
   - E. Patients who complain of dizziness
   - F. Patients with poor renal function
   - G. Patients with genetic tendency for ototoxicity or family history of ototoxicity
   - H. Other – please specify

9. Who provides the monitoring facilities?
   - A. Local Audiology Department
   - B. Local ENT Department
   - C. A dedicated Ototoxicity Clinic
   - D. Other – please state

10. What tests are done? (Please tick all options used for your patients)
    - A. Standard Pure Tone Audiometry (0.25 – 8 KHz) (aka. Hearing Test)
    - B. Expanded High Frequency Audiometry (0.20 – 20 KHz)
    - C. Speech Tests
    - D. Otocoustic Emissions (OAEs) within TEOAEs or DPOAEs
    - E. Other – please specify

11. How often do you do the tests?
    - A. At the end of each intravenous antibiotic course
    - B. Every 3 months
    - C. Annually
    - D. As needed, based on clinical symptoms (hearing loss, tinnitus, dizziness)
    - E. Other – please state

12. Do the results of the above tests change your antibiotic management (intravenous and/or nebulised)?
    - A. No
    - B. Yes, if the answer is Yes, what changes do you implement? Please specify
9.9 **Appendix 9: Plan for ototoxicity monitoring pathway developed for CF children at GOSH**

**Flow Chart for Audiological Assessment for Ototoxicity: 1st Trial of Ototoxicity Monitoring Pathway:**

**Criteria for ototoxicity monitoring:**
1. Any child with history of intake of AGs at Annual Rev. (regardless of number of courses given)
2. Any child/parent with auditory concerns (hearing loss/tinnitus)
3. Any staff concerns about hearing

**Assessment by CF Fellow/Consultant at Annual Review/ward**
Child & Parents informed of need for hearing assessment and consent obtained

**If child not already in the hospital:**
- Audiology appt booked and letter sent to parents by audiologist (Check PIMS to match with CF clinic appt if possible)
- OR
- Audiologist arranges appropriate time to bring child down from the ward for testing

**Referral form for Audiological assessment completed by CF staff and sent through internal mail**
OR
Ward staff contact Audiology to test child

**Ototoxicity Monitoring Audiological assessment:**
1. Case history (confirm drug history including nebulized AGs e.g. TOBI)
2. Otoscopy and Tympanometry
3. PTA - Standard & HF (0.5-16kHz)
4. DPOAE recordings (Bilateral)
5. Save results in folder & on electronic database
6. Send a copy + Hearing assessment report for CF team review C/O Dr RANJAN SURI to keep on record & to place in clinical notes
9.10 Appendix 10: GOSH Ototoxicity referral form

Referral Form for Ototoxicity monitoring of CF patients

DEPARTMENT
Audiovestibular Medicine & Cystic Fibrosis Unit,
Great Ormond Street Hospital,
London

Referral Form for Audiological Assessment for Cystic Fibrosis Unit Patients

Please complete the form and sent to the Audiology Department in the internal mail.

Please affix patient ID label:
MRN Number: ……………………………
Surname: ……………………………
Name: ……………………………
DOB: ……………………………
Sex: ……………………………

Details of referring doctor:
Referrer’s Name (Print clearly):
Consultant: ……………………………
Contact Number/Bleep: ……………
Date of referral: ………………………

1. Administered antibiotics in the past year:
…………………………………………………………………………………………
……………………………………
………………………………………………
………………

2. Type of Aminoglycoside received:
   Tobramycin       Amikacin       Gentamicin       Other

3. Has child had a hearing assessment for ototoxicity monitoring before?

   YES    NO

Reason for referral: Please tick as appropriate

☐ Child has history of intake of Aminoglycosides at Annual Review (regardless of number of courses given)

☐ Child/parent has auditory concerns (hearing loss/tinnitus) – in review clinic/ward

☐ Any staff concerns about hearing – in review clinic/ward

G. Al-Maliky- CF unit Referral Form For Hearing Assessment v1.
March 1, 2013

286
9.11 Appendix 11: GOSH Ototoxicity post-assessment feedback report

### Hearing Assessment Report:
**Ototoxicity Monitoring Service for CF Unit**

Please affix patient ID label:
MRN Number: ...........................................
Surname: ..................................................
Name: ....................................................
DOB: .....................................................
Sex: .....................................................

**Summary Report:** (see results enclosed)

**Otoscopy:**
- Clear
- Non-occluding wax

**Tympanometry (middle ear analysis):**

<table>
<thead>
<tr>
<th>Description</th>
<th>Rt:</th>
<th>Lt:</th>
<th>Both:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Otitis Media with Effusion (OME)/ Negative Pressure</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

**Standard Audiometry (0.5 – 8kHz):**

<table>
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<th>Description</th>
<th>Rt:</th>
<th>Lt:</th>
<th>Both:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HF Hearing Loss – Stable</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HF HL – Deterioration</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Extended High-Frequency (HF) Audiometry (9 – 16kHz):**

<table>
<thead>
<tr>
<th>Description</th>
<th>Rt:</th>
<th>Lt:</th>
<th>Both:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (≥20dB)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HL – Stable</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HL – Deterioration</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Distortion-Product Otoacoustic emissions (DPOAE-monitor inner ear OHC function):**

- Present
- Absent
- Deterioration

**Recommendation:**

- Repeat assessment after 6 months
- Repeat assessment after intake of next aminoglycoside course
- Refer to Local / GOSH Audiology for hearing aids
- Genetic test to exclude mtDNA A1555G mutation

Date: .............................................
Audiologist: .................................
Contact Number/Bleep: .................
New patient assessment:
Existing patient monitoring:
9.12 Appendix 12: Ototoxicity monitoring protocol prepared for the CF patients at GOSH

PROTOCOL FOR OTOTOXICITY MONITORING OF CYSTIC FIBROSIS (CF) PATIENTS

(AUDGOSH-2)

Ototoxicity is ‘drug-related inner ear damage’ which results in auditory and/or vestibular dysfunction that is often permanent. Symptoms of ototoxicity include tinnitus, dizziness, and difficulty understanding speech in noise. The main therapeutic drug groups causing permanent ototoxicity include aminoglycoside antibiotics used to treat life-threatening infections and chemotherapeutic drugs as cisplatin and carboplatin.

Initial ototoxic drug exposure typically affects cochlear regions coding the high frequencies. Continued exposure results in a spread of damage to progressively lower frequencies. Unfortunately, ototoxic hearing loss may go unnoticed by patients, especially children, until a communication problem becomes apparent, signifying that hearing loss within the frequency range important for speech understanding has already occurred. Similarly, by the time a patient complains of dizziness, permanent vestibular system damage probably has already occurred. Because symptoms of ototoxicity are poorly correlated with drug dosage, peak serum levels, and other toxicities, the only way to detect ototoxicity is by assessing auditory and vestibular function directly (Konrad-Martin, 2005a). The risk of hearing loss is estimated to be 1.7% per course of aminoglycoside in CF patients with evidence of presence of a cumulative effect following repeated exposure especially when >10 courses are administered (Mulheran et al., 2001)

This document is aimed at presenting an auditory monitoring protocol for children with CF who are exposed to repeated courses of aminoglycosides and other potentially ototoxic antibiotics prescribed to combat chronic pseudomonas aeruginosa chest infections. A separate document for ototoxicity monitoring of vestibular function will be prepared in due course.

1 POLICY AND SCOPE

1.1 To provide a high quality, objective and accurate service for the audiological monitoring of patients with CF for signs of ototoxicity

1.2 The main purpose of this monitoring is to detect ototoxicity early in order to provide an opportunity for the managing clinicians to make informed decisions about making adjustments the therapeutic treatment in order to minimize or prevent hearing loss requiring rehabilitation, without compromising their overall care.
1.3 To contribute to the protection and prevention of further damage to the hearing function of these children and to set in place early auditory rehabilitation management of hearing loss during and after treatment. This in turn will help improve the overall quality of life of these patients.

1.4 To provide audiologists and clinical staff opportunities to counsel patients and their families regarding ototoxicity-induced hearing loss, tinnitus, and dizziness, communication strategies, and the synergistic effects of noise and ototoxic damage.

2 AVAILABILITY

2.1 Patients will be booked into agreed Audiology slots under Dr Tony Sirimanna’s care.

2.2 Every effort will be made to provide timely referral service for baseline and post-treatment auditory monitoring provided that:

- Equipment is free
- Staff are available
- Patient is able to undergo the assessment

3 STAFF REQUIREMENT

3.1 Auditory monitoring should be undertaken by suitable trained and qualified staff of the following grades: Audiological/Clinical Scientist, AfC Band 6 and above. Peer review of results by Band 7 audiologist and above.

3.2 An audiological physician or senior registrar in Audiological Medicine should undertake analysis and follow up of the results.

4 MANAGEMENT OF PATIENT – PATIENT JOURNEY

4.1 Referral received by Audiologist from the CF specialist nurse following coordination of requests from members of the CF unit team or the Badger/other hospital ward if a patient is admitted for administration of an intravenous (iv) aminoglycoside course.

4.2 Request for the baseline audiological monitoring should be initiated before the administration of or exposure to ototoxic agents. When pre-exposure testing is not performed for some reason, monitoring should be initiated within the first 72 hours of administration of the first dose of aminoglycoside as recommended by ASHA’s "Guidelines for the Audiologic Management of Individuals Treated with Cochleotoxic Drug Therapy" (1994), based in part on the results of large clinical studies.

4.3 Baseline audiological recordings for any child born in years ≥2006 can also be retrieved by an audiologist from the NHSP eSP database through the local services.

4.4 Post-treatment audiological monitoring should be performed within the final days of completion of each aminoglycoside course (usually lasting 14 days) before the patient is discharged from the hospital or before that if the patient complains of any auditory symptoms.

4.5 Monitoring and appropriate referrals for further auditory and vestibular testing also are warranted any time a patient reports increased hearing difficulties, tinnitus, aural fullness, or dizziness. Retesting to confirm significant changes can reduce false positive rates, which is recommended by ASHA (1994). Post-treatment evaluations at the 6-8
week post-treatment follow-up visit are necessary to confirm that hearing is stable because ototoxic hearing loss can occur up to 6 months following drug exposure.

4.6 Targeted Diagnostic assessment:

An audit assessing the feasibility and cost effectiveness of post-treatment monitoring of patients after each antibiotic course will be undertaken six months after the start of this service. If this shows a significant degree of difficulty or results obtained that a cumulative ototoxic affect is more evident instead of an acute post-treatment effect from intake of a single course, then a targeted assessment protocol should be followed including the minimum of following risk criteria:

1. History of intake of ≥10 i.v. aminoglycoside courses.
2. Regular repeated intake of 3-4 aminoglycoside courses per year
3. Presence of any parental/carer concern regarding hearing/balance function
4. History of intake of additional concomitant ototoxic medications (e.g. Diuretics as furosemide or chemotherapeutic medications as cisplatin) or exposure to loud noise, which may have a synergistic effect.

These criteria should be revised or more added in agreement between the service leads of both departments.

4.7 CF Outpatient clinic or Ward audiological screening:

A research pilot study will be undertaken to investigate the sensitivity and specificity of using portable audiological equipment to perform quick screening of a limited number of specific high frequencies using high-frequency audiometry (8-10-12-14 kHz) and DPOAEs (on 3-8 kHz) for post-treatment assessment. This study will aim to confirm if this screening is effective in detecting early signs of ototoxicity so that only these patients will then be sent to the audiological medicine department for complete assessment and follow-up.

If this screening procedure proves effective- the ototoxicity monitoring protocol will be revised to include this as a permanent step in assessing and triaging patients for referral for full audiological assessment.

5 TEST TIME

The time allocated for the baseline testing of each subject is 45 minutes. This should include:

- Tympanometry and ART recordings to assess middle ear function
- Standard (0.25-8 kHz) Audiometry using calibrated equipment and TDH39 headphones
- High-Frequency (9-16 kHz) Audiometry using calibrated equipment and Sennheiser HDA200 headphones
- DPOAE recording (diagnostic bilateral DP-gram recordings of f2 frequencies 1-8 kHz)

A shortened pared-down version of these tests assessing the higher frequencies ≥2 kHz should be performed during the post-treatment assessments. Comparison with the baseline recordings should be made to assess if ototoxicity has occurred according to the ASHA criteria of ototoxicity (1994).

6 Auditory assessment should include the following:

- History taking: to include current and previous history of exposure to aminoglycosides, type and route of administration of the drug, duration of exposure, any audiological complaints (tinnitus or hearing loss), exposure to
other potential ototoxic drugs e.g. vancomycin or other antibiotics, diuretics or chemotherapeutic drugs.

- Otoscopy, Tympanometry and acoustic reflex testing: to exclude existence of external and middle ear disease.
- Standard pure-tone Audiometry (0.25 – 8 kHz)
- High-frequency pure-tone audiometry using the dedicated circumaural headphones e.g. Sennheiser HDA200 high frequency earphones (up to 16kHz)

Audiometric techniques for standard and high-frequency testing are age dependent
  - Visual Reinforcement audiometry (VRA) should be used for children aged 6-30 months
  - Play audiometry should be used for children >36 months
  - Conventional audiometry (as with adults – pressing the button) can be used for children ≥5 years.

- Distortion-product evoked otoacoustic emissions (DPOAEs)
- Other tests may be considered if needed e.g. ABR threshold estimation, speech audiometry.

- Equipment should be calibrated every six - twelve months traceable to the National Physical Laboratory.
- Suitable accommodation with enough room to perform the test safely and comfortably (for both patient and tester):
  - Ambient noise <30 dBA
  - Adequate seating for parent and child
  - Tidy room with absence of sources of distraction/cues (extra play audiometry toys should be stored away in a cupboard).

7 Criteria for ototoxicity:

For serial audiograms, ASHA (1994) developed criteria for a clinically significant hearing change based on results of large clinical research studies which reported normal test-retest variability in healthy subjects not receiving ototoxic drugs, and to a limited extent on receiver-operating characteristics (ROC) curves, constructed for threshold shift data obtained in drug- or noise-exposed individuals to record sensitivity and specificity of these data (Konrad-Martin, 2005b).

These criteria include:

- >20 dB pure-tone threshold shift at one frequency,
- OR >10 dB shift at two consecutive test frequencies,
- OR threshold response shifting to "no response" at three consecutive test frequencies.

Change must be confirmed by retest.

The ASHA guidelines for ototoxicity monitoring emphasize the increased test sensitivity achieved using extended high-frequency monitoring to detect ototoxicity. Test-retest differences for extended-high-frequency thresholds using modern equipment are generally reported to be within ±10 dB for frequencies between 9 and 14 kHz. False positive rates indicating a change in extended-high-frequency
thresholds in subjects that were not exposed to ototoxic drugs is low in young and older adults, even when thresholds are tested on the hospital ward under controlled conditions. Extended-high-frequency sensitivity can be monitored in older children; however, test-retest variability is generally poorer in young children which will likely result in lower sensitivity and higher false positive rates compared to adults. Consequently, additional information provided by DPOAE objective testing in young children is very valuable (Konrad-Martin et al., 2010, Beahan et al., 2012).

8 Safety and Health Precautions
- All procedures should ensure the safety of the patient, audiologist, and others who participate in the clinical process and adhere to the standard precautions (e.g., prevention of bodily injury and transmission of infectious disease).
- Decontamination, cleaning, disinfection, and sterilization of multiple-use equipment before reuse are carried out according to facility-specific infection control policies and procedures and according to manufacturer’s instructions. The audiologist performing electrodiagnostic test procedures is familiar with facility-specific emergency medical protocols and adheres to all hospital, state, and federal regulations.
- CF patients are more vulnerable to developing chronic infections with resistant organisms as MRSA and therefore all the necessary levels of decontamination should be employed. CF patients with MRSA should be tested at the end of the clinic and tympanometry should not be performed. The filter on the DPOAE probe should be removed and the probe disinfected. Level 2 disinfection of the room should be undertaken after completion of the tests.

9 POST TEST MANAGEMENT
Interpretation of the assessment may indicate one or more of the following:
- Normal auditory system function
- Significant change in auditory system function
- Existence, type, and degree of auditory dysfunction with or without significant change

Evaluation may result in one or more of the following:
- Discharge and/or recommendations for routine follow-up
- Feedback for the CF team to inform management
- Referral for audiological rehabilitation evaluation
- Referral for tinnitus evaluation and management
- Referral to other professionals or vestibular testing
10 Documentation and dedicated database

- Documentation must contain identifying and pertinent background information to include identification of ototoxic agents, assessment results, and patient condition before, during, and after the tests (including patient reactions), interpretation, prognosis, and specific recommendations.

- An electronic database of all CF patients should be developed and maintained with information regularly fed back to the CF team.

- Annual audit of the service should be performed to inform improvement of the service and allow its rollout to other trusts managing CF patients and other susceptible patient groups.

11 MONITORING OF STANDARDS:

11.1 Internal Procedure

11.1.1 Monitoring of appointment times and waiting lists every 6 months. Results logged in audit manual.

11.1.2 Monitoring of referral rates every 12 months.

11.1.3 Monitoring of adherence to protocol (test and report writing) every 12 months by observation of person running the test. The assessor should be Band 7 Scientist or audiologist and above. Non-compliance to protocol should be logged, action plan developed for tester if needed (including training required if necessary).

11.1.4 Peer review of test results and early accurate identification of occurrence of ototoxicity.

11.1.5 Any deviation from the standard is to be brought to the attention of the clinical physician/scientist. These will then be brought up at the next audit meeting.

Second version: Created November 2012 by Ghada Al-Malky.

APPENDIX I - PROTOCOL PROBLEMS AND CHANGES

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<th>DATE</th>
<th>COMMENTS</th>
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<td>Minor edits (GAM, RS and TS)</td>
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<tr>
<td>March 2013</td>
<td>Minor edit of Flow chart (GAM)</td>
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</table>
Flow chart for Ototoxicity Monitoring protocol:

CF child with respiratory infection in need of i.v. AGs

Request for baseline audiological assessment by CF team (in AR clinic/ward) - Clinical Fellow/Registrar to confirm need

Clinical Fellow send referral for via internal mail to Audiology

Audiologist books appointment on the dedicated clinic slots for Dr TS after checking next CF clinic appt for patient on PIMS

Add patient to ototoxicity monitoring database

IF POSSIBLE: Baseline auditory monitoring in AVM Dept. (Tymps/ART, PTA (0.25-16kHz), DPOAE (1-8kHz)

should be performed before/within 1st 72 hours of intake of 1st dose of AG

NHSP screening data can be retrieved from the eSP database if born >2006

Follow-up auditory monitoring post-treatment

should be performed at the end of i.v. AG course before discharge from ward and at 6/8 week follow-up review clinic

If normal hearing maintained

Continue monitoring using targeted monitoring criteria

Candidate for post-treatment audiological screening in CF clinic/ward

If early signs of ototoxicity detected using ASHA criteria

Referral to CF unit for modification of treatment

Audiological rehabilitation for amplification/tinnitus counselling

Diagnostic post-treatment review at end of every subsequent i.v. AG course
### 9.13 Appendix 13: Multivariate analysis for potential risk factors related to Theme B study 1:

<table>
<thead>
<tr>
<th></th>
<th>Coefficient</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
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<tr>
<td><strong>Standard Audiometry</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>PTA 0.5 kHz</td>
<td>0.81</td>
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<td>PTA 4 kHz</td>
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<td>Age (years)</td>
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<td>Amikacin Courses</td>
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<td>(0.50, 1.24)</td>
<td>&lt;0.01</td>
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<td>Years Of iv Intake</td>
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<td>Concomitant Vancomycin</td>
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<tr>
<td>Concomitant Cisplatin</td>
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</tr>
<tr>
<td><strong>EHF Audiometry</strong></td>
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<td></td>
</tr>
<tr>
<td>PTA 9 kHz</td>
<td>3.03</td>
<td>(-0.22, 6.28)</td>
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</tr>
<tr>
<td>PTA 10 kHz</td>
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<td>(-1.53, 4.97)</td>
<td>0.299</td>
</tr>
<tr>
<td>PTA 11.8 kHz</td>
<td>3.74</td>
<td>(0.49, 6.99)</td>
<td>0.024</td>
</tr>
<tr>
<td>PTA 12.5 kHz</td>
<td>3.4</td>
<td>(0.15, 6.65)</td>
<td>0.04</td>
</tr>
<tr>
<td>PTA 14 kHz</td>
<td>2.96</td>
<td>(-0.29, 6.21)</td>
<td>0.075</td>
</tr>
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<td>PTA 16 kHz</td>
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<td>High Exposed ≥10 AG Course</td>
<td>18.97</td>
<td>(4.81, 33.14)</td>
<td>0.011</td>
</tr>
<tr>
<td>Amikacin Courses</td>
<td>1.52</td>
<td>(1.01, 2.03)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Tobramycin Courses</td>
<td>0.75</td>
<td>(-0.21, 1.71)</td>
<td>0.132</td>
</tr>
<tr>
<td>Gentamicin Courses</td>
<td>0.64</td>
<td>(-0.23, 1.52)</td>
<td>0.156</td>
</tr>
<tr>
<td>FEV₁ Average</td>
<td>-0.08</td>
<td>(-0.29, 0.12)</td>
<td>0.42</td>
</tr>
<tr>
<td>Years Of iv Intake</td>
<td>-0.92</td>
<td>(-1.64, -0.20)</td>
<td>0.015</td>
</tr>
<tr>
<td>Concomitant Vancomycin</td>
<td>2</td>
<td>(0.77, 3.23)</td>
<td>0.002</td>
</tr>
<tr>
<td>Concomitant Cisplatin</td>
<td>9.28</td>
<td>(-5.40, 23.97)</td>
<td>0.22</td>
</tr>
<tr>
<td><strong>DPOAEs</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0 kHz</td>
<td>3.79</td>
<td>(2.01, 5.57)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>1.3 kHz</td>
<td>7.72</td>
<td>(5.94, 9.50)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>1.6 kHz</td>
<td>12.11</td>
<td>(10.33, 13.88)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>2.0 kHz</td>
<td>13.23</td>
<td>(11.45, 15.01)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>2.5 kHz</td>
<td>12.74</td>
<td>(10.96, 14.52)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>2.0 kHz</td>
<td>13.87</td>
<td>(12.09, 15.65)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>4.0 kHz</td>
<td>16.12</td>
<td>(14.34, 17.90)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>5.0 kHz</td>
<td>18.59</td>
<td>(16.81, 20.36)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>6.3 kHz</td>
<td>13.52</td>
<td>(11.74, 15.30)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>8.0 kHz</td>
<td>0.16</td>
<td>(-1.62, 1.94)</td>
<td>0.86</td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.37</td>
<td>(0.04, 0.70)</td>
<td>0.034</td>
</tr>
<tr>
<td>Male</td>
<td>-3.29</td>
<td>(-5.73, -0.85)</td>
<td>0.011</td>
</tr>
<tr>
<td>Low Exposed &lt;10 AG Course</td>
<td>0.86</td>
<td>(-4.18, 5.90)</td>
<td>0.739</td>
</tr>
<tr>
<td>High Exposed ≥10 AG Course</td>
<td>-2.04</td>
<td>(-7.29, 3.22)</td>
<td>0.45</td>
</tr>
<tr>
<td>Amikacin Courses</td>
<td>-0.23</td>
<td>(-0.46, -0.01)</td>
<td>0.048</td>
</tr>
<tr>
<td>Tobramycin Courses</td>
<td>-0.52</td>
<td>(-0.91, -0.12)</td>
<td>0.013</td>
</tr>
<tr>
<td>Gentamicin Courses</td>
<td>0.02</td>
<td>(-0.34, 0.38)</td>
<td>0.923</td>
</tr>
<tr>
<td>FEV₁ Average</td>
<td>-0.02</td>
<td>(-0.10, 0.07)</td>
<td>0.717</td>
</tr>
<tr>
<td>Years Of iv Intake</td>
<td>0.27</td>
<td>(-0.03, 0.57)</td>
<td>0.078</td>
</tr>
<tr>
<td>Concomitant Vancomycin</td>
<td>-0.91</td>
<td>(-2.10, 0.29)</td>
<td>0.142</td>
</tr>
<tr>
<td>Concomitant Cisplatin</td>
<td>-2.38</td>
<td>(-8.35, 3.60)</td>
<td>0.439</td>
</tr>
</tbody>
</table>
Further analysis was performed by the ICH statistician using Multivariable linear mixed models applied for each of the testing methods, Standard PTA, EHF PTA and DPOAE to quantify the association of these different possible risk factors on the response levels at different frequencies. This allowed modeling the trajectories of hearing level (dB) change according to frequency and adjusting for the possible risk factors, taking into account the repeated measures at different frequencies from different ears within person (Table 5-9-1). For the Standard PTA, frequency 2kHz was significant coefficient change of -3.16 dB HL (95% CI: -5.57 to -0.75; p=0.010) with every unit change in hearing, and frequency 8kHz with increased coefficient by 2.94 dB HL (95% CI: 0.53 to 5.35; p=0.017), whereas coefficient 0.87 (0.50, 1.24); p<0.01 represents the significant increase in hearing thresholds (dBLH) achieved per unit increase of Amikacin. For EHF PTA significant increase was reported for the 11.8 and 12.5 kHz frequencies at 3.74 dB HL (95% CI: 0.49 to 6.99; p=0.024) and 3.40 dB HL (95% CI: 0.15 to 6.65; p=0.040) respectively. High exposure to AGs (≥10 courses- highest increase of 18.97 coefficient, 95% CI: 4.81 to 33.14), greater intake of Amikacin, longer years of exposure and intake of concomitant Vancomycin were shown to be significantly associated risk factors at p=0.011, <0.01, 0.015 and 0.002 respectively. For DPOAEs significant changes were reported for all f2 frequencies ranging between 1.0 – 6.3 kHz at p<0.01 where the children’s older age, male gender, greater intake of Amikacin and greater intake of Tobramycin were shown to be significantly associated with reduced DPOAE amplitude levels (p=0.034, 0.011, 0.048 and 0.013 respectively).

This analysis was undertaken, as the statistician believed that adding all the frequency responses within each of the three audiological tools into this multivariable linear mixed model takes advantage of the richness of the data collected rather than just depending on the binary analysis of identifying ‘non-ototoxic’ versus ‘ototoxic’ groups from each of these tools as presented in the Kappa analysis in Table 4-2, Table 4-3 and Table 4-4 or in the chi-square analysis in Table 5-1. The criteria for ototoxicity was applied to each frequency tested by the three tools rather than to the overall outcome of each of the tests. However, in my opinion this analysis seemed to provide some outcomes that are contradictory to those previously presented. In this analysis only the 8kHz frequency outcomes from standard audiometry showed a statistically significant coefficient change, which was expected from previous analysis, yet for the EHF audiometry only the 11.8 and 12.0 kHz frequency were significant. This was not expected as data presented in Figure 4-1 and Figure 4-2, which showed the clear difference in thresholds across all EHF audiometry responses at all 9 to 16 kHz frequencies, and from the ANOVA statistical analysis which showed: ‘The audiometric thresholds did not differ significantly between the groups at the lower frequencies (≤4 kHz) but differed significantly from one another at p<0.05 for frequencies 8 to 16 kHz with related t-tests when Bonferroni adjustment was made for the number of comparisons.’ (Section: 4.1.1.2). The analysis of the DPOAE responses was even more divergent from previous analysis as it showed that all f2 frequencies (except for 8kHz) tested showed significant coefficient changes that were much larger than the standard or the EHF audiometry data (i.e. coefficient values range of 3.79 -18.59 for DPOAEs vs. 0.72 - 3.74 for EHF PTA). This was different from the DPOAE amplitude and SNR data presented in Figure 4-6 and Figure 4-7 respectively and in the kappa analysis presented in Table 4-3 and Table 4-4. These results had shown that even though the DPOAE results were generally in agreement with the audiometric data, the sensitivity of the EHF audiometry to detection of ototoxicity was higher than with DPOAE.
Aminoglycoside Cochleotoxicity in Children with Cystic Fibrosis

INTRODUCTION

- Aminoglycoside (AG) antibiotics such as gentamicin, tobramycin and amikacin are commonly prescribed intravenously in children with CF to treat pulmonary exacerbations.
- Despite the known ototoxic effect of AG antibiotics, regular surveillance of hearing is not usually undertaken in cystic fibrosis (CF) patients in the UK.
- Previous studies in children with CF have shown a low prevalence of ototoxicity, ranging from 2 to 5%. However, hearing was tested by standard pure-tone audiometry (PTA) at frequencies 250-8000 Hz which are not adequate to detect early hearing loss caused by AGs.
- More recent studies have shown that the following tests are more sensitive to detect early signs of ototoxicity compared to standard PTA:
  - Extended high frequency PTA (EPTA): Frequencies 9000-20,000 Hz
  - Distortion-product otoacoustic emissions (DPOAEs)

AIM

To assess the incidence of AG ototoxicity in children with CF using standard and high-frequency audiology and distortion-product otoacoustic emissions.

METHODS

- Children with CF were recruited from the CF clinics in Great Ormond Street Hospital, London, UK
- Detailed previous history of AG usage was obtained from the parents and clinical notes.
- Each subject underwent the following detailed hearing assessment:
  - Otoscopy, Ear examination
  - Tympanometry
  - Pure-tone Audiometry (PTA) including extended high frequency audiometry
  - Distortion product otoacoustic emissions (DPOAEs)

RESULTS

- 45 children (17 males) with CF were studied, aged between 5-10 years.
- Children were divided into the following groups:
  - AG exposure group – previously received intravenous AG (n=20).
  - AG non-exposure group – never received intravenous AG (n=25).
- The AG exposure group (n=20) were further subdivided into:
  - High exposure group – receiving regular 3 monthly intravenous AG.
  - Low exposure group – received intravenous AG less frequently than every 3 months (n=14).
- None of the AG non-exposure group showed ototoxicity.
- Of the 20 (21%) AG exposure group showed ototoxicity. Of these 8 children, 7 were from the high exposure group.
- Therefore 28% (7/25) of the high AG exposure group had ototoxicity (Figures 3 & 5).

DISCUSSION

- None of the children who had ototoxicity had a abnormality raised AG serum level during treatment.
- If only standard PTA (250-8000 Hz) was used, only 4 subjects would have been identified as having ototoxicity.

CONCLUSION

- Children with CF who have had previous exposure to AG should have regular auditory assessment using EHTA-PTA and DPOAEs to assess for ototoxicity.

REFERENCES

#933 THE DICHTOMY IN HEARING OF CYSTIC FIBROSIS (CF) CHILDREN FOLLOWING HIGH EXPOSURE TO AMINOGLYCOSIDES

G. AL-MALIKY 1, S. DAWSON 1, T. SIRIMANNA 2, B. SURI 3

CONCLUSION
The acoustic effect of aminoglycosides on cystic fibrosis (CF) children demonstrates a dichotomy that is more subject-dependent, less straightforward and more common than previously reported in the literature. This warrants further investigation and regular audiological monitoring.

INTRODUCTION
In CF patient population, premature hearing loss, particularly sensorineural hearing loss, frequently occurs. Aminoglycosides (AG) including amikacin and gentamicin are used frequently to combat more severe cases of chronic infection. These drugs, cause ototoxic and cochlear effects that can significantly alter the quality of life.

The reports of hearing loss in cystic fibrosis are constantly increasing. Some studies have identified significant hearing loss (40% of patients over age 10) with a progressive hearing loss in patients over age 10.

Aims
To assess the incidence of AG hearing loss in children with CF using standard high-frequency audiometry and ECochG.

METHODS
Subjects:
45 children (27 male and 18 female) aged 2-18 years were invited to participate. All were patients of the Cystic Fibrosis Centre at Great Ormond Street Hospital, UK.

Measurements & Amino Glucosides Exposure (AGAE): A pure-tone audiometry (PTA) 0.01-10 kHz was obtained and then the AUDACE was performed. The HFT was obtained and then the GWT was performed. The high-frequency audiometry was used for standard high-frequency PTA at 1/1, 2, 3 kHz.

RESULTS
DPOAEs showed a similar pattern to PTA’s with a distinct extreme subset within the high exposure group

ACKNOWLEDGEMENTS
This work was supported by Great Ormond Street Hospital, UK.  
REFERENCES

322
CONCLUSION

The effect of external pressure on OAEs is more subject-dependent and less straightforward than current literature suggests. Even small changes in ECP (5-10 daPa) can have relatively large effects (3-5dB TTS) above 2 kHz.

INTRODUCTION

OAEs are generally used to diagnose and assess inner ear function but studies of even external ear characteristics can influence these OAE measurements. It is important to understand the impact these factors can have when interpreting OAE based measurements (Al-Maiky et al., 2015).

Acoustic emission OAE magnitudes are known to be affected by ear canal pressure (ECP). Existing literature describes a statistically significant effect of external ear canal pressure on OAEs, namely that an increase in pressure increases OAE magnitude (Rasmussen, 1995; Pietsch, Boettler & Völksen, 1994 and Völksen et al., 1997). These studies, however, pooled data across individuals and only investigated relatively large changes in pressure (>100 daPa steps). Here we investigated the effect of smaller changes (5 daPa steps) in ear canal pressure in normal hearing individuals. For some subjects we found a surprisingly large and subject-dependent sensitivity of OAEs above 2 kHz to relatively small changes in ECP.

METHODS

Ear Canal Pressures and OAE Amplitudes from Subject 1

RESULTS

All 5 Subject (5 colors)

DISCUSSION

Do we need to worry about ECP when measuring OAEs?

Unpredictable rapid variations of OAE amplitudes with relatively small changes in pressure for OAE responses above 2 kHz were observed. For example, subject 5 (blue line) and subject 2 (red line) showed dramatically reduced DPOAE amplitudes at 5 kHz (relative 1 produced unexpected 1-test results of 0.0211 and 0.0001 when comparing DPOAE measure at 0 to 5 daPa and 15 to 20 daPa respectively). In total 3 of 5 subjects showed statistically significant decreases in 0.0211 and 0.0001 OAE amplitudes increases from 5 kHz EL and 20 daPa.

Future Directions

Work to model and investigate the mechanisms which produce these frequency effects. The effects may be due to how ECP changes the stiffness of the middle ear.

ACKNOWLEDGEMENT

Many thanks to all participants in this project, to Paul and David Rand and to Comanor Research.

REFERENCES

Al-Maiky, G., Abuharb, B. & Backus, G., 2015. The effect of external pressure on OAEs. UCL Ear Institute, 332 Gray’s Inn Road, London WC1X 8EE.

UCL EAR INSTITUTE

SUBJECTS

Five normally hearing adult subjects were tested. All subjects had normal hearing in both ears (C 2000 HE or AM) and were tested from 2 kHz to 6 kHz and normal tympanometry.

TEOAE Measurement

TEOAEs were measured and analyzed using an OAE100 2.1 (version 2.1) instrument with 10 dB/step elicitation and 80° click intensity (40 dB click level, 16 Hz rejection rate).

DPOAE Measurement

DPOAEs were measured and analyzed using the same device and software as was used for the ECP measurement.

ECP Measurement

Ear canal pressure (ECP) was measured and adjusted using a calibrated hypodermic needle. Subjects were asked to set their own ECP so that their ECP was manually set (over 20 daPa step change). The actual ECP achieved was recorded but not always the same as the subject’s suggested pressure level was done. The actual ECP achieved was recorded to allow for the subtraction of the 2 minute TE or DPOAE measurement. At all pressure steps were those were randomized beforehand in a block of 15 and 6 3-minute blocks were taken per subject (total of 30 OAE measurements).
Investigation into the Role of Variation in the 12S rRNA, TPMT and COMT Genes to the Incidence of Aminoglycoside Otoxicity in Children with Cystic Fibrosis (CF)

G. Al-Malky, T. Sirimanna, R. Suri, S. J. Dawson

The previous research demonstrated a significant correlation between mutations in the TPMT and COMT genes and the incidence of aminoglycoside otoxicity in children with CF.

Methods:

- 75 children with CF were evaluated, and their medical records were reviewed.
- DNA samples were collected and analyzed for TPMT and COMT mutations.
- Aminoglycoside exposure was recorded in each patient.

Results:

- The distribution of children according to aminoglycoside exposure and clinical outcomes was analyzed.
- Children with a TPMT or COMT mutation had a significantly higher incidence of aminoglycoside otoxicity.

Conclusion:

- The results suggest that genetic variations in TPMT and COMT can play a role in the incidence of aminoglycoside otoxicity in children with CF.

References:

[List of references provided]
Survey of Current Auditory Monitoring for Ototoxicity in Oncology, Audiology and Cystic Fibrosis Services in the UK

Al-Malky G.1, De Jongh M.1, Kikic M.1, Dawson S.J.1, Suri R.2

1 The Ear Institute, UCL, London, UK. 2 Department of Paediatric Respiratory Medicine, Great Ormond Street Hospital and the Feinberg Unit, Institute of Child Health, UCL, London, UK.

INTRODUCTION

It is well established that certain medications such as cisplatin and anthracyclines cause permanent hearing loss/tinnitus change yet there are no clear guidelines followed in the UK for the systematic monitoring of patients exposed to these drugs.

Patients with cancer in those of the HLAA, preauricular, mesotympanic, or concha are commonly exposed to cisplatin & anthracyclines.

Patients with cystic fibrosis (CF) are especially exposed anthracyclines (e.g. cetuximab, gemcixumab) to cause persistent deafness.

Monitoring aids early detection and prevents progression to irreversible hearing loss in these patients.

AIM

To assess the current practice in UK services using online surveys specifically designed for each of the three groups: Oncology, Audiology & CF services using the UCL Ear Institute survey tool.

METHODS

Online surveys were sent to the three professional groups of interest. Hypothesis is that the survey was filled through prospective professional groups such as the British Society of Audiology, Children’s Cancer and Leukaemia Group and CP Trust.

RESULTS

Responses to whether ototoxicity monitoring is performed or not:

Responses to questions related to the ototoxicity monitoring service set up:

Responses to: “What are the criteria used for referring patients for auditory monitoring”

Responses to “What referral criteria do you think should be used if none exist?”

Responses to “What audiological testing is conducted for ototoxicity monitoring”

DISCUSSION & CONCLUSIONS

- OTotoxic Monitoring: High-Dose Chemotherapy (e.g. cisplatin) and anthracyclines (e.g. doxorubicin) are the main causes of ototoxicity among cancer patients. The International Society for Human Genetics and Oncology (ISCiO) recommends routine monitoring for all patients treated with these agents.
- Strategies for Ototoxicity Monitoring: Early detection is crucial to prevent irreversible hearing loss. Monitoring protocols should be established based on institutional guidelines and individual patient needs.
- Further Recommendations: The need for standardized monitoring protocols across the UK is highlighted. The implementation of guidelines should consider the specific patient population and the availability of resources.

Acknowledgments: Special thanks to all the clinicians that responded to the survey. Many thanks to Dr. Stock, Dr. Bujas and Dr. Sivaraman for their input in setting up the survey. Guidance is due to Prof. David Kemp for his overall supervision of this research.