The Conversion of Ocular Hypertension to Early Glaucoma: Epidemiology, Risk factors, Early Detection and Treatment

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Abstract

Background
The management of ocular hypertension (OHT) has considerable practical and financial implications for ophthalmology services in the U.K.

Previous prophylactic treatment trials for OHT have so far been inconclusive (Kass, 1980, Epstein, 1989, Schulzer, 1991). New methods of detecting early glaucomatous damage are needed to identify ocular hypertensive patients at greatest risk of developing glaucoma, so that appropriate treatment may be targeted at those individuals.

Objectives
1. To determine the effect of betaxolol on the conversion rate of OHT to early glaucoma.
2. To identify possible risk factors for conversion.
3. To evaluate methods of early detection of glaucoma, as compared to gold-standard methods.

Methods
356 ocular hypertensives were randomised to treatment with betaxolol drops or placebo, and followed 4 monthly for 2-6 years with visual field testing, intraocular pressure (IOP) measurement and optic disc and retinal nerve fibre layer (RNFL) imaging. Conversion was defined using visual field criteria.

Results
1. No overall protective effect of betaxolol against conversion was found as compared to placebo.
2. The converters had significantly higher pre-and post-treatment IOPs than the group of non-converters. Betaxolol had a smaller hypotensive effect on the mean pre-treatment IOP level of the converters.

3. Sequential HRT analysis demonstrated glaucomatous optic disc change, prior to reproducible visual field change in the converters. Some non-converters demonstrated optic disc change despite maintaining normal visual fields.

**Conclusions**

Betaxolol did not affect the conversion rate as compared to placebo, despite having a statistically significant IOP lowering effect.

Higher IOP levels are a risk factor for conversion. Betaxolol appeared to have a smaller hypotensive effect in the converting group, and it is possible that these less responsive patients are therefore at greater risk of conversion.

The HRT is a useful tool for the early detection of glaucomatous optic disc damage and may identify patients at risk of developing visual field loss.
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Publications arising from work included in this thesis


Author's Contribution

The betaxolol Vs placebo trial in ocular hypertension.

The author was the co-ordinator of this study during the period 1996-1998 after which the trial was terminated. She was responsible for the day to day running and administration of the trial and all the clinical care of the trial patients, along with the glaucoma patients and normal subjects, during this time.

All statistical analysis of the trial data was carried out by the author with advice from Miss C Bunce, medical statistician with the Glaxo Unit of Epidemiology at Moorfields Eye Hospital.

Methods of Early Detection of glaucoma.

All imaging for the studies included in this section was carried out by trained technicians attached to the trial. All image and data analysis was carried out by the author herself, with statistical advice from Miss C. Bunce.
The World Health Organisation (WHO) estimates the number of people world-wide with glaucoma to be 33 million (World Bank, 1993), with half of this number suffering from undiagnosed disease (Quigley, 1996). Glaucoma is currently the third leading cause of blindness in the world with a prediction that 6.7 million people may be bilaterally blind by the millennium (Quigley, 1996). WHO estimates of the global prevalence of raised intra-ocular pressure are considerably higher, at 105 million. It is clear from these figures and others gained from prospective clinical trials (Kitazawa, 1977, Epstein, 1989, Schulzer, 1991) that raised intra-ocular pressure is only one of several risk factors that make an individual susceptible to glaucomatous damage. Our definition of what the terms glaucoma and ocular hypertension mean are changing as our methods for the detection of early structural and functional glaucomatous damage improve (Drance, 1992). The two disorders require a different management approach, with both financial (Worthen, 1980) and practical implications for our challenged health service. As yet, there is no conclusive evidence that prophylactic treatment may reduce the number of ocular hypertensive patients who develop evidence of glaucomatous damage (Kass, 1980, Epstein, 1989, Schulzer, 1991). Indeed there is still conjecture as to whether treatment can alter the course of even established glaucoma (Rossetti, 1993).

In this thesis I will report on two main areas of interest in the concept of the conversion of ocular hypertension to early glaucoma. The first is the betaxolol Vs placebo treatment trial in ocular hypertension – a prospective, longitudinal, double-blind, placebo controlled trial carried out to determine
whether betaxolol can delay or prevent conversion in a group of ‘at risk’ patients. The second relates to the development of new methods for the early detection of glaucomatous damage, as compared to currently used ‘gold standard’ techniques.

In the review of the literature I will discuss some of the previous work which has been carried out in these and other relevant areas, highlighting weaknesses and strengths of important preceding studies.
Section 1 A Critical Review of the Literature
1.1 General aspects of ocular hypertension and early glaucoma

1.1.1 Definitions

1.1.1.1 Ocular hypertension

The definition of ocular hypertension (OHT) is controversial. Several early studies investigating the course of OHT (Armaly, et al., 1980, Kitazawa, et al., 1977, Quigley, et al., 1994, Schulzer, et al., 1991, Walker, 1974, Wilensky, et al., 1974) have included an initial definition based on intra-ocular pressure (IOP) and visual field characteristics alone. Using these criteria a patient is defined as having OHT if the IOP is consistently greater than 21mmHg on applanation tonometry, and automated perimetry shows no glaucomatous defect.

Others have included a normal optic disc appearance as a diagnostic criterion (Kass, 1983, Phelps, 1980) and this is probably the most controversial area of discussion. The optic disc appearance of the normal population shows a wide range of variability (Jonas, et al., 1988). Only a few studies have attempted to define the parameters of the normal optic discs (Britton, et al., 1987 Jonas, et al., 1988). Current techniques of optic disc analysis and assessment used in clinical practice have limited objectivity, and agreement amongst even expert observers has limitations (Lichter, 1976 Varma, et al., 1992). New techniques of objective optic disc analysis such as the Heidelberg Retina Tomograph, which we will discuss later in detail, show promise and in the future should help to allow us to more precisely define and differentiate between OHT and early glaucoma (Wollstein, et al., 1997).
For the purposes of this thesis, the following definition of OHT has been used.

1. The intra-ocular pressure (IOP) as measured by contact Goldmann tonometry is consistently above 21 mmHg on two or more separate readings.

2. Normal open angles on gonioscopy.

3. No glaucomatous defect is present on repeated visual field testing using threshold automated static perimetry. All visual field testing in this study was performed using the 24-2 program of the Humphrey perimeter. Performance on visual field testing is subject to a significant learning effect (Gordon, 1999), so an initially abnormal field may ‘become’ normal on subsequent testing and this should be taken into account when faced with an initial abnormal test result.

4. There is no predisposing previous ocular history such as topical steroid use, uveitis or ocular trauma leading to angle recession.
1.1.1.2 Early Glaucoma.

The definition of early glaucoma (POAG) is less controversial, and is generally made on visual field and optic disc criteria. The inclusion of IOP criteria is not as important as more than one third of patients with glaucoma have IOPs consistently within the normal statistical range (Bengtsson, 1981, Hollows and Graham, 1966, Klein, et al., 1992, Shiose, 1991, Tielsch, et al., 1991). The definition of early glaucoma used in this thesis is as follows:

1. The presence of a typical glaucomatous defect on visual field testing. The Advanced Glaucoma Intervention Study (AGIS) classification (AGIS Investigators, 1994) was used to define early glaucomatous field loss with an AGIS score of 1-5. This system will be discussed in more detail in the methods section.


3. The untreated IOP may be raised above the upper limit of normal (21mmHg) or may be within the normal range.
1.1.2 Epidemiology of OHT and glaucoma

The prevalence of an average IOP above the level of 21mmHg in the non-glaucomatous population of the United States and Europe ranges from 2.1% (Bonomi, et al., 1998, Giuffre, et al., 1995) to 9.4% (Wilson and Martone, 1996). Recent population-based studies examining the prevalence of POAG in Caucasians have produced over-all figures ranging from 1.1% to 2.1% (Coffey, et al., 1993, Tielsch, et al., 1990, Bonomi, et al., 1998, Dielemans, et al., 1994). However, there is a marked increase in the prevalence of POAG with increasing age rising to 3.5% (Tielsch, et al., 1990) in the over 70 age group and 4.7% in the over 75s (Klein, et al., 1992).

Estimates of the incidence of POAG in the population are lacking as many of the recent population studies were designed to yield only prevalence data. The Swedish prevalence study from Dalby, however, found an annual incidence rate of 0.24% (Bengtsson, 1989).

A summary of prevalence data from the major population based studies is given below.

<table>
<thead>
<tr>
<th>Location</th>
<th>Age group</th>
<th>Racial group</th>
<th>Number of participants (% eligible)</th>
<th>Number of cases of POAG (% prevalence)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baltimore Tielsch, 1990</td>
<td>≥40</td>
<td>2913 white</td>
<td>5308 (79%)</td>
<td>32 (1.29%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2395 black</td>
<td></td>
<td>100 (4.74%)</td>
</tr>
<tr>
<td>Beaver Dam Klein, 1992</td>
<td>43-84</td>
<td>White</td>
<td>4926 (83%)</td>
<td>104 (2.1%)</td>
</tr>
<tr>
<td>West of Ireland Coffey, 1993</td>
<td>≥50</td>
<td>White</td>
<td>2186 (99%)</td>
<td>41 (1.9%)</td>
</tr>
<tr>
<td>Barbados Leske, 1994</td>
<td>40-84</td>
<td>Mainly black</td>
<td>4709 (84%)</td>
<td>309 (6.6%)</td>
</tr>
<tr>
<td>Rotterdam Dielemans, 1994</td>
<td>≥55</td>
<td>White</td>
<td>3062 (71%)</td>
<td>34 (1.1%)</td>
</tr>
</tbody>
</table>

The WHO estimates the global population of people with raised IOP to be 105 million with 13.5 million having POAG (World Bank, 1993). The worldwide incidence of POAG is estimated at 2.4 million per year. It is predicted that the number of people with POAG will be 33 million by the year 2000 (World Bank, 1993), with half of this number suffering from undiagnosed disease.
Glaucoma is the third commonest cause of blindness world-wide, and it is thought that 6.7 million people will be considered bilaterally blind by the millennium (Quigley, 1996). POAG is more common in Afro-Caribbean populations (Mason, et al., 1989, Tielsch, et al., 1991) and occurs at an earlier age than in Caucasians (Mason, et al., 1989) giving a higher burden of disease amongst the younger age groups.

It is clear from these figures that OHT is more prevalent than POAG and the epidemiology of OHT conversion to POAG will be considered separately.
1.1.3 Epidemiology of the conversion of OHT to POAG.

The term ‘conversion’ refers to the development of glaucomatous damage, in a patient who had been previously been classified as having OHT. From the previous discussion about the difficulties inherent in defining these basic entities, it becomes clear that defining the onset of conversion will pose equal problems for the researcher and clinician alike. Concepts of conversion will change, as new technologies become available for detecting earlier glaucomatous structural and functional damage in the individual patient.

There are several studies that have followed OHT patients over time, both on and off treatment. These have examined different parameters when looking for glaucomatous change, including visual fields, optic disc morphology and IOP level.

Estimates of the number of patients who ‘convert’ from OHT to early glaucoma over time range widely depending on patient selection criteria and the end-points used to determine conversion.

An ideal study that sets out to determine the rate of conversion from OHT to early glaucoma should be prospective, with well-defined end-points. The end-points chosen will partly determine the conversion rate.

Most studies that have used visual field criteria to define the onset of glaucoma give estimates of conversion in the region of 10% and these are discussed below. Kitazawa et al. (Kitazawa, et al., 1977), carried out a prospective study to determine the course of untreated OHT in 75 patients who had no initial visual field defects on Goldmann perimetry. 7 patients (9.3%)
developed reproducible glaucomatous visual field defects over an average of 9.5 years, giving an annual conversion rate of less than 1%. David et al. (David, et al., 1977) followed 61 treated and untreated patients for an average of 40.7 months and found that 10.2 % (or 3.3% per annum) of patients developed glaucomatous defects.

Later, in the Collaborative Glaucoma Study, Armaly (Armaly et al. 1980), reported on a prospective 13 year study to identify factors that influence the development of visual field defects in OHT patients. The initial recruitment of the study also included initially normotensive blood relatives of patients with POAG. Almost 6,000 eyes were followed up but only 98 (1.7%) went on to develop a visual field defect on Goldmann perimetry. There was a high drop-out rate in the study and the inter-centre variation in clinical assessment of cup: disc ratios meant that the assessment of risk factors for conversion was limited. The factors found to be important, although with a low predictive value, included age, IOP and cup-disc ratio as measured at baseline. 98.54% of patients with base-line IOPs less than 20 mmHg continued to have normal visual fields during the course of the trial, as compared to 93.4% of those with an IOP level greater than 20 mmHg.

Quigley et al. (Quigley, et al., 1994) prospectively followed 647 untreated and treated OHT patients over 12 years, and 68 or 10.5% developed reproducible Goldmann visual field defects. Patients were initially included on the basis of an IOP level >21mmHg, and normal base-line visual fields.

Other studies have included structural change end-points, such as glaucomatous optic disc and retinal nerve fibre layer (RNFL) change and the occurrence of optic disc haemorrhages. In general, these have reported much
higher conversion rates. Schulzer et al. (Schulzer, et al., 1991) prospectively followed 143 OHT patients over 6 years after randomisation to either treatment with timolol or no treatment. Defined trial end-points were the development of reproducible visual field defects on automated (Peritest) perimetry, the occurrence of optic disc haemorrhages or optic disc change documented by stereophotography. A total of 42 (29.4%) patients developed early glaucoma by these criteria, giving a conversion rate of 4.9% per year.

Epstein et al. (Epstein, et al., 1989) randomly assigned 107 OHT patients with normal Goldmann fields and no clinical evidence of optic nerve damage to groups on either treatment with timolol or no treatment. Patients were followed over an average of 56 and 51 months respectively. In total, 26 patients (24.3%) reached an end-point according to pre-determined 'failure' criteria. These included: an IOP rise above 32 mmHg on two or more occasions during follow-up; optic disc progression documented by sequential stereo-photographs; or the onset of a reproducible visual field defect. A patient was also deemed to have failed if the examiner felt that the patient was 'at risk' of visual failure despite not meeting any of the above criteria, and felt ethically bound to remove them from the trial. Conversion rates were 4-7% per year in this study.

Yablonski et al. (Yablonski, et al., 1980) retrospectively examined data from 102 patients with OHT defined by raised IOPs and normal Goldmann visual fields. 27% had developed visual field loss over a 5-year period, with an annual conversion rate of 5.4%. However, the authors acknowledge that their subjects represented a highly selected sample as inclusion criteria for the retrospective study required that disc photographs had been taken before the onset of visual field defects. They point out that not all OHT patients in their unit routinely
undergo optic disc photography, and therefore those that did were likely to have had larger cup: disc ratios or higher IOPs than those who did not. They suggested that this selection bias is therefore responsible for the high rate of conversion in their study.

A table summarising of some of the conversion studies is illustrated below. Those studies that investigated the effect of topical hypotensive treatment will be discussed in detail later.

Summary of previous OHT trials – end-points and rates of conversion

<table>
<thead>
<tr>
<th>Study</th>
<th>Mean follow up</th>
<th>Number of eyes/pts</th>
<th>Conversion criteria</th>
<th>% visual field converters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kitazawa 1977</td>
<td>9.5 years</td>
<td>75</td>
<td>Goldmann visual fields</td>
<td>9.3</td>
</tr>
<tr>
<td>David 1977</td>
<td>40 months</td>
<td>117</td>
<td>Goldmann visual fields</td>
<td>10.2</td>
</tr>
<tr>
<td>Kass 1989</td>
<td>5-8 years</td>
<td>62</td>
<td>Visual fields, Optic disc change</td>
<td>17.7 treatment effect</td>
</tr>
<tr>
<td>Epstein 1989</td>
<td>54 months</td>
<td>107</td>
<td>IOP&gt;32, Visual fields, optic disc change</td>
<td>6.5 treatment effect</td>
</tr>
<tr>
<td>Schulzer 1991</td>
<td>6 years</td>
<td>137</td>
<td>Peritest visual field, Optic disc haemorrhage, Optic disc change</td>
<td>21.4 No treatment effect</td>
</tr>
<tr>
<td>Quigley 1994</td>
<td>12 years</td>
<td>647</td>
<td>Goldmann visual fields</td>
<td>10.5</td>
</tr>
</tbody>
</table>

In summary, these studies demonstrate that there is a wide range of conversion rates overall, reflecting the differing ‘end-point’ criteria used by various investigators.
The validation of new techniques for detecting early structural damage against the ‘gold standard’ of perimetric methods is still ongoing. Until we have a ‘gold standard’ method of detecting early optic disc or RNFL change, then the debate on how to define conversion will continue. From these figures it is clear that the majority of patients with OHT do not go on to develop POAG, and it is therefore important to determine if there are specific risk factors operating in the small proportion that do. These factors will be considered in a following section.

One major limitation of all these studies is the time length over which they were carried out. It might be expected that the number of OHT patients who convert to early POAG may rise as the length of time over which they are followed increases. Study practicalities limit the time a patient is followed for, but it is possible that longer studies may demonstrate higher conversion rates.
1.1.4. Risk Factors for the conversion of OHT to early glaucoma

The term ‘risk’ refers to the probability that a disease will occur. Risk factors are inherited or in-born characteristics, environmental exposures, or behavioural aspects that influence the probability of an ‘at risk’ individual developing the disease. There has been much interest in this area of glaucoma, and there is evidence to support the existence of several risk factors more than others.

1.1.4.1 IOP

One of the most important risk factors determining who may convert to early glaucoma is the degree of elevation of the IOP (Armaly, et al., 1980 David, et al., 1977 Quigley, et al., 1994). A causal role for the level of the IOP in glaucomatous damage is supported by experimental studies of induced pressure elevation leading to typical glaucomatous cupping in animals (Quigley, 1980). Clinical support is provided by evidence that asymmetrical IOP levels lead to asymmetrical cupping (Cartwright and Anderson, 1988, Crichton, et al., 1989). Many of the population based prevalence studies have found higher rates of POAG with increasing levels of IOP (Armaly, et al., 1980 David, et al., 1987 Sommer, et al., 1991).

One of the clearest overviews of the relationship between IOP and the risk of glaucoma is illustrated below. The data was obtained through recalculation of incidence data from previous studies (Wilson and Martone, 1996).
Kass et al. (Kass, et al., 1976) carried out a retrospective review of 31 patients in whom there was bilateral elevation of the IOP, but only unilateral visual field loss. They found that 62% of the fellow eyes with IOP levels > 26 mmHg developed visual field loss, compared to only 6% of those with lower IOPs. The mean IOP level in the group developing visual field loss was 33.1 mmHg and significantly different to the mean of 24.8 mmHg in the group that did not. However, these patients had the additional risk factor of having functional glaucomatous loss in the other eye.

Kitazawa (Kitazawa, et al., 1977), found that an IOP level greater than 30 mmHg had the highest specificity of all the factors analysed, in predicting which OHT patients would develop glaucomatous visual defects. However, the sensitivity of this measure was comparatively low.

David et al. (David, et al., 1977) found that the risk of conversion in patients with an initial untreated IOP of less than 25 mmHg was 2.7%, rising to 41% in those with a level greater than 31 mmHg.

Quigley et al. (Quigley, et al., 1994) found in a large prospective study of OHT over a 12 year period that the higher the IOP, the greater the risk of developing visual field loss. Initial IOP levels greater than 30 mmHg gave a risk ratio of 1.91. Interestingly, eyes receiving glaucoma therapy were at greater risk of developing visual field loss, presumably because they had been initially selected for treatment by the clinician because of perceived increased risk.

The pattern of the IOP level over time in OHT has also been studied. Schwartz (Schwartz and Tolusan, 1980) retrospectively noted three basic patterns over time in a group of 60 OHT patients receiving no treatment:
stability, a downward trend, and an upward trend, and these findings were confirmed by Linner (Linner, 1980). These studies have suggested that those patients in whom the IOP follows an upward trend are at an increased risk of developing POAG over time.

Although the level of IOP has been identified as one of the major risk factors for glaucomatous damage, the observation that not all OHT patients with higher IOPs convert, and that not all POAG patients have raised IOP indicates the role of other risk factors. These may act independently or interact with the IOP level in the individual patient.

Other well supported risk factors for the conversion of OHT to early glaucoma include;

b) Age of the patient.

As discussed previously, many studies have confirmed that the older the individual, the higher the prevalence of POAG (Bengtsson, 1981 Bengtsson, 1981 Coffey, et al., 1993 Hollows and Graham, 1966 Klein, et al., 1992 Tielsch, et al., 1990).

Quigley et al. (Quigley, et al., 1994) found that increasing age was a risk factor for the development of visual field defects in OHT, with as risk ratio of 1.47.

The Collaborative Glaucoma Study (Armaly, et al., 1980), identified age as the major predictor of onset of glaucomatous field damage; defects were 7 times more likely in patients > 60 years old compared with patients < 40 years of age.
There is also evidence of a trend towards higher IOP with increasing age (Armaly, 1965 Klein, et al., 1992 Leibowitz, et al., 1980), which may be a contributing factor to the increased risk. Again, on the basis of the previous discussion, IOP level may interact along with underlying structural factors such as a decline in supportive optic disc tissue (Quigley, et al., 1994, Quigley, et al., 1991), or a generalised neuro-degeneration associated with increasing age.

c) Race

Some studies have found that Afro-Caribbean race is a highly significant risk factor (David, et al., 1978 Wilson, et al., 1987 Yablonski, et al., 1980) for conversion, but others have not (Quigley, et al., 1994). POAG is more prevalent in the Afro-Caribbean population as found in some of the large population based eye surveys (Leske, et al., 1994 Tielsch, et al., 1991), but it is not yet clear whether black patients with OHT are more at risk of developing early glaucoma. In an early prospective study of OHT, David et al. (David, et al., 1978) found a conversion rate of 18% over a ten year period in the Afro-Caribbean group - more than three times higher than for the Caucasians.

d) Family History.

The presence of a positive family history of glaucoma in a patient with ocular hypertension is accepted as a risk factor for conversion but good evidence to support this is lacking. The collection of data on family history relies heavily on the self-reporting by patients. Often their understanding of what the term glaucoma means is limited, and recollections of relatives who lost their sight may erroneously be put down to glaucoma, once their own diagnosis is made. Combined with the earlier assertion that up to 50% of POAG may be
undiagnosed, the investigation of family history as a risk factor is fraught with
difficulty.

The Baltimore Eye study specifically examined the data for any association
between family history and an increased prevalence of glaucoma (Tielsch, et al.,
1994). They found an increased age-adjusted odds ratio of 3.69 for siblings with
less significant values for parental or sibling relationships. The authors, however,
also felt that selection bias may have affected their results, as the odds ratios
were higher in the group that had prior knowledge of their diagnosis. The study
also found higher odds ratios in black subjects, indicating that family history may
be more important in this racial group – a finding confirmed by others (Leske, et
al., 1995).

Quigley et al. (Quigley, et al., 1994) did not find an increased relative risk
associated with a positive family history in their study.

e) Optic disc features

Asymmetric optic disc cupping has been noted to be a predictor for the
development of visual field loss (Fishman, 1970, Yablonski, et al., 1980,
Quigley, et al., 1994 Quigley, et al., 1992). However, such an association may be
misleading, as asymmetry may simply reflect early disease in one eye. As the
definitions of OHT Vs early glaucoma become clearer, according to the detection
techniques that come into use, then the reference to this as a risk factor rather
than an early sign may change.

Increased cup/disc area ratio at baseline examination has been found to have
prognostic significance for future visual field loss by some groups (Armaly, et
1992, Yablonski, et al., 1980), but again, this probably represents pre-perimetric glaucomatous change. Also in the earlier studies quoted here, the methods for assessing the optic disc were mainly clinically based and subjective, limiting the amount of useful conclusions that may be gained from them.

f) RNFL features

Moderate or severe RNFL atrophy at baseline examination was shown by Quigley and co-workers (Quigley, et al., 1994) to give an increasing risk ratio with increasing severity of the changes. Again the same arguments as described above apply to this observation.

g) Systemic risk factors

The influence of systemic risk factors on the future development of visual field loss in OHT has been studied but no conclusive evidence exists for an association with diseases such as hypertension, diabetes mellitus, or vascular disease (Kass, et al., 1980 Wilensky, et al., 1974).

A combined analysis of such risk factors is probably necessary to obtain the best possible predictive value when considering which OHT patients are likely to convert to early glaucoma (Wilensky, et al., 1974).
1.1.5 Conversion of OHT to early glaucoma: Changes in optic disc and RNFL morphology

a) Progressive optic disc change

It has been well documented that optic disc changes may precede both kinetic and automated static visual field perimetric changes by several years (Airaksinen, et al., 1985, Pederson and Anderson, 1980, Sommer, et al., 1979); (Quigley, et al., 1982 Quigley, et al., 1989 Quigley, et al., 1992). The earliest changes occurring in the optic disc and the techniques available for detecting them will be considered in detail.

i) Optic disc cup parameters

Pederson and Anderson (Pederson and Anderson, 1980) retrospectively studied the serial optic disc photographs of 259 OHT and early glaucoma patients over a period of up to 15 years. 29 of these eyes showed progressive enlargement of the cup, with the majority showing generalised enlargement and the minority showing vertical or horizontal cup extension. 27 out of the total 29 eyes had normal Goldmann visual fields at the time of the initial photography. Progressive optic disc cupping was noted before the development of visual field defects in 9 eyes over the time course of observation, with an average separation of one year between the two events. The main criticism of this study was the lack of detail about the precise methods of optic cup measurements used, and if any allowance was made for possible differences in magnification of the photographs between examinations. Photographs for inclusion into the study were also selected on the basis of observable optic disc change, and other characteristics such as visual field appearance and IOP level were looked at secondarily.
Sommer et al. (Sommer, et al., 1979) selected serial optic disc photographs taken prior to the development of Goldmann visual field defects in 12 OHT patients, undergoing yearly routine examination. Subjects were chosen on the basis of visual field and IOP characteristics rather than the appearance of the optic disc. These were compared with serial photographs from 35 matched control subject eyes, and all were analysed in a randomised-masked fashion using a measurement grid. The results showed that in 10 of the 12 OHT eyes progressive changes in the optic disc cup occurred by the time that glaucomatous field loss first appeared, with most of the changes taking place during the initial years of follow-up. In the remaining 2 OHT eyes examined, progressive pallor of remaining optic disc tissue, rather than a change in the contours of the optic cup was noted. 4 out of the 35 ‘control’ eyes demonstrated change in the optic cup dimensions. This observation may in part be explained by the fact that some of the ‘controls’ were blood relatives of the OHT patients, and may therefore have been at an increased risk of developing the disorder themselves.

Yablonski et al. (Yablonski, et al., 1980) conducted a 5 year study and examined stereoscopic disc photographs of 102 OHT patients taken prior to the development of any visual field loss on Goldmann perimetry. Subjects were selected on the basis of IOP, visual field and gonioscopic characteristics rather than initial optic disc appearance. Over the time of follow-up, 2 groups emerged - those retaining normal visual fields (75 patients) and those who developed a visual field defect (27 patients), and their results were compared. The study found that larger cup-disc ratios (>0.6) were present in eyes that subsequently developed field loss. If this measurement was combined with an initial IOP level > 28mmHg, then the ability to predict the onset of visual field loss was
improved. Progressive optic disc cupping in the visual loss group was found to occur in those discs with larger cups than in the no visual loss group. The authors concluded that progressive disc cupping, especially in an eye with an already enlarged cup is an important prognostic sign for future visual field loss.

Motolko and Drance (Motolko and Drance, 1981) prospectively examined stereoscopic disc photographs of 16 ‘pre-glaucomatous’ eyes that developed visual field defects on kinetic perimetry with photos from 38 eyes that retained normal visual fields over an average of 4.5 years follow-up. The definition of ‘pre-glaucoma’ used in the study included an IOP >22 mmHg, and absence of visual field defects on screening with the modified method of Armaly (Armaly, 1972). Conversion was defined as the development of reproducible defects on two occasions with 3 to 6 monthly intervals between testing. The stereo-photographs were analysed in both a non-masked, sequential manner and a masked randomised manner for both groups and these methods were compared. With both methods of subjective disc analysis, 31 % of the converting discs were shown to demonstrate an enlargement of the cup, agreed by two independent observers, compared to only 8 % of the stable group. There was noted to be a higher frequency of larger cups in the converter group at the baseline examination. 12 % of converters however, had an initial cup-disc ratio of less than 0.4 and did not demonstrate any subsequent enlargement prior to visual field loss. 44% of converters developed an optic disc haemorrhage at some point during follow-up, compared to one eye in the stable group.

In a more recent study, Quigley et al. (Quigley, et al., 1992) compared optic disc and RNFL photographs in 2 age-matched subgroups taken from 813 OHT eyes. One group comprised 37 eyes that converted on visual field criteria
using Goldmann perimetry, and the other was made up of 37 OHT patients who retained normal visual fields. Each group had undergone annual photography and sequential photographs were analysed in a masked fashion. Optic disc measurements were made using a micrometer overlay in the stereo-viewing device. Glaucomatous progression was defined by an enlargement in cup size or a change in rim slope. This part of the study was performed with the viewer both masked and unmasked to the temporal sequence of the photographs. They found that the initial mean cup-disc ratio was higher in a group of converters defined on the basis of visual field loss than in the non-converting OHT group. The cup:disc ratio was found to qualitatively increase in 7 out of 37 (19%) of converting eyes but only 1 out of 37 stable OHT eyes. Further quantitative assessment of change provided a specificity level of 85% and a sensitivity level of 88% for this method of optic disc analysis. The authors, however, concluded that the value of using a ‘cut-off’ cup: disc ratio of greater than 0.55 was limited in predicting which OHT patients would convert to early visual field loss.
ii) Neuro-retinal rim parameters

An early study by Airaksinen et al. (Airaksinen, et al., 1985) found highly significant differences in the neuro-retinal rim areas between groups of normals, OHT patients and glaucoma patients. The study found that 95% of the normal subjects had rim areas greater than 1.09 mm$^2$; 30% of the subjects with ocular hypertension and 73% of the patients with glaucoma had rim areas less than 1.09 mm$^2$.

This finding was followed by a further study (Airaksinen, et al., 1992) which examined sequential stereoscopic optic disc photographs of 75 OHT patients, 43 patients with early glaucoma and 5 normal controls, over 5 to 10 years of follow up. In this study, the glaucoma patients were selected on the basis of having typical glaucomatous visual fields (on Freidmann automated perimetry) and optic disc characteristics. The OHT patients had IOP levels above 21 mmHg and normal visual fields and optic discs. The controls had IOP levels < 21 mmHg with normal visual fields and optic discs. Optic disc analysis was carried out using computer-assisted planimetry, with corrections made for magnification errors. The rate of change of the neuro-retinal rim area was the parameter examined on sequential photographs, using linear regression analysis to determine the rate of change for each group. The results showed that none of the controls showed a statistically significant decrease in the neuro-retinal rim area, whereas 79% of the glaucoma and 57% of the OHT patients did. In the latter group, 28% of these patients in whom a decrease in the neuro-retinal rim area was demonstrated retained normal visual fields. Rate of rim area change was weakly but significantly correlated with the age of the patient and higher IOPs in the OHT group and with initial neuro-retinal rim area in the glaucoma group. The
yearly rate of loss of rim area was found to be 0.23% for the normal control group, 0.47% for the OHT group who maintained normal visual fields, 2.75% in the OHT group who developed visual field defects, and 3.47% in patients with glaucoma. The nature of the loss was linear in the majority (49%) of patients showing a significant decrease but also followed an episodic (22%) or curvilinear (29%) pattern. The main criticism of these studies is the introduction of the inherent selection bias by including subjects into the different groups on the basis of their initial optic disc appearance. The definition of ‘normality’ is not discussed, and the ‘acceptable’ ranges of optic disc appearance for each group are not described.

iii) RNFL changes

Several authors have demonstrated that RNFL changes precede both optic disc and visual field changes as one of the earliest signs of glaucomatous damage (Tuulonen and Airaksinen, 1991, Tuulonen, et al., 1992).

In an early study, Sommer et al. (Sommer, et al., 1977) examined stereoscopic fundus photographs of 14 OHT eyes that converted to early glaucoma on the basis of visual fields. Each eye that lost visual field demonstrated consistent abnormalities of the RNFL, beginning as early as 5 years (mean, 1.5 years) before the development of glaucomatous visual field defects on routine Goldmann perimetry. Only 9% of the matched controls showed similar RNFL changes, but these were inconsistent and non-reproducible. A second study by the same group (Sommer, et al., 1979) set out to determine the sensitivity and specificity of certain RNFL and optic disc parameters as screening criteria for future visual field loss in a cross-sectional
study. A variety of disc parameters including horizontal and vertical cup: disc ratios, ‘width of the narrowest remaining rim’ and presence of RNFL defects was examined. The latter was the only parameter found by this group to be sensitive and specific enough to provide promise as a clinical screening tool.

A further larger study from Sommer’s group (Sommer, et al., 1991) retrospectively performed masked examination of red-free RNFL photographs of 1344 eyes with elevated IOPs but initially normal visual fields on Goldmann perimetry. 83 (6.2 %) of these went on to develop visual field defects, and these were compared with normal control eyes and those OHT eyes that maintained normal visual fields. RNFL defects were detected in 88% of readable photographs at the same time that the field loss occurred. 60 % of eyes had detectable RNFL defects 6 years prior to visual field loss. The RNFL was considered to be abnormal in 11% of normal and 26% of non-converting OHT eyes. Sommer’s work is particularly useful because of the absence of selection bias that has affected other studies.

Caprioli et al. (Caprioli, et al., 1996) carried out a later study to compare the rates of structural glaucomatous damage by four methods, in a prospective longitudinal study:

a) Qualitative evaluation of stereoscopic colour optic disc photographs, b) qualitative evaluation of monochromatic nerve fibre layer photographs, c) manual stereo-planimetric measurements of optic disc rim area, and d) computerised measurement of peripapillary nerve fibre layer height. One eye of 193 patients with glaucoma or ocular hypertension was evaluated at the beginning and end of a mean follow-up period of 3.3 years. The rates of structural change measured by these techniques and the rate of visual field
change measured with threshold automated perimetry were determined. Twenty-nine (15%) of 193 eyes progressed by qualitative optic disc evaluation; 14 (7.2%) eyes progressed by qualitative nerve fibre layer evaluation; seven (3.6%) eyes progressed by stereo-planimetry; and 24 (13.2%) eyes progressed by measurement of nerve fibre layer height. Visual field deterioration was detected in 12 (5.2%) of patients and correlated best with qualitative optic disc and nerve fibre layer evaluations. Evaluation by stereo-planimetry and RNFL height measurement detected change in eyes with primarily diffuse structural damage, a pattern not well detected by qualitative methods.

In Quigley’s study (Quigley, et al., 1992) comparing methods of examination of the optic disc and RNFL mentioned previously, red-free photographs of the RNFL were examined using a magnifier and qualitatively assessed as normal, as showing wedge-shaped or diffuse atrophy. Initial RNFL assessment was thought to be abnormal in 57% of those converters with readable photographs, and 35% of stable OHT control eyes. The RNFL defects were deemed to worsen in 49% of the former group and only 5% of the latter group. The most common description of an initial RNFL defect was diffuse atrophy, whereas newly developing defects were described as being wedge shaped. RNFL defects were therefore detected more frequently in this group than optic disc changes.
1.1.6 The optic nerve in ocular hypertension: histological findings

Quigley and co-workers have provided us with much of the histological evidence of possible pathological mechanisms underlying glaucomatous damage. On the basis of histological studies (Quigley and Addicks, 1981, Quigley, et al., 1981, Quigley and Green, 1979 Quigley, et al., 1983 Quigley, et al., 1987), Quigley has postulated that susceptible regions of the RNFL form the shape of an hourglass at the poles of the optic disc. By using the scanning electron microscope he has determined that the normal lamina cribrosa in the polar regions have larger pores for the passage of nerve fibre bundles, providing less structural support than in the temporal and nasal regions. This anatomical arrangement makes the fibre bundles at the poles more susceptible to the distorting forces associated with a raised intra-ocular pressure, and hence more damage is caused in these regions. There is considerable evidence to suggest that the larger ganglion cell nerve fibres sub-serving the magno-cellular pathway are relatively more susceptible to glaucomatous damage (Quigley, et al., 1987).

An early study (Quigley, et al., 1982) presented the histological findings in 5 OHT or ‘glaucoma suspect’ eyes compared with those from 5 normal eyes, and 13 glaucomatous eyes with established visual field defects. Manual estimates of axon counts were made using high-powered photomicrographs. An estimated 40% of nerve fibres were lost in one of the OHT eyes, in a fairly diffuse pattern but mainly at the inferior and superior poles. This was in an eye with previously normal visual fields on Goldmann perimetry. The other OHT eyes had relatively lesser degrees of nerve fibre loss. The glaucoma eye with the least extensive visual field loss had lost 50% of the estimated one million normal number of axons. The authors concluded that more than 40% of nerve fibres might be lost
before detectable change in Goldmann perimetry. Damage to the axons in the OHT and glaucoma eyes was greatest at the poles, corresponding to the pattern of arrangement of connective tissue sheets in the lamina cribrosa. The pre-mortem clinical appearance of the optic disc is not given in this report.

A later study (Quigley, et al., 1989) correlated the degree of retinal ganglion layer atrophy with the degree of loss of sensitivity on automated perimetry (including Octopus and Humphrey perimetry) in 6 glaucomatous eyes compared to 5 eyes from age-matched controls. A reduction in normal numbers of axons by 20% in the glaucomatous eyes was found to correlate with a 5-dB sensitivity loss in the central 30 degrees of the retina, and a 40% loss was required to cause a 10-dB loss.

The most recent post-mortem work was presented by Quigley at ARVO 1999 (Quigley, 1999), describing a study in a larger number of 17 eyes of 13 glaucoma patients with threshold visual field data were examined histologically. The locations of 28 tested points in the visual field were matched to specific retinal locations and the retinal ganglion cell count was estimated at these locations for each eye. Data from the glaucoma subjects was compared to 17 age-matched controls with no past ocular history. The results found that a 5-dB loss in sensitivity correlated to a 25% retinal ganglion cell count loss. An estimate of an age-related loss of 7,205 cells per year, between the ages of 55 to 95 was derived from examination of the normal eyes. This study also confirmed some of Quigley’s earlier work that found that the larger diameter axons were preferentially lost in glaucoma.

Differing results were found in a histological study carried out by Mikelberg et al. (Mikelberg, et al., 1995) which examined 8 eyes of 5 OHT
patients at post mortem. 3 patients had Goldmann perimetry ranging from 1 to 32 months prior to death, and 2 patients had Humphrey 30-2 threshold visual field testing 4-12 months prior to death. Automated image analysis techniques were used to calculate the total axon counts for each nerve head. Patients had a range of IOPs both on and off treatment from 16-38mmHg during life. The authors compared the counts of these nerves to those expected for age-matched controls, estimated from previous histological studies. This study found no significant differences between axon numbers counted in the OHT patients and those expected for the controls. Pre-mortem information on the optic disc appearance was given only for 3 of the patients, with only one of the eyes documented as having a clinically increased cup: disc ratio.

The number of eyes used in these studies are very small, and therefore unlikely to represent the full range of disc appearances that may be observed in OHT patients who have maintained normal visual fields.

Differing results from the two studies probably reflect the relative differences in the samples of eyes examined. Much larger histological studies on optic nerves from OHT patients would be of great value in determining how many axons are lost prior to the development of perimetric defects, particularly with current methods of automated threshold perimetry.
1.2 Measuring optic disc and RNFL change: Methods

From some of the studies mentioned above, it is clear that there is a wide variation in the methods used to detect glaucomatous change in both the optic disc and RNFL. Many of the older studies used mainly subjective, qualitative methods of analysis. The lack of agreement about the usefulness of such qualitative methods has limited the possibility of detecting pre-perimetric glaucoma.

Several authors have therefore recently attempted to provide objective methods to quantify change and these will be discussed in detail in this section.

1.2.1 Quantifying glaucomatous change in the optic disc

1.2.1.1 Analysis of stereophotographs.

The aim of stereo-photography is to provide images of the optic disc that when viewed stereoscopically give an impression of depth, and therefore allow evaluation of cup and rim parameters.

In planimetric measurement of optic disc parameters, the photograph is viewed stereoscopically and the edge of the optic disc and the optic cup are traced either manually, or on a computer screen. Computerised analysis techniques are then to measure rim area, and cup-disc area ratios (Airaksinen, et al., 1985 Caprioli, et al., 1996). This method requires the observer to map out the limits of the disc and cup and there may a degree of subjectivity involved with this.
1.2.1.2 Analysis of confocal scanning laser ophthalmoscopic (SLO) images.

This technique was relatively recently developed by groups in both the United States and Heidelberg, Germany. The system uses confocal optics comprising an imaging system with a known focal length and a detector located in a plane conjugate to the focal plane of the imaging system. A low-power diagnostic helium-neon laser beam is scanned point-wise line by line across the fundus and optic nerve. The reflected light from the illuminated area of fundus is deflected from the optical path of the laser by a beam splitter to a detector that measures the intensity of this reflected light. A diaphragm with a pinhole is placed in front of the detector to ensure that only light reflected from the focal plane of the laser is measured. Cross-sectional images of the optic disc are obtained by varying the focal plane of the laser and these images are reconstructed to create a three dimensional form. The Heidelberg Retina Tomograph (HRT, Heidelberg Engineering, Heidelberg, Germany) is the more widely used type of SLO used in both research and clinical settings. The methodology of the technique will be discussed in detail (Chauhan, et al., 1994, Rohrschneider, et al., 1993). The HRT acquires 32 equally spaced confocal images along the ‘Z’ axis that is perpendicular to the optical axis, and has an image resolution of 256 X 256 pixels. Scan areas can be set to 10 X 10 degrees, 15 X 15 degrees, or 20 X 20 degrees, and the scan depth can be varied from 0.5 to 4.0mm. A reflectivity image is generated by summing reflectance measurements along the Z-axis for each aligned pixel. The location along the Z-axis where the maximum reflectance is measured is assumed to be the height of the location and this information is used in the generation of a topography image.
Reflectivity of the image is measured on a relative percentage scale, and topography is measured in micrometers from the focal plane of the eye. In most examinations, three good quality image series are acquired per eye per sitting, through an undilated pupil. Integral software is then used to generate the topography of each of these series and once this has been obtained, the mean topographical image is generated. This mean image is then used for the final optic disc analysis. A mouse-drawn contour line is used to delineate Elschnig's ring or the optic disc edge. HRT software then analyses disc parameters both globally and segmentally. All analysis is done relative to a standard reference plane that is 50μm below the mean retinal height of a temporal segment of the rim. The following parameters are generally analysed: rim area, rim volume, cup area, cup volume, disc area. Third moment (cup-shape measure) and cup-disc area ratio may be calculated from the cup area and disc area measurements. Global or segmental analysis of these parameters is possible. There is also an option on the latest HRT software version 2.01 to analyse each area of the optic disc in 10 degree segments.

Several authors have examined the reproducibility of the HRT in optic disc analysis. Dreher et al. (Dreher, et al., 1991) carried out a study using 8 normal eyes and 8 glaucomatous eyes, acquiring five 15 by 15 degree images per eye. The mean height and standard deviation of each of 65,536 pixels over the five images was calculated. In normal eyes, the standard deviation for measurements in the optic nerve head was 41.2μm and in glaucomatous eyes this was 49.4μm with no significant difference between the two groups.

Chauhan et al. (Chauhan, et al., 1994) studied the test-retest variability of topographical measurements of the optic disc in 30 normal controls and 30
glaucoma patients. Again, the variability was tested at the individual pixel level by examining mean standard deviations of the topographical measurements at each point. Values of 25.94 μm for the controls and 31.20 μm for the glaucoma patients were obtained, and the difference in the variability between the two groups reached the level of significance. This study found that variability increased with the age of the subject. A high level of reproducibility of measurements taken with the HRT is obviously important if the technique is to be used for sequential analysis of optic disc images over time, in order to assess glaucomatous change.

Cioffi et al. (Cioffi, et al., 1993) investigated the reproducibility of depth measurements in 30 normal eyes. 30 separate images of each optic nerve head were aligned and specific regions compared in each image. Variability of depth measurements for the whole image was within 102μm (95% confidence interval). The highest level of variability was found where the rim sloped at the edge of the optic cup.

Other studies have examined the ability of the HRT to detect glaucomatous damage. One study (Iester, et al., 1997), investigated the relationship between optic disc parameters as measured by the HRT and visual field indices on the 30-2 programme of the Humphrey field analyser. For this study, optic disc appearance was unfortunately used to select both normals and controls. A statistically significant correlation was found between cup area, cup/disc area ratio, rim area, rim volume, Third moment and RNFL cross-sectional area with the mean deviation and corrected pattern standard deviation values on the visual field indices.
Zangwill et al. (Zangwill, et al., 1996) did a cross-sectional study comparing measurements of optic disc parameters made with the SLO in normal, glaucomatous and ocular hypertensive eyes. The results showed that although there was considerable overlap between study groups for all measurements made, the values obtained for the ocular hypertensive eyes were intermediate between those for normal and glaucoma eyes. Recruitment for this study was on the basis of clinical optic disc assessment, and the results should therefore be interpreted with caution. The normal and OHT eyes were judged to have ‘normal-appearing’ optic discs on stereoscopic viewing at the slit-lamp with a 78D lens, and on expert examination of stereo-photographs.

Uchida et al. (Uchida, et al., 1996) carried out a study to determine which structural optic nerve head parameters as measured with the SLO best discriminated between normals and POAG patients. They found that the Third Moment or cup shape measure best discriminated between groups, with a specificity of 86% and a sensitivity of 93%. Using the parameter values for cup-disc ratio, values of 77% and 93% respectively were obtained.

Hatch et al. (Hatch, et al., 1997) evaluated the use of the HRT in the assessment of the optic disc in OHT and glaucoma. Three subject groups were studied: OHT patients with normal optic discs on slit-lamp examination, subjects with glaucomatous optic discs on clinical examination, but normal visual fields, and patients with glaucomatous optic discs and visual fields. Three HRT parameters were investigated - rim volume, Third Moment, and the variation in relative RNFL height at the optic disc margin. ANOVA statistical analysis was used to determine if there were any significant differences between the three groups, and when these were present, the student t test was used to determine
differences between two individual groups. Not surprisingly, significant differences were found between the group of OHT patients with normal optic discs and those with glaucomatous discs but normal visual fields. Measurements in the glaucomatous disc groups with or without normal visual fields also differed significantly.

A German study (Mardin, 1999), evaluated the ability of the HRT to detect glaucomatous damage at the optic disc in OHT eyes without visual field defects. A large number of subjects were studied - 50 normals, 61 POAG patients with glaucomatous visual fields and 102 'pre-perimetric' OHT patients with clinically glaucomatous optic discs (assessed by stereo-photography). The authors found a great deal of overlap between the groups, but significant differences between the normal and OHT eyes. With the specificity set to 95%, a low level of sensitivity of the technique was obtained for detecting the OHT group using a multivariate approach, mainly because of the degree of overlap.

The main criticism of the studies discussed here that use optic disc appearance as an inclusion criterion, is that the results are likely to have been influenced by selection bias (Garway-Heath, 1998). The groups have been pre-selected on the basis of their optic disc appearance, which is the very parameter that is under investigation. It is therefore not surprising that differences are found between such groups that have been selected in this way.

One study which set out to eliminate such selection bias was carried out by Mikelberg et al. (Mikelberg, et al., 1995) in which they studied 45 normal and 46 eyes with early glaucomatous field loss. Optic disc appearance was not an inclusion criterion. The sensitivity and specificity of the HRT for detecting the early glaucoma cases was examined using discriminant function analysis and
values of 87% and 84% respectively were obtained. Important factors for detecting early damage were rim volume, third moment and maximum depth measurements.

Jester et al (Jester, et al., 1996) evaluated the ability of the HRT to detect glaucomatous field loss, and examined the relationship between optic disc size to diagnostic classification. This study did not include either POAG patients or normal controls on the of optic disc appearance, but rather on visual field and IOP characteristics. The authors assessed the sensitivity and specificity of parameters including cup shape measure, rim volume, and height variation contour in discriminating between the two groups, using a pre-determined formula combining all these factors along with the age of the patient. 60 normal and 93 POAG eyes were included in total. Significant differences were found between the groups. The sensitivity, specificity and diagnostic precision of the formula used were 74%, 88% and 80 respectively in discs of all sizes, with a trend for the sensitivity to increase as the disc size increased above 2 mm^2. The authors concluded that their discriminant analysis formula showed promise in allowing the detection of early glaucomatous damage, but that small optic discs with an area <2mm^2 may pose more of a diagnostic problem.

A more recent study (Wollstein, et al., 1997) also used a group of normals and patients with early field loss to determine the ability of the HRT to identify early glaucoma cases. Again, selection of subjects was not based on optic disc appearance. The highest levels of sensitivity and specificity for separating the two groups were obtained by using the 98% prediction interval from the linear regression between the optic disc area and the log of the neuro-retinal rim. Values of 92.5% and 86.3% were obtained respectively.
1.2.1.3 Comparison of Methods

Some studies have compared assessment of optic disc parameters using the HRT and photographic measurements. Dichtl et al. (Dichtl, et al., 1996) examined the optic discs of 25 normal eyes and 32 glaucomatous eyes with both the HRT and stereo-photographs using manual planimetry. They looked at only two parameters: the area and width of the neuro-retinal rim were determined as percentages of the optic disc diameter and area respectively. The study found that analysis with the HRT gave larger values than the corresponding measurements made by planimetry. The differences between the two methods were most marked in the nasal part of the disc, and increased with an increasing degree of glaucomatous damage in the optic discs examined. Using the HRT, the rim in the nasal part of the normal discs was widest whereas using the planimetric method the rim in the temporal segment was widest. One of the explanations given by the authors for this discrepancy included an erroneous inclusion of the nasal blood vessel trunk in the calculations made by the HRT, thus leading to an increased value. They also noted that it is easier to trace the edge of the disc in normal eyes on stereo-photographs than on HRT images. This lack of distinction between the different tissues of the optic disc head is therefore a potential problem with the HRT if cross-sectional data is analysed to determine if early glaucomatous change is present or not. An over-estimation of the neuro-retinal rim area by including blood vessels in the analysis may miss minor changes in the configuration of the rim. A more recent study (Jonas, 1998) conversely found that the HRT made smaller optic disc area measurements than manual planimetry, but there were no other significant differences between measures made by the two techniques.
Garway-Heath et al. (Garway-Heath DF, 1999) compared the intra-and inter-observer variation in the analysis of optic disc images using the HRT and computer assisted planimetry. Variability was determined by calculating the standard deviation of differences as a percentage of the mean. The study found that the overall inter observer agreement was better using the HRT, for measurement of all the parameters examined. Neuroretinal rim area measurements using planimetry gave variability values of 16.3% as opposed to 8.1 % using HRT. Intra-observer variability levels were similar for the two methods - 4.0 % for planimetry and 4.5 % for HRT. Greater levels of variability (2.4 % compared with 1.5% for planimetry), were obtained when the same observer repeatedly delineated the same disc edge using the HRT, a result supporting the earlier suggestion that the disc edge is more easily seen on photographs.

In conclusion, the HRT has been demonstrated to show high levels of reproducibility in the analysis of normal and glaucomatous optic discs, and relatively good levels of specificity and sensitivity in separating groups of patients. It provides a more objective way of examining optic disc parameters than previously used methods, although current techniques in which the optic disc edge is outlined manually may give rise to a degree of variability (Orgul, et al., 1997).

The studies described so far have evaluated the use of the HRT on a cross-sectional level. The most important potential use of the HRT is in the follow up of the OHT or POAG over time. Work presented as part of this thesis was the first such longitudinal study to be published (Kamal DS, 1999) – ‘Detection of
optic disc change with the Heidelberg Retina Tomograph prior to confirmed visual field change in ocular hypertensives converting to early glaucoma’.

The main drawbacks to the widespread use of the HRT in the clinical setting are its cost, the requirement of experienced personnel to acquire the image series, and the time required for the generation and analysis of topographical images. The interpretation of the results of many of the studies cited above has been limited by the inherent selection bias introduced by including both normals and patients on the basis of optic disc appearance. The studies reported in this thesis have eliminated this source of selection bias. All the subjects investigated were selected on the basis of IOP and visual field criteria, rather than optic disc appearance. Normal controls were only included if they had no family history of POAG or OHT. All subjects demonstrated the absence of any other ophthalmic disease.
1.2.2. Quantifying glaucomatous change in the RNFL

Examination of the RNFL has up until recently been a mainly subjective exercise. Stereoscopic clinical examination at the slit-lamp using red-free light and a fundus lens has provided qualitative information about the presence of RNFL defects and whether they are localised or diffuse. Post-hoc examination of the RNFL is achieved by the use of red-free photography, allowing a qualitative assessment about the type of defect.

1.2.2.1 Semi-quantitative methods.

Some authors have recently attempted to introduce techniques to quantify RNFL assessment. Quigley et al. (Quigley, 1993) described a semi-quantitative grading method used to interpret red-free RNFL photographs. Defects were graded on the basis of brightness of the RNFL reflex, their ‘texture’ and the degree to which the RNFL obscured the view of retinal blood vessels. RNFL defects were then given one of 4 possible grades on the basis of these factors - normal, mild, moderate and severe. Observers of different levels of experience were trained to use this method over a 4-hour period. Subsequent inter-observer agreement between 5 newly trained observers and one expert observer was found to be high. Reproducibility of gradings made by the expert was also high. Good correlation between the grade of RNFL defect and other parameters such as optic disc change and visual field indices was also found. Although the authors have made some attempt to semi-quantify RNFL defects, there is still a great deal of subjectivity involved with this method of assessment.
Another group developed a standardised grading system using a reference set of 25 red-free photographs (Niessen, 1995, Niessen, 1998), with different grades of RNFL thickness scores ranging from 25 to 0. These photographs were simultaneously viewed along with the photograph under examination, and a score was given. Good levels of intra- and inter-observer reliability, by both experienced and inexperienced observers were demonstrated. Clinical validity, in which the scores for upper and lower halves of photographs were compared with the mean deviation of the Humphrey 30-2 visual field program, was also good.

1.2.2.2 Quantitative measures.

The HRT does not measure RNFL thickness directly but measures retinal height and calculates the retinal nerve fibre layer cross-sectional area. This calculation is made on the assumption that the mean thickness of the RNFL at a distance of 1.5 disc diameters from the centre of the disc is 50 \( \mu\text{m} \) - a measurement obtained from studies in monkey rather than human eyes (Weinreb, et al., 1990). The measurement is also made relative to the standard reference plane of 50\( \mu\text{m} \) below the mean retinal height of a temporal segment of the rim contour. It is assumed that this measure is stable in-patients with early glaucoma, but variability must exist in all individuals. Values of the RNFL cross-sectional area also include the area of glial cells. The HRT provides a measurement of RNFL thickness relative to a standard reference plane, rather than an absolute and independent measure (Weinreb, et al., 1995).

The Nerve Fibre Analyser (NFA) was therefore developed by Laser Diagnostic Technologies, San Diego, with the purpose of providing a direct, objective and quantitative assessment of RNFL thickness. The machine
incorporates a polarimeter within SLO technology. The technique of scanning laser polarimetry (Weinreb, et al., 1995) depends on the principle that the RNFL has bi-refringent properties. Each nerve fibre has a diameter less than the wavelength of polarised light emitted by the SLO that is incident on the RNFL. The light source consists of a 780nm-diode laser in which the state of polarisation is modulated. The polarised light enters the RNFL, and is partially reflected from the deeper layers of the retina. Thus the degree of polarisation of this light is changed depending on the thickness of the RNFL. Light emerging from the eye is separated from the illuminating light beam by a non-polarising beam splitter. The change in polarisation of the incident light is termed 'retardation' and the polarimeter detects the amount of retardation caused by the RNFL. The cornea and lens also have bi-refringent properties that may produce measurement artefacts, therefore newer generation machines have polarisation compensators to neutralise the polarisation effects of the anterior segment. An SLO image of the optic disc and peri-papillary retina is acquired through an undilated pupil, and the NFA produces a topographical retardation map representing the RNFL thickness in specific areas. This map is made up of a total of 65, 536 retinal locations that are singly tested over a 15 degree retinal area with a spatial resolution of approximately 17 x 17μm. NFA software is used to convert retardation figures into RNFL thickness values, using Fourier analysis. Each degree of retardation is equivalent to 7.4μm of RNFL thickness (Weinreb, et al., 1990). No reference plane is required for the calculations and the NFA theoretically gives an absolute measure of RNFL thickness. The outputs of the polarimeter are digitised and stored by the computer for later analysis. In order to carry out the analysis, a contour line is fitted to the disc edge as closely as
possible using the mouse. An area of peripapillary retina equivalent to 1.5 disc diameters is automatically included in the area of analysis. NFA software is used to calculate RNFL thickness in 4 main segments: superior, inferior, nasal and temporal and a mean total value is also given. Recent advances in the software (GDx manual 1998) also give the option of calculating ratios of measurements within the quadrants. The GDx is the latest available version of the NFA, and is the machine used in the study reported in this thesis.

1.2.2.3 Previous studies using the NFA or GDx

Although the technique of scanning laser polarimetry has been only relatively recently introduced, several studies have investigated its use in the examination of normal subjects and patients with OHT and POAG. Weinreb et al. (Weinreb, et al., 1995) carried out a study to evaluate the association between quantitative RNFL measurements using the NFA and visual field loss in patients with POAG. They found an association between a decreasing RNFL thickness, and global indices of visual field loss such as mean deviation and corrected pattern standard deviation. The test-retest variability of 5 independent measurements of 5 normal eyes obtained on 5 separate days was low with a mean coefficient of variation of 4.5%.

Niessen et al. (Niessen, et al., 1996) compared the results of RNFL assessment using scanning laser polarimetry and standardised photography techniques, in 60 POAG or OHT patients and 24 healthy subjects. They also determined if there was any correlation between the structural findings and visual
field indices by the two methods. The reproducibility of scanning laser polarimetry was found to be 6.6%. Photography was performed under red-free conditions, and a standardised grading system previously devised by them (Niessen, 1995) was used in the analysis. The study found a poor correlation between the polarimetry values and the photographic scores. Correlation of the mean deviation scores of the visual field results with the polarimetry results was lower than with the photographic method. This study was limited by the method of selection of subject eyes. This was done on the basis of visual field, IOP and ophthalmoscopic optic disc findings for the OHT and POAG group, but only on history and examination for the control group of normals - no visual field testing was performed in this group if no abnormality was found. Even fellow eyes of glaucomatous eyes without clinical evidence of early damage were included as ‘normals’.

More recent studies have compared results obtained with the NFA for RNFL thickness in normals, POAG and OHT patients. One group (Tjon Fo Sang, et al., 1996) compared RNFL thickness values in the superior and inferior quadrants in 210 normals and 100 patients with OHT. The mean values for the OHT group were significantly lower than for the controls.

Hollo et al. (Hollo, et al., 1997) evaluated the clinical use of the GDx in POAG and ‘capsular glaucoma’. Polarimetry was performed on one eye of 30 patients with POAG, 25 patients with capsular glaucoma and 35 normal controls. The results found that the mean RNFL thickness in the superior and inferior quadrants as well as along the total circumference was significantly lower in both glaucoma groups than the normals. There were no significant differences between measurements in the nasal and temporal quadrants. Again, subjects were
selected on the basis of the normality/abnormality of their optic disc appearance. Reproducibility of measurements was also examined for the three groups and was found to be comparable, with the coefficient of variation ranging from 3.0% to 8.9%. The greatest variability was found in the temporal segment measurements.

A study from Hong Kong (Lee, 1999) assessed RNFL thickness with the GDx in 80 normal and 75 age-and race-matched eyes. They analysed the data on the basis of ratios of measurements between the segments. They found that the superior/nasal and inferior/nasal ratios were the most sensitive parameters to differentiate between the groups. Another study (Xu, 1998) evaluated a new method of data analysis to distinguish between groups of 36 normals, 69 glaucoma eyes, and 19 OHT eyes. Modulation parameters were used in which the average of the nasal and temporal minimum retardation was subtracted from the retardation in the superior or inferior segments. They found that these modulation parameters gave the best level of differentiation between groups. This study used data that was obtained from an area 1.8 disc diameters away from the centre of the disc, an area previously shown to provide less reproducible measurements. The use of data from the nasal and temporal quadrants is also questionable in these studies, as the RNFL is thinnest in these regions (Weinreb, et al., 1995).

Weinreb et al. (Weinreb, 1998) compared GDx measurements from 84 eyes with those from 83 eyes with early to moderate glaucomatous loss. Differentiation was limited by overlap, but the best discriminant function was established using total average thickness, ellipse modulation and average ellipse thickness, all parameters measured directly by GDx software.
Some investigators have found that there is a progressive decline in the RNFL thickness as measured by the NFA with increasing age. One study (Chi, et al., 1995) examined 75 eyes of 75 normal volunteers aged 20-66 years using the first generation NFA. Patients were divided into 5 age groups, and significant differences were found in the RNFL thickness by age group. All normal subjects had normal automated perimetric results, but also were selected on the basis of having clinically normal optic discs. The authors estimated that the RNFL thickness as evaluated by the NFA decreased linearly with increasing age by 0.2μm per year. The reproducibility of measurements made with the NFA was assessed in a separate part of the study on a small number of normals and patients with POAG. The most reproducible diameter ring of examination used the NFA was the 1.5 disc diameter area, with a mean coefficient of variation of 3.59 +/- 2.14% for normals, and 5.65 +/- 2.44 % for the POAG patients.

Pinoosawmy et al. (Pinoosawmy, et al., 1997) also found an age-related decline in the RNFL thickness in a group of 150 normal volunteer patients. Values obtained from the eyes of Afro-Caribbean patients were also compared with those obtained from Caucasians, and significantly lower RNFL thicknesses were found for the former group. The rate of decline of RNFL thickness was estimated by this study to be in the region of 0.38μm per year. The relative difference between the ethnic groups was thought to reflect a difference in the optic disc size between the two groups. If measurements of RNFL thickness are made at a distance of 1.5 disc diameters, then this would be expected to be further towards the periphery in eyes with larger optic discs.
In summary, preliminary studies that have investigated the use of the NFA or GDx in the analysis of RNFL thickness have found that significant differences do exist between groups of subjects, although there is considerable overlap in values. Levels of reproducibility are good, and the age-related decrease in thickness should be taken into account during the individual assessment of results. The analysis of The RNFL in Afro-Caribbean patients should take into account morphological differences in optic disc size, which may not be provided by the available normative databases. However, as for many of the SLO studies, the main criticism of some of those cited above is the selection bias introduced by adding disc and RNFL appearance to the inclusion criteria for subjects. It is important to assess whether differences in RNFL thickness may be detected by the NFA or GDx between groups included on visual field and IOP characteristics alone (Kamal, 1999).
1.3 The Treatment of OHT

1.3.1. Topical β-Blockers.

This class of drugs has formed the first-line treatment for raised IOP since the introduction of timolol in the late 1970s. The mode of action of topical β-blockers is to reduce the production of aqueous by the blockade of β₂ receptors on the non-pigmented epithelial cells of the ciliary body (Bromberg, 1980). If given chronically, they have been shown to have a long lasting effect for up to 4 weeks (Schlecht and Brubaker, 1988). Unlike the miotics they have little effect on pupil size or accommodation, and unlike the epinephrine compounds they do not cause mydriasis or reactive hyperaemia. There are two main classes of β-blockers; non-selective, affecting cardiac β₁ receptors and respiratory β₂ receptors; and β₁-selective compounds that have little effect on respiratory receptors. Timolol, carteolol and levobunolol drops are non-selective and therefore can affect both these systems. Most β-blockers have little or no capacity to stimulate β-receptors apart from carteolol which does have some intrinsic sympathomimetic activity or ISA. This should theoretically result in fewer systemic side effects but this has not been proven clinically (Stewart, 1994). Administration of all the beta-blocker preparations is on a twice-daily basis. Timolol and levobunolol are available in preservative free preparations.

Betaxolol is relatively β₁cardio-selective, with a lesser effect on the respiratory receptors. It was first introduced in the early 1980’s (Berrospi, 1982).
The mode of action of betaxolol is still slightly puzzling. It is thought to reduce aqueous production by the ciliary body via a blockade of the $\beta$-receptors, but as mentioned before these are thought to mainly comprise $\beta_2$ receptors. Other explanations have been put forward (Feghali and Kaufman, 1985) including; betaxolol may also have $\beta_2$ blocking capacity; $\beta_1$ receptors may also mediate aqueous production; betaxolol may influence aqueous production by a mechanism unrelated to $\beta$-blockade.

Several trials have shown that betaxolol is less effective than timolol (Stewart, et al., 1986; Collignon-Brach, 1992) or levobunolol (Long, et al., 1988). However, one randomised study found that betaxolol and timolol were similarly effective in reducing the intra-ocular pressure (Berry, et al., 1984). Betaxolol is commercially available as Betoptic, manufactured by Alcon Laboratories in a 0.5% solution or a 0.25% suspension. Onset of action is noted in 20 minutes with a peak IOP lowering effect at 2 hours and a single dose lasts for about 12 hours. Betaxolol appears to be absorbed into the systemic circulation via the nose about 4 times less than timolol (Vuori, 1993).

Several studies have demonstrated that betaxolol may have an enhancing effect on ocular blood flow as compared to other $\beta$-blockers. Boles-Carenini et al. (Boles Carenini, et al., 1994) followed 14 glaucomatous eyes on timolol and 11 eyes on betaxolol over a 12 month period with pulsatile ocular blood flow (POBF) measurements. The POBF fell over time in the timolol group but not in the betaxolol group, despite a similar IOP lowering effect in the two groups. The authors concluded that the role of ocular blood flow on the pathogenesis of glaucoma remained to be clarified, as 90% of the POBF may be due to the choroidal circulation, with an unclear significance.
Gupta et al. (Gupta, et al., 1994) randomised each eye of 10 ocular hypertensive patients to treatment with either betaxolol or hypromellose as the placebo. Retinal blood flow was measured by means of laser Doppler velocimetry just prior to the instillation of drops and two hours later. The measurements from the two eyes of the patients were then compared. The study reported an increase in retinal blood flow of 15% in the betaxolol treated eyes with no significant change in the placebo treated eyes. A 27.7% reduction in IOP was achieved by betaxolol leading to a 16.9% increase in perfusion pressure compared to an 8.4% increase in the placebo treated eyes. The systemic absorption of betaxolol and its effect therefore on the pressure and blood flow dynamics of the fellow eye were likely to have played a role in this effect.

Pillunat (Pillunat and Stodtmeister, 1988) found no effect of either timolol or betaxolol on ocular perfusion pressure but they achieved a much more modest IOP lowering than the Gupta study. Another study (Harris, et al., 1995) used a one month double-blind cross-over design study of 13 normal tension glaucoma patients to determine any differential effects on the retinal blood flow of timolol Vs betaxolol as measured by Doppler colour imaging. Interestingly, they found that betaxolol did not significantly lower the IOP, but that it did increase the end-diastolic velocity measured in the vessels under study, and lowered the vasculature resistance index in contrast to timolol which did lower the IOP.

Hester et al. (Hester, et al., 1994) confirmed a positive effect on ocular blood flow of betaxolol as compared to timolol and carteolol in the porcine posterior ciliary artery and suggested that this was due to an increased ability of betaxolol to inhibit calcium ion mediated vascular spasm. It has been suggested
that the reason for the differential effects of these beta-blockers on ocular blood flow is their relative inhibitory influence on $\beta_2$ receptor mediated dilatation in the retinal and choroidal vasculature (Collignon-Brach, 1994). Betaxolol has a less marked blocking effect on these receptors, and ocular blood flow is therefore maintained.

Several studies have shown that betaxolol may provide long-term preservation of visual field sensitivity compared to timolol. Kaiser et al. (Kaiser, et al., 1994) followed 17 glaucoma patients treated with timolol and 12 treated with betaxolol over a four year study period with automated perimetry. They found an increased mean sensitivity and a lower mean deviation at 3, 6, 12 and 18 months for the betaxolol group, but effect was lost with further follow-up. The authors suggested that a larger group was needed to further validate these results.

Collignon-Brach et al. (Collignon-Brach, 1994) also carried out a 4 year prospective study on 19 OHT or POAG patients randomised to treatment with either betaxolol or timolol to compare the effects on IOP and the mean retinal sensitivity (as measured by Octopus automated perimetry). Despite the fact that timolol was found to produce a statistically more significant reduction in IOP than betaxolol, but the mean sensitivity index in the betaxolol group showed a relative increase at the 12, 24, 36 and 48 month visits.

Drance (Drance, 1998) compared the effects of betaxolol, timolol and pilocarpine on the visual function of patients with POAG by means of a randomised trial. Betaxolol had a lesser hypotensive effect than the other medications, but despite this there was no differential effect on visual function as assessed by visual fields, motion detection and contrast sensitivity. The time
course of the study was rather short at 24 months, perhaps insufficient to demonstrate any difference.

A possible neuro-protective effect of betaxolol was demonstrated by Osborne et al. (Osborne, 1997) who performed in vitro and in vitro studies in rabbit and rat eyes. Retinal ischaemia was induced by elevating the intra-ocular pressure for 45 minutes followed by a period of re-perfusion. Electro-retinograms showed a marked reduction in the b-wave induced by the ischaemia. The effect of injecting betaxolol prior to the period of IOP elevation and then once or twice daily was compared to a control injection of normal saline. The results found that the betaxolol treated eyes showed a complete recovery of the b-wave amplitude, with the twice-daily betaxolol having an even greater effect. The authors suggested that the observation might be mediated by a calcium channel blocking effect of betaxolol to prevent the influx of Ca\(^{2+}\) ions into cells. The release of agents mediating ischaemic damage is therefore limited. Further evaluation of a possible neuro-protective effect is needed to augment these results.

The \(\beta_1\)-selective action of betaxolol has been shown to cause less systemic and respiratory side-effects as compared to other non-selective topical \(\beta\)-blockers (Diggory, 1994, Diggory, et al., 1995 Frishman, et al., 1994, Goldberg and Goldberg, 1995), making it a safer choice of drug for a randomised clinical trial. However, up to 41% of the elderly population may have some form of respiratory disease, much of it undiagnosed (Diggory and Franks, 1996), and the prescription of a \(\beta\)-blocker should always be accompanied by a warning to the patient to stop their use should any adverse effects be noted. The use of beta-blockers is contra-indicated in patients with respiratory symptoms or
bradycardia. Other well-documented side effects of β-blockers include heart failure, hypotension and impotence.

In summary, topical betaxolol is a cardio-selective β₁-blocking agent, which is an effective ocular hypotensive agent. There is reasonable evidence that it is a less effective hypotensive than the gold standard β-blocker timolol, but independent beneficial effects on ocular blood flow may offset this. At the start of the clinical trial reported in this thesis, betaxolol was a relatively new topical agent for use in the treatment of glaucoma. For the reasons discussed previously, this particular drug was chosen as the active agent in the trial, for comparison against a placebo.
1.3.2 Previous treatment / no treatment trials

The pros and cons of prophylactic treatment of ocular hypertension have been investigated by several major treatment trials.

One of the first studies which examined the effect of topical hypotensive treatment on the outcome of OHT was carried out by David et al. (David, et al., 1977). However, this was not a randomised-controlled trial and patients treated with ocular hypotensives were selected on the basis of being at increased risk for conversion to early glaucoma. 61 patients (117 eyes) with OHT were followed over a period of 1 -11 years. OHT was defined using the following criteria: mean untreated IOP over 3 readings with Goldmann tonometry ≥ 21 mmHg; normal Goldmann visual fields; normal appearance of the optic disc on clinical assessment; open filtration angle. 50 eyes were treated in total and this group consisted of 3 subsets: those with IOPs ≥35mmHg; those with OHT in an only eye; and a separate group treated as part of the prospective study. Treatment was with pilocarpine (2,3 or 4%) 3-4 times per day, with adjunctive adrenaline 2% twice daily if the IOP was not controlled on monotherapy. The response to treatment was considered to be ‘good’ if a decrease in IOP ≥ 11mmHg occurred, ‘fair’ with a drop of 6-10mmHg and ‘poor’ if the drop was < 5 mmHg. 48 eyes (41%) were followed up for more than 42 months, 69 (59%) for more than 3 years, and 29 (25%) for more than 5 years. The mean age at diagnosis was 61.2 years. 12 eyes in total (10.2%) converted to glaucoma on the basis of optic disc or visual field criteria over the follow up period. 9 of these were on treatment when conversion occurred. Most of these eyes had an initial mean IOP level > 31
mmHg. The authors concluded that the level of the initial mean IOP was the most important risk factor for predicting the development of POAG. The risk for patients with an IOP level < 25mmHg was 2.7%, and in those with an IOP level >31 mmHg, this rose to 41.2%. Most of the eyes that developed POAG had a good response to treatment, and it was concluded that the initial untreated IOP level was a more important factor than the treated IOP, in determining risk. The higher rate of conversion in the treated group reflects the greater incidence of risk factors in these patients, and the inherent selection bias in the study.

A later study by Epstein et al. (Epstein, et al., 1989) carried out a prospective, randomised treatment Vs no treatment trial with timolol drops. Inclusion criteria for OHT subjects included open angles; no previous anti-glaucoma therapy; an IOP of 22-28mmHg in at least one eye; cup asymmetry between the two eyes of less than 0.2; normal Goldmann fields; absence of any abnormal disc cupping; and no contraindications to treatment with β-blockers. Patients were randomly assigned to treatment with timolol 0.5% twice daily, or to no treatment, and reviewed every 3 months. The failure criteria for this trial have been described earlier and included IOP, visual field and optic disc change end-points. The statistical analysis methods of this study can be criticised in that analysis was based on patients rather than eyes. Data from the two eyes were therefore averaged, except when one eye met any of the failure criteria. It cannot be assumed that both eyes will respond to treatment in the same way, and there may be a degree of initial asymmetry between the two eyes. IOP data was averaged over all the follow up visits over a 3-month period, to avoid including patients more than once if they had attended more frequently. Continuous variable data was compared between the two groups using the independent t test.
Much of the data was analysed by computing Kaplan-Meier survival curves, and by using Cox proportional hazards models to give adjusted risk ratios with 95% confidence intervals. An 'intent to treat' analysis was used to analyse patient data in their originally assigned group, even if for some reason they stopped taking the treatment. An alternative analysis or ‘treatment analysis’ was also carried out in which patients who discontinued timolol were only included for 6 months after stopping and then treated as if they had stopped attending.

In total, 109 patients were recruited to the study over a 6-year period. 2 were randomised but never attended for follow up and were therefore treated as if they had never joined the study. 53 patients were randomly assigned to the timolol treatment group (14 of these had only one eye assigned), and 54 patients were assigned to the no treatment group (12 of these had only one eye assigned). Follow up was for a mean of 56± 29 months in the treatment group and 51± 29 months in the no treatment group. The authors report that the two groups were similar for baseline IOP (24±1.3 mmHg for treated, 23.9±1.6 mmHg for non-treated); baseline cup/disc ratio (0.34±0.14 Vs 0.39±0.16); and age (60± 11 years Vs 59± 12 years). 10 patients had adverse reactions to timolol therapy, including bradycardia, asthma, dizziness and palpitations, and confusion. The treatment group had overall quarterly IOP levels that were significantly lower than the non-treated group but this decreased over time as more of the treated group were withdrawn. 26 patients reached one or more end-points and were deemed to have failed. A large number of patients (23) were lost to follow-up with equal numbers from the two groups. 58 patients were still being followed up at the end of the study.
The table below summarises the reasons for ‘failure’.

<table>
<thead>
<tr>
<th>Reason</th>
<th>Treatment group</th>
<th>Non-treatment group</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of failures</td>
<td>9</td>
<td>17</td>
</tr>
<tr>
<td>IOP too high</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>VF change</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>Optic disc change</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Other (examiner decision)</td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>

6 of the 9 failures in the ‘treatment group’ occurred after patients had stopped using timolol because of adverse reactions. In the intent-to-treat analysis the treatment group had an adjusted risk ratio (ARR) of 0.38 (p=0.03). In the treatment analysis, it was found that treatment with timolol was highly protective, with an ARR of 0.25 (p=0.008). Baseline IOP asymmetry of ≥ 3mmHg was a strong risk factor for failure (ARR=5.2, p=0.0003). Cup/disc ratio ≥ 0.5 was also a significant risk factor (ARR= 4.1, p=0.0008). The authors concluded that the results demonstrated a favourable influence on the clinical course of OHT, and in the discussion they advocated the earlier treatment of patients with mildly elevated IOP.
The study was one of the first randomised controlled trials to evaluate the effect of treatment on the course of OHT. It may be criticised in that the number of subjects included was relatively small, especially after the dropout rate was considered. This reduced the power of the study to demonstrate an effect of treatment on disease outcome. The data of patients who stopped treatment because of adverse effects were still included in the treatment group analysis for 6 months after the cessation. End-points for failures included 'definite' optic disc changes that were poorly defined, allowing room for subjective error. A patient could also be deemed to have failed for ethical reasons if the examiner believed their vision was 'at risk', again a poorly defined criterion. Finally, the study was obviously not 'double-blind', as the control group had no treatment rather a placebo drop. This may have influenced some of the measures made in the trial.

Kass et al (Kass, et al., 1989) carried out a randomised double blind prospective study to investigate whether treatment with timolol drops was effective in delaying or preventing the onset of glaucomatous damage in moderate risk OHT patients. Entry criteria included the following; baseline IOP>21mmHg<35mmHg; IOP asymmetry between the 2 eyes of <3 mmHg; age >40 years; normal Goldmann visual fields; normal optic discs; open angles on gonioscopy. 62 patients with both eyes fitting these criteria were entered into the study, and one eye per patient was randomly allocated to receive timolol drops while the other eye received placebo drops. Each eye initially received drops in a bottle labelled strength ‘1’- containing timolol 0.25% or placebo. If the IOP was found to be greater than 20mmHg at any point during the study, then the treatment in that eye was increased to strength ‘2’ - containing timolol 0.5% or placebo. Investigators and technicians performing the visual field tests and optic
Disc examinations were masked to the treatment modality. Patients were seen every 4 months and underwent IOP and visual field examinations at each visit. Stereoscopic disc photographs were taken every year.

The primary end-point of the study was the development of a reproducible visual field defect on 3 consecutive tests. Automated Octopus or Humphrey perimetry became available during the course of the study and was performed on each patient once or twice a year. If the visual field became consistently abnormal, the treatment code was broken for that patient, and the affected eye treated according to standard clinical practice. However, follow-up on the fellow eye receiving timolol drops or placebo continued in an unmasked fashion. The treatment of the fellow eye was not changed unless a reproducible visual field defect also developed.

The secondary end-point of the study was the demonstration of progressive optic disc cupping from serial stereoscopic disc photographs. The definition of change was rather unclear, and based on a qualitative assessment of any generalised or localised thinning of the neuroretinal rim by a panel of masked clinicians. Patients with progressive disc change were not withdrawn from the study or treated unless they also developed visual field loss. IOP level was not a study endpoint.

Baseline IOP levels were calculated by averaging all measurements taken over the year prior to entry into the study. The effect of treatment on IOP level over time was analysed using the ANOVA statistical test. The McNemar test for paired observations was used to assess whether treatment with timolol reduced the incidence of glaucomatous optic disc and visual field damage.
Follow-up was from 5 to 8 years, with a mean of $61 \pm 26.5$ months. 65 patients were recruited in total, with 3 patients judged to be ineligible, leaving 62 patients for inclusion in the study. 5 patients developed adverse reactions, including bronchitis and asthma, and treatment was discontinued. 2 patients died from causes unrelated to treatment factors. 18 further patients were withdrawn for other reasons, and 11 patients reached the study endpoint of glaucomatous visual field change.

Baseline characteristics of the eyes in the timolol and placebo groups were very similar, probably because they were in the same patient. IOP levels were $26.4 \pm 4.4$ mmHg, and $26.6 \pm 4.5$ mmHg respectively. Mean cup/disc ratio was $0.48 \pm 0.2$ for the timolol group and $0.49 \pm 0.21$ for the placebo group, and mean ages were comparable. The mean difference in IOP between the groups during the course of the study was $2.3 \pm 2.6$ mmHg and this had a significance level of $p<0.0001$.

The number of patients reaching the study end-points are summarised below:

<table>
<thead>
<tr>
<th></th>
<th>Timolol treated group</th>
<th>Placebo treated group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visual field change</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>Optic disc change</td>
<td>4</td>
<td>8</td>
</tr>
</tbody>
</table>

The mean base-line IOPs of the 14 eyes that developed visual field loss were $31.2 \pm 7.2$ mmHg and $25.9 \pm 3.6$ mmHg for the 110 eyes that did not. The
authors concluded that medical treatment reduced the incidence of glaucomatous visual field loss and optic disc cupping in patients with OHT, due to a reduction in the IOP level. The study was an expedited publication.

There are several major criticisms of this study, which essentially invalidate the results. It is not clear why each eye of the same patient was randomised to the different treatment groups. Treatment with topical timolol may lead to a reduction in the IOP of the contralateral eye via systemic absorption, and indeed this effect was observed by the authors. Therefore, the results of the study have been confounded by this effect. Secondly, although the investigators were masked to the initial treatment groups of the eyes in the studies, the codes were broken when the endpoint was reached and the masking was removed to the treatment of both eyes. This factor may also have confounded the results. Thirdly, the number of patients who left the study was large, reducing the power of its findings. As pointed out by Schulzer et al. (Schulzer, et al., 1991) in the discussion of their subsequent study, the statistical design of the Kass study assumed that pressure reduction would either be protective or not, and did not allow for the possibility that treatment might in some way be detrimental. They suggested that a two-tail test of significance would have been more appropriate, and that if this had been used then none of the results would have reached significance.

Schulzer et al. (Schulzer, et al., 1991) then reported on a prospective 6 year clinical trial in which 143 OHT patients were randomised to either treatment with timolol or no treatment. Recruitment criteria included an initial IOP level of 22 mmHg or higher on at least 3 separate occasions, normal Goldmann visual fields on at least 2 consecutive occasions, and no obvious signs of acquired optic
disc change characteristic of glaucoma. Patients were excluded if they had other ocular disease, any contra-indications to the use of beta-blockers, poor reliability indices on visual field performance, or a VA of less than 20/70 due to cataract. Base-line investigations included ophthalmoscopy, colour stereo-photography, 3 automated visual field examinations (initially on the Perimetron machine and later on the Peritest automatic perimeter) performed at least 2 days apart, and ECG recording. Each patient was re-evaluated once a month for 4 months during the initial recruitment phase. Stereo-disc photography was performed annually and assessed in a masked fashion.

Each of the recruited patients underwent at least 6 years of follow-up. 73 out of the total 143 recruited were assigned to the untreated control group and 70 were assigned to the treated group. 2 recruited patients were found to be ineligible and 4 failed to complete the baseline investigations. Therefore, 137 patients (70 controls, 67 treated) were followed in total, but 36 of these were lost to follow-up during the course of the study.

Study end-points included the development of reproducible visual field defects on automated perimetry, optic disc haemorrhage, or documented optic nerve head change on stereophotography. A visual field was determined to be abnormal on the Peritest if any two adjacent points were depressed by 6 dB or more; if a single point in the central 30° was depressed by 10 dB or more; if any point immediately above or below the horizontal meridian was depressed by 0.6 dB or more; or if any point within 5° of fixation was depressed by 0.4dB or more. For a visual field defect to be confirmed, the same points had to be abnormal on a second repeat test, and then repeated on visual field testing on the Octopus or Humphrey perimeter.
42 patients reached study end-points. 28 of these developed visual field defects by the above criteria, 8 developed documented optic disc change, and 6 patients had optic disc haemorrhages. 20 patients in total were in the treatment group and 22 were in the control untreated group. A further breakdown of the results is given in the table below.

Summary of results from Schulzer et al. 1991

<table>
<thead>
<tr>
<th></th>
<th>Treated</th>
<th>Untreated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total reaching study end-point</td>
<td>20</td>
<td>22</td>
</tr>
<tr>
<td>Development of VF defects</td>
<td>15</td>
<td>13</td>
</tr>
<tr>
<td>Optic disc change</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Optic disc haemorrhage</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Mean IOP during study (mmHg)</td>
<td>21.77</td>
<td>26.28</td>
</tr>
<tr>
<td>Mean survival time (days)</td>
<td>2061</td>
<td>1942</td>
</tr>
</tbody>
</table>

No statistically significant differences were found between the two groups for any of these factors.

Survival analysis was carried out within the two groups to examine if there was any correlation between any of the end-point criteria and any of the co-variates, including baseline cup-disc ratio, baseline IOP level, mean follow-up IOP level, or mean IOP level change. No correlation was found for any of these co-variates in the treated groups but significant correlations were found in the untreated group. For the end-point of optic disc change, a highly significant
correlation was found between survival time and the overall mean IOP level during the study, with shorter survival times correlating to higher IOPs. This end-point was also correlated to the mean IOP change during the study, with longer survival times correlating to a mean fall in IOP from base line. The end-point of visual field failure showed a highly significant correlation to the baseline cup-disc area ratio, with a larger ratio correlating to a shorter survival time. There was no correlation with the mean IOP level during follow-up, but a shorter survival time correlated with a mean fall in IOP level from baseline.

39 out of the 42 patients who reached the study end-points were followed up after the end of the study. 26 of these were clinically labelled as having glaucoma on the basis of further confirmed visual field progression, optic disc changes, or changes in these parameters occurring after the development of optic disc haemorrhages. In the remaining 13 patients, no visual field progression occurred in defects described as being ‘slight’, or no visual field defects or optic disc change occurred following the end-point of optic disc haemorrhage.

In the study discussion, the authors gave an overall annual incidence of 4.9% for the development of glaucomatous end-points. They acknowledged that this was higher than previous studies had found, but pointed out that their OHT patients were initially on entry into the trial, at high risk for the subsequent development of glaucoma. 31% had a family history of POAG, 29% had a baseline C/D area ratio of 0.5 or greater, and 20% had a baseline entry pressure of 30mmHg or greater.

They pointed out that although there was a significant reduction in the mean IOP level by treatment with timolol, there was no significant difference in the incidence of glaucomatous visual field defects between the groups. They
suggested that the pressure reduction obtained might not protect the susceptible individual from the development of localised visual field defects. The authors concluded that the results of the study did not confirm the previous findings of Kass et al. (Kass, et al., 1989), or Epstein et al. (Epstein, et al., 1989) and they could therefore not recommend blanket prophylactic treatment of all OHT patients.

The study by Schulzer et al. was probably the most rigorously executed of the ones discussed here. The relatively high conversion rate reflects both the patient characteristics and the range of end-points chosen for the study. The number of patients in the study was higher than in the others, but still limited. Examiner or patient bias may have influenced the results, in that the treatment status was not masked to either group. However, the assessment of optic disc photographs and visual field printouts was masked, on a post hoc basis.

Rossetti et al. (Rossetti, et al., 1993) addressed the question whether randomised clinical trials on the medical treatment of glaucoma were appropriate to guide clinical practice. They carried out a systematic quantitative and qualitative overview of previously published clinical trials to assess the outcome of treatment in patients with POAG and OHT. They found that only 16% of 102 randomised clinical trials were controlled (i.e. compared to a placebo or no treatment group). Pooled analysis showed a statistically significant reduction in IOP for the treated groups as compared to control groups. Of the trials included in the analysis, only 3 provided data on long-term visual field changes, and these failed to show a significant protective effect of treatment when statistically combined. The review excluded those trials (the majority) that had compared the effectiveness of one treatment with another. The authors concluded that the
effectiveness of treatment on the outcome of glaucoma is still not fully
determined, and they suggested guidelines for improving the design of clinical
trials. These included the comparison of a treatment group with a control group,
well-defined end-points other than IOP level (such as visual field change), and a
time span long enough to assess any possible effect. A major criticism of many
of the studies was their short duration.

The largest treatment trial in OHT is the currently ongoing multi-centre
'Ocular Hypertension Treatment Study' in the United States. The design protocol
and base-line characteristics of the participants was reported in early 1999
(Gordon, 1999). The study aim is to evaluate the safety and efficacy of topical
hypotensive medication in preventing or delaying the onset of visual field loss
and/or optic nerve damage in OHT patients. It is the largest trial conducted so
far to address the question of prophylactic treatment for OHT, and 1637 subjects
have been randomised to either treatment or close observation between 1994 and
1996. The mean age of the overall group is 55.4 ± 9.0 years and 56.9% of the
subjects are female. 25% of the group are African-American. The original
protocol of the trial defined 2 consecutive abnormal visual fields as confirmation
of a glaucomatous defect. This was later revised to include 3 abnormal
reproducible fields, due to a high proportion of initially abnormal fields that
became normal on re-testing. The learning effect on visual field performance
(Johnson, Nelson and Quigg, 1993), and an increased inter-test reliability
fluctuation for OHT and glaucoma patients (Katz, et al., 1991) has been well
documented. All patients have performed at least 2 baseline Humphrey 30-2
visual fields during the initial eligibility assessment. Treatment goals in the
medication group are an IOP of 24mmHg or less and at least a 20% reduction in
IOP from the average baseline level. The clinician can use any available single drop or combined therapy to achieve this goal, mimicking more closely the real-life clinical situation. This is the great strength of the study, along with the large number of subjects involved. However, the study design has several weaknesses: initial inclusion criteria included a normal optic disc appearance on stereoscopic photograph assessment; the control arm is close follow up only, so the two groups are unmasked; and optic disc change is defined on the basis of subjective assessment, albeit by two masked experienced optic disc 'readers'. Other ancillary studies including the prospective longitudinal assessment of optic disc change with the HRT, and the assessment of sequential testing with short wavelength perimetry will also be carried out. The study aims to include at least 5 year of follow-up for each patient. The findings are eagerly awaited.
The betaxolol Vs placebo treatment trial in ocular hypertension described in this thesis was designed to eliminate some of the problems listed above. The primary aim of the study was to determine whether treatment with the topical hypotensive agent betaxolol was able to delay or prevent the conversion from ocular hypertension to early glaucoma. This double-blind, prospective, longitudinal study randomised a large number of eligible patients to treatment with either betaxolol or placebo drops with a follow up period of 2 to 6 years. The well-defined end-point of the study was the development of reproducible glaucomatous visual field defects on automated perimetry. Ancillary studies included the analysis of change in sequential HRT images of the optic disc in groups of subjects included in the trial.

The aims, methodology and results of the trial will be presented in subsequent chapters, along with a discussion of the findings and the relevant conclusions that may be drawn from the work.
Section 2 Aims
2.1 The betaxolol Vs placebo treatment trial for ocular hypertension.

2.1 Primary aims

1. To determine if treatment with topical betaxolol can prevent or delay the onset of glaucomatous visual field loss in a group of ocular hypertensive patients, by means of a randomised, prospective, longitudinal, double-blind, placebo controlled clinical trial.

2. To compare IOP characteristics of the betaxolol and placebo treated groups over time.

3. To assess IOP levels and demographic data, as possible risk factors for conversion.
2.2 Secondary aims

2.2.1 Evaluation of methods for the early detection of glaucomatous damage:

2.2.1.1 Use of the Heidelberg Retina Tomograph to detect optic disc change prior to confirmed visual field change in ocular hypertensives converting to early glaucoma.

The aim of this study is to determine whether analysis of sequential optic disc images obtained with the Heidelberg Retina Tomograph is able to demonstrate glaucomatous change prior to the development of reproducible field defects in a group of ocular hypertensive patients converting to early glaucoma.

2.2.1.2 Use of the Heidelberg Retina Tomograph to detect change at the optic disc in ocular hypertensives at risk of developing glaucoma.

The aim of this study is to determine if the analysis of sequential HRT optic disc images is able to demonstrate glaucomatous change in individual OHT patients who maintain normal visual fields.
2.2.1.3 Use of the GDx to Detect Differences in the RNFL Thickness Between Normal, Ocular Hypertensive and Early Glaucomatous Eyes

The aim of this study is to determine if differences in the RNFL thickness between normal, ocular hypertensive and glaucomatous eyes could be detected using the GDx.
Section 3 Patients, Materials and Methods
3.1 Patients

3.1.1 Ocular hypertensive trial patients: Inclusion criteria

a) Age greater or equal to 35 years

b) Caucasian.

c) Initial IOP level on Goldmann tonometry consistently greater or equal to 22
   mmHg and less than 35mmHg in one or both eyes, off all hypotensive topical
   treatment for a wash-out period of at least 6 weeks, measured on at least 2
   separate occasions.

d) Open angles on gonioscopy

e) Corrected Snellen visual acuity greater or equal to 6/12.

f) Refractive error not exceeding the range –6.0D to +6.0D.

g) Normal visual field testing (AGIS score = 0)(AGIS Investigators, 1994) on
   the Humphrey 24-2 strategy with acceptable reliability indices, after a
   learning period of 3 successive tests.

h) No previous intra-ocular surgery.

i) No concurrent ocular disease that could affect visual field performance or
   IOP level during the course of the study

j) No history of pulmonary or cardiac disease contra-indicating the use of
   topical β-blockers.

k) No systemic β-blocker use

l) No concurrent pregnancy in female patients
m) No history of diabetes mellitus

n) Willingness to participate in a longitudinal study with a commitment to comply with treatment.
3.1.2 Normal volunteers: inclusion criteria

Normal volunteers participated in the ‘evaluation of methods of early detection’ part of the study.

a) Age greater or equal to 35 years

b) Caucasian.

c) IOPs on Goldmann tonometry consistently less than or equal to 21 mmHg in both eyes, on at least 2 separate occasions.

d) Corrected Snellen visual acuity greater or equal to 6/12.

e) Refractive error not exceeding the range –6.0D to +6.0D.

f) Normal visual field testing (AGIS score =0) on the 24-2 Humphrey strategy after a learning period of 3 successive visual field tests.

g) No history of diabetes mellitus, or systemic beta-blocker use.

h) No concurrent ocular disease likely to effect visual field performance or IOP level.

i) No family history of glaucoma or ocular hypertension.

j) No previous intra-ocular surgery.

k) Willingness to participate in a longitudinal study.
3.1.3 Early glaucoma patients

Early glaucoma patients participated in the ‘evaluation of methods of early detection’ part of the study.

a) Age greater or equal to 35 years

b) Caucasian.

c) Initial untreated IOP on Goldmann tonometry greater than 21 mmHg in one or both eyes on at least two separate occasions.

d) Open angles on gonioscopy

e) Snellen visual acuity greater or equal to 6/12.

f) Refractive error not exceeding the range –6.0D to +6.0D.

g) Reproducible glaucomatous visual field defects on at least three consecutive 24-2 Humphrey visual fields. All visual field defects were classified as showing early glaucomatous change with AGIS scores of 1-5.

h) No co-existent ocular disease likely to effect visual field performance or IOP level.

i) No previous intra-ocular surgery.

j) Willingness to participate in a longitudinal study.
3.2 Methods I (Betaxolol Vs placebo treatment trial: primary aims)

The betaxolol Vs placebo treatment trial in ocular hypertension was given full ethical approval by the Ethics Committee of the Moorfields Eye Hospital NHS Trust in 1991.
3.2.1 Recruitment

Ocular hypertensive patients were recruited from the Moorfields Eye Hospital glaucoma service, after initial referral from local optometrists, primary care clinics, and other speciality services, over the period from 1992-1996.

Eligibility of recruits was assessed over an initial period of 3 visits spread over 4-6 weeks, within which patients underwent repeated visual field testing on the
Humphrey 24-2 strategy, and repeated Goldmann tonometry to assess the baseline IOP level. The initial consultation included detailed history taking, including details of past ocular history, a review of systemic disease and symptoms and a drug history. Informed consent was then obtained from eligible patients for participation in the trial.

3.2.2. Drop preparations

All drops used in the study were manufactured and labelled by Alcon laboratories. Both the betaxolol and placebo preparations contained preservative and were administered on a twice-daily basis. Bottle labelling was on the basis of A, B, C or D, so that continuity of the same code could be checked by both pharmacy and the patient when repeat prescriptions were collected. A and C labelled bottles were betaxolol preparations; B and D labelled bottles were placebo preparations.

3.2.3 Randomisation.

The chief pharmacist at MEH, who had no other direct involvement with the trial, randomised patients to treatment with either betaxolol drops or placebo drops by means of randomisation tables. A trial number was allocated to each patient that corresponded to a treatment code for drops A, B, C or D. All clinicians and patients participating on the trial were blind to all treatment codes until the end of the study when these were broken for analysis. Pharmacists responsible for dispensing the drops were able to access only the drop codes
associated with each trial number. The chief pharmacist revealed the treatment
codes at the end of the trial.

3.2.4 Clinic visits

Routine trial patient appointments were scheduled for every 4 months.

At each visit, each patient underwent the following procedures:

a) IOP testing by Goldmann tonometry.

b) Visual field testing on the 24-2 strategy of the Humphrey Analyser

c) Other tests undergoing evaluation for the early detection of glaucoma
   including optic disc imaging on the Heidelberg Retina Tomograph
   (HRT), and RNFL imaging by the GDx. Both these techniques are
   described in detail below.

3.2.5 Visual field analysis and scoring of defects developed over
time.

Visual field analysis during the course of the study was performed using
the AGIS scoring system (Advanced Glaucoma Intervention Study) (AGIS
Investigators, 1994). The AGIS visual field defect score is based on the number
and depth of depression of clusters of adjacent points as compared to age-
matched normal results, in the upper and lower hemi-fields and the nasal area of
the total deviation print-out of the 24-2 Humphrey print-out. Test locations above
and below the centre of the physiologic blind spot are excluded leaving 52 test
locations for scoring - 23 in each hemifield and 6 in the nasal field. For a defect in the hemi-fields to be considered as significant, 3 or more adjacent points must be affected. 2 points are defined as being adjacent if they are side by side horizontally, vertically or obliquely. 3 or more points are adjacent if each location in the cluster is adjacent to at least one other in the cluster. The amount of depression in decibels that (denoted by displayed figures) defines a test site to be defective varies with its location as illustrated below.

A transparent template was made to aid the scoring of each individual visual field. The template consists of 5 sequential ‘cut-out’ areas representing different parts of the total deviation plot on the Humphrey 24-2 print out. Next to each cut-out area, the lower limit for depression points in decibels is displayed. The 5 different cut-out areas can then be moved sequentially over the entire plot, marking each point falling below the normal limit. To complete the scoring, the
points fitting AGIS criteria for abnormality are then viewed over the entire plot. A cluster of 3 or more adjacent depressed test sites in the nasal field constitutes a nasal defect. In this case the cluster may cross the horizontal mid-line. One or more depressed test locations in the nasal field either above or below the horizontal mid-line in the absence of depression of any of the nasal locations on the opposite side of the mid-line constitute a nasal step. A cluster of 3 or more depressed sites in a hemifield constitutes a hemifield defect.

The scoring system is applied as follows:

1. For a nasal defect or step, 1 is added to the score, and if 4 or more of the 6 nasal locations are depressed by 12 dB or more, 1 more is added to the score.

2. 1 is added to the score if there are 3-5 depressed sites in the clusters depressed within each hemifield. 2 are added if there are 6-12 depressed sites; 3 if there are 13-20; and 4 if there are more than 20.

3. If half or more of the defective points in a hemi-field are depressed 28 dB or more, 5 are added to the score. 4 are added if the same proportion are depressed 24 dB or more; 3 are added if these points are depressed 20 dB or more; 2 is added if they are depressed by 16dB or more and 1 is added if they are depressed by 12 dB or more.

4. If a hemifield lacks a cluster of 3 adjacent depressed test sites but contains 2 adjacent depressed test sites one of which is depressed by 12 dB or more, then 1 is added to the score.
The total AGIS score is then summated over the whole field, with a possible range from 0 to 20. A score of 0 represents no significant defect, and a score of 20 represents end-stage glaucoma, with all points being deeply depressed. A score of 1 to 5 represents early glaucoma.

Reliability of visual field performance in the study was defined according to the following criteria:

a) Less or equal to 20% fixation losses.

b) Less or equal to 33% false-positive or false-negative responses.

By definition, all the trial patients eligible for the trial had starting base-line visual field with an AGIS score of 0.
3.2.6 Definition of conversion to early glaucoma on the basis of visual field criteria

Conversion to early glaucoma on the basis of visual field criteria was defined as the development of a reproducible glaucomatous visual field defect with an AGIS score of greater or equal to 1, on 3 consecutive reliable visual fields.

If an individual trial patient developed a defect fitting the AGIS criteria noted above, then they were asked to return for repeat visual field testing within a 4-week period. If the same defect was reproduced on a second reliable visual field test, then the patient was asked to return for repeat testing for a third time. If the defect was reproduced on this third consecutive reliable visual field, then the entire set of the individual’s visual fields from the baseline visual field onwards were sent to an independent glaucoma expert for assessment and confirmation of conversion.

If conversion was confirmed, then the patient was withdrawn from the trial and started on an appropriate topical hypotensive agent other than betaxolol with the aim of reducing the IOP to an appropriate therapeutic level. The treatment code of the converting patient remained unbroken until the end of the study, to maintain the double blind status. If the defect was present but the reliability indices fell outside those described above, then the test was repeated again within 1 month. If a field defect was not reproduced on the second or third attempts, then a routine perimetric appointment was given for 4 months time.
Three consecutive research fellows each on a 2-year assignment were responsible for the daily running of the trial. The research fellow performed routine examination and IOP measurement whereas visual field testing and optic disc/retinal nerve fibre analysis was performed by 3 experienced technicians, attached solely to the trial.

3.2.7 Criteria for removing eligible subjects from the trial

The clinician was able to remove patients from the trial under the following circumstances:

1. Conversion on the basis of visual field criteria.

2. Patient choice – if the patient no longer wished to participate in the trial or take the drops.

3. Commencement of systemic β-blockers by a general physician. This was done in order to avoid a possible ocular hypotensive effect of the systemic treatment. A letter was sent to the physician stating that such medication would lead to withdrawal from the trial and requesting the consideration of an alternative treatment.

4. Persistent failure to attend appointments regularly led to withdrawal from the trial.

5. Patients who moved away or found the travelling to the clinic too onerous were withdrawn, and alternative follow-up in a more convenient unit was arranged.

6. Patients with IOP levels greater than 35mmHg were withdrawn and started on an ocular hypotensive agent other than betaxolol.
7. Patients who developed medical conditions that limited their attendance at the clinic were withdrawn. Any patient who developed a visual field defect secondary to a cerebro-vascular accident, was withdrawn.

8. Patients who experienced systemic or local side effects from the trial drops were withdrawn.

Wherever possible, withdrawn patients continued to be followed up in the OHT clinic. Following withdrawal, the treatment codes remained unbroken until the end of the trial. Any patients who required topical ocular hypotensive treatment following withdrawal were started on drops other than betaxolol. The choice of treatment was guided by the reason for withdrawal.

3.2.8 Data collection and recording

For each individual patient visit, the following data was collected and recorded by the examiner onto a data sheet in the front of the patient’s trial notes. The values were then entered by one of two trial technicians onto an excel workbook, with a separate worksheet for each visit:

a) Date of the visit

b) IOP level - this was measured with the examiner blind to the preceding measurements, and repeated 3 times to obtain the mean IOP level for that visit.
c) Details of whether optic disc or RNFL imaging was performed on that visit were recorded.

Verbal checking of drop compliance was also checked and recorded. If drops had not been instilled as per instructions, (e.g. forgotten on the morning of the visit) then IOP measurements were not entered onto the database and the patient was brought back for an earlier appointment, emphasising the need for careful compliance.

All imaging performed during the trial was analysed some time after the acquisition of the data and was entered into separate excel databases. For the purposes of analysis, the datasheets were then converted into SPSS (version 7.0) worksheets directly from the excel format.

The treatment code remained unbroken until the end of the trial for any patient who was withdrawn during its course.
3.2.9. **Statistical methods**

Statistical analysis of all data was performed using SPSS for Windows software version 7.0 by the author. Excel workbooks containing the data were converted into SPSS worksheets.

The mean pre-treatment IOP was calculated from two readings made during the eligibility assessment of the patients. The mean post-treatment IOP level was calculated from all the IOP readings taken after entry onto the trial, once drops had been instituted.

Kaplan-Meier survival analysis was used to determine if the numbers of patients converting to early glaucoma differed between the betaxolol and placebo groups.

Differences in age and IOP characteristics between the betaxolol Vs placebo groups, and the converter Vs non-converter groups were determined using an analysis of co-variance, and independent samples student – t test statistics.
3.3 Methods II (Betaxolol Vs placebo treatment trial - secondary aims; evaluation of methods of early detection).

3.3.1 Detection of optic disc change with the Heidelberg Retina Tomograph prior to confirmed visual field change in ocular hypertensives converting to early glaucoma

3.3.1.1 Patients

a) 21 converters as defined above.

b) 21 normal volunteers as defined above.

3.3.1.2 Methodology of the HRT

The Heidelberg Retina Tomograph is a type of confocal scanning laser ophthalmoscope, developed by Heidelberg Engineering, Heidelberg, Germany, for the objective analysis of optic disc parameters. The system (Chauhan, 1994, Rohrschneider, 1994) uses confocal optics comprising an imaging system with a known focal length and a detector located in a plane conjugate to the focal plane of the imaging system. A low-power diagnostic helium-neon laser beam is scanned point-wise line by line across the fundus and optic nerve. The reflected light from the illuminated area of fundus is deflected from the optical path of the laser by a beam splitter to a detector that measures the intensity of this reflected light. A diaphragm with a pin-hole is placed in front of the detector to ensure that only light reflected from the focal plane of the laser is measured. Cross-sectional images of the optic disc are obtained by varying the focal plane of the laser and
these images are reconstructed to create a three dimensional form. The HRT acquires 32 equally spaced confocal images along the ‘Z’ axis that is perpendicular to the optical axis, and has an image resolution of 256 X 256 pixels. Scan areas can be set to 10 X 10 degrees, 15 X 15 degrees, or 20 X 20 degrees, and the scan depth can be varied from 0.5 to 4.0mm. A reflectivity image is generated by summing reflectance measurements along the Z-axis for each aligned pixel. The location along the Z axis where the maximum reflectance is measured is assumed to be the height of the location and this information is used in the generation of a topography image. Reflectivity of the image is measured on a relative percentage scale, and topography is measured in micrometers from the focal plane of the eye.

In most examinations, three good quality image series are acquired per eye per sitting, in order to generate a mean image. Integral software is then used to generate the topography of each of these series and once this has been obtained, the mean topographical image is generated. This mean image is then used for the final optic disc analysis. A mouse-drawn contour line is used to delineate Elschnig’s ring or the optic disc edge. HRT software then analyses disc parameters both globally and segmentally. The following parameters are generally analysed: rim area, rim volume, cup area, cup volume, disc area, Third moment (cup-shape measure) and cup-disc area ratio may be calculated from the cup area and disc area measurements.
3.3.1.3 HRT imaging and analysis

Each subject included in the study had undergone yearly optic disc imaging with the HRT. One eligible eye was included per subject.

The HRT used in this study was modified using a specially designed ‘range-finder’, to ensure that all eyes being examined were 15mm away from the SLO, in order to eliminate possible magnification errors caused by differences in the head position between sittings (Kotecha et al, 1997).

Optic disc analysis on the Heidelberg Retina Tomograph was performed yearly from 1994 onwards when the technology became available to us.

For each HRT imaging session, three 10 by 10-degree images were obtained for each eye through an undilated pupil by one of two experienced technicians. Images with significant movement artefact, as assessed by observing the ‘movie’ of the imaging session at the end of acquisition, were rejected. All analysis on the HRT was performed using HRT software version 2.01. The mean topography of the 3 images was generated by integral software and the optic disc edge was delineated on the mean image by a single observer (D.K.), using a mouse drawn contour line. The contour line was then exported from the first HRT mean image (HRT1) onto the second HRT mean image (HRT2) of the same optic disc acquired sequentially. In house studies have demonstrated reproducibility levels similar to those previously described with inter-observer variability measures of neuro-retinal rim values of 8.1%, and intra-observer
variability measures of 4.5% (Garway-Heath D F, 1998). HRT software was used to analyse both global and segmental optic disc parameters.

The following optic disc parameters were analysed using HRT software: disc area, cup area, cup volume, rim volume and Third moment (cup shape measure) relative to the standard reference plane of 50\(\mu\)m below the mean retinal height of a temporal segment of the rim contour. Cup/disc (C/D) area ratio was calculated by dividing the cup area by the disc area. Rim area was calculated by subtracting the cup area from the disc area.

The following 6 pre-defined optic disc segments were analysed: nasal, supero-nasal, infero-nasal, temporal, supero-temporal, infero-temporal. Global parameters were also analysed.

Images obtained by the HRT are illustrated in the appendix.

For each subject included in the study, 2 sequential HRT images were analysed. Only those subjects with good quality HRT images i.e. without any movement artefact and a mean topography standard deviation < 20\(\mu\)m were included in the study analysis. Images from 21 converters and 21 normal subjects were therefore included in the study.

3.3.1.4 Statistical methods

All statistical analysis was performed by the author using the statistical software SPSS for Windows, version 7.0.

The sequential HRT image data was not distributed normally (as demonstrated by histogram representation of the results). The non-parametric Wilcoxon Signed Ranks statistical test was therefore used to determine if there were any statistically significant differences between the parameter values for
HRT1 and HRT2. A result was considered to be statistically significant if the p value was <0.05.
3.3.2 Use of sequential Heidelberg Retina Tomograph images to identify changes at the optic disc in ocular hypertensive patients at risk of developing glaucoma

3.3.2.1 Patients

a) 21 converters as defined above

b) 21 normal volunteers as defined above

c) 164 ‘non-converter’ OHT trial patients maintaining normal visual fields, as defined above.

3.3.2.2 HRT Imaging and analysis

As described above

3.3.2.3 Statistical Methods

1. Establishing the normal limits of variability

The mean magnitude of difference in parameter values between HRT1 and HRT2 for the group of normal subjects was established and the 95% limit of normal variability was determined by multiplying the standard deviation of this mean difference by 1.96.
2. Analysis of individual optic discs to identify those demonstrating change outside the normal level of variability

All the optic discs in the three subject groups were analysed individually to determine which discs showed a magnitude of change greater than that limit established from the analysis of the normal data. Change in both directions was examined i.e. if parameter change was in the glaucomatous direction, this was labelled as ‘getting worse’ represented by (−) and if parameter change was in the opposite direction it was labelled as ‘getting better’ represented by (+) Figure 6 to Figure 12.
3.3.3 Use of the GDx to determine differences in the RNFL thickness between normal, ocular hypertensive and glaucomatous eyes.

3.3.3.1 Patients

a) 33 Normal subjects as described above.
b) 145 OHT patients as described above.
d) 44 POAG patients as described above.

3.3.3.2 GDx imaging and analysis

Clinical optic disc and RNFL appearance on slit-lamp biomicroscopy were not inclusion criteria for any of the subject groups. One eye per subject was entered into the study. When both eyes fitted the above criteria, then the study eye was chosen at random. The mean ages of the three groups were calculated and compared. Optic disc area measurements were also obtained for each individual from HRT image analysis performed at the same time as the GDx examination, to determine if any inter-group differences in optic disc size existed. Three HRT images of the study eye disc were acquired through an undilated pupil, and a mean topography was generated by HRT software version 2.01. The optic disc edge was then delineated using a mouse-drawn contour line, and the software was used to automatically calculate the disc area.
3.3.3.3 Methodology of the GDx

The GDx is a scanning laser polarimeter that was developed by Laser Diagnostic Technologies, San Diego, with the purpose of providing a direct, objective and quantitative assessment of RNFL thickness. The machine incorporates a polarimeter within scanning laser ophthalmoscope technology. The technique of scanning laser polarimetry depends on the principle that the RNFL has bi-refringent properties (Weinreb, 1995, Neissen, 1996). Each nerve fibre has a diameter less than the wavelength of polarised light emitted by the SLO that is incident on the RNFL. The light source consists of a 780nm-diode laser in which the state of polarisation is modulated. The polarised light enters the RNFL, and is partially reflected from the deeper layers of the retina. Thus the degree of polarisation of this light is changed depending on the thickness of the RNFL. Light emerging from the eye is separated from the illuminating light beam by a non-polarising beam splitter. The change in polarisation of the incident light is termed ‘retardation’ and the polarimeter detects the amount of retardation caused by the RNFL.

The cornea and lens also have bi-refringent properties that may produce measurement artefacts, therefore newer generation machines have polarisation compensators to neutralise the polarisation effects of the anterior segment. An SLO image of the optic disc and peri-papillary retina is acquired through an undilated pupil, and the GDx produces a topographical retardation map representing the RNFL thickness in specific areas. This map is made up of a total of 65, 536 retinal locations that are singly tested over a 15 degree retinal area with a spatial resolution of approximately 17 x 17μm. GDx software is used to
convert retardation figures into RNFL thickness values, using Fourier analysis. Each degree of retardation is equivalent to 7.4μm of RNFL thickness (Weinreb, 1990).

No reference plane is required for the calculations and the GDx theoretically gives an absolute measure of RNFL thickness. The outputs of the polarimeter are digitised and stored by the computer for later analysis. In order to carry out the analysis, a contour line is fitted to the disc edge as closely as possible using the mouse. An area of peri-papillary retina ranging from 1.5 to 1.8 disc diameters in size can be included in the area of analysis. GDx software is used to calculate RNFL thickness in 4 main segments: superior, inferior, nasal and temporal and a mean total value is also given. Recent advances in the software also give the option of calculating ratios of measurements within the quadrants.

Retinal nerve fibre layer analysis using the GDx was performed at every routine visit from 1997 onwards, when the technology became available to us.

All the imaging was performed through an undilated pupil. 3 good quality 15 by15 degree images of the RNFL and optic disc were obtained for each eye, by one of two experienced technicians. The individual images were all analysed by a single experienced operator (D.K). A contour line was fitted as closely as possible to the disc edge, using the mouse. An area of retina 1.5 disc diameters from the centre of the optic disc was then analysed using GDx software. RNFL thickness was measured in 4 quadrants in this area around the circumference of the disc: superior, inferior, temporal and nasal. The software also calculated a
total circumferential RNFL thickness. The total and quadrantic values for all the
three images per eye were then averaged to produce a mean value and this was
used in the final analysis.

Images obtained by the GDx are included in the appendix.

3.3.3.4 Statistical Methods

SPSS for Windows version 7.0 was used by the author to perform all analysis for
this study.

Histogram representation demonstrated skewed data and non-parametric
tests were therefore used in the statistical analysis. The Kruskal-Wallis one-way
analysis of variance was used to determine if there were any overall differences
between the groups in each of the quadrantic and total RNFL thickness values.
Further specific inter-group analysis was performed using the Mann-Whitney U
test, to determine which specific groups showed significant differences between
the total or each of the quadrantic RNFL thickness.
Section 4 Results
4.1 The betaxolol Vs placebo treatment trial

428 patients were recruited over the period from 1992-1996. 72 of these were ineligible: 37 because of abnormal base-line visual fields; 12 because of IOPs less or equal to 22mmHg, or greater or equal to 35mmHg; 2 patients were on systemic β blockers; 1 patient had a visual acuity less than 6/12; 1 patient was under 35 year of age; 13 patients refused entry into the trial; previous notes were unavailable for 5 patients; and 1 patient failed to attend prior to randomisation;

356 eligible patients were randomised to treatment with betaxolol or placebo from 1992 - 1996. 183 were randomised initially to placebo, and 173 were randomised to treatment with betaxolol.

101 patients were withdrawn during the course of the study, and the reasons for withdrawal are summarised in Table 1 below.
### Table 1 Reasons for withdrawal

B=betaxolol  P=placebo

<table>
<thead>
<tr>
<th>Reason for withdrawal</th>
<th>Number of subjects</th>
<th>Specific reasons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Did not attend</td>
<td>10B 12P</td>
<td></td>
</tr>
<tr>
<td>Medical Reasons</td>
<td>9B 11P</td>
<td>Oral β-blockers, stroke, diabetes, chemotherapy, CABG, myocardial infarct</td>
</tr>
<tr>
<td>Moved away</td>
<td>3B 4P</td>
<td></td>
</tr>
<tr>
<td>Died</td>
<td>8B 8P</td>
<td>B-myocardial infarct, leukaemia, melanoma, alcohol, murder</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P-3 myocardial infarct, Ca prostate</td>
</tr>
<tr>
<td>Drop intolerance</td>
<td>8B 7P</td>
<td></td>
</tr>
<tr>
<td>Cataract surgery</td>
<td>3B 2P</td>
<td></td>
</tr>
<tr>
<td>IOP&gt;35mmHG</td>
<td>3B 1P</td>
<td></td>
</tr>
<tr>
<td>Side effects</td>
<td>3B 4P</td>
<td></td>
</tr>
<tr>
<td>Patient choice</td>
<td>1B 2P</td>
<td></td>
</tr>
<tr>
<td>POAG other eye</td>
<td>1B 1P</td>
<td></td>
</tr>
</tbody>
</table>
255 patients completed follow-up until the end of the trial. 134 of these were on betaxolol drops and 121 patients were on placebo drops Figure 1 below.

Figure 1 Proportions of OHT subjects on either betaxolol or placebo drops
The mean age and sex characteristics of the overall group, and of the converter vs non-converter groups are shown in Table 2.

### Table 2 Age and sex characteristics of whole OHT group, converter and non-converter groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Sex</th>
<th>Age years (SD)</th>
<th>Independent t test (mean age)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>male</td>
<td>Female</td>
<td>male</td>
<td>Female</td>
</tr>
<tr>
<td>Whole OHT group</td>
<td>157</td>
<td>97</td>
<td>65.9 (9.9)</td>
<td>64.3 (9.8)</td>
</tr>
<tr>
<td>Converters</td>
<td>17</td>
<td>11</td>
<td>68.3 (9.4)</td>
<td>65.5 (13.1)</td>
</tr>
<tr>
<td>Non-converters</td>
<td>140</td>
<td>86</td>
<td>65.7 (9.9)</td>
<td>64.1 (9.4)</td>
</tr>
</tbody>
</table>
61.6% of the subjects in the trial were male and 38.4% were female. This relative proportional difference is also reflected in converting group, with 60.7% of converters being male and 39.3% were female. There was no significant inter-sex difference in the ages of any of the groups. (Table 3).

Table 3 Age characteristics of betaxolol Vs placebo groups, and converter Vs non-converter groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean age in years (SD)</th>
<th>Independent t-test p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Betaxolol</td>
<td>65.9 (9.9)</td>
<td>P=0.39</td>
</tr>
<tr>
<td>Placebo</td>
<td>64.8 (9.9)</td>
<td></td>
</tr>
<tr>
<td>Converters</td>
<td>67.2 (10.9)</td>
<td>P=0.6</td>
</tr>
<tr>
<td>Non-converters</td>
<td>65.1 (9.8)</td>
<td></td>
</tr>
</tbody>
</table>
Mean follow-up was 56.7 months (SD 13.8). Figure 2 shows the overall distribution of follow-up time.

Figure 2 Histogram to show distribution of follow-up time (months) for OHT subjects on the betaxolol vs placebo trial.
28 patients converted to early glaucoma on the basis of our visual field criteria: 16 were on placebo, and 12 were on betaxolol.

Kaplan-Meier survival analysis did not show any overall significant difference in the numbers converting or the survival times between the two groups (Figure 3), with a log rank statistic of 0.92 (p=0.34).

**Figure 3** Kaplan-Meier survival curves for betaxolol Vs placebo groups. B=betaxolol group, P=placebo group.
Overall, the mean drop in IOP for the betaxolol group was 4.7 mmHg. However, a mean drop in IOP was also seen in the placebo group of 1.9 mmHg. An analysis of covariance showed a significant IOP lowering effect of betaxolol as compared to placebo of 2.6 mmHg (p<0.0001).

IOP analysis using the student ‘t’ test showed that the betaxolol group had statistically higher mean pre-treatment and post-treatment IOP levels than the placebo group. These results are summarised in Table 4. Similarly, the group of converters had significantly higher pre-treatment and post-treatment IOPs than the non-converters Table 4

<table>
<thead>
<tr>
<th></th>
<th>betaxolol</th>
<th>placebo</th>
<th>t-test</th>
<th>converters</th>
<th>Non-converters</th>
<th>t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p-value</td>
<td>p-value</td>
<td>p-value</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-treatment IOP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mmHg) SD</td>
<td>26.3 (2.34)</td>
<td>25.6 (2.19)</td>
<td>&lt;0.01</td>
<td>26.8 (2.46)</td>
<td>25.8 (2.26)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Post-treatment IOP</td>
<td>21.6 (2.88)</td>
<td>23.7 (2.85)</td>
<td>&lt;0.0001</td>
<td>24.9 (3.35)</td>
<td>22.3 (3.03)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>(mmHg) SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
A comparison of the converters Vs non-converters within the betaxolol group (Table 5) showed a significantly higher pre-treatment IOP, and a significantly higher post treatment IOP for the converters. Betaxolol had a less marked effect on IOP reduction in the converters as compared to the non-converters, but the difference was not statistically significant.

Table 5 Comparison of pre-and post-treatment IOPs for converters and non-converters in betaxolol group.

<table>
<thead>
<tr>
<th></th>
<th>Converters</th>
<th>Non-converters</th>
<th>t-test p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean pre-treatment IOP mmHg (SD)</td>
<td>27.71 (2.67)</td>
<td>26.15 (2.27)</td>
<td>0.03</td>
</tr>
<tr>
<td>Mean post- treatment IOP mmHg (SD)</td>
<td>24.29 (3.15)</td>
<td>21.34 (2.80)</td>
<td>0.001</td>
</tr>
<tr>
<td>IOP reduction (mmHg)</td>
<td>3.42 (3.37)</td>
<td>4.81 (2.73)</td>
<td>0.1</td>
</tr>
</tbody>
</table>
Figure 4 & Figure 5 illustrate the pattern of IOP over time starting from the two pre-treatment visits, for betaxolol and placebo respectively.

Figure 4 Graph to show mean IOP over follow-up time for betaxolol group

Dotted line represents time at which drops were commenced

Figure 5 Graph to show mean IOP over time for placebo group

Dotted line represents time at which drops commenced
Evaluation of methods of early detection

4.2.1 Detection of optic disc change with the Heidelberg Retina Tomograph prior to confirmed visual field change in ocular hypertensives converting to early glaucoma

21 converters and 21 normal volunteer subjects with good quality sequential HRT images were included in the study analysis. Group characteristics of the OHT group included in the next part of the study are also illustrated here for ease of comparison. The mean ages of the groups and the time between HRT1 and HRT2 are shown in Table 6. There were no significant differences in age between the groups (p = 0.4).

Table 6 Mean time interval from HRT1 to HRT2 with standard deviations and mean ages of subject groups

<table>
<thead>
<tr>
<th></th>
<th>Normals</th>
<th>OHT</th>
<th>Converters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Time(months)</td>
<td>21.5</td>
<td>16.6</td>
<td>16.3</td>
</tr>
<tr>
<td>from HRT1 to HRT2 (SD)</td>
<td>(8.5)</td>
<td>(9.7)</td>
<td>(8.5)</td>
</tr>
<tr>
<td>Mean Age (years)</td>
<td>65.1</td>
<td>66.2</td>
<td>67.9</td>
</tr>
<tr>
<td>(SD)</td>
<td>(10.1)</td>
<td>(9.8)</td>
<td>(8.9)</td>
</tr>
</tbody>
</table>
For the converters, HRT2 was performed before the third conversion confirming visual field (VF3), in all but three subjects when the imaging was completed on the same day as VF3. The mean time from HRT2 to VF3 was 5.4 months (SD 5.1).

The initial CD area ratio values calculated from HRT1 for the normals and the converters are shown in Table 7. Data for the OHT group included in the next part of the study are also included for comparison. The CD area ratio was larger (p<0.001) and rim area ratio was smaller (p<0.001) for the converter group as compared to the normal and OHT groups. The same parameters did not differ significantly between the normal and OHT groups (p=0.52 and p=0.85) respectively.

Table 7 Comparison of CD area ratio taken from HRT1 for each group of subjects

<table>
<thead>
<tr>
<th></th>
<th>Converters</th>
<th>OHT</th>
<th>Normals</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD area ratio</td>
<td>0.29 (0.09)</td>
<td>0.31 (0.16)</td>
<td>0.47 (0.15)</td>
</tr>
<tr>
<td>(SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rim area mm²</td>
<td>0.93 (0.27)</td>
<td>1.22 (0.28)</td>
<td>1.22 (0.17)</td>
</tr>
<tr>
<td>(SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
4.2.1.2 Normal Group

No parameters showed significant change within the normal group. (See Table I in appendix).

4.2.1.3 Converter Group

The following segmental and global parameters demonstrated glaucomatous change ('getting worse') on the Wilcoxon analysis of the converter data:

a. Cup area - global, supero-nasal, and supero-temporal.

b. Cup volume- global, infero-temporal, supero-nasal, supero-temporal.

c. Rim area - global, supero-nasal, and supero-temporal.

d. Rim volume - global, infero-temporal, infero-nasal, supero-nasal, and supero-temporal. See Table 8.
Table 8 Optic disc parameters showing glaucomatous change in converters.

N/S=non-significant.

<table>
<thead>
<tr>
<th>Region</th>
<th>Cup area p value Magnitude (SD) mm²</th>
<th>Cup volume p value Magnitude (SD) mm³</th>
<th>Rim area p value Magnitude (SD) mm²</th>
<th>Rim Volume p value Magnitude (SD) mm³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Global</td>
<td>=0.002 0.08 (0.1)</td>
<td>=0.001 0.032 (0.037)</td>
<td>=0.002 0.08 (0.11)</td>
<td>&lt;0.001 0.045 (0.056)</td>
</tr>
<tr>
<td>Infero-temporal</td>
<td>N/S 0.009 (0.023)</td>
<td>=0.003 0.006 (0.009)</td>
<td>N/S 0.009 (0.023)</td>
<td>=0.001 0.004 (0.005)</td>
</tr>
<tr>
<td>Infero-nasal</td>
<td>N/S 0.009 (0.018)</td>
<td>=0.006 0.003 (0.005)</td>
<td>N/S 0.009 (0.020)</td>
<td>=0.002 0.007 (0.010)</td>
</tr>
<tr>
<td>Supero-temporal</td>
<td>&lt;0.001 0.023 (0.030)</td>
<td>&lt;0.001 0.008 (0.007)</td>
<td>&lt;0.001 0.023 (0.03)</td>
<td>&lt;0.001 0.006 (0.007)</td>
</tr>
<tr>
<td>Supero-nasal</td>
<td>&lt;0.001 0.01 (0.02)</td>
<td>N/S 0.004 (0.006)</td>
<td>=0.004 0.012 (0.020)</td>
<td>&lt;0.001 0.007 (0.009)</td>
</tr>
</tbody>
</table>
4.2.2 Use of sequential Heidelberg Retina Tomograph images to identify changes at the optic disc in ocular hypertensive patients at risk of developing glaucoma

The limit of normal variability of sequential HRT optic disc images was calculated using the same normal data set as described in the preceding section. Normal variability was determined by multiplying the standard deviation of the mean difference in parameter values from HRT1 to HRT2 by 1.96. The values of normal variability for each of those parameters found to change on analysis of the converter group data are shown in Table 9.
Table 9 95% limit of normal variability

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal limit of variability (mm^2/mm^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Global cup area</td>
<td>0.160</td>
</tr>
<tr>
<td>Infero-nasal cup area</td>
<td>0.023</td>
</tr>
<tr>
<td>Supero-nasal cup area</td>
<td>0.020</td>
</tr>
<tr>
<td>Supero-temporal cup area</td>
<td>0.020</td>
</tr>
<tr>
<td>Global cup volume</td>
<td>0.056</td>
</tr>
<tr>
<td>Temporal cup volume</td>
<td>0.027</td>
</tr>
<tr>
<td>Infero-temporal cup volume</td>
<td>0.008</td>
</tr>
<tr>
<td>Infero-nasal cup volume</td>
<td>0.004</td>
</tr>
<tr>
<td>Supero-nasal cup volume</td>
<td>0.010</td>
</tr>
<tr>
<td>Supero-temporal cup volume</td>
<td>0.013</td>
</tr>
<tr>
<td>Global rim area</td>
<td>0.248</td>
</tr>
<tr>
<td>Infero-nasal rim area</td>
<td>0.025</td>
</tr>
<tr>
<td>Supero-nasal rim area</td>
<td>0.036</td>
</tr>
<tr>
<td>Supero-temporal rim area</td>
<td>0.033</td>
</tr>
<tr>
<td>Global rim volume</td>
<td>0.116</td>
</tr>
<tr>
<td>Temporal rim volume</td>
<td>0.014</td>
</tr>
<tr>
<td>Nasal rim volume</td>
<td>0.043</td>
</tr>
<tr>
<td>Infero-temporal rim volume</td>
<td>0.019</td>
</tr>
<tr>
<td>Infero-nasal rim volume</td>
<td>0.017</td>
</tr>
<tr>
<td>Supero-nasal rim volume</td>
<td>0.017</td>
</tr>
<tr>
<td>Supero-temporal rim volume</td>
<td>0.014</td>
</tr>
</tbody>
</table>
Data obtained from the analysis of sequential individual HRT optic disc images in the 3 groups of subjects were then examined to determine which ones showed change above this limit of normal variability. As described previously, change could be in the direction of 'getting worse' (+) or 'getting better' (-). 13 out of the 21 converters showed change in the direction of 'getting worse'. None of the converter discs showed change in the direction of 'getting better'

1 of the normal control discs showed change in the direction of 'getting worse' and 2 discs showed change in the direction of 'getting better'

47 of the 164 OHT discs showed change in the direction of 'getting worse' and 15 discs showed change in the direction of 'getting better'. These results are illustrated in Figure 6

Figure 6 Direction of change in one or more parameter for individual optic discs in all groups

<table>
<thead>
<tr>
<th>Normals</th>
<th>Converters</th>
<th>OHT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/21 - worse</td>
<td>13/21 - worse</td>
<td>47/164 - worse</td>
</tr>
<tr>
<td>2/21 - better</td>
<td>0/21 - better</td>
<td>15/164 - better</td>
</tr>
</tbody>
</table>
For the converters and OHT group, the number of discs changing in the 'getting worse' direction was plotted against each of the significant parameters illustrated in Table 8. The 4 most frequently changing parameters were the same for both groups of subjects illustrated in Figure 7 & Figure 8.

Figure 7 Parameters change showing glaucomatous change in individual converter discs.
The percentages of individual optic discs showing glaucomatous change were as follows:

Converters (% of 21 optic discs):

- Global cup volume (33.3%)
- Infero-temporal cup volume (28.5%)
- Infero-nasal cup volume (38.9%)
- Supero-temporal cup area (33.3%)
OHT group (% of 164 optic discs):

- Global cup volume (2.5%)
- Infero-temporal cup volume (4.3%)
- Infero-nasal cup volume (4.9%)
- Supero-temporal cup area (1.2%)

These 4 parameters were therefore examined in more detail to identify the number of optic discs that were 'getting better' as well as those 'getting worse'.

For the converters, no optic discs were demonstrated to be 'getting better'. For the OHT group, 4 discs were 'getting better' for the global cup-volume parameter, 2 discs were 'getting better' for the supero-temporal cup area, 3 discs were 'getting better' for the infero-temporal cup-volume and 8 discs were 'getting better' for the infero-nasal cup volume. These results are illustrated in Figure 9 to Figure 12.
Figure 9 change in global cup volume

Figure 10 Change in supero-temporal cup area
Figure 11 Change in infero-temporal cup volume

Figure 12 Change in infero-nasal cup volume
4.2.3 Use of the GDx to determine differences in the RNFL thickness between normal, ocular hypertensive and glaucomatous eyes.

No significant difference was found between the groups for either age or optic disc area Table 10.

**Table 10 Mean Age (SD) and mean optic disc areas (SD) of groups**

<table>
<thead>
<tr>
<th>Subject Group</th>
<th>Mean age in years (SD)</th>
<th>Range (years)</th>
<th>Disc Area in mm$^2$ (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>65.2 (8.0)</td>
<td>45-78</td>
<td>1.77 (0.35)</td>
</tr>
<tr>
<td>OHT</td>
<td>64.3 (10.0)</td>
<td>38 - 93</td>
<td>1.83 (0.48)</td>
</tr>
<tr>
<td>POAG</td>
<td>68.0 (12.0)</td>
<td>36 - 85</td>
<td>1.77 (0.28)</td>
</tr>
<tr>
<td>p-value (ANOVA)</td>
<td>$p = 0.42$</td>
<td></td>
<td>$p = 0.59$</td>
</tr>
</tbody>
</table>
The Kruskal-Wallis test demonstrated significant inter-group differences in the total, superior and inferior values of RNFL thickness **Table 11**.

**Table 11 Results of the Kruskal-Wallis one-way analysis of variance for total and quadrant measurements**

<table>
<thead>
<tr>
<th>Area of RNFL Measured</th>
<th>x² (2) value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>14.0</td>
<td>=0.001</td>
</tr>
<tr>
<td>Inferior</td>
<td>25.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Superior</td>
<td>10.4</td>
<td>=0.006</td>
</tr>
<tr>
<td>Nasal</td>
<td>2.0</td>
<td>=0.360</td>
</tr>
<tr>
<td>Temporal</td>
<td>2.1</td>
<td>=0.344</td>
</tr>
</tbody>
</table>
Using the Mann-Whitney U test, the following specific inter-group differences were found:

RNFL thickness was significantly reduced in the POAG eyes compared to the normal and OHT eyes for the total, superior and inferior quadrantic values. **Table 12** No significant differences were found between the normal and OHT group for the total or each of the quadrantic RNFL thickness values.

**Table 12 Results of the Mann-Whitney U Test comparing OHT, POAG and normal eyes.**

<table>
<thead>
<tr>
<th>Region of RNFL Measured</th>
<th>OHT eyes</th>
<th>p-value</th>
<th>POAG eyes</th>
<th>p-value</th>
<th>Normal eyes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>median (inter-quartile range) μm</td>
<td>median (inter-quartile range) μm</td>
<td>median (inter-quartile range) μm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>64.1 (58.6-71.7)</td>
<td>&lt;0.001</td>
<td>53.2 (53.2-63.3)</td>
<td>&lt;0.001</td>
<td>65.0 (60.2-73.4)</td>
</tr>
<tr>
<td>Inferior</td>
<td>76.1 (64.8-85.7)</td>
<td>&lt;0.001</td>
<td>63.7 (55.5-72.4)</td>
<td>&lt;0.001</td>
<td>76.2 (67.6-87.5)</td>
</tr>
<tr>
<td>Superior</td>
<td>67.7 (60.7-67.7)</td>
<td>&lt;0.001</td>
<td>60.6 (54.2-66.6)</td>
<td>&lt;0.001</td>
<td>72.5 (62.5-81.3)</td>
</tr>
<tr>
<td>Nasal</td>
<td>51.5 (46.5-56.7)</td>
<td>=0.106</td>
<td>48.1 (44.9-55.2)</td>
<td>=0.138</td>
<td>50.7 (47.0-58.3)</td>
</tr>
<tr>
<td>Temporal</td>
<td>45.6 (39.9-54.5)</td>
<td>=0.393</td>
<td>44.3 (37.0-52.2)</td>
<td>=0.885</td>
<td>42.7 (37.6-50.9)</td>
</tr>
</tbody>
</table>
Box plots of the results are illustrated in Figure 13, Figure 14, Figure 15. The box-plots also demonstrate clearly the large degree of overlap in measurements between the groups.

**Figure 13** Box plot to show results of Total RNFL thickness values (µm)

![Box plot for Total RNFL thickness](image)

**Figure 14** Box plots to show results of Inferior RNFL thickness values (µm)

![Box plot for Inferior RNFL thickness](image)
Figure 15 Box plots to show results of Superior RNFL Thickness values (μm)
Section 5 Discussion
5.1 The betaxolol Vs placebo treatment trial in ocular hypertension

The initial power calculation made at the beginning of the study (see methods section) assumed a relatively high conversion rate of 8.75% per year, in the untreated group, and that treatment should reduce this by at least 50% if the null hypothesis was to be rejected. Calculations were made on the assumption that the cohort of OHT patients included in the study were at moderate to high risk of conversion. Initial calculations were made assuming a 3 year trial period. The estimated drop-out rate was 20%, and this was allowed for in the calculations. The power calculation done on the basis of these assumptions gave a figure of 384 patients in each group for a 3-year trial period. The conversion rate of treated patients observed in this study was 0.72% per year of all patients remaining in the trial. The overall annual conversion rate for both treated and untreated patients was 1.83%. The observed drop-out rate was in fact higher than initially estimated at 28.4%. It is clear from these figures that the initial power calculation overestimated the conversion rate in the untreated group and the possible effect of treatment. On the basis of this, the study may be criticised for not having sufficient power to be able to demonstrate any effect of treatment. However, despite this, greater numbers of patients were included in each of the groups than most of the previous treatment studies that have already been discussed.

Additional criticism can also be made about the use of a one-tail test of significance for the calculation, on the assumption that treatment would have no detrimental effect on the conversion rate. A more ideal estimate may have been
made using a two-tail test of significance, allowing that treatment may have an adverse effect on outcome.

A relatively high withdrawal rate was observed during the course of the study, but this was partly because of a strict trial protocol. The non-attendance rate was relatively small, and patients showed considerable commitment to completing their participation in the trial. The numbers of patients withdrawing for each of the specific reasons was roughly equal for the betaxolol and placebo groups. The incidence of drop intolerance was very similar for the two groups, reflecting the use of preservative in both drop preparations. The withdrawal of patients for medical reasons included the commencement of oral β-blockers for hypertension, the development of visual field defects following a stroke, or general debility secondary to chronic illness.

Overall, placebo produced a similar side-effect profile as betaxolol, including the onset of asthma and chest pain. It has been previously well established that patients on placebo frequently report side-effect symptoms similar to those of active drugs used in clinical trials (Weikrauch, 1999).

Survival analysis did not demonstrate any overall protective effect of betaxolol against conversion as compared to placebo over the time course of our study. However, if the pattern of the Kaplan-Meier curves is examined rather than overall survival statistics, then the curves appear to diverge slightly during the interval from 40-63 months of follow-up. It could be argued that this might represent a trend for betaxolol to delay conversion relative to placebo during this period, although this effect is small and not statistically significant. It should be pointed out that little useful information can be gained from the latter parts of the
survival curves illustrated, due to the small numbers of patients who have been followed up for longer than 60 months.

The baseline IOP levels for the betaxolol and placebo groups were comparable to the baseline mean IOP of the OHT subjects included in the multi-centre Ocular Hypertension Treatment Study (Gordon, 1999) reported as 24.9 +/- 2.7 mmHg. The authors of this initial paper from the study describing the design and base-line characteristics of subjects concluded that this level of initial IOP was sufficiently high to be able to demonstrate potential benefit of ocular hypotensive treatment in preventing or delaying glaucomatous damage.

Our results show that betaxolol significantly lowered the IOP of a group of ocular hypertensives as compared to placebo, but the effect was clinically small. The IOP lowering effect of betaxolol that we found is similar to that reported in previous studies (Boles Carenini, et al., 1994, Long, et al., 1988, Collingnon-Brach, 1994). The mean post-treatment IOP of the betaxolol group, although lower than for the placebo group, still remained just above the upper limit of the ‘normal’ range of IOP at 21.6 mmHg (+/-2.88). The pattern of the mean IOP over time (Figure 4) shows an initial drop from the base-line IOP once treatment was started and this fall was well maintained through the first 60 months of the trial.

The design of the trial in comparing monotherapy with placebo limits the scope for the clinician to set a target pressure for individual patients. In the clinical situation, when a decision has been made to control the IOP, then a borderline or slightly raised pressure may not be acceptable, and treatment made be changed in order to optimise the response. Treatment trials in which a target IOP lowering is
set and achieved (Gordon, 1999) may give us more useful information on possible benefits of prophylactic treatment.

It is interesting to note that even in the placebo group there was an overall lowering of the IOP, with a gradual reduction over time. This observation may be explained by the well-described ‘placebo effect’ (Kaptchuk, 1998, Weikrauch, 1999), or a tendency for IOPs in some OHT patients to reduce over time (Linner, 1980, Schwartz and Tolusan, 1980). This effect also explains why the IOP lowering effect of betaxolol was reduced when compared to the effect of placebo.

Other studies have reported that the height of the base-line IOP level is a risk factor for conversion (David, et al., 1977, Quigley, et al., 1994, Schulzer, et al., 1991). This is confirmed by our study as the converters had significantly higher pre-and post-treatment IOPs compared to the non-converters. From the results it is evident that the converters overall had a smaller fall in the IOP (1.9 mmHg) than the non-converters (3.5mmHg). On analysis of the betaxolol group alone, the converters showed a less marked drop in IOP compared to the non-converters, although the difference did not reach statistical significance. It is possible that the group of converters was comprised of individuals who responded less well to treatment, and were therefore at greater risk.

If a protective effect by betaxolol had been observed during the time course of the trial despite the clinically small IOP effect, then this may have been partly attributed to a possible ‘neuro-protective’ effect of the agent. In view of the results presented here, there is no evidence to support such an effect of betaxolol.
field conversion criteria (David, et al., 1977 Kitazawa, et al., 1977 Quigley, et al., 1994). However, with the establishment of better methods for the detection of pre-perimetric optic disc change (Kamal DS, 1999), a well recognised early sign of the onset of glaucoma (Caprioli, et al., 1996, Pederson and Anderson, 1980, Sommer, et al., 1979), then similar prospective OHT studies will report higher conversion rates. Our conversion criteria were based on the gold standard methods of visual field analysis available at the start of the study, and these were continued throughout its course. Subjects on the trial underwent yearly optic disc imaging on the HRT from 1994 onwards. Work is currently under way to analyse all the consecutive HRT images of trial subjects for evidence of conversion on the basis of optic disc conversion criteria. It is likely that by using these alternative criteria the number of subjects who are classified as converters will increase, and a reanalysis of the data may produce different results to this study.

The results of this study may apply only to the Caucasian sub-group, as this was an inclusion criterion for entry into the trial. We did not collect information on the prevalence of family history of glaucoma amongst the trial patients, because of the difficulties associated with self-reporting. This, however, may have provided some additional information on relevant risk factors. We did not find any significant difference between the converters and non-converters for age, previously identified as a risk factor for conversion.

A summary flow diagram of the trial results is illustrated below.
Flow diagram to describe the progress of patients through the betaxolol Vs placebo treatment trial in ocular hypertension.

Eligible patients n=356

Randomisation

Placebo n=173
- Withdrawn n=52
  - Converted n=16
    - Completed trial n=121

Betaxolol n=183
- Withdrawn n=49
  - Converted n=12
    - Completed trial n=134

Withdrawn n=52

Converted n=16

Completed trial n=121

Completed trial n=134
5.2 Evaluation of methods of early detection

5.2.1 Detection of optic disc change with the Heidelberg Retina Tomograph prior to confirmed visual field change in ocular hypertensives converting to early glaucoma

Four global optic disc parameters were found to change in the converters over time: cup area, C/D area ratio, and rim area and cup volume. These results correspond to what we would expect to observe in glaucomatous change (Airaksinen and Drance, 1985 Armaly, 1969 Armaly, 1970).

The other significant results were found on segmental analysis of optic disc parameters. Interestingly, the largest magnitude of change was found in the supero-temporal cup area and supero-temporal rim area. The next largest change was found in the supero-nasal cup and rim area, segmental parameters not often documented to show change. We did find significant change of a smaller magnitude in the infero-temporal rim and cup parameters, a segment previously described as the most common site of clinically detected early glaucomatous change (Jonas et al. 1988). One possible reason for this finding is that on subjective clinical optic disc examination, the infero-temporal segment is the easiest segment to assess, whereas the nasal segments may be obscured by blood vessels. Objective assessment using the HRT may facilitate the detection of change in these regions.

The results indicate that segmental as well as global analysis of optic disc images is required for detection of glaucomatous change, and suggest that the
HRT may be able to detect change in areas such as the supero-nasal, infero-nasal and supero-temporal segments which may not be detectable clinically.

It may be argued that by analysing several parameters in several segments some significant results may be found simply by chance. However no significant results were found in the normal control group using the same method of analysis. In addition, all the parameters that changed did so in the glaucomatous direction.

The Wilcoxon statistical test is a non-parametric test that analyses data as a group rather than on an individual basis. The results obtained reflect the general direction of change, if present, for the group as a whole. Thus, for those segmental parameters that did show change over time from HRT1 to HRT2 the majority of the optic discs within the group changed in that direction but a number remained stable. Therefore, in the analysis of the mean magnitude of change, the standard deviation values are relatively large, reflecting the wide range of individual values within the group.

Variability in measured topographical parameters may arise from variability in the contour line alignment when the contour line export facility is used (Orgul et al., 1996) This may contribute to some of the variability between HRT1 and HRT2 parameter values, but not to the overall direction of change.

Another possible criticism of the method used here is that the examiner was un-masked to the subject group or time sequence of the optic disc images examined in the study, thus introducing a potential source of bias. However, we do not feel that the lack of masking would influence the results to a significant degree as once the initial contour line has been drawn around the disc edge, all further analysis including the export of the contour is fully automated by HRT.
software. The design of the equipment makes masking impossible as dates of examination details are displayed along with each image on the screen.

A recent study (Iester et al., 1997) which examined the correlation between visual field indices and HRT parameters in glaucomatous patients found that the neuro-retinal rim area was the most important predictor of mean deviation and corrected pattern standard deviation values, by multiple regression analysis. Cup area, C/D area ratio, rim area, rim volume, Third moment and the RNFL cross-sectional area were also found to correlate with these indices. Other cross-sectional studies have also found that the Third moment may be of value in demonstrating group differences between normal, glaucomatous and ocular hypertensive eyes (Hatch, et al., 1997, Uchida, et al., 1996, Zangwill, et al., 1996). Third moment was not found to be useful in detecting change by longitudinal analysis of the same optic discs in our study. We have not included an analysis of the correlation between the location of the visual field defects and the location of optic disc change. The analysis method used in this study examined the data for each of the groups as a whole and therefore it would not be appropriate to examine the position of the field defects for individual converters.

Optic disc appearance was not a restriction criterion for entry into the study for either the OHT group or the normal group. Subjects were therefore not chosen on the basis of their optic disc appearance, eliminating an important and often overlooked source of selection bias from our study. The HRT1 mean values for the CD area ratio in the converters were significantly greater than the values for the normal and OHT subjects. Also, the mean values for the rim area in HRT1 were less in the converting group than in the normal and OHT groups. There were no differences in both these parameters between the OHT group and
the normals. The wide range of CD area ratio and rim area values in the converter group is reflected by the large standard deviations, but smaller in the normal group. This may be explained by the fact that a number of the converters had ‘glaucomatous optic discs’ at the time of HRT1, despite having normal visual fields. In this group, the HRT was able to detect further glaucomatous change in an already abnormal disc, before the development of confirmed visual field loss. For the group of converters the time interval between HRT1 and the final conversion confirming visual field was short (only 5.4 months ± 5.2). This was due to the relative infrequent HRT imaging that was possible within the constraints of the trial. Work is currently underway to determine if more frequent imaging on a four monthly basis is able to detect change over a shorter period of time.

One of the major challenges in the management of glaucoma is the early detection of disease. This study, although small scale, suggests that the HRT may be of use in detecting glaucomatous change in the optic nerve head over time, prior to confirmed visual field change. Although other clinical methods have also demonstrated similar changes, this study provides, for the first time, actual measured data of optic disc changes. This allows quantification of the changes as well as the identification of the specific optic disc regions where it occurs.
5.2.2 Use of sequential Heidelberg Retina Tomograph images to identify changes at the optic disc in ocular hypertensive patients at risk of developing glaucoma

Using the normal control data, this study has determined the normal limit of variability of optic disc change, and used this value in the analysis of individual disc data. It is apparent on individual analysis that the majority of converter discs (13/21) do show change above that expected for normal variability but in 8 cases this could not be detected by this method of HRT analysis which is therefore limited. However, no converter discs show change in the direction of 'getting better'. The change occurring may have been too small to be detectable by this method of HRT analysis. The converters that demonstrated disc change did so prior to visual field change.

The OHT group maintained normal visual fields but 47 individual discs were shown to be ‘getting worse’ and follow up of these patients is continuing to determine whether they develop reproducible visual field defects. On the basis of the previous converter data, these individual subjects are at risk of developing future visual field loss, and they represent a group of ocular hypertensives that may benefit from early IOP lowering treatment. 15 OHT discs also showed evidence of ‘getting better’ suggesting that there is some inherent variability in the analysis of sequential images of HRT, as it is unlikely that these discs showed real improvement. It is interesting to note that the initial HRT1 cup and rim parameters of the OHT group did not differ significantly from those of the normal group, unlike the values for the converter group. Optic disc damage is therefore occurring in a disc which is fairly healthy at base-line. This may
explain the delay in functional loss observed in this group, assuming that they
will eventually develop visual field defects.

Cross-sectional studies such as those that have been mentioned previously,
are useful in demonstrating the use of the HRT in the detection of glaucomatous
damage at a point in time, but the real use of the technique will be in the
longitudinal follow-up of the individual patient. There has not yet been any other
published longitudinal HRT data on OHT patients with normal visual fields with
which we may compare our results. At ARVO 1999 Chauhan et al. (Chauhan B
C, 1999) presented their method for detecting change in sequential HRT images
in glaucoma patients by means of change probability maps that use data analysed
on a pixel basis. Preliminary results found that this method was able to identify
95% of glaucoma eyes with early visual field defects determined to be
progressing on optic disc photography. Publication of further work using change
probability maps is awaited.

In conclusion, this study has for the first time identified those optic discs
in a group of OHT patients that demonstrate change (above the
level expected for normal variability) over time. OHT patients who show such
change are at risk of conversion and may benefit from treatment to prevent the
development of functional visual field loss. Follow up of these patients will
continue to determine whether they go on to develop visual field defects and over
what time scale. Potential selection bias has again been eliminated by excluding
optic disc appearance in the inclusion criteria for each of the subject groups. The
use of the HRT in clinical practice awaits resolution of the issue of
'improvement' noted in a subset of our OHT patients. Refinement of analysis
methods may also resolve the issue of those converter eyes in which no change could be detected prior to the development of visual field defects.
5.2.3 Use of the GDx to determine differences in the RNFL thickness between normal, ocular hypertensive and glaucomatous eyes.

The results of this study have demonstrated that the relatively new technology utilised by the GDx is able to detect differences between glaucomatous eyes as compared with normal and OHT eyes that were initially grouped on the basis of visual field and IOP characteristics. This study has therefore also eliminated the selection bias that has influenced results from other studies that have included subjects on the basis of their optic disc and RNFL appearance (Anton, 1997, Hollo, et al., 1997, Weinreb, et al., 1995). A study by Tjon-Fo-Sang et al. (Tjon Fo Sang, et al., 1996) compared the RNFL thickness obtained using the NFA in normals and OHT subjects with similar characteristics to the groups described here and found significant differences in the mean superior and inferior values, in contrast to these results. They did, however, also find a large degree of overlap between the groups, and this has been confirmed by later studies using the GDx (Lee, 1999, Weinreb, 1998, Xu, 1998). The study presented here did not find any difference in the RNFL thickness values between the normals and OHT groups, despite the well recognised fact that optic disc and RNFL damage pre-date perimetric change (Quigley H A, 1999, Quigley, et al., 1982 Sommer, et al., 1991). The GDx may therefore be limited in its ability to identify early structural damage in those subjects at risk of developing glaucoma.

This study did not set out to determine the relative specificity and sensitivity of the technique, and the considerable degree of overlap found between groups of subjects would limit the value of this. Another study by the Dutch group however, (Tjon-Fo-Sang, 1997) specifically addressed the issue of
specificity and sensitivity and found surprisingly high values of 93% and 96% respectively. However, these results have not been reproduced, and their original results may have been affected by technician observer bias (Lemij-Personal Communication to Professor Hitchings, Gullstrand meeting Stockholm May 1999). The upgrade in software used by the new generation GDx machine, has limited the influence of such bias on the acquisition of RNFL images.

A more recent study (Weinreb, 1998) has investigated the ability of the GDx to detect glaucomatous damage, and produced more realistic figures for specificity and sensitivity of 62% and 82% using the GDx software, with the figures rising to 74% and 92% using the authors’ best discriminant function. Overlap of values between groups remains the largest obstacle to the use of this tool in population based screening.

Previous studies (Chi, et al., 1995, Poinosawmy, et al., 1997) have found a progressive decline in RNFL thickness values with increasing age, the loss ranging from 0.2 – 0.38μm per year. The mean ages of our subject groups were very similar and it is unlikely that this was a factor influencing our results, but age should be taken into account when individual data are analysed.

A comparison was made only of the basic mean quadrantic and total RNFL thickness values in this analysis. Other groups have found analysis of a combination of parameters such as supero-nasal and infero-nasal ratios (Lee, 1999), and ‘modulation’ parameters (Xu, 1998) to have greater discriminating ability. Further studies are needed to determine which are the most useful parameters to differentiate between normals, OHT and glaucoma patients, and further work is currently being carried out on larger numbers of subjects using
the same criteria to determine levels of specificity, sensitivity and discriminant ability.

Good reproducibility (Hollo, et al., 1997, Niessen, et al., 1996, Waldocks A., 1998) of the technique may allow use of this system for longitudinal studies and preliminary work is promising (Pinoosawmy D., 1999).

In summary, this study has demonstrated that the GDx may detect differences in RNFL thickness between POAG patients as compared to OHT and normal subjects, not previously selected on the basis of optic disc and RNFL criteria. The technique shows promise in the detection of early glaucomatous damage but requires further validation before it can be of value in the clinical setting.
Section 6 Summary and Conclusions
The results of the betaxolol treatment Vs placebo trial reported here have provided important information on the natural history of both treated and untreated ocular hypertension. The study design was rigorous in comparison with some previous OHT treatment trials (Kass, 1980, Epstein, 1989, Schulzer, 1991). An ‘ideal’ clinical trial investigating the effect of a specific treatment on the course of a disease such as OHT or glaucoma should be prospective, randomised, double-blind and controlled (Rossetti, 1993) – all criteria met by this study. These factors help to eliminate sources of bias that can influence results and lead us to make mis-guided conclusions. The time span of this study was comparable to previous ones (Kass, 1980, Epstein, 1989, Schulzer, 1991, Quigley, 1994). The practical aspects of conducting a clinical trial as well as limited resources will ultimately dictate the follow-up time. It has been well established that the incidence of glaucoma increases with increasing age (Tielsch, 1990, Tielsch, 1991, Klein, 1992) so an ‘ideal’ OHT study would be considerably longer than is actually feasible. We would expect a larger number of patients in our study to convert over time, with an extended period of follow-up, and this must be considered when interpreting the results for general clinical practice. We did not find that betaxolol had a beneficial effect on the rate of conversion from ocular hypertension to glaucoma, over the time course of our study. With a longer period of follow-up, and possibly larger numbers of patients, this result may have been different. One factor for the observation is the clinically small effect of betaxolol on the IOP level, as compared to placebo. With the advent of more powerful IOP lowering drops such as latanaprost (Greve, 1997, Watson, 1998, Camras, 1998), future OHT treatment trials achieving a greater drop in IOP may demonstrate a protective effect. One major criticism of placebo controlled
treatment trials designed simply to investigate the effect of single medications is that investigators are unable to set IOP lowering targets. A more informative and conclusive trial may incorporate the setting of, for instance, a 20% target reduction in base-line IOP, allowing the clinician to add or change topical treatments as required (Gordon, 1996). This would mimic more closely the situation found in everyday clinical practice, with the clinician obtaining the best possible medical control of IOP.

On the basis of the OHT treatment trials conducted so far, there is still no conclusive evidence that blanket prophylactic administration of topical hypotensive agents can alter the natural course of ocular hypertension. Individual assessment of the risk of conversion, accessibility to follow-up and patient preference will therefore continue to guide clinical practice. However, there seems no justification on the basis of current evidence to prophylactically lower the IOP levels of all OHT patients, when the treatment itself may cause adverse effects. Treatment should continue to be targeted at those perceived to be at the greatest risk of conversion, with individual tailoring of treatment regimes. This policy may also help to lessen the burden of OHT management on ophthalmology services, allowing the follow-up of stable untreated OHT by experienced optometrists with access to applanation tonometry and automated perimetry.

The development of methods for the early detection of structural glaucomatous damage in the optic disc or RNFL may lead to earlier, more effective hypotensive treatment of those at greatest risk of functional loss. The methods of detecting optic disc change with the HRT presented here are fairly basic, and only analyse two sequential images. A more ideal method would look
at change over several images, obtained over shorter intervals of time. Despite these limitations, results from this study formed the basis of the first publication examining longitudinal optic disc change by means of the HRT in the literature. Currently use of the HRT is limited to specialist centres and research settings due to the costs involved in purchasing machines and training technicians to become proficient in the technique. The cost of such technology will gradually fall over the next few years, widening the settings in which the HRT may become clinical tool. There is a need for integral HRT analysis software to detect change in optic disc parameters over time, so that this may be easily and quickly demonstrated in the clinical setting, and this should be developed once a suitable method has been validated.

The GDx requires further investigation before may be utilised in the clinical setting. The study presented here was published (Kamal, 1999) on the basis that it provided important data on groups of patients not preselected on optic disc or RNFL criteria. Despite the overlap in measurements between the groups, it is encouraging that differences could be detected in groups selected on visual field and IOP criteria.
**Future Work**

There is still much work to be done on the data of the group of OHT patients included in these studies. This includes the longitudinal analysis of all the HRT and GDx data that has been collected over the last few years for all groups of subjects. It will be interesting to determine whether treatment with betaxolol had an effect on the optic disc or RNFL parameters noted over time, for both the converting and non-converting groups. The continued follow-up of those stable OHT patients who demonstrated optic disc change despite maintaining normal visual fields will reveal how many develop visual field defects and within what time-scale. The vast majority of all these patients still continue with their follow-up in the OHT research based clinic at Moorfields Eye Hospital. A further study is also underway to analyse longitudinal Humphrey visual field data by a different computerised method (Fitzke 1996), to determine if the converters may be picked up at an earlier stage than by the AGIS criteria that were used in this study. Future work will reflect a refinement of some of the techniques described here, so that the detection of early glaucomatous damage and its progression may be simplified and made more objective.
References


# Glossary of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNFL</td>
<td>Retinal Nerve Fibre Layer</td>
</tr>
<tr>
<td>GDx</td>
<td>Nerve Fibre Analyser (latest version)</td>
</tr>
<tr>
<td>HRT</td>
<td>Heidelberg Retina Tomograph</td>
</tr>
<tr>
<td>IOP</td>
<td>Intra-ocular pressure</td>
</tr>
<tr>
<td>NFA</td>
<td>Nerve Fibre Analyser</td>
</tr>
<tr>
<td>OHT</td>
<td>Ocular Hypertension</td>
</tr>
<tr>
<td>POAG</td>
<td>Primary open angle glaucoma (glaucoma)</td>
</tr>
</tbody>
</table>
Appendix
Images obtained by the HRT. The image on the right is the reflectivity image, and the mean topography is on the left. Patient is from the group of converters.
Images with contour line drawn around optic disc edge for analysis.
Values of global and segmental parameters on screen print-out

<table>
<thead>
<tr>
<th>Parameter</th>
<th>(mm²)</th>
<th>Global</th>
<th>temp sup</th>
<th>temp inf</th>
<th>nasal</th>
<th>nsl sup</th>
<th>nsl inf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area</td>
<td>2.217</td>
<td>0.551</td>
<td>0.280</td>
<td>0.295</td>
<td>0.555</td>
<td>0.273</td>
<td>0.264</td>
</tr>
<tr>
<td>Effect. Area</td>
<td>1.912</td>
<td>0.498</td>
<td>0.236</td>
<td>0.282</td>
<td>0.467</td>
<td>0.233</td>
<td>0.197</td>
</tr>
<tr>
<td>Area bel Refr</td>
<td>1.283</td>
<td>0.378</td>
<td>0.215</td>
<td>0.287</td>
<td>0.181</td>
<td>0.199</td>
<td>0.103</td>
</tr>
<tr>
<td>Height in Cont [mm]</td>
<td>0.533</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MeanHeight Cont [mm]</td>
<td>0.141</td>
<td>0.256</td>
<td>0.207</td>
<td>0.103</td>
<td>0.087</td>
<td>0.109</td>
<td>0.021</td>
</tr>
<tr>
<td>PeakHeight Cont [mm]</td>
<td>-0.013</td>
<td>0.198</td>
<td>0.126</td>
<td>0.028</td>
<td>0.065</td>
<td>0.070</td>
<td>-0.013</td>
</tr>
<tr>
<td>Height Var Cont [mm]</td>
<td>0.203</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vol bel Surface [cm³]</td>
<td>0.879</td>
<td>0.189</td>
<td>0.266</td>
<td>0.241</td>
<td>0.138</td>
<td>0.161</td>
<td>0.091</td>
</tr>
<tr>
<td>Vol abo Surface [cm³]</td>
<td>0.811</td>
<td>0.002</td>
<td>0.002</td>
<td>0.000</td>
<td>0.003</td>
<td>0.001</td>
<td>0.002</td>
</tr>
<tr>
<td>Vol bel Refr [cm³]</td>
<td>0.628</td>
<td>0.145</td>
<td>0.137</td>
<td>0.096</td>
<td>0.073</td>
<td>0.120</td>
<td>0.056</td>
</tr>
<tr>
<td>Vol abo Refr [cm³]</td>
<td>0.135</td>
<td>0.010</td>
<td>0.007</td>
<td>0.012</td>
<td>0.060</td>
<td>0.013</td>
<td>0.034</td>
</tr>
<tr>
<td>Maximum Depth [mm]</td>
<td>0.961</td>
<td>0.852</td>
<td>0.951</td>
<td>0.946</td>
<td>0.942</td>
<td>0.970</td>
<td>0.989</td>
</tr>
<tr>
<td>Third moment []</td>
<td>-0.043</td>
<td>-0.118</td>
<td>0.271</td>
<td>0.031</td>
<td>-0.271</td>
<td>0.343</td>
<td>0.818</td>
</tr>
<tr>
<td>Mean radius [mm]</td>
<td>0.840</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **Area** = disc area
- **Area below reference** = cup area
- **Volume below reference** = cup volume
- **Volume above reference** = rim volume
Images taken from the GDx: Scanning laser ophthalmoscope image with corresponding retardation map. The patient is from the normal group.
The images below show contour line fitted to optic disc edge, with an area of 1.5 disc diameters from the centre included for RNFL thickness analysis.
Comparison of data from HRT1 and HRT2 for normal group. Mean difference between images for each parameter and p-value.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean difference (SD)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Global rim volume</td>
<td>-0.007 (0.059)</td>
<td>0.59</td>
</tr>
<tr>
<td>Nasal rim volume</td>
<td>0.0003 (0.022)</td>
<td>0.10</td>
</tr>
<tr>
<td>Infero-nasal rim volume</td>
<td>-0.002 (0.009)</td>
<td>0.30</td>
</tr>
<tr>
<td>Supero-nasal rim volume</td>
<td>-0.0001 (0.009)</td>
<td>0.10</td>
</tr>
<tr>
<td>Temporal rim volume</td>
<td>-0.002 (0.007)</td>
<td>0.25</td>
</tr>
<tr>
<td>Infero-temp rim volume</td>
<td>-0.002 (0.01)</td>
<td>0.29</td>
</tr>
<tr>
<td>Supero-temp rim volume</td>
<td>-0.001 (0.007)</td>
<td>0.46</td>
</tr>
<tr>
<td>Global cup area</td>
<td>0.003 (0.082)</td>
<td>0.20</td>
</tr>
<tr>
<td>Nasal cup area</td>
<td>-0.03 (0.015)</td>
<td>0.88</td>
</tr>
<tr>
<td>Infero-nasal cup area</td>
<td>0.03 (0.018)</td>
<td>0.97</td>
</tr>
<tr>
<td>Supero-nasal cup area</td>
<td>0.0003 (0.010)</td>
<td>0.93</td>
</tr>
<tr>
<td>Temporal cup area</td>
<td>0.0003 (0.038)</td>
<td>0.78</td>
</tr>
<tr>
<td>Supero-temp cup area</td>
<td>-0.0002 (0.01)</td>
<td>0.94</td>
</tr>
<tr>
<td>Infero-temp cup area</td>
<td>0.004 (0.014)</td>
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</tr>
<tr>
<td>Global C/D area ratio</td>
<td>0.0009 (0.050)</td>
<td>0.88</td>
</tr>
<tr>
<td>Nasal C/D area ratio</td>
<td>-0.004 (0.040)</td>
<td>0.24</td>
</tr>
<tr>
<td>Infero-nas C/D area ratio</td>
<td>0.008 (0.049)</td>
<td>0.78</td>
</tr>
<tr>
<td>Sup-nas C/D area ratio</td>
<td>0.0003 (0.050)</td>
<td>0.44</td>
</tr>
<tr>
<td>Temp C/D area ratio</td>
<td>0.003 (0.093)</td>
<td>0.10</td>
</tr>
<tr>
<td>Sup-temp C/D area ratio</td>
<td>-0.03 (0.050)</td>
<td>0.21</td>
</tr>
<tr>
<td>Inf-temp C/D area ratio</td>
<td>-0.16 (0.060)</td>
<td>0.32</td>
</tr>
<tr>
<td>Global disc area</td>
<td>-0.017 (0.076)</td>
<td>0.54</td>
</tr>
<tr>
<td>Nasal disc area</td>
<td>-0.004 (0.027)</td>
<td>0.34</td>
</tr>
<tr>
<td>Infero-nas disc area</td>
<td>-0.001 (0.005)</td>
<td>0.44</td>
</tr>
<tr>
<td>Supero-nas disc area</td>
<td>-0.002 (0.010)</td>
<td>0.19</td>
</tr>
<tr>
<td>Temporal disc area</td>
<td>-0.006 (0.021)</td>
<td>0.42</td>
</tr>
<tr>
<td>Sup-temp disc area</td>
<td>-0.009 (0.05)</td>
<td>0.42</td>
</tr>
<tr>
<td>Inf-temp disc area</td>
<td>0.004 (0.01)</td>
<td>0.49</td>
</tr>
<tr>
<td>Global rim area</td>
<td>-0.019 (0.126)</td>
<td>0.80</td>
</tr>
<tr>
<td>Nasal rim area</td>
<td>-0.002 (0.034)</td>
<td>0.20</td>
</tr>
<tr>
<td>Inf-nas rim area</td>
<td>-0.004 (0.012)</td>
<td>0.61</td>
</tr>
<tr>
<td>Sup-nas rim area</td>
<td>-0.002 (0.020)</td>
<td>0.51</td>
</tr>
<tr>
<td>Temp rim area</td>
<td>0.007 (0.050)</td>
<td>0.10</td>
</tr>
<tr>
<td>Inf-temp rim area</td>
<td>0.007 (0.019)</td>
<td>0.80</td>
</tr>
<tr>
<td>Sup-temp rim area</td>
<td>0.0007 (0.012)</td>
<td>0.22</td>
</tr>
<tr>
<td>Global Third moment</td>
<td>0.006 (0.030)</td>
<td>0.26</td>
</tr>
<tr>
<td>Nasal Third moment</td>
<td>-0.002 (0.053)</td>
<td>0.07</td>
</tr>
<tr>
<td>Inf-nas Third moment</td>
<td>0.02 (0.063)</td>
<td>0.98</td>
</tr>
<tr>
<td>Sup-nas Third moment</td>
<td>0.09 (0.371)</td>
<td>0.10</td>
</tr>
<tr>
<td>Temp Third moment</td>
<td>0.01 (0.024)</td>
<td>0.21</td>
</tr>
<tr>
<td>Inf-temp Third moment</td>
<td>0.0002 (0.040)</td>
<td>0.35</td>
</tr>
<tr>
<td>Sup-temp Third moment</td>
<td>0.023 (0.062)</td>
<td>0.07</td>
</tr>
<tr>
<td>Global cup volume</td>
<td>0.008 (0.030)</td>
<td>0.98</td>
</tr>
<tr>
<td>Nasal cup volume</td>
<td>0.001 (0.005)</td>
<td>0.11</td>
</tr>
<tr>
<td>Inf-nas cup volume</td>
<td>0.001 (0.002)</td>
<td>0.07</td>
</tr>
<tr>
<td>Sup-nas cup volume</td>
<td>0.001 (0.005)</td>
<td>0.39</td>
</tr>
<tr>
<td>Temporal cup volume</td>
<td>0.003 (0.014)</td>
<td>0.30</td>
</tr>
<tr>
<td>Inf-temp cup vol</td>
<td>0.0008 (0.004)</td>
<td>0.38</td>
</tr>
<tr>
<td>Sup-temp cup vol</td>
<td>0.002 (0.007)</td>
<td>0.21</td>
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