Title page

QT prolongation in patients treated for drug-resistant tuberculosis

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[redacted]

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Declaration page

I, Gareth Hughes confirm that the work presented in my thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

……

Dr Gareth Hughes

……..

Date

Abstract

Introduction

STREAM (Standardised Treatment Regimen of Anti-TB Drugs for Patients with MDRTB) Stage 1 demonstrated non-inferior efficacy of a Short regimen for rifampicin-resistant TB compared to the WHO-recommended Long regimen. Cardiac safety of participants was a concern due to the composition of the Short (study) regimen. It was unclear how many patients would be affected, who would be affected, the best monitoring strategy and how to identify those at risk of developing QT prolongation.

Methods

An analysis of new data created from STREAM Stage 1 ECGs was performed to investigate cardiac safety. In total, 424 participants (282 Short: 142 Long) were randomised. Data were analysed to identify risk factors for clinically relevant QT prolongation (QT/QTcF ≥500ms), how it evolved over time, whether a monitoring strategy could be refined, if machine readings were reliable and whether differences between groups for T-wave morphology abnormalities existed.

Results

The risk of developing QT or QTcF prolongation ≥500ms was higher for participants on the Short regimen (11%) vs the Long regimen (5%) (HR 95% CI: 2.31 (1.02-5.26)), p=0.0470. QT/QTcF prolongation ≥500ms was more frequent in patients from Mongolia (10/22 45.5%) compared with 3.5-11.9% at other sites, as well as those

with higher baseline values (QTcF ≥400ms; OR 5.99, 95% CI 2.04-17.62). Compared with manual readings, machine readings overestimated the QT interval by a mean of 22.5ms. Nearly half (90/200) of ECGs reviewed had T-wave morphology abnormalities by 3 months or more of treatment.

Conclusion

Though a sizeable number developed clinically relevant QT prolongation, the Short regimen in STREAM Stage 1 was safe and tolerated by most participants. This thesis has shown who was at greater risk, how they were managed and how early ECG readings can identify them for more targeted monitoring. This will inform clinicians and policy makers involved in rifampicin resistant tuberculosis (RR-TB) treatment programmes.

Impact statement

Over the last decade there has been a paradigm shift from long complex regimens for patients with rifampicin-resistant tuberculosis (RR-TB) to simplified shorter regimens. Much work has been done investigating the efficacy and safety of newer regimens which include some novel and repurposed medications. It could be argued that three of the commonest adverse events associated with RR-TB regimens are ototoxicity, hepatotoxicity, and cardiotoxicity. With the move away from injectable regimens, ototoxicity will become less of a concern, however the other two remain problems when managing these patients. The focus of this thesis is on cardiotoxicity through the measurement of the QT interval in ECG monitoring for participants in the STREAM Stage 1 trial. Each data chapter (except Chapter 3) is either published or has been presented as an abstract at a major international conference.

My thesis has shown that most participants tolerated a RR-TB regimen from a cardiac perspective, though a sizeable number required treatment modification. Our understanding of baseline risk factors, ethnic differences, and evolution of QT interval prolongation over time has improved following the work in chapter 1 (published).

In trial settings with greater resources, patient monitoring is easier than in programmatic settings. The work for chapter 2 (published) has shown which groups are at higher risk of QT prolongation based on their intervals in the first month of treatment which could allow policy makers to reduce patient monitoring for those at lower risk without missing higher risk patients, which may save resources whilst benefiting patients with reduced visits for those at lower risk.

Many clinicians and patients understandably have anxiety about a prolonged QT interval, particularly once it reaches 500ms as the risk of ventricular arrythmias and sudden death is higher. Chapter 3 (abstract accepted) details how patients in the STREAM Stage 1 trial were managed and shows that development of QT prolongation, that often-needed treatment modification, did not result in poorer outcomes compared to those who did not develop QT prolongation.

In cardiology settings there is much data to support the accuracy of manual readings over machine readings of the QT interval at a single point in time. This is not practical though in most other settings like TB programmes where automated machine readings that can be performed with little training would be preferential. The work in chapter 4 (oral abstract) demonstrated that in most circumstances the machine readings can be considered reliable over several months of treatment with drugs known to cause cardiotoxicity. This has not been shown before and not only supports clinicians and other stakeholders in using machine readings but identifies in which circumstances the readings are less reliable with some guidance on how to identify these.

The final chapter (oral abstract) is novel in that, to the best of my knowledge, it has shown for the first-time patients receiving DR-TB treatment frequently develop abnormalities in their T wave morphology which are consistent with disruption to the voltage gated potassium channels in the myocardium. Importantly the work showed that these abnormalities occur early, so could have implications for policy makers and clinicians who might utilise these abnormalities to identify high risk patients and increase their cardiac monitoring or modify their treatment.

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GH and HB designed and led the analyses and wrote the first draft of the manuscript. All authors provided input in study design, commented on analysis methods, and wrote the manuscript with GH and HB. AJN and SKM were chief investigators of the STREAM trial; IDR was the sponsor representative. All authors read and approved the final manuscript.

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GH and HB designed and led the analyses and wrote the first draft of the manuscript. All authors provided input in study design, commented on analysis methods, and wrote the manuscript with GH and HB. AJN and SKM were chief investigators of the STREAM trial; IDR was the sponsor representative. All authors read and approved the final manuscript.		
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Acknowledgements

I am incredibly grateful to Professor Meredith for providing me with the opportunity to work and study at the Medical Research Council Clinical Trials Unit (MRC CTU) over the last few years and for also providing invaluable patience and feedback during the writing of this thesis. I am equally grateful to Professor Crook for her comments and feedback on this thesis and for helping a non-statistician understand some basic statistical principles.

I would like to acknowledge Professor Lambiase for his expert cardiology advice and suggestions in developing research questions relating to the ECG data as well as for providing training in manual QT interval calculation and T wave morphology categorisation. I am grateful to Dr Esmail for his comments and suggestions for several aspects of my thesis.

I am also indebted to Henry Bern for all his help with the statistical analysis for the various pieces of work that went into this thesis, his patience with my endless questions and prompt replies. Dr Young also deserves acknowledgement for being instrumental in helping with the ECG training for manual QT interval calculation and T wave morphology categorisation.

Finally, I would not have been able to complete this work without the help and support of my wife Mekhala and the welcome distractions provided by my two children Aashriya and Kalyaan.

Contents

Abbreviations

BA – Bland Altman Plot

CI – Confidence Interval

DAIDS – Division of Acquired Immunodeficiency Syndrome

DS-TB – Drug sensitive tuberculosis

FDA – United States, Food and Drug Administration

hERG - Human Ether-a-go-go Related Gene

HIV - Human immunodeficiency virus

KG - Kilogram

LOA – Limits of agreement

LQTS – Long QT syndrome

MDR-TB - Multi-drug resistant tuberculosis

MG - Milligram

NPV – Negative Predictive Value

NTP – National Treatment Programme

PK - Pharmacokinetics

PPV – Positive Predictive Value

QTc – Corrected QT interval

QTcB - Corrected QT interval using Bazett's formula

QTcF – Corrected QT interval using Fredericia's formula

ROC – Receiver Operating Characteristic

RR-TB – Rifampicin resistant tuberculosis

SD – Standard deviation

SE – Standard Error

STREAM – Standard treatment regimen of anti-tuberculosis drugs for patients with Rifampicin-resistant Tuberculosis

TB - Tuberculosis

TdP – Torsades de Pointes

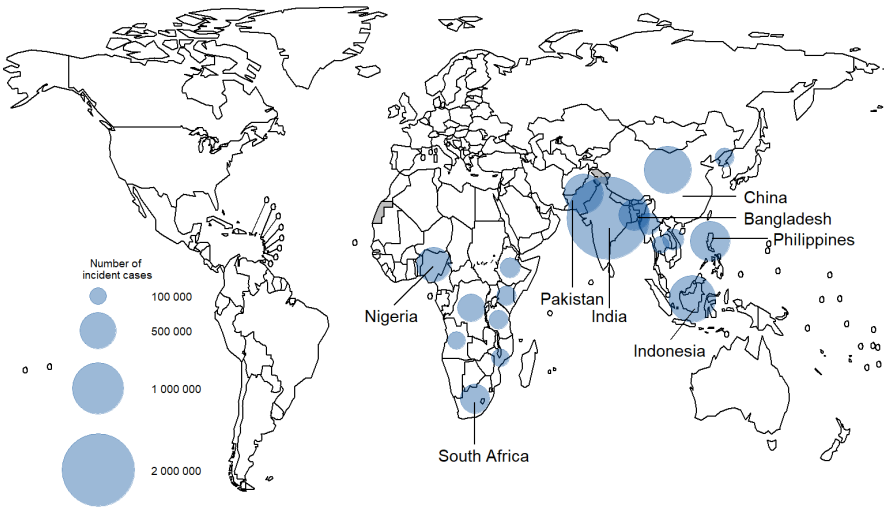
WHO – World Health Organization

XDR-TB – Extensively drug resistant tuberculosis

Introduction

Tuberculosis (TB) continues to cause significant morbidity and mortality and remains one of the top 10 causes of death worldwide with an estimated 1.28 million (range, 1.21–1.36 million) TB deaths among HIV-negative people and 214 000 deaths (range, 187 000–242 000) among HIV-positive people in 2020. The total worldwide population who was estimated to have become ill with TB in the same year was 10 million.[1]

Figure 1. Estimated TB incidence in 2020 for countries with ≥100,000 incident cases (taken from the WHO Global Report 2021).[1]



The eight countries labelled in Figure 1 account for two thirds of global cases in 2020 and are ranked in terms of numbers of cases.

Drug susceptible Tuberculosis and shortening of regimen

The first clinical trial for tuberculosis was reported in 1948.[2] The next thirty years saw a few new drugs developed and regimens were tested. The replacement of streptomycin with pyrazinamide in the 1980's to a regimen including rifampicin, isoniazid and ethambutol led to an all-oral regimen of 6 months duration.[3] This regimen for drug susceptible TB (DS-TB) has remained largely unchanged for several decades since then.

In the last decade, there has been renewed interest in trying to reduce the duration for DS-TB using newer agents. The OFLOTUB, REMoxTB and RIFAQUIN trials all individually aimed to reduce treatment duration to 4 months with the inclusion of a fluoroquinolone, but all were unable to demonstrate non-inferiority using a pre-defined margin.[4–6] A pooled analysis of these trials later showed non-inferiority for patients with an "easy-to-treat" phenotype.[7] The authors defined this as negative or 1+ smear with no cavitation. The TRUNCATE-TB trial has shown treatment for DS-TB can be shortened to two-months in some patients using new regimens and RIFASHORT will help answer whether higher doses of rifampicin can allow treatment to be shortened to four-months. [8, 9] Study 31/A5349 investigated the efficacy of two different four-month regimens containing high-dose rifapentine; one with moxifloxacin and one without for the treatment of DS-TB.[10] The trial demonstrated non-inferiority of the 4-month regimen containing high-dose rifapentine, isoniazid, pyrazinamide and moxifloxacin for 8 weeks followed by rifapentine, isoniazid and moxifloxacin for 9 weeks compared with the existing standard of care. This was the first successful regimen in nearly 40 years for DS-TB that showed treatment could be shortened.

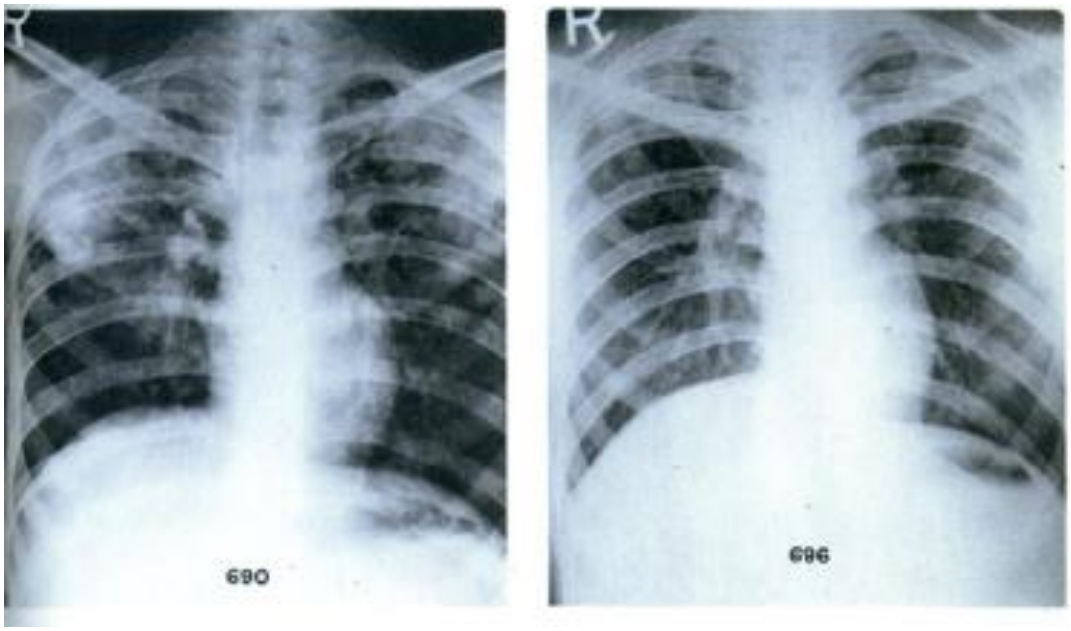


Figure 2. Chest X-ray pre and post treatment with Streptomycin [2]

Drug-resistant Tuberculosis

The 2022 WHO report estimated 10.6 million people fell ill with TB in 2021 with 1.6 million deaths attributed to TB. There was an increase in drug-resistant TB with 3% more estimated cases between 2020 to 2021 and 450, 000 new cases of RR-TB in 2021.[11] There are numerous problems associated with the development of drug resistant TB including reduced efficacy of existing drugs, longer duration of treatment and increased toxicity with some of the regimens. Treatment success globally for RR/MDR-TB (50%) and XDR-TB (30%) has historically been far lower than that of DS-TB (83%).[12] There are numerous reasons for this such as less effective drugs that need to be given for a longer duration, which will inevitably affect patient adherence either leading to treatment failure or development of further resistance.

Shortening of regimens for drug-resistant Tuberculosis

As with DS-TB, there has been interest over the last decade or so in shortening regimens for drug resistant tuberculosis (DR-TB). It was demonstrated through a series of six successive prospective treatment cohorts in Bangladesh, that the most promising regimen assessed resulted in relapse free cure of 87.9% (95% confidence interval, 82.7–91.6) among 206 patients.[13] The regimen given for a minimum of 9 months included gatifloxacin, clofazimine, ethambutol, and pyrazinamide throughout treatment and supplemented with prothionamide, kanamycin, and high-dose isoniazid during an intensive phase for a minimum of 4 months. The promising results from the study in Bangladesh led to the development of the STREAM Stage 1 trial.[14]

STREAM Stage 1 trial development

The Short (study) regimen developed for STREAM Stage 1 was similar to that described in the Bangladesh study by Van Deun et al [13], but moxifloxacin replaced gatifloxacin as there was limited supply globally manufactured to good manufacturing practice when STREAM Stage 1 was being developed.[14] The reason for this was a withdrawal of the drug for safety concerns. In 2008, the FDA announced that gatifloxacin (Tequin) was withdrawn from sale for reasons of safety or effectiveness.[15] A couple of years earlier the company manufacturing the product, Bristol-Myers-Squibb, decided to stop selling Tequin as it was associated with potentially fatal dysglycaemia. One of the key papers providing evidence to support this decision used two population based nested case-control studies in a Canadian population aged 66 years or over.[16] The first showed that compared with other antibiotics, including other fluoroquinolones, gatifloxacin was associated with an

adjusted odds ratio of 4.3; (95% confidence interval 2.9 to 6.3) for hypoglycaemic events within 30 days of receiving gatifloxacin compared with other antibiotics in a sample of 788 patients. The second study showed an adjusted odds ratio of 16.7; (95% confidence interval 10.4 to 26.8) for hyperglycaemic events within 30 days of receiving gatifloxacin compared to other antibiotics in a sample of 470 patients. Other fluoroquinolones were considered for the Short regimen in STREAM Stage 1 with moxifloxacin chosen due to similar bactericidal activity.[14,17]

STREAM Stage 1 protocol

STREAM Stage 1 was a phase 3 non-inferiority randomised controlled trial for patients with RR/MDR-TB. The Short (study) regimen (detailed in table 1) was compared to the Long (control) regimen as recommended by WHO in 2011. Patients were randomised in a 2:1 ratio in favour of the Short regimen. Patients were recruited from July 2012 to June 2015 with 689 screened of whom 424 were randomised; 282 participants were assigned to the Short regimen and 142 to the Long regimen. Patients were recruited from seven sites in four countries (Ethiopia 126; Mongolia 33; South Africa 165 and Vietnam 100). The Short regimen is shown in Table 1. The intensive phase could be extended by up to 8 weeks depending on smear status at weeks 16 and 20. Patients on the Long regimen (20-24 months) were given medication according to their country's National Treatment Programme (NTP) for Tuberculosis consistent with the WHO 2011 recommendations.[18] No Long regimen patients were given clofazimine though they did all receive a fluoroquinolone at standard dose.

Table 1. Drugs and doses given in the Short regimen.

Phase and duration	Drug	Weight group		
		Less than 33 kg	33 kg to 50 kg	More than 50 kg
Intensive and continuation (40 weeks)	Moxifloxacin	400 mg	600 mg	800 mg
	Clofazimine	50 mg	100 mg	100 mg
	Ethambutol	800 mg	800 mg	1200 mg
	Pyrazinamide	1000 mg	1500 mg	2000 mg
Intensive (16 weeks)	Isoniazid	300 mg	400 mg	600 mg
	Prothionamide	250 mg	500 mg	750 mg
	Kanamycin*	15 mg per kilogramme (kg)body weight (maximum 1g)		

* Kanamycin given three times weekly after 12 weeks

Cardiac safety in drug-resistant TB treatment

Due to the known safety profile of moxifloxacin and clofazimine, close cardiac monitoring was undertaken for all patients up to 52 weeks of follow up. The trial demonstrated that the Short 9–11-month regimen was non-inferior to a Long 20-month regimen with a favourable status of 78.8% in the Short versus 79.8% in the Long (95% confidence interval [CI], −7.5 to 9.5) (P=0.02). STREAM Stage 2 is ongoing with all patients recruited having now completed treatment and undergoing follow up. The primary outcome in STREAM Stage 2 is to assess whether the proportion of participants with a favourable efficacy outcome at Week 76 on Regimen C (an all-oral regimen including bedaquiline) is non-inferior to that on Regimen B (included in Table 1 above).

One of the concerns with RR/MDR-TB regimens is the adverse effect profile. In STREAM Stage 1, adverse events of DAIDS ≥ Grade 3 occurred in 45.4% of participants in the Long-regimen group and in 48.2% in the Short regimen group.

One of the most important adverse effects monitored for was QT interval prolongation which is associated with an increased risk of tachyarrhythmia and sudden cardiac death once a threshold of 500 milliseconds (ms) has been reached. The QT interval is affected by heart rate and needs to be corrected (QTc). A heart rate of 60 beats per minute would result in a QT and QTc of identical values. A tachycardia would increase the QTc value and bradycardia would reduce the QTc value. There are a number of different correction formulae that can be applied. For STREAM Stage 1, Fredericia's formula was used (QTcF) as recommended by the FDA.[19] The formula to calculate the QTcF is the QT interval divided by the cubed root of the R-R interval. Analysis of all patients who received at least one dose of trial medication (safety analysis population) showed a higher number of patients exceeding a maximum QT or QTcF of ≥500 ms after start of treatment in the Short regimen (11%) compared with the Long regimen (6.4%) but the difference was not statistically significant p=0.14 [20]. This highlights the difficulty in balancing patient safety with effective anti-tuberculous drug therapy in the treatment of RR/MDR-TB.

Fluoroquinolone use in TB treatment

Fluoroquinolones were initially developed as broad spectrum anti-microbial agents with the first-generation agents such as ciprofloxacin receiving regulatory approval in the 1980's.[21] At the time of their approval, it had been nearly two decades since any

new anti-tuberculous drugs had been used. Given their broad-spectrum nature against a range of Gram positive and Gram-negative bacteria and their favourable bioavailability following oral administration, a number of studies started to investigate and demonstrate in-vitro efficacy against *Mycobacterium spp*. including *Mycobacterium tuberculosis* (MTB).[22,23] Some studies also demonstrated in-vitro efficacy against strains of MTB resistant to first line agents such as rifampicin and isoniazid prompting interest that they may be useful for the treatment of RR/MDR-TB.[22] Fluoroquinolones were initially often added to failing regimens rather than forming part of a new regimen. Whilst there was some response to begin with, resistance often developed.[24] They were shown to distribute well into lung and alveolar macrophages [25,26] and also shown to maintain bactericidal activity against strains resistant to first line agents.[26] In-vitro evidence suggests fluoroquinolones have both bactericidal activity against actively dividing bacilli as well as sterilising activity against semi-dormant persister cells.[27] Early studies demonstrated that addition of a fluoroquinolone to a patients' regimen in DS-TB resulted in increased bactericidal activity and sterilising effect potentially allowing reduced treatment duration.[28]

Gillespie et al have previously summarised the different stages of *M. tuberculosis* growth, the evolution of resistance and how the different first line agents vary in their activity depending on the stage of growth.[29] For example, isoniazid is critical early in treatment in reducing actively dividing bacilli when the pulmonary cavity environment is mainly aerobic whereas pyrazinamide is active at low pH inside caseous necrotic foci. Ideally, anti-tuberculous regimens should include a combination of drugs that have high early bactericidal activity (i.e., actively dividing bacilli) as well as high sterilising effect (i.e., slow growing bacilli with low metabolic activity). The

fluoroquinolones have both bactericidal and sterilising effects against MTB making them an important drug group.[4] A phase 2 study investigating the sterilising activities of a range of fluoroquinolones in drug sensitive TB showed moxifloxacin and gatifloxacin improved the sterilising activity of a standard regimen and had the potential to reduce treatment duration.[17]

The efficacy seen in the “Bangladesh regimen” was based on a high dose of gatifloxacin with up to 600 - 800mg used for patients dependent on their weight. Moxifloxacin replaced gatifloxacin in the Short regimen in STREAM Stage 1 and the same weight dependent higher doses were used to reduce the risk of resistance and improve efficacy. The standard dose of moxifloxacin is 400mg. Higher doses of 600 or 800mg dependent on patient weight were used in STREAM as it was felt more likely to suppress the risk of drug resistance. Gumbo et al has previously shown through an in-vitro infection model using monte-carlo simulation that moxifloxacin at doses of 600mg and 800mg would suppress drug resistance in 86% and 93% of patients respectively compared with 60% of patients at the standard 400mg dose.[30]

Moxifloxacin is well known to cause QT prolongation and is often used as a positive control in thorough QT studies evaluating other drugs. A review of 642 individuals comprising healthy volunteers and patients from 8 studies by Khan et al found the mean QT interval prolongation was 11.5 – 19.5ms after administration of moxifloxacin.[31] The same study found five case reports of prolongation >500ms in which four of the patients developed the arrhythmia Torsade de Pointes (TDP) which is linked to a prolonged QT interval, though all had cardiac co-morbidities. Whilst all fluoroquinolones are known to cause some degree of QT prolongation through inhibition of the voltage gated potassium channels and delayed repolarisation, their potency varies and appears to be dose dependent. Falagas et al reviewed a number

of studies and case reports describing the pro-arrhythmic effect of fluoroquinolones.[32] They concluded that moxifloxacin appeared to be the most potent and there was a dose dependent effect particularly with moxifloxacin and levofloxacin, though the risk of TdP appeared to be rare.

Clofazimine use in TB treatment

Clofazimine was first developed in 1957 and showed initial promise in vitro and in vivo as an anti-tuberculous agent.[33] Later studies showed mixed results against MTB and with the introduction of the more effective anti-tuberculous drugs isoniazid and pyrazinamide (1950s) along with rifampicin and ethambutol (1960s) it fell out of favour. It was shown to be effective against *M. leprae* (another Mycobacterium) and after undergoing a number of clinical trials in the late 1960s has remained a key drug in the treatment of Leprosy.[34,35] Efficacy against other clinically important Mycobacterium makes clofazimine an important drug in treatment of many non-tuberculous Mycobacterium (NTM) [36] though not universally.[37] With the growing problem of drug resistant TB over the last two decades coupled with limited new available drugs, clofazimine rose to prominence again in the treatment of RR/MDR-TB.[38,39]

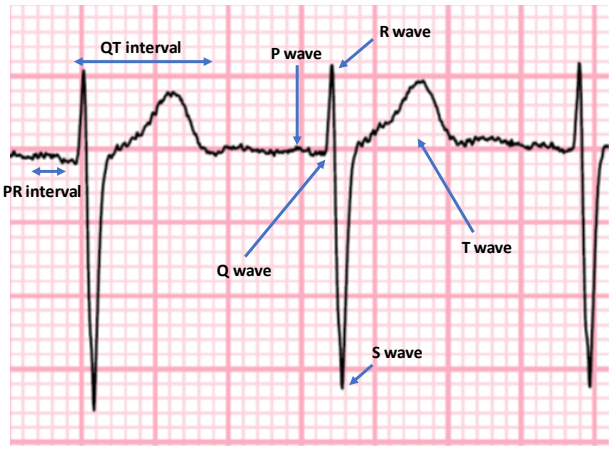
One of the commonest adverse effects with clofazimine is skin discolouration. Data on the less common but more serious adverse effect of QT prolongation and cardiac arrhythmias were previously limited to case reports.[40, 41] However, recent studies have demonstrated this cardiac effect in larger patient groups. A phase 2 randomised open label study demonstrated a mean increase of 17ms from baseline in the QT interval after 14 days for patients receiving clofazimine alone, 21ms when combined with bedaquiline and no change in the QT interval when neither drug was

given.[42] In another phase 2 open label single arm trial looking at the safety and efficacy of bedaquiline, higher QT intervals in those also taking clofazimine as part of their regimen was demonstrated.[43] The two patients who experienced an increase in the QTcF interval >500ms were both taking clofazimine and the mean maximum increase from baseline at week 24 was 31.9ms in those taking clofazimine (n=17) compared with 12.3ms for those not taking clofazimine as part of their regimen (n=177). A retrospective observational study from Brazil found higher mortality in patients receiving a clofazimine containing regimen for MDR-TB but did not contain ECG data.[44] Finally, a review of the safety of clofazimine in NTM treatment found 10 out of 17 patients who had ECG monitoring developed QT prolongation (defined as QTc ≥450 ms in men and ≥470 ms in women) with one patient exceeding 500ms.[45]

QT interval prolongation and why it is of concern

The electrocardiogram (ECG) was first developed by the Dutch physician Willem Einthoven in the early 20th Century and later won him the Nobel prize in 1924 for Physiology or Medicine. [46] The ECG captures the electrical activity of the heart displaying voltage against time measuring a cardiac cycle through depolarisation of the cardiac muscle to repolarisation. Einthoven's early ECGs used only three leads though were remarkably similar to those of the 12 lead ECG in current use. [47] The QRS complex on the ECG (Figure 2) represents ventricular depolarisation, after which the ventricles contract. The QT interval, measured from the beginning of the Q wave to the end of the T wave, is reflective of ventricular depolarisation and repolarisation marking the beginning of ventricular relaxation.

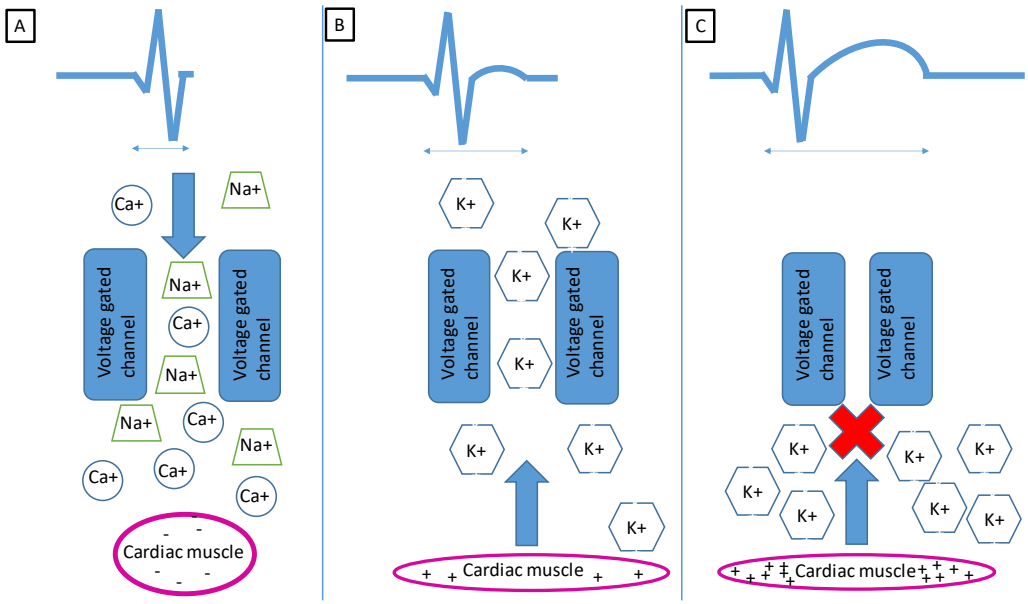
Figure 2. ECG complex showing the different parts of the waveform



During depolarisation, the voltage gated sodium and calcium channels open allowing an influx of positively charged cations into the cell resulting in a change from negative to positive charge. During repolarisation the calcium and sodium channels are inactivated, and the potassium channels open allowing an efflux of potassium ions and a return to a negative charge.[48-49]

The illustration in Figure 3 (A) shows that to begin with the cardiac muscle is relaxed which allows the ventricles to fill with blood. The negative charge then becomes positive as the voltage gated ion channels allow an influx of positively charged sodium (Na) and calcium (Ca) ions into the cardiac muscle. Immediately after this stage the ventricles become depolarised, after filling with positively charged ions, and contract. This electrically charged contraction which pumps blood out from the ventricles is represented by the QRS complex in the ECG trace at the top of section A. The next illustration in Figure 3 (B), shows that the cardiac muscle is contracted due to positively charged ions. In order for the ventricles to repolarise and relax, which then allows blood to fill the ventricles again, the cardiac muscle needs to reduce its positive charge.

Figure 3. Illustration of voltage gated ion channels and the relationship with the QT interval



This is facilitated by the voltage gated potassium channels opening, which allows an efflux of positively charged potassium ions out of the cardiac muscle and is represented by the length of the QT interval in the ECG trace above. The final illustration in Figure 3 (C), shows what happens when the voltage gated potassium channels are inhibited from opening. The depolarised, contracted cardiac muscle is unable to reduce its positive charge as the efflux of positively charged potassium ions is unable to take place. This causes a prolonged repolarisation, represented by the prolonged QT interval above the graphic which can eventually lead to tachyarrhythmias such as Torsades de Pointes and ventricular fibrillation which in turn can lead to death. Drugs that affect the h*ERG* potassium channels such as moxifloxacin and clofazimine can lead to the pathology seen in section C of the graphic.

The duration of the QT interval is dependent on the patient's heart rate, becoming longer with slower heart rates and shorter with faster heart rates. Following the first description of the effect of heart rate on QT interval by Warren Lombard and Otis

Cope [49], formulae were developed to calculate a QT interval corrected for the patient's heart rate (QTc). Henry Bazett whilst working at the University of Oxford developed the eponymous formula (QTcB) that calculates the QT interval from the square root of the R-R interval (time between two ventricular depolarisation complexes).[50] In the same year as Bazett, the Danish physician Louis Fredericia developed another formula (QTcF) for calculating a corrected QT interval based on the cubed root of the R-R interval.[51] Whilst there are other formula that can be applied to calculate a corrected QT interval the QTcB and QTcF are the most commonly used. There is considerable variability in the QT interval that can be affected by several factors such as biological differences (time of day the ECG was performed, gender, electrolytes and drug metabolism) as well as technical differences (intra and inter reader variability, abnormal T wave morphology and presence of U waves).[52,53] The risk factors for drug-induced QT prolongation are well described and include drug type, dose, gender, age, electrolyte imbalance, hypothyroidism, bradycardia, structural heart disease, renal and hepatic impairment.[47, 54–56]

A normal QT interval is defined as <450ms in males and <460 or <470ms in females. [57, 58] Several large-scale population studies have provided the basis for these definitions. Mason et al looked at 79,743 healthy volunteers and noted that the range of QT values were as follows QT, 325 to 452ms; QTcB, 361 to 457 ms; and QTcF, 359 to 445 milliseconds with higher values in females compared to males.[59] Taggart et al studied the distribution of QTc values among patients with and without mutation-proven congenital long QT syndrome (LQTS). Whilst the average QTc for the LQTS cohort was 482ms and the maximum was 760ms, the minimum was 365ms in a bell-shaped distribution showing that many patients with proven LQTS

can have QT values lower than some healthy patients.[60] Vink et al also showed that whilst patients with LQTS had higher values than controls there was some overlap.[61] The significance of a prolonged QT interval is that there is an increased risk of developing the arrhythmia Torsade de Pointes which may progress to ventricular fibrillation and ultimately sudden death.[47,62] TdP was first described by the French cardiologist François Dessertenne in 1966 as a polymorphic ventricular tachycardia associated with a prolonged QT interval.[54] QT prolongation is not a risk in itself but acts as a proxy for arrhythmogenic risk. Not all QT prolongation will lead to arrhythmia, an example being the effect of the class III anti-arrhythmic agent amiodarone on the QT interval. Due to amiodarone's effect on blocking the potassium channel and delaying repolarisation it is well-known to cause QT prolongation but rarely leads to TdP.[63,64]

Much of the data on risk of arrhythmia and sudden cardiac death in relation to QT prolongation comes from patients with congenital LQTS. Sauer et al carried out one of the largest studies to date on patients with congenital LQTS looking at clinical course and risk factors for 812 mutation-confirmed LQTS patients aged 18 years or older.[65] They found patients with QTc values of 500-549ms had a roughly three-fold higher risk of aborted cardiac arrest or LQTS related death ((HR 3.34 95% CI 1.49-7.49 ($p<0.01$)) compared with patients ≤499ms. This risk increased further with QTc values ≥550ms (vs. ≤499ms) ((HR 6.35 95%CI 2.82-14.32 ($P<0.01$)). A few years earlier Priori et al had also shown patients with QT prolongation >498ms had a higher risk of a cardiac event (syncope, cardiac arrest or sudden death) than patients below this level. They looked at 647 patients with mutation proven congenital LQTS and found for those with type 1 and 2 LQTS the risk of cardiac events in patients with a QTc 469-498ms was increased by a factor of 5.34 (95% CI 2.82-10.13) with a

higher risk for those with a QTc >498ms 8.36 (95% CI 2.53-27.21) compared with those ≤446ms.[66] Another large data set of patients found to have a QTc ≥500ms from hospital records (without suffering from congenital LQTS) showed a 3-year survival of 60% compared to 90% for those with normal QTc values.[67] A review of 101 studies within a 33-year period involving QT prolongation and risk of TdP in patients receiving non-cardiac medications found 107 of 116 cases of TdP (92.2%) where the QTc value exceeded 500ms and only 9 cases when the QTc was <500ms.[46] As a result of this higher risk of TdP and sudden cardiac death with more severe QT prolongation both the FDA [19] and EMA [68] state a QTc >500ms or >60ms above baseline to be of particular concern.

There are important differences in the management of congenital LQTS and drug-induced LQTS and the focus of this thesis is on drug-induced LQTS. The first descriptions of sudden death associated with a prolonged QT interval appeared in the middle of the 20th Century. The first description of congenital LQTS was published in 1957, by coincidence the same year that clofazimine was first discovered. Jervell and Lange-Nielson described four children from the same family in Tonsburg, Norway who suffered from congenital deafness along with frequent "fainting attacks".[69] All four children died suddenly and whilst post-mortem findings revealed no evidence of cardiac anomalies, three of the children were noted to have QT intervals of up 600ms during medical exams that took place prior to death though the significance of the QT interval length was not known at the time. As no known risk factors had been identified for a prolonged QT interval, a hereditary cause was postulated. Shortly after, similar descriptions appeared of sudden death in children with congenital deafness and a prolonged QT interval.[70] Romano and Ward later described patients with congenital LQTS in the absence of deafness.[71] The

underlying defect for these conditions was later found to be the human ether-a-go-go-related gene (HERG) showing a genetic basis for the condition through disrupted potassium channels.[72] In the same year Wang et al also described a different chromosomal abnormality (SCN5a) which was linked to disruption of the sodium channels.[73]

Prior to the first descriptions of congenital LQTS and arrhythmias and also before the significance of a prolonged QT interval was known, there were descriptions of patients collapsing after receiving the anti-arrhythmic agent quinidine.[74] Later non-cardiac drugs were becoming implicated in sudden collapse and death of patients with no pre-existing cardiac abnormalities and post-mortem reports that did not explain the cause of death.[75-76] Some of these patients were also noted to have a prolonged QT interval on ECGs prior to death. This suggested there might be a similar mechanism between patients with congenital LQTS and drug induced LQTS. T wave morphology abnormalities seen in patients with congenital LQTS were also reproducible in those with drug-induced LQTS further emphasising this link.[77-79] Psychiatric patients are one of the groups most at risk of drug-induced LQTS and it is now well recognised within the specialty.[80-81]

Whereas the management of congenital LQTS is often an implantable cardiac defibrillator and beta-blockers, drug induced LQTS often resolves after suspension or cessation of the offending drug. The British Heart Rhythm Society guidelines group the anti-psychotics based on risk of QT prolongation and advise stopping the drug and switching to one with lower effect on the QT interval should it become a clinical concern.[80] This is less straightforward in an MDR-TB setting as regimens containing several drugs are required. The WHO recently updated their guidelines

for drug resistant TB and divided drugs into 3 categories (A-C) in order of recommendation.[82] These recommendations were largely based on an individual patient data meta-analysis of 50 studies published over a 7-year period that included 12, 030 patients with MDR-TB and looked at treatment success and reduced mortality with regimens that included these drugs. The optimum number of drugs for the intensive and continuation phase was also assessed but no adverse event assessment was performed which adds weight to the importance of my thesis. [83] From the WHO guideline, of the seven drugs in Category A and B, four of them (moxifloxacin, levofloxacin, bedaquiline and clofazimine) are known to prolong the QT interval. Given the efficacy of these drugs in treating TB and the limited alternatives, the benefits and risks should be carefully assessed, and the QT prolongation associated with these drugs needs to be monitored for and managed rather than avoided altogether.

Cardiac monitoring in STREAM Stage 1

The data for this thesis comes from the cardiac monitoring that took place in STREAM Stage 1. QT monitoring in STREAM Stage 1 was based on the automated reports from the ECG machines provided for the trial. All sites in the study used a MAC 800 (GE Healthcare) machine to record their ECGs using a paper speed of 25mm/s and voltage gain of 10mm/mV. All machines were calibrated at baseline with the 12SL measurement and interpretation algorithm used to calculate the QT interval.

When recruitment began in July 2012, patients deemed eligible for randomisation had ECGs performed for monitoring at the following time points: baseline (pre-dose),

2 hours and 4 hours (post first dose), weeks 1-4, 12, 24 and 36. The protocol was amended after 20 months (March 2014) to require ECGs every 4 weeks from week 4 to week 52 to allow detection of QT prolongation earlier in follow-up. A final protocol amendment in December 2014, removed the 2-hour (post first dose) ECG. As cardiac safety was a concern at the beginning and the investigators didn't know what they would find, 24-hour cardiac Holter monitors were performed on a sub-set of participants. If the QTc for pre- and post-dose ECGs at enrolment is between 450-499msec then a Holter was performed at week 1. It was also performed later in follow-up for enrolled patients whose ECG went above 450msec for the first time. This requirement was later dropped in the December 2014 protocol amendment, roughly 6 months before the end of recruitment, so not all eligible patients would have undergone a Holter if they met the original criteria.

This thesis will discuss the following five research areas using data from the STREAM Stage 1 trial.

Chapter 1 - Predictive factors for QT prolongation and evolution of QT prolongation over time

Although a higher proportion of participants exceeded a maximum QT or QTcF interval of ≥500 ms on the Short regimen (11%) compared with the Long regimen (6.4%), it was unclear if this was solely because of the drugs, particularly as most patients on the Short regimen did not experience severe prolongation. This Chapter will aim to explore where there were other factors that could predict which participants were more likely to experience QT or QTcF interval of ≥500 ms on the Short regimen. The Chapter will also explore the evolution of QT/QTcF prolongation over time for the participants in each regimen.

Chapter 2 - Can the QT interval early in treatment be used to predict which patients will develop severe QT prolongation?

Due to concerns regarding cardiac safety of the drugs, frequent ECG monitoring was employed in STREAM Stage 1. This might be challenging for treatment programmes worldwide. The aim of this Chapter was to determine whether those at higher risk of severe QT prolongation could be identified early within the first month of treatment for more targeted monitoring, whilst at the same time allowing less frequent monitoring for lower risk participants.

Chapter 3 – Severe QT prolongation: detection, clinical management and patient outcome

This Chapter aimed to investigate how many patients developed a QT or QTcF interval ≥500ms on either a 12-lead ECG or 24-hour Holter ECG monitor and to provide a descriptive analysis of how patients were managed when they did. It will also assess whether development of a QT or QTcF of ≥500ms affected patient outcome.

Chapter 4 - Are automated machine readings a reliable and valid method of monitoring for QT prolongation compared to manual measurements?

The regular ECG monitoring participants underwent utilised automated machine readings to identify QT or QTcF prolongation. This Chapter aimed to investigate whether the machine QT interval readings could be relied upon in all circumstances when compared to the gold standard manual reading.

Chapter 5 - Did T wave morphology abnormalities consistent with *hERG* potassium channel pathology occur in STREAM Stage 1 participants and if so was there a difference between treatment regimen and time on treatment?

T wave morphology abnormalities have been shown to identify patients with congenital Long QT syndrome. Similar abnormalities have been demonstrated in healthy patients when exposed to a limited duration of drugs, such as moxifloxacin, which can disrupt the voltage gated potassium channels. It is not clear what effect fluoroquinolones and clofazimine have on T wave morphology over several months of treatment, whether certain groups are more likely to display T wave abnormalities and whether they could be used to predict patients at risk of severe QT prolongation. This Chapter aimed to determine whether T wave morphology abnormalities consistent with *hERG* potassium channel disruption occurred in STREAM Stage 1 and if so whether there was a relationship between regimen, time on treatment and risk of severe QT prolongation.

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Chapter one: Predictive factors for QT prolongation and evolution of QT prolongation over time

Background: STREAM Stage 1 demonstrated non-inferior efficacy of a shortened regimen for RR-TB compared to the contemporaneous WHO-recommended regimen. Severe QT prolongation was more common on the Short regimen; in this chapter I analysed the risk factors for QT prolongation with the Short regimen.

Methods: Data from patients allocated the Short regimen (n=282) were analysed to determine risk factors for severe QT prolongation (QT/QTcF ≥500ms or ≥60ms increase in QTcF from baseline).

Results: 94 (33.3%) patients on the Short regimen developed severe QT prolongation:31 QT/QTcF ≥500ms; 92 ≥60ms QTcF increase from baseline. Median time to QT/QTcF ≥500ms was 20 [IQR: 8, 28] weeks and to ≥60ms increase from baseline was 18 [8, 28] weeks. Prolongation ≥500ms was most frequent in patients from Mongolia (10/22, 45.5%) compared with 3.5-11.9% at other sites p <0.001. Higher baseline QTcF was associated with increased risk of prolongation to ≥500ms (QTcF ≥400ms: OR 5.99 (95% CI 2.04-17.62)).

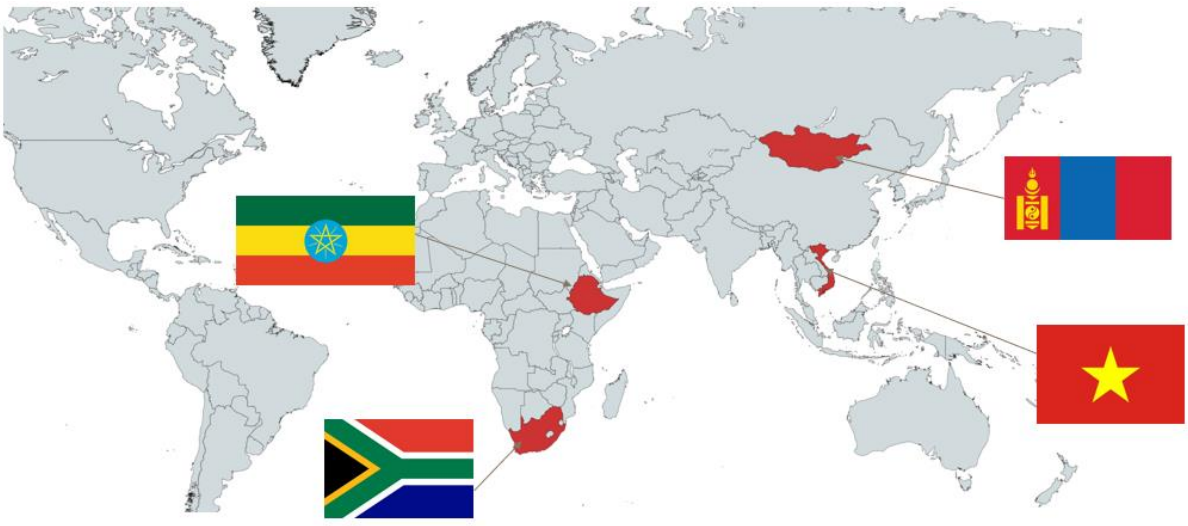
Conclusion: One third of patients on the Short regimen developed severe QT prolongation. QT/QTcF ≥500ms was more common in patients from Mongolia and in those with a higher baseline QTcF. These findings may have implications for implementation of RR-TB treatment.

Introduction

The treatment of drug resistant tuberculosis has improved over recent years with the development of shorter and more effective regimens. There may, however, be a trade-off between treatment improvement and risk of adverse events.[1] The STREAM Stage 1 trial was a phase 3 non-inferiority randomised controlled trial for patients with rifampicin-resistant pulmonary tuberculosis (RR-TB); it demonstrated that a “Short” 9 month regimen was non-inferior to a “Long” 20 month regimen that was standard of care.[2-3]

Patients were randomised in a 2:1 ratio in favour of the Short regimen which was based on that studied in Bangladesh [4] replacing gatifloxacin with moxifloxacin as the product was no longer available to good manufacturing practice.[5] Patients were recruited from four countries (Ethiopia, Mongolia, South Africa and Vietnam) between 2012 to 2015 (figure 1.1); 424 were randomised - 282 to the Short regimen and 142 to the Long regimen.

Figure 1.1 Map of countries which recruited participants in STREAM Stage 1



The Short regimen includes moxifloxacin and clofazimine, both known to independently cause QT prolongation; in addition, high dose moxifloxacin was used to reduce the development of resistance and improve efficacy.[6] Although all fluoroquinolones may affect QT, moxifloxacin has been shown to have the greatest effect [7] which is more pronounced at higher doses.[8] Clofazimine has also been associated with QT prolongation [9,10] and the arrhythmia Torsades de Pointes (TdP). [11] The QT prolongation effect of these two drugs is thought to result from the inhibition of the *hERG* potassium channel.[12,13] Therefore, particular attention to cardiac safety in the trial was warranted. Patients allocated the Long regimen received a standard dose of moxifloxacin or levofloxacin, both of which are known to prolong the QT interval although the risk was considered lower.[14–16] No Long regimen participants received clofazimine. Frequent electrocardiogram (ECG) monitoring was undertaken in all trial patients up to week 52.

Patients that develop a QT or QTcF ≥500 milliseconds (ms) are at increased risk of tachyarrythmias such as TdP and there is also a lesser risk for patients who experience ≥60ms increase in QTcF over baseline.[17] It is estimated that there is a 5% increased risk of arrhythmia for every 10ms increase in QT interval above the upper limit of normal.[18] Patients with a corrected QT (QTc) interval ≥500ms during screening were ineligible for trial inclusion.

ECG monitoring

A baseline 12-lead ECG was performed at enrolment and repeated 4 hours after the first dose of trial medication. Initially ECGs were performed weekly until week 4 and then at 12, 24 and 36 weeks. The protocol was amended after 18 months (January

2014) to require ECGs every 4 weeks from week 4 to week 52. We used Fredericia's formula (QTcF) to correct the QT interval for patients' heart rate, as recommended in FDA guidance.[19] All sites in the study used a MAC 800 (GE Healthcare) machine with a paper speed of 25mm/s and voltage gain of 10mm/mV. All machines were calibrated at baseline and the machine estimate of the QT and QTcF interval were recorded.

Analysis of all patients in STREAM stage 1 who received at least one dose of trial medication (safety analysis population) showed a higher number of patients exceeded a maximum QT or QTcF of ≥500ms after start of treatment in the Short regimen (11%) compared with the Long regimen (6.4%) (p=0.14).[2]

If a patient had QT or QTcF prolongation ≥500ms treatment was reviewed and adjusted if required. No treatment changes were advised because of an increase in QTcF of ≥60ms from baseline value alone which is also a DAIDS Grade 3 event. Of the 31 (11%) patients who received the Short regimen and developed a QT or QTcF prolongation ≥500ms, seven continued treatment with no modifications. Four had a permanent dose reduction of moxifloxacin alone. Twenty had either moxifloxacin and/or clofazimine treatment interruption, dose reduction and/or a change of drug. Moxifloxacin was replaced by Levofloxacin in eleven patients and three patients permanently discontinued clofazimine.[2]

Torsade de Pointes was not seen in 12-lead ECG recordings for any patient. Four cases of sudden death occurred at home during treatment (three on the Short regimen; one on the Long regimen), though only two were attributed to tuberculous treatment (one on each regimen) at independent review of deaths. In two of the

cases on the Short regimen, a QT or QTcF of ≥500ms and an increase in QTcF ≥60ms from baseline had been recorded.

The Short regimen is shown in Table 1 of the introductory chapter. The intensive phase could be extended by up to 8 weeks if the smear was still positive at 16 or 20 weeks of treatment. Patients on the Long regimen were given medication according to their country's National Treatment Programme (NTP) for Tuberculosis. The intensive phase could be extended beyond 8 months if the smear was still positive, consistent with the WHO 2011 recommendations.[3]

Known risk factors for QT prolongation such as hypokalaemia, renal and hepatic impairment were monitored with routine bloods collected monthly during the intensive phase. Thyroid function, hypomagnesaemia and hypocalcaemia were not routinely checked.

The objective of this chapter is to describe the evolution of QT prolongation on treatment over time in both the Short and Long regimen and to identify factors predictive of increased risk of developing QT or QTcF of ≥500ms or ≥60ms QTcF increase from baseline on the Short regimen.

Methods

Statistical analysis

A retrospective analysis was undertaken of QT and QTcF intervals during follow-up. Statistical analyses were conducted using STATA v15.1.

The timing and value of the maximum QT or QTcF was identified for each patient, with ECG data censored at the point two or more drug changes occurred, indicating the patient was no longer taking the allocated regimen. The cumulative probability of maximum QT/QTcF exceeding 500ms over time was estimated using a Kaplan-Meier curve and compared between treatment arms using a log-rank test. The effect of treatment arm on the hazard of maximum QT/QTcF exceeding 500ms was estimated using a Cox proportional-hazards model.

Patients allocated to the Short regimen were classified according to whether they had experienced severe QT prolongation at any time up to their week 52 visit.

Univariable comparisons of baseline characteristics between patients with and without severe QT prolongation were conducted using Chi-square or Fisher's exact tests for categorical variables and t-tests or Mann-Whitney rank tests for continuous variables. Those comparisons found to be significantly associated with severe QT prolongation at the 10% level were included in a multivariable logistic regression model in addition to the a priori factors of age, baseline QTcF reading and baseline potassium levels. Backwards elimination, with exit probability p=0.05, was employed to select the final model. The association between HIV status and severe QT prolongation was explored in analyses restricted to countries with at least 5% of participants HIV positive (South Africa and Ethiopia) which together had 98% of HIV positive patients in the trial.

Results

Evolution of QT/QTcF prolongation over time by regimen

Figure 1.2 shows the mean and standard error of the QTcF by visit and treatment arm. The difference in patient numbers assessed at each visit are largely explained by the protocol-mandated ECG monitoring in place at the time. By week 4 there is a clear separation between the two arms which increased at week 8, after which time there is little change until Week 40 when the QTcF on the Short regimen declined, reflecting the completion of treatment for most patients on that regimen. Patients who received the Long regimen experienced a median maximum change in QTcF from baseline of 30 [22, 41] ms compared to 50 [36, 65] ms on the Short regimen. A maximum difference of 24ms between the two arms was seen at week 28. A similar pattern was seen for the mean change in QTcF from baseline (Fig 1.3).

Figure 1.2. Mean QTcF by visit treatment arm

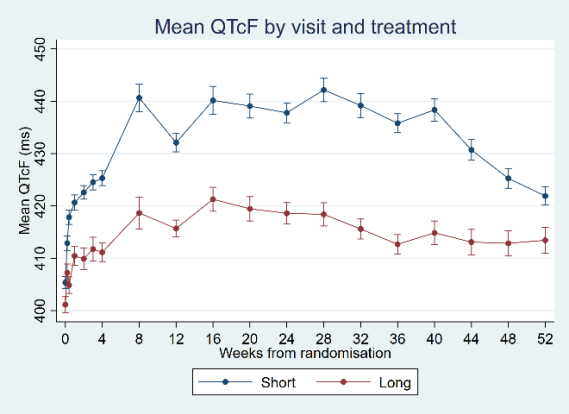
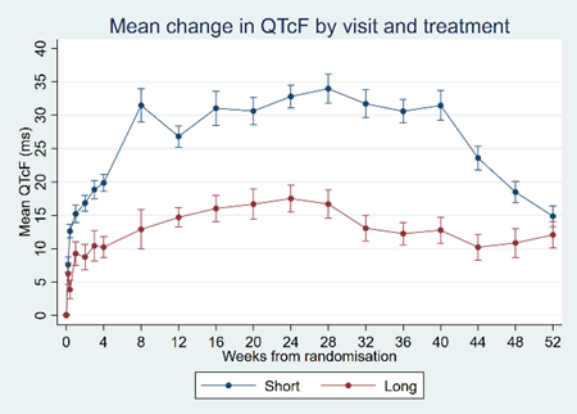


Figure 1.3. Mean change in QTcF from baseline



Mean QTcF and mean change in QTcF presented with error bars detailing ± 1 standard error of the mean.

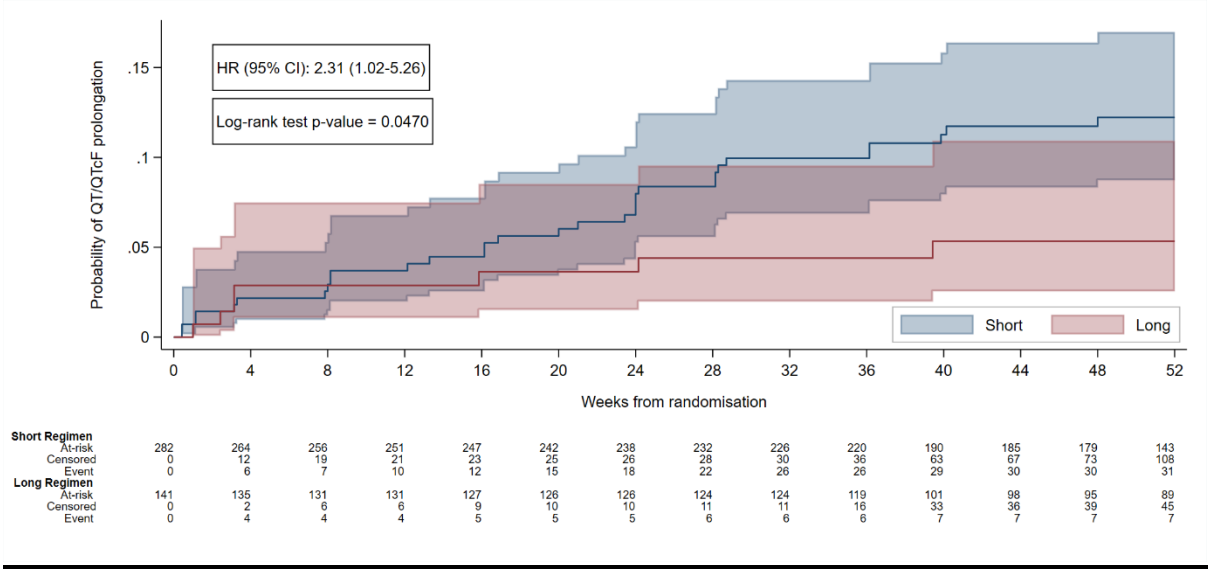
Figure 1.4. Number of participants with ECG readings across visits by treatment arm

Number of participants with ECG reading across visits by treatment arm

	Baseline	2 Hours	4 Hours	Week 1	Week 2	Week 3	Week 4	Week 8	Week 12	Week 16	Week 20	Week 24	Week 28	Week 32	Week 36	Week 40	Week 44	Week 48	Week 52
Short regimen	282	280	280	270	272	270	267	152	259	155	160	253	180	188	244	198	192	193	194
Long regimen	141	138	138	137	136	136	137	72	130	76	77	128	86	89	127	95	88	96	97

Thirty-one (11%) participants on the Short regimen reached a maximum QT/QTcF of 500ms versus seven (5%) on the Long regimen up until 52 weeks of follow up with routine ECG monitoring. We observed a significant difference between treatment regimens in time to QT/QTcF≥500ms (Figure 1.5, p=0.047); participants taking the Short regimen had a higher risk of reaching a maximum QT/QTcF ≥500ms (HR 2.31 (95% CI: 1.02-5.26)).

Figure 1.5. Kaplan-Meier plot of time to exceeding maximum QT/QTcF of 500ms post baseline.



Factors predictive of QT prolongation on the Short regimen

Of the 282 participants on the Short regimen, 94 (33.3%) had severe QT prolongation on at least one ECG. QT/QTcF of ≥500ms occurred in 31 participants, at a median time of 20 [IQR 8, 28] weeks and 92 had ≥60ms increase in QTcF from baseline at a median time of 18 [8, 28] weeks. The median QTcF change was 102 [81, 137] ms in those with QT/QTcF ≥500ms and 72 [66, 94] ms in those with a QTcF ≥60ms increase from baseline.

No statistically significant association ($p>0.1$) was observed between severe QT prolongation and gender or baseline smoking status, diabetes, height, weight, body mass index, moxifloxacin and clofazimine dose, liver function tests, glucose or potassium (Tables A4-A5). Analyses restricted to South African and Ethiopian participants showed no significant association between HIV status and severe QT prolongation.

A significant association between both country and baseline QTcF with the development of QT prolongation ≥500ms remained after adjustment for other variables (Table 1.2). Mongolia showed the greatest association (10/22, 45.5%) compared with 3.5-11.9% of patients at the other sites ($p <0.001$); baseline QTcF ≥400ms was associated with increased risk compared to <400ms (OR 5.99 (95% CI 2.04-17.62)).

There was some evidence of an association with increased risk of QT/QTcF ≥500ms for participants with lower baseline creatinine levels and increased age on univariable analysis, but not after adjustment for other factors in the multivariable analysis. There was no evidence that baseline hypokalaemia increased the risk of severe QT prolongation at baseline.

Of the factors explored, only country was significantly associated with an increase in QTcF of ≥60ms from baseline on univariable analysis, with Mongolia having a significantly higher proportion of cases p<0.001 (Table 1.3); country remained independently associated with QTcF increase in the multivariable analysis. In addition, there was evidence suggesting that a lower baseline QTcF interval significantly raised the risk of having an increase in QTcF of ≥60ms from baseline. Age did not appear to be associated with risk of developing a ≥60ms increase in QTcF from baseline.

Table 1.2. Univariable and multivariable analysis of risk factors for a QT/QTcF ≥500 ms in the Short regimen arm

		% with QT/QTcF ≥500ms at any point	n / N	OR (95% CI)	P-value	Multivariable analysis adjusted OR (95% CI)	P-value
Baseline QTcF	0-399ms	**3.3**	4 / 122	**1.00** (base)	**0.001**	**1.00** (base)	**0.007**
	≥400ms	**16.9**	27 / 160	**5.99** (2.04-17.62)	-	**4.77** (1.54-14.71)	-
Age (10 additional years)	-	-	-	**1.51** (1.09-2.09)	**0.013**	**1.43** (0.98-2.08)	**0.063**
Age category	18-24	**4.7**	3 / 64	**1.00** (base)	**0.092**	-	-
	25-34	**8.4**	8 / 95	**1.87** (0.48-7.33)	-	-	-
	35-44	**14.9**	10 / 67	**3.57** (0.93-13.62)	-	-	-
	45+	**17.9**	10 / 56	**4.42** (1.15-16.98)	-	-	-
Baseline Potassium	≥3.5 mmol/L	**11.2**	28 / 249	**1.00** (base)	**0.614**	**1.00** (base)	**0.353**
	<3.5 mmol/L	**15.0**	3 / 20	**1.39** (0.38-5.05)	-	**1.98** (0.47-8.34)	-
Country	Ethiopia	**3.5**	3 / 85	**1.00** (base)	**<0.001**	**1.00** (base)	**<0.001**
	Vietnam	**7.6**	5 / 66	**2.24** (0.52-9.74)	-	**1.01** (0.21-4.76)	-
	South Africa	**11.9**	13 / 109	**3.70** (1.02-13.44)	-	**2.48** (0.65-9.52)	-
	Mongolia	**45.5**	10 / 22	**22.78** (5.48-94.74)	-	**15.45** (3.45-69.24)	-
Baseline Creatinine (Additional 10 µmol/L)	-	-	-	**0.78** (0.63-0.97)	**0.022**	-	-

Table 1.3. Univariable and multivariable analysis of risk factors for a QTcF ≥60 ms increase from baseline in the Short regimen arm

		% with QTcF ≥60ms from baseline	n / N	OR (95% CI)	P-value	Multivariable analysis adjusted OR (95% CI)	P-value
Baseline QTcF	0-399ms	**37.7**	46 / 122	**1.00** (base)	**0.113**	**1.00** (base)	**0.014**
	≥400ms	**28.7**	46 / 160	**0.67** (0.40-1.10)	-	**0.49** (0.27-0.87)	-
Age *(10 additional years)*	-	-	-	**1.11** (0.89-1.39)	**0.352**	**1.02** (0.79-1.33)	**0.880**
Age category	18-24	**34.4**	22 / 64	**1.00** (base)	**0.373**	-	-
	25-34	**27.4**	26 / 95	**0.72** (0.36-1.43)	-	-	-
	35-44	**31.3**	21 / 67	**0.87** (0.42-1.81)	-	-	-
	45+	**41.1**	23 / 56	**1.33** (0.63-2.79)	-	-	-
Baseline Potassium	≥3.5 mmol/L	**32.5**	81 / 249	**1.00** (base)	**0.496**	**1.00** (base)	**0.554**
	<3.5 mmol/L	**40.0**	8 / 20	**1.38** (0.54-3.52)	-	**1.36** (0.49-3.74)	-
Country	Ethiopia	**20.0**	17 / 85	**1.00** (base)	**<0.001**	**1.00** (base)	**<0.001**
	Vietnam	**40.9**	27 / 66	**2.77** (1.34-5.71)	-	**3.17** (1.40-7.15)	-
	South Africa	**28.4**	31 / 109	**1.59** (0.81-3.12)	-	**1.74** (0.84-3.63)	-
	Mongolia	**77.3**	17 / 22	**13.60** (4.39-42.10)	-	**22.86** (6.42-81.40)	-

Just over half the patients who developed severe QT prolongation were taking the higher 800mg dose of moxifloxacin, (18/30 participants who developed QT/QTcF ≥500ms and 47/92 participants who had ≥60ms QTcF increase from baseline). The dose of moxifloxacin and clofazimine at baseline, expressed as mg/kg, was not found to significantly increase the risk of severe QT prolongation (Fig A1, Tables A4-A5). Baseline moxifloxacin (mg/kg) mean (SD) was 13.7 (1.4) in those who did not develop severe QT prolongation versus 13.5 (1.5) in those with QT/QTcF ≥500ms. Baseline clofazimine (mg/kg) mean (SD) was 2.0 (0.3) in the no prolongation group versus 1.9 (0.4) in the QT/QTcF ≥500ms group. This was similar for participants with ≥60ms QTcF increase from baseline.

To investigate further the effect of dose of moxifloxacin and clofazimine per kg patients were separated by weight band (33-50kg and >50kg, allocated 600mg and

800mg moxifloxacin respectively). Patients 33-50kg who developed QT/QTcF ≥500ms (n=13) received higher mg/kg doses of both drugs than those who did not at weeks 8, 12 and 16 ($p \leq 0.05$ for all three time points). However, this relationship was not found in the >50kg weight band (Tables A1-A3). Although total drug dose remained constant through these time points, patients' weight changed affecting the mg/kg dose. Results were similar for moxifloxacin and clofazimine at the same time point as total drug dose remained constant (Tables A2-A3).

No association was observed between mg/kg dose of moxifloxacin or clofazimine and ≥60ms QTcF increase from baseline, either when weight bands were combined or separated with p values >0.1 for all.

Discussion

The evolution of QTcF over time showed a clear difference between regimens after the first month of treatment. Participants allocated the Short regimen experienced a greater increase in QTcF than those on the Long regimen. Mean QTcF values fell sharply after treatment completion at week 40 on the Short regimen; this suggests that moxifloxacin with a much shorter half-life than clofazimine [20,21], plays a key role in QT prolongation. However, the increase in QTcF values at the end of treatment compared to baseline may be due to persistence of clofazimine. The small increase in QTcF with the Long regimen, still present at week 52 when patients were still on treatment, is likely to be due to the standard-dose fluoroquinolone in regimen. It is also possible that other drugs could have caused QT prolongation through secondary adverse effects such as hypothyroidism with prothionamide and renal dysfunction with kanamycin [1] which were used in the Long regimen.

The risk factors for development of QT/QTcF ≥500ms on the Short regimen included being from the Mongolian site and having a baseline QTcF ≥400ms. The latter is not surprising since such patients were closer to the threshold of 500ms. Case reports of levofloxacin induced QT prolongation and Torsades de Pointes have found patients had a high baseline value [22,23] and a review of 900 patients admitted to a cardiac care unit found a baseline QTc >450ms was an independent risk factor for severe QTc prolongation.[24]

The increased risk of severe QT prolongation (both development of QT/QTcF ≥500 ms and ≥60ms QTcF increase from baseline) in patients from the Mongolian site was unexpected and may represent genetic or environmental differences that affected the pharmacokinetics (PK) of the trial medications. Genes such as *KCNQ1* and

SCN5A (associated with congenital long QT syndrome) have been described in Mongolia and the relative genetic isolation has been proposed as a possible cause for QT prolongation in this population.[25–29] Single Nucleotide Polymorphisms in the *UGT1A* gene are known to affect the metabolism of moxifloxacin.[30] The absence of increased risk in the Vietnamese patients in STREAM Stage 1 would support regional variation amongst Asian populations. Amongst trial sites hypothermia (a known cause of QT prolongation) was a risk specific to Mongolia with winter temperatures averaging -15 to -30 degrees Celsius which may have affected some patients.[31] Excess alcohol consumption has been associated with cardiac arrythmias and alcohol consumption is known to be high amongst some groups in Mongolian society. [32, 33] Anecdotally during the STREAM trial alcohol consumption appeared to be higher amongst Mongolian participants compared to other sites and countries. Though data was not routinely collected to confirm this, it is possible that different levels of alcohol consumption contributed to a higher proportion of participants developing QT prolongation. Environmental differences may also have contributed. For example, chronic arsenic exposure from contaminated drinking water has been associated with QT prolongation in an Inner Mongolian population. [34] The next steps to investigate this unexpected site/country specific difference might be to conduct a prospective study in the same countries for participants with MDR-TB which might allow an exclusion of a-LQTS causes as contributory factors. These would include electrolyte abnormalities, thyroid dysfunction, alcohol consumption, concomitant medications, structural cardiovascular disorders e.g. heart failure and alcohol or other illicit drugs. Collection of this data over a longer time frame during treatment and post treatment would allow correlation with the QT interval. If Mongolia still had a higher proportion of patients affected by QT

prolongation and there were no significant factors that contributed to this then the next step would be to investigate for c-LQTS amongst the same participants with genetic testing for the relevant genes e.g. *KCNQ1* and *SCN5A*.

Whilst kanamycin can cause QT prolongation indirectly through renal impairment and electrolyte abnormalities [(1)] this analysis showed a lower mean creatinine at baseline in patients who developed QT/QTcF ≥500ms on the Short regimen. However, this was not seen at later time points and does not seem clinically plausible. The apparent lack of association between hypokalaemia and risk of QT prolongation was unexpected. However, my ability to examine the relationship was limited by lack of testing except during the intensive phase.

Increasing age is associated with QT prolongation [(35)], but although I found some evidence of association between age and QT/QTcF ≥500ms in univariable analysis it was not maintained in the multivariable model and not seen in relation to risk of ≥60ms QTcF increase from baseline. However, the population was relatively young with most participants less than 45 years of age.

Moxifloxacin (at standard dose) is known to prolong the QT interval and is often used as a positive control in thorough QT studies for this reason. [(36, 37, 38)] High dose moxifloxacin in combination with clofazimine in the Short regimen is likely to explain the differences in QT prolongation between regimens, however the lack of relationship between weight-adjusted dose (mg/kg) and maximum QTcF was surprising. Although we found that those allocated 600mg moxifloxacin who developed QT/QTcF ≥500ms were more likely to receive a higher mg/kg dose this was not seen in the higher weight band >50kg who were allocated 800mg moxifloxacin.

This study had limitations. First, this is a post-hoc analysis of the trial data so the probability of any significant findings is increased and may still be due to chance. Second, safety bloods were only recorded routinely during the intensive phase. It is possible that risk factors for QT prolongation such as hypokalaemia and hypothyroidism may have been prevalent during the continuation phase. Third, no PK studies were undertaken in STREAM Stage 1 which may have enlightened the relationship between weight-adjusted dose and maximum QTcF.

This study has a number of strengths. First, the QT data presented are from routine ECG monitoring that took place at the same time point for all patients and was not modified by the clinical situation. Second, participants were randomised from multiple sites in several different countries with different ethnic groups; this adds weight to the generalisability of the findings. Third, this is the largest analysis to date of QT prolongation in the Short regimen from a randomised controlled trial.

The majority of patients on the Short regimen did not experience severe QT prolongation, and higher dose fluoroquinolone in combination with clofazimine could be used safely in most patients. As patients with baseline QTcF ≥400ms appeared to be at greater risk, they may need closer monitoring in programmatic settings. Most patients who reached the 500ms threshold did so ≥3 months after start of treatment suggesting ECG monitoring may be required throughout treatment with this regimen. Fluoroquinolones and clofazimine are now frequently used in regimens alongside bedaquiline and/or delamanid, such as in the WHO recommended all-oral regimen.[39] This could potentiate the QT prolongation seen in STREAM Stage 1.[40-41] The results from STREAM Stage 2 will provide important information on the safety of an all-oral regimen.

Appendix – 1

Table A1. QT or QTcF prolongation 500ms predictors – mg per kg – all weight groups

	No Prolongation (N=251)	Prolongation (N=31)	P-Value	Overall (N=282)
Baseline Moxifloxacin per kg, Mean (SD)	13.7(1.4)	13.5(1.5)	0.644	13.7(1.4)
Week 4 Moxifloxacin per kg, Mean (SD)	13.6(1.4)	13.5(1.4)	0.814	13.6(1.4)
Week 8 Moxifloxacin per kg, Mean (SD)	13.5(1.5)	13.5(1.4)	0.961	13.5(1.5)
Week 12 Moxifloxacin per kg, Mean (SD)	13.4(1.5)	13.3(1.5)	0.615	13.4(1.5)
Week 16 Moxifloxacin per kg, Mean (SD)	13.3(1.4)	13.2(1.5)	0.862	13.3(1.4)
Baseline Clofazimine per kg, Mean (SD)	2.0(0.3)	1.9(0.4)	0.636	2.0(0.3)
Week 4 Clofazimine per kg, Mean (SD)	1.9(0.3)	1.9(0.4)	0.983	1.9(0.3)
Week 8 Clofazimine per kg, Mean (SD)	1.9(0.3)	1.9(0.4)	0.817	1.9(0.3)
Week 12 Clofazimine per kg, Mean (SD)	1.9(0.3)	1.9(0.4)	0.612	1.9(0.3)
Week 16 Clofazimine per kg, Mean (SD)	1.8(0.3)	1.9(0.4)	0.607	1.9(0.3)

Table A2. QT or QTcF prolongation 500ms predictors – mg per kg – 33-50kg

	No Prolongation (N=114)	Prolongation (N=13)	P-Value	Overall (N=127)
Baseline Moxifloxacin per kg, Mean (SD)	13.5(1.2)	13.8(1.7)	0.444	13.5(1.3)
Week 4 Moxifloxacin per kg, Mean (SD)	13.4(1.3)	14.0(1.1)	0.131	13.5(1.2)
Week 8 Moxifloxacin per kg, Mean (SD)	13.3(1.2)	14.1(1.0)	**0.026**	13.3(1.2)
Week 12 Moxifloxacin per kg, Mean (SD)	13.2(1.2)	14.0(1.3)	0.050	13.3(1.3)
Week 16 Moxifloxacin per kg, Mean (SD)	13.1(1.1)	14.0(1.2)	**0.013**	13.2(1.2)
Baseline Clofazimine per kg, Mean (SD)	2.3(0.2)	2.3(0.3)	0.444	2.3(0.2)
Week 4 Clofazimine per kg, Mean (SD)	2.2(0.2)	2.3(0.2)	0.131	2.2(0.2)
Week 8 Clofazimine per kg, Mean (SD)	2.2(0.2)	2.3(0.2)	**0.026**	2.2(0.2)
Week 12 Clofazimine per kg, Mean (SD)	2.2(0.2)	2.3(0.2)	0.050	2.2(0.2)
Week 16 Clofazimine per kg, Mean (SD)	2.2(0.2)	2.3(0.2)	**0.013**	2.2(0.2)

Table A3. QT or QTcF prolongation 500ms predictors – mg per kg – over 50kg

	No Prolongation (N=136)	Prolongation (N=18)	P-Value	Overall (N=154)
Baseline Moxifloxacin per kg, Mean (SD)	13.8(1.5)	13.4(1.4)	0.241	13.8(1.5)
Week 4 Moxifloxacin per kg, Mean (SD)	13.7(1.5)	13.2(1.6)	0.201	13.7(1.6)
Week 8 Moxifloxacin per kg, Mean (SD)	13.6(1.6)	13.1(1.5)	0.155	13.6(1.6)
Week 12 Moxifloxacin per kg, Mean (SD)	13.5(1.6)	12.7(1.4)	0.058	13.4(1.6)
Week 16 Moxifloxacin per kg, Mean (SD)	13.3(1.6)	12.7(1.5)	0.104	13.3(1.6)
Baseline Clofazimine per kg, Mean (SD)	1.7(0.2)	1.7(0.2)	0.241	1.7(0.2)
Week 4 Clofazimine per kg, Mean (SD)	1.7(0.2)	1.7(0.2)	0.201	1.7(0.2)
Week 8 Clofazimine per kg, Mean (SD)	1.7(0.2)	1.6(0.2)	0.155	1.7(0.2)
Week 12 Clofazimine per kg, Mean (SD)	1.7(0.2)	1.6(0.2)	0.058	1.7(0.2)
Week 16 Clofazimine per kg, Mean (SD)	1.7(0.2)	1.6(0.2)	0.104	1.7(0.2)

Table A4. Univariable analysis of risk factors for QT/QTcF ≥500 ms in the Short regimen arm

	No Prolongation (N=251)	Prolongation (N=31)	P-Value	Overall (N=282)
Age, Median (IQR)	31.8(25.0,41.5)	40.2(27.8,48.9)	**0.011**	32.8(25.6,42.2)
Age Group, N (%)			**0.072**	
18-24	61(95.3)	3(4.7)		64
25-34	87(91.6)	8(8.4)		95
35-44	57(85.1)	10(14.9)		67
45+	46(82.1)	10(17.9)		56
Sex, N (%)			0.629	
Male	149(89.8)	17(10.2)		166
Female	102(87.9)	14(12.1)		116
Country, N (%)			**0.000**	
Ethiopia	82(96.5)	3(3.5)		85
Vietnam	61(92.4)	5(7.6)		66
South Africa	96(88.1)	13(11.9)		109
Mongolia	12(54.5)	10(45.5)		22
HIV Status[1], N (%)			0.863	
Negative	85(91.4)	8(8.6)		93
Positive	93(92.1)	8(7.9)		101
Smoking Status, N (%)			0.746	
Never	162(88.5)	21(11.5)		183
Smoker	88(89.8)	10(10.2)		98
Diabetes, N (%)			0.810	
No	229(88.8)	29(11.2)		258
Yes	19(90.5)	2(9.5)		21
Height *(cm)*, Mean (SD)	164.7(8.5)	163.4(11.0)	0.445	164.6(8.8)
Weight *(kg)*, Mean (SD)	52.3(9.4)	53.6(10.2)	0.457	52.4(9.5)
BMI *(kg/m^2)*, N (%)			0.710	
<16	32(91.4)	3(8.6)		35
16-18.4	81(91.0)	8(9.0)		89
18.5-24.9	122(87.8)	17(12.2)		139
25+	15(83.3)	3(16.7)		18
Moxifloxacin Dose, N (%)			0.872	
800mg	136(88.3)	18(11.7)		154
600mg	114(89.8)	13(10.2)		127
400mg	1(100.0)	0(0.0)		1
Moxifloxacin *(mg/kg)*, Mean (SD)	13.7(1.4)	13.5(1.5)	0.644	13.7(1.4)
Clofazimine *(mg/kg)*, Mean (SD)	2.0(0.3)	1.9(0.4)	0.636	2.0(0.3)
QTcF, N (%)			**0.000**	
Less than 400ms	118(96.7)	4(3.3)		122
Greater than or equal to 400ms	133(83.1)	27(16.9)		160
ALT, Mean (SD)	20.5(16.3)	20.8(14.8)	0.903	20.5(16.1)
AST, Mean (SD)	27.5(19.0)	29.1(18.0)	0.649	27.7(18.9)
Creatinine, Mean (SD)	67.9(19.7)	59.1(20.0)	**0.021**	66.9(19.9)
Glucose, Mean (SD)	5.9(3.3)	5.6(2.7)	0.687	5.9(3.2)
Potassium, N (%)			0.613	
3.5mmol/L and above	221(88.8)	28(11.2)		249
Under 3.5mmol/L	17(85.0)	3(15.0)		20

[1] *Ethiopia & South Africa only*

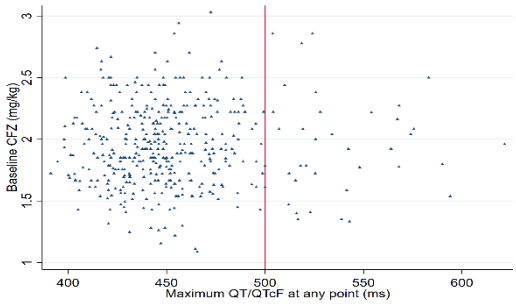
Table A5. Univariable analysis of risk factors for a QTcF ≥60 ms increase from baseline in the Short regimen arm

	No Prolongation (N=190)	Prolongation (N=92)	P-Value	Overall (N=282)
Age, Median (IQR)	32.0 (26.0,41.3)	33.8 (25.4,45.6)	0.353	32.8 (25.6,42.2)
Age Group, N (%)			0.369	
18-24	42 (65.6)	22 (34.4)		64
25-34	69 (72.6)	26 (27.4)		95
35-44	46 (68.7)	21 (31.3)		67
45+	33 (58.9)	23 (41.1)		56
Sex, N (%)			0.968	
Male	112 (67.5)	54 (32.5)		166
Female	78 (67.2)	38 (32.8)		116
Country, N (%)			**0.000**	
Ethiopia	68 (80.0)	17 (20.0)		85
Vietnam	39 (59.1)	27 (40.9)		66
South Africa	78 (71.6)	31 (28.4)		109
Mongolia	5 (22.7)	17 (77.3)		22
HIV Status[1], N (%)			0.319	
Negative	67 (72.0)	26 (28.0)		93
Positive	79 (78.2)	22 (21.8)		101
Smoking Status, N (%)			0.578	
Never	121 (66.1)	62 (33.9)		183
Smoker	68 (69.4)	30 (30.6)		98
Diabetes, N (%)			0.127	
No	177 (68.6)	81 (31.4)		258
Yes	11 (52.4)	10 (47.6)		21
Height *(cm), Mean (SD)*	165.0 (8.4)	163.6 (9.5)	0.212	164.6 (8.8)
Weight *(kg), Mean (SD)*	52.8 (9.8)	51.7 (8.8)	0.354	52.4 (9.5)
BMI *(kg/m^2)*, N (%)			0.349	
<16	27 (77.1)	8 (22.9)		35
16-18.4	56 (62.9)	33 (37.1)		89
18.5-24.9	92 (66.2)	47 (33.8)		139
25+	14 (77.8)	4 (22.2)		18
Moxifloxacin Dose, N (%)			0.271	
800mg	107 (69.5)	47 (30.5)		154
600mg	83 (65.4)	44 (34.6)		127
400mg	0 (0.0)	1 (100.0)		1
Moxifloxacin *(mg/kg)*, Mean (SD)	13.7 (1.5)	13.6 (1.3)	0.900	13.7 (1.4)
Clofazimine *(mg/kg)*, Mean (SD)	2.0 (0.3)	2.0 (0.3)	0.661	2.0 (0.3)
QTcF, N (%)			0.112	
Less than 400ms	76 (62.3)	46 (37.7)		122
Greater than or equal to 400ms	114 (71.3)	46 (28.7)		160
ALT, Mean (SD)	21.5 (17.8)	18.4 (11.9)	0.121	20.5 (16.1)
AST, Mean (SD)	28.3 (19.9)	26.3 (16.6)	0.409	27.7 (18.9)
Creatinine, Mean (SD)	67.2 (20.1)	66.2 (19.6)	0.688	66.9 (19.9)
Glucose, Mean (SD)	5.6 (2.6)	6.3 (4.1)	0.102	5.9 (3.2)
Potassium, N (%)			0.495	
3.5mmol/L and above	168 (67.5)	81 (32.5)		249
Under 3.5mmol/L	12 (60.0)	8 (40.0)		20

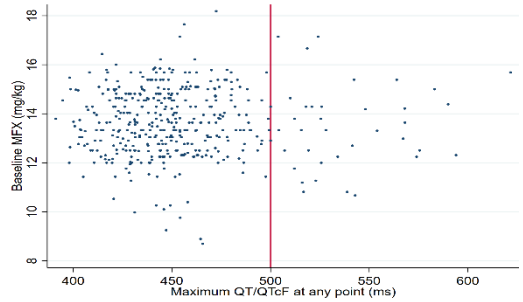
[1] *Ethiopia & South Africa only*

Figure A1. Baseline clofazimine (A+C) and moxifloxacin (B+D) dose (mg/kg) and relationship to maximum QT/QTcF (A+B) and maximum QTcF increase (C+D) on the Short regimen.

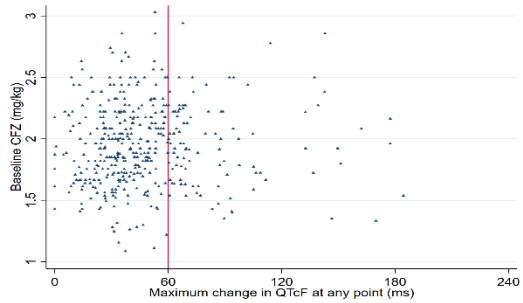
A Baseline CFZ exposure by maximum QT/QTcF within one year



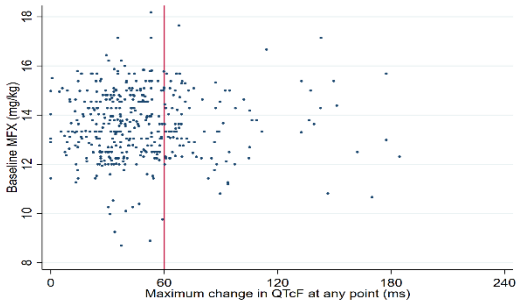
B Baseline MFX exposure by maximum QT/QTcF within one year



C Baseline CFZ exposure by maximum increase in QTcF within one year



D Baseline MFX exposure by maximum increase in QTcF within one year



The red line indicates a maximum QT/QTcF of 500 ms (A+B) or a maximum increase in QTcF of 60 ms (C+D) within 52 weeks of follow-up, with patients to the right of this line exceeding the threshold. A non-linear relationship is seen in all four graphs plotting baseline mg/kg of clofazimine (A+C) and moxifloxacin (B+D) with maximum QT or QTcF value.

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Chapter two: ECG monitoring for participants receiving the Short regimen in STREAM Stage 1: Can we identify those at increased risk of QT prolongation?

Background

STREAM Stage 1 was a randomised trial of a Short (9 month) regimen for RR-TB. QT or QTcF prolongation ≥500ms occurred in 31 (11%) of 282 Short regimen participants. The frequent ECG monitoring employed might be challenging for treatment programmes. This chapter aimed to determine whether those at higher risk of clinically relevant QT prolongation could be identified early for more targeted monitoring.

Methods

Data from the first month of treatment were used to investigate whether it was possible to predict which participants were at risk of developing a QT or QTcF interval ≥500ms. QTcF increases from baseline at different time points were examined. Absolute QTcF measurements were categorised in 5ms increments at each time-point. The most discriminating time points and QTcF cut-offs were combined to optimise sensitivity and specificity. The same process was applied to Long regimen participant data for absolute QTcF values to assess validity of this strategy in a different regimen.

Results

Absolute QTcF values were more discriminating than size of increase from baseline. More participants who developed QT/QTcF ≥500ms had a QTcF ≥425ms and ≥430ms at 4 hours and week 3 respectively ($p<0.05$) than those who did not. Combining QTcF values ≥425ms at 4 hours and ≥430ms at week 3 identified high-

risk participants with 97% sensitivity and 99% negative predictive value. A combination of the same absolute QTcF cut-off values at the same time-points gave a sensitivity of 75% and specificity of 81% with a negative predictive value of 98% for Long regimen participants.

Conclusion

Reduced ECG monitoring may be possible for many Short regimen participants without missing patients at higher risk. This would allow a more targeted approach in settings with limited resources.

Background

The background to STREAM Stage 1 was described in the introduction and chapter one.[1] As both regimens had the potential to cause QT prolongation in participants, frequent ECG monitoring took place.

The higher frequency of QT prolongation on the Short regimen may be due to high-dose moxifloxacin and clofazimine in combination. Chapter 1 presented analyses of predictive factors for QT prolongation in this population which identified country (Mongolia) and baseline values as independent predictors.[2] Most participants on the Short regimen did not develop a QT/QTcF ≥500ms and may not need the intensive ECG monitoring used in the trial. On the other hand, some "high-risk" participants, did develop a QT/QTcF ≥500ms and benefited from close monitoring so that treatment could be modified if needed.

Guidance on cardiac monitoring during treatment for RR-TB

The TB drug monographs website [3] recommends ECGs at baseline, 2 weeks and then every 3 months for participants taking clofazimine or moxifloxacin containing regimens. The South African Department of Health recommends routine monitoring with ECGs at baseline, monthly for the intensive phase and then 3 monthly in the continuation phase for RR-TB.[4] The frequency of monitoring has implications for both financial and staff resources and may negatively affect adherence. The cost of managing a RR-TB patient in the UK has been estimated to be roughly ten times that of a drug susceptible TB patient.[5] Part of this cost is likely to be due to increased patient monitoring, which would be more difficult in a resource-limited setting. Regular visits to a health facility for ECG monitoring may affect adherence by increasing anxiety, frequency of clinic visits, waiting times and travel difficulties.[6]

The pharmacokinetics (PK) of moxifloxacin and clofazimine influence risk and timing of QT prolongation. Oral moxifloxacin is rapidly absorbed, reaching a maximum serum concentration (T_{max}) in 0.75–3.5 hours with a half-life of 6-12 hours.[7] Clofazimine has unusual PK properties being highly protein bound and lipophilic with a long half-life (approximately 25-34 days), reaching a steady plasma state after 18 - 21 weeks.[8,9] Several weeks of treatment may occur before participants reach a QT/QTcF ≥500ms. Analysis in chapter 1 found most participants who experienced QT or QTcF prolongation ≥500ms did so ≥12 weeks into treatment.[2] Predicting who these participants are from early QT readings would allow monitoring to be targeted to those most at risk. Whilst QT or QTcF interval prolongation ≥500ms is categorised as severe, smaller increases are also of concern. The FDA considers drugs that increase the mean QT/QTc interval by >20ms in thorough QT studies, more likely to be proarrhythmic[10] and a 5% increased risk of arrythmia for every 10ms increase in QT interval above the upper limit of normal has been shown in congenital long QT patients.[11] Patients with higher baseline readings are more likely to experience QT prolongation ≥500ms.[12–14] Analysis of STREAM Stage 1 data showed participants on the Short regimen with a QT/QTcF ≥500ms were nearly six times more likely to have a baseline QTcF reading ≥400ms than <400ms.[2] However, most participants with baseline readings ≥400ms did not later develop QT or QTcF ≥500ms. A monitoring strategy that separates patients into high and lower risk groups cannot rely on baseline readings alone. The aim of this chapter was to determine whether QTcF measurements early on during treatment would distinguish between participants at “high” and “lower” risk of QT or QTcF prolongation and could be used as the basis for development of a simplified monitoring strategy for programmatic settings.

Methods

The data for this chapter came from the STREAM Stage 1 trial.[1] Participants were included if they were randomised to the Short regimen and had at least four out of six ECG measurements between baseline and week 4. Participants with missing ECGs at particular time points were excluded from analyses at that time.

A series of exploratory analyses were undertaken to assess association with subsequent QT/QTcF prolongation:

1. Increase from baseline of 5, 10, 15, 20 and 40ms in participants' QTcF from 4 hours post first dose up to week 4.

2. Absolute QTcF values at baseline, 4 hours post first dose and weeks 1-4 were analysed to see whether a cut-off value could be identified below which no participants developed a QT/QTcF ≥500ms. The range of cut-off values considered (410ms up to 450ms in 5ms increments) reflected the Interquartile Range of QTcF readings from Short regimen participants during follow-up (409.7, 445.5).

3. The most discriminating absolute QTcF cut-off values from different time-points were combined to optimise sensitivity and specificity.

Participants allocated the Short regimen were classified according to whether they had experienced QT/QTcF ≥500ms at any time up to week 52 ("high-risk") or not ("lower-risk").

Short regimen participants were also classified according to whether they had reached a QTcF of ≥60ms from baseline or not as this is also classified as clinically relevant QT prolongation. Time points from 4 hours post first dose to week 4 were assessed using three cut-off values; <20ms, 20-40ms and >40ms.

Steps 1 to 3 were also applied to the Long regimen participant data to assess whether the strategy was valid in a different dataset to the Short regimen.

The number and proportion of “high-risk” participants whose QTcF exceeded the cut-off value (either QTcF increase from baseline or absolute value) at each time-point were calculated. The proportions identified were compared across categories using Fisher’s exact test. Sensitivity, specificity, and positive and negative predictive values (PPV, NPV) for QT prolongation ≥500ms, were calculated.

The optimal cut-off at each time point was selected based on the distance from the ideal scenario of 100% sensitivity and 100% specificity, with values closer to zero being best and to one being worst. Combinations of cut-off values and time points were amalgamated to assess the sensitivity and specificity of different combinations.

Receiver Operating Characteristic (ROC) curves, relating to QT/QTcF prolongation ≥500ms, were plotted based upon QTcF cut-off values ranging from 385 to 470ms, assessing sensitivity and specificity at each 5ms interval. Additionally, ROC curves were plotted for time points from week 1 to 4, restricting the analysis to high and lower-risk participants with a QTcF below 425ms at their 4-hour reading (or were missing this result).

Statistical analyses were conducted using STATA v15.1.

Results

Of 282 participants allocated the Short regimen, seven were excluded as they had fewer than four ECG readings to week 4.

The size of increase in QTcF interval from baseline was a poor predictor of subsequent QT/QTcF ≥500ms; the best was ≥40ms at week 4, but twenty-two participants who went on to experience severe prolongation were missed and only eight correctly classified (Table 2.1). The size of increase in QTcF interval from baseline was also a poor predictor of patients that had a subsequent QTcF ≥60ms from baseline using the cut-off values <20ms; 20-40ms and >40ms (Table A10). Though the majority of patients who were >40ms from baseline at all time points examined were in the “high-risk” group, many of this “high-risk” group were also missed.

Absolute baseline values performed better than change from baseline, although the best cut-off (410ms) on its own missed a third of high-risk participants (Table A1).

ROC curves from 4 hours post-first dose to week 4 are displayed (Fig 2.1-2.2). The ROC curve analysis in Figure 2.2 shows how different time points (4 hours up to week 4) performed as a screening tool for high-risk participants using QTcF cut-off values of 410-450ms. Time points of 4 hours and week 3 appeared best when looking at the same population for each point (Fig 2.1, Table A4). The cut-off value of 425ms at 4 hours gave a sensitivity of 83.3% and missed only five high-risk participants with a false-positive rate to 32.8%. A cut-off value of 430ms at the later time point of week 3 was equally good with a sensitivity of 83.9% and false positive rate of 32% (Fig 2.1, Table A4).

Figure 2.2 shows ROC curves for time-points weeks 1-4 in the population whose QTcF at 4 hours was <425ms. The best time point appeared to be week 3 and using a cut-off QTcF of 430ms (the same second-best scenario as in the whole population). All five high-risk participants with 4-hour readings <425ms are identified, with a false-positivity rate of 21% (Table A5). The sensitivity was 83.3% as one high-risk patient was missed using this approach with a week 3 QTcF <430ms, though their 4-hour reading was unavailable, so they were not included in analysis at that time point.

Performance statistics of different QTcF cut-offs at 4 hours post first dose in the whole study population (Table A2), at week 3 amongst participants with a QTcF <425ms at 4 hours (Table A3)**,** and for the best scenarios from the two time points combined (Table 2.2). Using the combination of time points and cut-offs described, 47% of participants were correctly identified as lower risk, 11% correctly identified as high-risk, 41% incorrectly identified as high-risk and less than 1% incorrectly identified as lower risk. This equates to a 97% sensitivity, 53% specificity, 21% PPV and 99% NPV.

The performance of different QTcF cut-off values for Long regimen participants at the same time points as for the Short regimen are shown in Tables A6-A9. Of the eight patients identified as reaching the 500ms threshold within 52 weeks of follow up, six would have been captured using the same monitoring strategy described here for the Short regimen participants. Two participants had 4-hour readings ≥425ms with four participants showed week 3 readings ≥430ms. There were two participants missed at both time points using the cut-offs described who crossed 500ms at week 24 and week 40 respectively. Using the same strategy of combining 425ms at 4 hours post first dose and 430ms at week 3, it was possible to identify the

high-risk Long regimen participants with a sensitivity of 75%, specificity of 81%, PPV of 19% and NPV of 98% (Table 2.3, A11-12).

Table 2.1. Increase from baseline QTcF in the first 4 weeks of treatment based on a 40ms threshold comparing Short regimen participants who subsequently developed QT/QTcF prolongation ≥500ms and those who did not.

Time point	Increase from baseline	Total assessed[1] (n=275)	Prolongation ≥500ms (n=31)	P-value[2]
4 hours	<40ms	260	26 (86.7%)	0.054
	≥40ms	14	4 (13.3%)	
Week 1	<40ms	248	24 (77.4%)	0.017
	≥40ms	26	7 (22.6%)	
Week 2	<40ms	243	26 (83.9%)	0.368
	≥40ms	31	5 (16.1%)	
Week 3	<40ms	241	24 (77.4%)	0.064
	≥40ms	31	7 (22.6%)	
Week 4	<40ms	229	22 (73.3%)	0.103
	≥40ms	42	8 (26.7%)	

[1] *Participants with missing ECG results at a particular time point are excluded from that analysis.*
[2] *Fisher's exact test*

Figure 2.1. Receiver operating characteristic curve analysis using QTcF cut-off at 4 hours and weeks 1- 4 to identify a diagnosis of QT/QTcF prolongation ≥500ms in Short regimen participants.

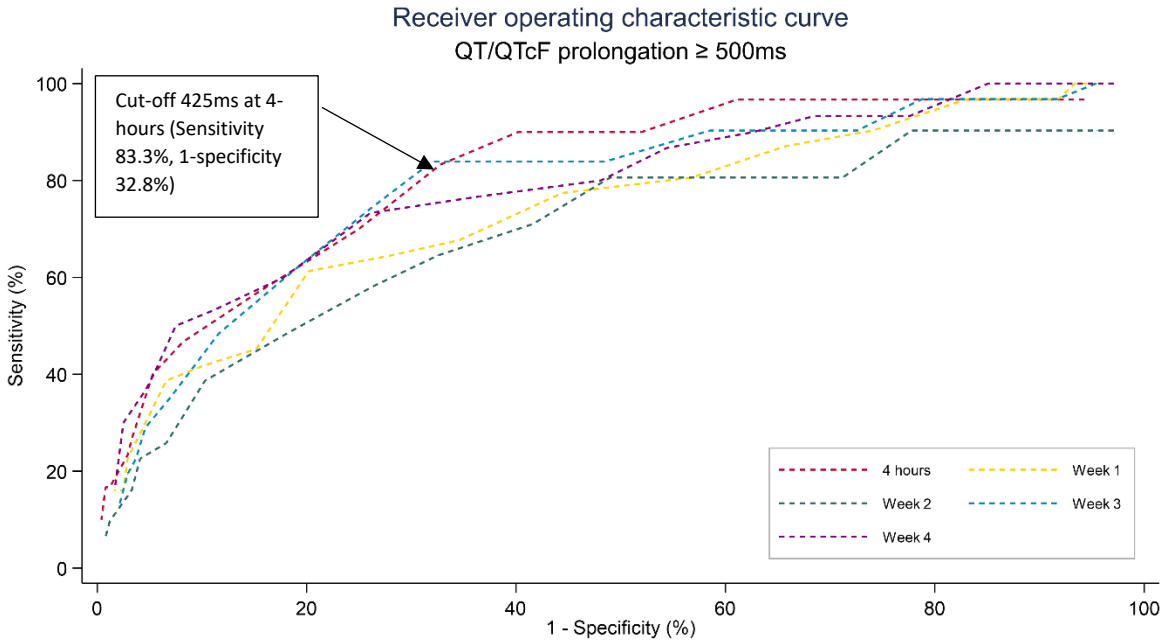

Figure 2.2. Receiver operating characteristic curve analysis for high and lower-risk Short regimen participants with a 4-hour QTcF <425ms using QTcF cut-offs at weeks 1- 4 to identify a diagnosis of QT/QTcF prolongation ≥500ms

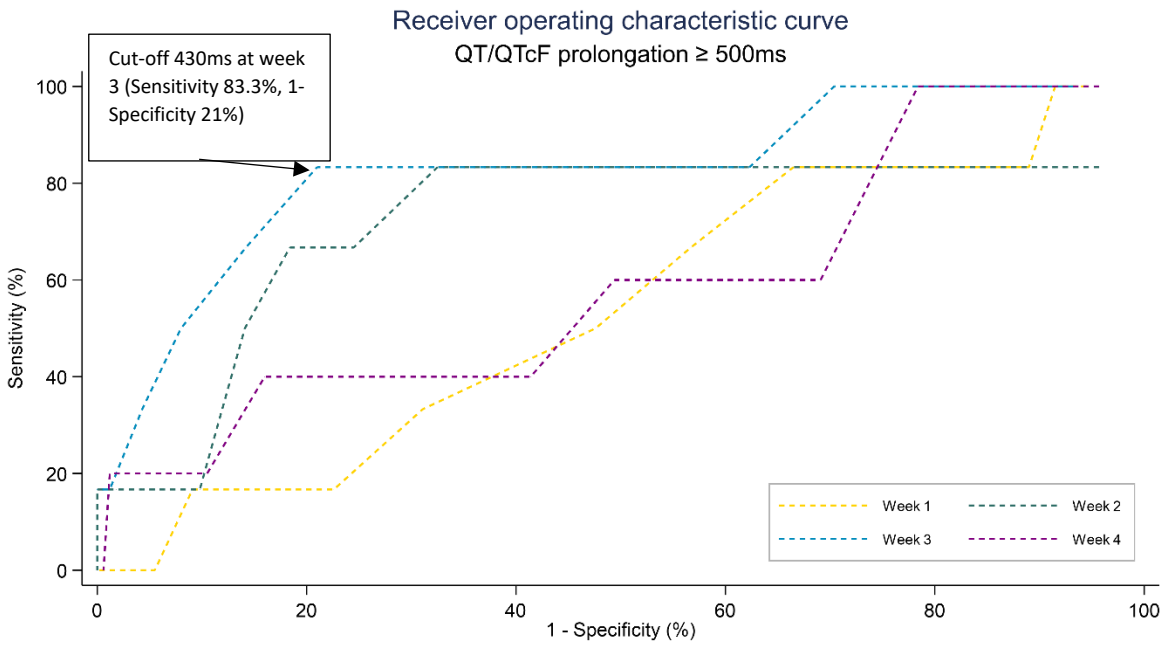

Table 2.2. Combined performance using a cut-off of 425ms at 4 hours and 430ms at week 3 to identify Short regimen participants with a diagnosis of QT/QTcF prolongation ≥500ms during ECG monitoring

Sensitivity	Specificity	PPV	NPV	Distance
96.77%	52.89%	20.83%	99.22%	0.4722

Table 2.3. Combined performance using a cut-off of 425ms at 4 hours and 430ms at week 3 to identify Long regimen participants with a diagnosis of QT/QTcF prolongation ≥500ms during ECG monitoring

Sensitivity	Specificity	PPV	NPV	Distance
75.00%	80.62%	19.35%	98.11%	0.3163

Although management and outcome of the patients is not covered in this analysis it is worth briefly mentioning what happened to some of these patients. Two of the

thirty-one patients on the Short regimen who reached a QT/QTcF ≥500ms during monitoring had done so by their 4-hour reading; in each treatment was modified. One had a further reading ≥500ms later in the trial; the other did not. Of the five patients missed at the 4-hour time point using a cut-off of 425ms; two had a single episode of QTcF prolongation ≥500ms (500 and 512ms respectively) late in treatment that required no changes to be made; one had a single episode (512ms) at week 3 requiring a brief interruption in treatment and two reached the 500ms threshold later in treatment at week 8 (550ms) and week 28 (536ms). Both these patients had further episodes >500ms requiring more than one modification to their treatment.

Finally, the one patient not captured by the combination of time-points and QTcF cut-offs described missed their 4-hour reading and was therefore not included in the earlier analysis so it is possible that they might have been identified. They had a single QT interval of 500ms exactly at week 28 of follow up, resulting in a dose reduction of moxifloxacin. The patient then completed the regimen with no further episodes of QT/QTcF ≥500ms.

Discussion

In programmatic settings with limited resources and/or large catchment areas it would be useful for clinicians to know early on which patients are more likely to develop clinically relevant QT prolongation and who are at lower risk so that monitoring strategies can be adjusted, and better use be made of scarce resources. I analysed STREAM Stage 1 ECG data to see whether increases in QTcF from baseline or absolute QTcF values at different time-points in the first month of treatment identified Short regimen participants with prolongation ≥500ms at any point during monitoring as well as those who developed an increase in QTcF of ≥60ms from baseline during monitoring. At 4-hours after the first dose 95% (260/274) of participants had QTcF measurements within 40ms of their baseline reading; this includes 234 participants who never went on to reach a QT/QTcF of 500ms. However, this also includes 26 out of 31 high-risk participants who did. At week 4, fewer of the lower-risk group had a QTcF increase of ≥40ms from baseline value and more high-risk participants are captured but 22 of those who developed QT/QTcF ≥500ms were missed.

Though an increase in QTcF of ≥60ms from baseline is also considered clinically relevant, the risk of developing life-threating arrythmias is lower than if the absolute value reaches ≥500ms. The analysis showed that the majority of the “high-risk” group that we now know went on to reach a QTcF increase of ≥60ms during follow-up, were below 40ms after one month of treatment. The analysis in Table A10 shows that after 4 weeks of treatment nearly a third of the 91 patients on the Short regimen who eventually reached a QTcF of ≥60ms higher than their baseline value, had already reached ≥40ms at this point. However, a third were between 20-40ms above their baseline value and importantly a third were still within 20ms of their baseline

value. This suggests an increase from baseline in the QTcF within 4 weeks of treatment was not a useful predictor of high-risk patients. As treatment was not modified for an increase from baseline QTcF of ≥60ms alone, predicting who these patients are likely to be from early monitoring is perhaps of less importance than predicting those who are likely to develop absolute QT or QTcF interval values of ≥500ms.

Absolute QTcF values performed better as a screening tool than increase from baseline in predicting those who reached a QT or QTcF interval ≥500ms as they discriminated high and lower risk participants to a greater degree. The analysis showed using a low QTcF threshold of <410ms at 4 hours meant only one patient who later developed a QT/QTcF ≥500ms would be missed (Table A4). However, this comes at a cost of more intense monitoring for 61% of participants in the lower-risk group. Similarly picking a QTcF cut-off value of >450ms would mean only having to perform extra monitoring on 3% of participants in the lower-risk group but would miss 22 participants who later developed a QT/QTcF >500ms which is unsatisfactory. A cut-off value of 425ms at the 4-hour time point would allow reduced monitoring for 67% of lower-risk participants and capture 25 of the 30 (83.3%) high-risk participants. All but one of the high-risk participants were identified using a combination of QTcF cut-offs; 425ms at 4 hours and 430ms at week 3. Of the 162 lower-risk participants in the analysis at week 3, 79% could have had reduced monitoring, as they were below the 430ms threshold.

The high sensitivity of 97% and NPV of 99% demonstrates nearly all participants in our data set were correctly predicted to have reached a QT/QTcF ≥500ms during trial follow up and a QTcF <425ms at 4 hours and <430ms at week 3 was fairly reliable at excluding participants who subsequently developed QT/QTcF

prolongation ≥500ms. The low specificity of 53% was disappointing and the PPV of 21% led to a relatively large number of false positives. However, as the aim was to determine whether a reduction in monitoring for lower-risk participants could be achieved without missing high-risk participants, this trade-off is still reasonable. The combination of early time-points and cut-off values would allow a reduction in ECG monitoring frequency in 47% of participants on the Short regimen from STREAM Stage 1. While 4 hours post first dose was used in the analysis as the first time-point, week 3 had similar sensitivity and false-positive rate (Table A4). As an ECG at a later time-point would be required to capture all higher-risk participants, I selected the 4-hour time point in preference to week 3 as participants are more likely to be easily available.

The Long regimen participants were also analysed to see whether the results differed or not using the same strategy that was applied to Short regimen participants. Most participants who reached 500ms during 52 weeks of follow-up were detected using this approach. As the regimen did not include high dose fluoroquinolone or clofazimine the mean QTcF for participants on this regimen was lower which possibly explains the improved specificity compared to the Short regimen analyses as there were less false positives using the same cut-offs. Two patients were missed using the strategy. Given these were isolated episodes occurring later in treatment, it may explain why they were missed at the earlier times points using the cut-off values described. Although the Long regimen has now largely been replaced, it was useful to see how the strategy compared in a different regimen that contained a fluoroquinolone at standard dose without clofazimine.

ECG monitoring allows patients who need treatment modification to be identified and managed appropriately. An ECG performed 4 hours after first dose of medication

would allow an early decision regarding follow-up. In the data shown, a monitoring strategy based on the 4-hour reading alone would have led to missing five high-risk participants who went on to develop a QT/QTcF ≥500ms. Though if participants with a QTcF <425ms at 4 hours were brought back within the first month of treatment additional high-risk participants who would benefit from increased ECG monitoring would be identified.

QT length may be affected by several factors in addition to drugs and treatment effects may be delayed. Torsades de Pointes developed >1 month after starting non-cardiac medication for 40% of participants in a large cohort.[15] This review of 249 patients with Torsades de Pointes related to non-cardiac medications found that of the 114 with details of the exact timing of the drug, 18% developed arrhythmia within 72 hours of starting the oral medication, 42% between day 3 and 30, and 40% occurred more than 1 month after starting the oral medication.[15] The analysis from Chapter 1, using the same population as for this Chapter, found that the median time to QT/QTcF prolongation ≥500ms was 20 [8, 28] weeks. Although most patients did not develop clinically relevant QT prolongation until week 12 or later, it was possible to predict all of these patients from values within the first month of treatment. The unusual PK properties of clofazimine, reaching a steady state in plasma after around 126 days [8], may partly explain why severe QT prolongation occurred more commonly later in treatment rather than in the first few weeks. One study found common risk factors such as female gender and hypokalaemia were also prevalent as was the finding that patients often had more than one risk factor.[15] Though this association was not found in the analyses presented in Chapter 1, which showed that patients who started with higher baseline QTcF values were more likely to develop clinically relevant QT/QTcF prolongation, although as already mentioned,

baseline values alone are not sufficient to identify all high-risk patients. There is evidence that patients who develop drug induced QT prolongation may have an underlying genetic predisposition. Itoh et al [16] found in a cohort of 188 patients with acquired long QT syndrome from Europe and Japan, a third carried mutations known to occur in congenital long QT syndrome families, with *KNCH2* being most common. In addition, whilst their baseline readings were lower than those with congenital long QT syndrome (478 ±46ms) they had higher baseline readings (453 ±39ms) compared to non-carriers (406 ±26ms).

Anti-psychotics are well known for QT effects, but the range of agents available with different side effect profiles [17-18] allows clinicians to switch should clinical concerns arise.[19] This is less straightforward in the MDR-TB setting; a treatment regimen of several agents is required and four of the seven drugs in Groups A and B recommended in WHO guidelines[20] (moxifloxacin, levofloxacin, bedaquiline and clofazimine) are known to prolong the QT interval. Given the efficacy of these drugs and the limited alternatives, QT prolongation needs to be monitored for and managed rather than being avoided altogether.

The proposed strategy described in this chapter has some limitations.

First, it is based on data from a single trial and has yet to be properly validated in a different data set (though this is planned for STREAM Stage 2). Second, new regimens are regularly being investigated. It would be a challenge and possibly impractical to test this monitoring strategy on every new regimen with different drugs. However, as the BPaLM and 9-month all oral regimen (investigated in STREAM Stage 2) is now recommended by WHO, it would be feasible to test the above ECG

monitoring strategy in only two new regimens, both of which contain the drugs likely to cause QT prolongation. So even if regimens were modified using other existing drugs it is unlikely they would significantly affect the QT interval and the monitoring strategy described if it was shown to have an acceptable sensitivity and specificity in these two regimens.

Third, other risk factors for QT prolongation, may affect the performance of this monitoring strategy e.g., renal or hepatic impairment could affect drug metabolism and vomiting could lead to electrolyte disturbance, potentiating QT interval prolongation. Concomitant medications that affect the QT interval or metabolism of moxifloxacin or clofazimine may also play a role. Fourth, the strategy requires lower risk participants to return for an extra ECG and some high-risk participants may still be missed.

The main strengths of this study are that STREAM Stage 1 is currently one of the largest trials in drug-resistant TB treatment and that regular ECG monitoring using a calibrated standardised machine was carried out for all participants, irrespective of regimen or QT interval. It was also applied to the Long regimen participants in STREAM Stage 1 with fairly good results.

Based on the findings from this chapter I would recommend that all patients taking the Short regimen (containing high dose moxifloxacin and clofazimine) have an ECG reading in clinic 4-hours after taking their first dose of treatment. They should then be brought back towards the end of their first month of treatment (week 3) for a repeat ECG. This would allow clinicians to categorise patients into high or low risk based on the thresholds described. High-risk patients should then return every 4 weeks on treatment for a repeat ECG whereas low-risk patients could have an ECG 12 weeks

into treatment and if there are no concerns about their QT interval, then a further 2 ECGs in the continuation phase should be sufficient. To give clinicians confidence in these recommendations, future work would need to validate the findings described in this chapter in a different population. Clinicians would also have to be mindful of other factors that may affect the validity of these results in their patients e.g. electrolyte abnormalities, concomitant medications that affect the QT interval. If other factors are present that could prolong the QT interval in low-risk patients then it would be prudent for the clinician to follow the ECG schedule recommended for the high-risk patients.

Conclusion

ECG monitoring in STREAM Stage 1 allowed patients who required treatment modification to be identified and managed appropriately. Using the strategy described, it may be possible to limit the number of ECG monitoring visits required in almost half the participants receiving the Short regimen. Such a targeted monitoring strategy could be useful in programmatic settings, particularly with limited resources or a large geographic spread of participants.

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Appendix – 2

Table A1. QTcF at baseline in participants who went on to develop QT/QTcF prolongation ≥500ms and those who did not by different cut-offs

Time point	QTcF	Total assessed (n=275)	Prolongation ≥500ms (n=31)	P-value[1]
Baseline	<410ms	169	10 (32.3%)	0.001
	≥410ms	106	21 (67.7%)	
	<415ms	194	12 (38.7%)	<0.001
	≥415ms	81	19 (61.3%)	
	<420ms	211	14 (45.2%)	<0.001
	≥420ms	64	17 (54.8%)	
	<425ms	235	17 (54.8%)	<0.001
	≥425ms	40	14 (45.2%)	
	<430ms	245	21 (67.7%)	<0.001
	≥430ms	30	10 (32.3%)	
	<435ms	257	24 (77.4%)	0.002
	≥435ms	18	7 (22.6%)	
	<440ms	264	25 (80.6%)	<0.001
	≥440ms	11	6 (19.4%)	
	<445ms	268	26 (83.9%)	<0.001
	≥445ms	7	5 (16.1%)	
	<450ms	270	27 (87.1%)	0.001
	≥450ms	5	4 (12.9%)	

[1] *Fisher's exact test*

Table A2. Performance of QTcF cut-off values 410-450ms at the 4-hour time point amongst all Short regimen participants

Cut-off	Sensitivity	Specificity	PPV	NPV	Distance
410	96.7%	38.9%	16.3%	99.0%	0.61
415	90.0%	48.0%	17.5%	97.5%	0.53
420	90.0%	59.8%	21.6%	98.0%	0.41
425	**83.3%**	**67.2%**	**23.8%**	**97.0%**	**0.37**
430	70.0%	75.0%	25.6%	95.3%	0.39
435	56.7%	84.8%	31.5%	94.1%	0.46
440	46.7%	91.8%	41.2%	93.3%	0.54
445	40.0%	94.7%	48.0%	92.8%	0.60
450	26.7%	96.7%	50.0%	91.5%	0.73

Table A3. Performance of QTcF cut-off values 410-450ms at the week 3 time point amongst Short regimen participants with a 4-hour reading <425ms

Cut-off	Sensitivity	Specificity	PPV	NPV	Distance
410	83.3%	37.7%	4.7%	98.4%	0.65
415	83.3%	54.3%	6.3%	98.9%	0.49
420	83.3%	64.8%	8.1%	99.1%	0.39
425	83.3%	72.2%	10.0%	99.2%	0.32
430	**83.3%**	**79.0%**	**12.8%**	**99.2%**	**0.27**
435	66.7%	85.8%	14.8%	98.6%	0.36
440	50.0%	92.0%	18.8%	98.0%	0.51
445	33.3%	95.7%	22.2%	97.5%	0.67
450	16.7%	98.8%	33.3%	97.0%	0.83

Table A4. Time points from 4 hours to week 4 of ECG monitoring showing number of high-risk and lower-risk Short regimen participants correctly identified using a range of QTcF Cut-off values from 410-450ms.

	4 Hours		Week 1		Week 2		Week 3		Week 4	
QTcF	Total assessed (n=274)	Increase to ≥500ms (n=30)	Total assessed (n=274)	Increase to ≥500ms (n=31)	Total assessed (n=274)	Increase to ≥500ms (n=31)	Total assessed (n=272)	Increase to ≥500ms (n=31)	Total assessed (n=271)	Increase to ≥500ms (n=30)
<410ms	96	1 (3.3%)	87	4 (12.9%)	76	6 (19.4%)	69	3 (9.7%)	78	2 (6.7%)
≥410ms	178	29 (96.7%)	187	27 (87.1%)	198	25 (80.6%)	203	28 (90.3%)	193	28 (93.3%)
<415ms	120	3 (10.0%)	111	6 (19.4%)	101	6 (19.4%)	103	3 (9.7%)	93	3 (10.0%)
≥415ms	154	27 (90.0%)	163	25 (80.6%)	173	25 (80.6%)	169	28 (90.3%)	178	27 (90.0%)
<420ms	149	3 (10.0%)	142	7 (22.6%)	130	6 (19.4%)	129	5 (16.1%)	114	4 (13.3%)
≥420ms	125	27 (90.0%)	132	24 (77.4%)	144	25 (80.6%)	143	26 (83.9%)	157	26 (86.7%)
<425ms	**169**	**5 (16.7%)**	169	10 (32.3%)	151	9 (29.0%)	149	5 (16.1%)	131	6 (20.0%)
≥425ms	**105**	**25 (83.3%)**	105	21 (67.7%)	123	22 (71.0%)	123	26 (83.9%)	140	24 (80.0%)
<430ms	192	9 (30.0%)	186	11 (35.5%)	175	11 (35.5%)	169	5 (16.1%)	160	7 (23.3%)
≥430ms	82	21 (70.0%)	88	20 (64.5%)	99	20 (64.5%)	103	26 (83.9%)	111	23 (76.7%)
<435ms	220	13 (43.3%)	206	12 (38.7%)	192	13 (41.9%)	186	8 (25.8%)	186	8 (26.7%)
≥435ms	54	17 (56.7%)	68	19 (61.3%)	82	18 (58.1%)	86	23 (74.2%)	85	22 (73.3%)
<440ms	240	16 (53.3%)	223	17 (54.8%)	215	16 (51.6%)	208	12 (38.7%)	210	12 (40.0%)
≥440ms	34	14 (46.7%)	51	14 (45.2%)	59	15 (48.4%)	64	19 (61.3%)	61	18 (60.0%)
<445ms	249	18 (60.0%)	236	18 (58.1%)	237	19 (61.3%)	229	16 (51.6%)	228	14 (46.7%)
≥445ms	25	12 (40.0%)	38	13 (41.9%)	37	12 (38.7%)	43	15 (48.4%)	43	16 (53.3%)
<450ms	258	22 (73.3%)	246	19 (61.3%)	250	23 (74.2%)	244	20 (64.5%)	238	15 (50.0%)
≥450ms	16	8 (26.7%)	28	12 (38.7%)	24	8 (25.8%)	28	11 (35.5%)	33	15 (50.0%)

Table A5 Time points from weeks 1-4 of ECG monitoring showing number of high-risk and lower-risk Short regimen participants[1] correctly identified using a range of QTcF Cut-off values from 410-450ms.

QTcF	Week 1		Week 2		Week 3		Week 4	
	Total assessed (n=170)	Increase to ≥500ms (n=6)	Total assessed (n=169)	Increase to ≥500ms (n=6)	Total assessed (n=168)	Increase to ≥500ms (n=6)	Total assessed (n=167)	Increase to ≥500ms (n=5)
<410ms	73	2 (33.3%)	68	1 (16.7%)	62	1 (16.7%)	72	2 (40.0%)
≥410ms	97	4 (66.7%)	101	5 (83.3%)	106	5 (83.3%)	95	3 (60.0%)
<415ms	89	3 (50.0%)	88	1 (16.7%)	89	1 (16.7%)	84	2 (40.0%)
≥415ms	81	3 (50.0%)	81	5 (83.3%)	79	5 (83.3%)	83	3 (60.0%)
<420ms	117	4 (66.7%)	111	1 (16.7%)	106	1 (16.7%)	98	3 (60.0%)
≥420ms	53	2 (33.3%)	58	5 (83.3%)	62	5 (83.3%)	69	2 (40.0%)
<425ms	132	5 (83.3%)	125	2 (33.3%)	118	1 (16.7%)	110	3 (60.0%)
≥425ms	38	1 (16.7%)	44	4 (66.7%)	50	5 (83.3%)	57	2 (40.0%)
<430ms	141	5 (83.3%)	135	2 (33.3%)	**129**	**1 (16.7%)**	124	3 (60.0%)
≥430ms	29	1 (16.7%)	34	4 (66.7%)	**39**	**5 (83.3%)**	43	2 (40.0%)
<435ms	154	5 (83.3%)	143	3 (50.0%)	141	2 (33.3%)	139	3 (60.0%)
≥435ms	16	1 (16.7%)	26	3 (50.0%)	27	4 (66.7%)	28	2 (40.0%)
<440ms	161	6 (100.0%)	152	5 (83.3%)	152	3 (50.0%)	149	4 (80.0%)
≥440ms	9	0 (0.0%)	17	1 (16.7%)	16	3 (50.0%)	18	1 (20.0%)
<445ms	164	6 (100.0%)	161	5 (83.3%)	159	4 (66.7%)	159	4 (80.0%)
≥445ms	6	0 (0.0%)	8	1 (16.7%)	9	2 (33.3%)	8	1 (20.0%)
<450ms	165	6 (100.0%)	165	5 (83.3%)	165	5 (83.3%)	163	4 (80.0%)
≥450ms	5	0 (0.0%)	4	1 (16.7%)	3	1 (16.7%)	4	1 (20.0%)

1 Data only from participants with a QTcF <425ms at the 4-hour time point

Table A6. Time points from 4 hours to week 4 of ECG monitoring showing number of high-risk and lower-risk Long regimen participants correctly identified using a range of QTcF Cut-off values from 410-450ms.

	4 Hours		Week 1		Week 2		Week 3		Week 4	
QTcF	Total assessed (n=137)	Increase to ≥500ms (n=8)	Total assessed (n=138)	Increase to ≥500ms (n=8)	Total assessed (n=137)	Increase to ≥500ms (n=8)	Total assessed (n=137)	Increase to ≥500ms (n=8)	Total assessed (n=139)	Increase to ≥500ms (n=8)
<410ms	83	3 (37.5%)	68	4 (50.0%)	69	3 (37.5%)	68	2 (25.0%)	67	2 (25.0%)
≥410ms	54	5 (62.5%)	70	4 (50.0%)	68	5 (62.5%)	69	6 (75.0%)	72	6 (75.0%)
<415ms	93	3 (37.5%)	85	4 (50.0%)	80	3 (37.5%)	83	2 (25.0%)	80	3 (37.5%)
≥415ms	44	5 (62.5%)	53	4 (50.0%)	57	5 (62.5%)	54	6 (75.0%)	59	5 (62.5%)
<420ms	107	3 (37.5%)	100	5 (62.5%)	96	3 (37.5%)	101	2 (25.0%)	98	3 (37.5%)
≥420ms	30	5 (62.5%)	38	3 (37.5%)	41	5 (62.5%)	36	6 (75.0%)	41	5 (62.5%)
<425ms	**122**	**6 (75.0%)**	112	5 (62.5%)	106	4 (50.0%)	109	3 (37.5%)	110	3 (37.5%)
≥425ms	**15**	**2 (25.0%)**	26	3 (37.5%)	31	4 (50.0%)	28	5 (62.5%)	29	5 (62.5%)
<430ms	131	6 (75.0%)	120	5 (62.5%)	119	4 (50.0%)	117	3 (37.5%)	119	3 (37.5%)
≥430ms	6	2 (25.0%)	18	3 (37.5%)	18	4 (50.0%)	20	5 (62.5%)	20	5 (62.5%)
<435ms	131	6 (75.0%)	127	5 (62.5%)	127	4 (50.0%)	124	3 (37.5%)	124	4 (50.0%)
≥435ms	6	2 (25.0%)	11	3 (37.5%)	10	4 (50.0%)	13	5 (62.5%)	15	4 (50.0%)
<440ms	134	7 (87.5%)	133	5 (62.5%)	128	4 (50.0%)	129	3 (37.5%)	131	4 (50.0%)
≥440ms	3	1 (12.5%)	5	3 (37.5%)	9	4 (50.0%)	8	5 (62.5%)	8	4 (50.0%)
<445ms	134	7 (87.5%)	135	6 (75.0%)	131	4 (50.0%)	130	3 (37.5%)	132	5 (62.5%)
≥445ms	3	1 (12.5%)	3	2 (25.0%)	6	4 (50.0%)	7	5 (62.5%)	7	3 (37.5%)
<450ms	136	7 (87.5%)	137	7 (87.5%)	132	4 (50.0%)	132	3 (37.5%)	134	5 (62.5%)
≥450ms	1	1 (12.5%)	1	1 (12.5%)	5	4 (50.0%)	5	5 (62.5%)	5	3 (37.5%)

Table A7 Time points from weeks 1-4 of ECG monitoring showing number of high-risk and lower-risk Long regimen participants[1] correctly identified using a range of QTcF Cut-off values from 410-450ms.

	Week 1		Week 2		Week 3		Week 4	
QTcF	Total assessed (n=123)	Increase to ≥500ms (n=6)	Total assessed (n=122)	Increase to ≥500ms (n=6)	Total assessed (n=122)	Increase to ≥500ms (n=6)	Total assessed (n=124)	Increase to ≥500ms (n=6)
<410ms	64	4 (66.7%)	68	3 (50.0%)	67	2 (33.3%)	66	2 (33.3%)
≥410ms	59	2 (33.3%)	54	3 (50.0%)	55	4 (66.7%)	58	4 (66.7%)
<415ms	79	4 (66.7%)	77	3 (50.0%)	80	2 (33.3%)	78	3 (50.0%)
≥415ms	44	2 (33.3%)	45	3 (50.0%)	42	4 (66.7%)	46	3 (50.0%)
<420ms	94	5 (83.3%)	91	3 (50.0%)	95	2 (33.3%)	95	3 (50.0%)
≥420ms	29	1 (16.7%)	31	3 (50.0%)	27	4 (66.7%)	29	3 (50.0%)
<425ms	104	5 (83.3%)	100	3 (50.0%)	101	2 (33.3%)	105	3 (50.0%)
≥425ms	19	1 (16.7%)	22	3 (50.0%)	21	4 (66.7%)	19	3 (50.0%)
<430ms	110	5 (83.3%)	110	3 (50.0%)	**106**	2 (33.3%)	112	3 (50.0%)
≥430ms	13	1 (16.7%)	12	3 (50.0%)	**16**	4 (66.7%)	12	3 (50.0%)
<435ms	116	5 (83.3%)	115	3 (50.0%)	111	2 (33.3%)	116	3 (50.0%)
≥435ms	7	1 (16.7%)	7	3 (50.0%)	11	4 (66.7%)	8	3 (50.0%)
<440ms	120	5 (83.3%)	116	3 (50.0%)	115	2 (33.3%)	120	3 (50.0%)
≥440ms	3	1 (16.7%)	6	3 (50.0%)	7	4 (66.7%)	4	3 (50.0%)
<445ms	121	5 (83.3%)	118	3 (50.0%)	116	2 (33.3%)	121	4 (66.7%)
≥445ms	2	1 (16.7%)	4	3 (50.0%)	6	4 (66.7%)	3	2 (33.3%)
<450ms	122	5 (83.3%)	118	3 (50.0%)	118	2 (33.3%)	122	4 (66.7%)
≥450ms	1	1 (16.7%)	4	3 (50.0%)	4	4 (66.7%)	2	2 (33.3%)

1 Data only from participants with a QTcF <425ms at the 4-hour time point

Table A8. Performance of QTcF cut-off values 410-450ms at the 4-hour time point amongst all Long regimen participants

Cut-off	Sensitivity	Specificity	PPV	NPV	Distance
410	62.50%	62.02%	9.26%	96.39%	0.5338
415	62.50%	69.77%	11.36%	96.77%	0.4817
420	62.50%	80.62%	16.67%	97.20%	0.4221
425	25.00%	89.92%	13.33%	95.08%	0.7567
430	25.00%	96.90%	33.33%	95.42%	0.7506
435	25.00%	96.90%	33.33%	95.42%	0.7506
440	12.50%	98.45%	33.33%	94.78%	0.8751
445	12.50%	98.45%	33.33%	94.78%	0.8751
450	12.50%	100.00%	100.00%	94.85%	0.875

Table A9. Performance of QTcF cut-off values 410-450ms at the week 3 time point amongst all Long regimen participants

Cut-off	Sensitivity	Specificity	PPV	NPV	Distance
410	66.67%	56.03%	7.27%	97.01%	0.5517
415	66.67%	67.24%	9.52%	97.50%	0.4674
420	66.67%	80.17%	14.81%	97.89%	0.3878
425	66.67%	85.34%	19.05%	98.02%	0.3641
430	66.67%	89.66%	25.00%	98.11%	0.349
435	66.67%	93.97%	36.36%	98.20%	0.3388
440	66.67%	97.41%	57.14%	98.26%	0.3343
445	66.67%	98.28%	66.67%	98.28%	0.3338
450	66.67%	100.00%	100.00%	98.31%	0.3333

Table A10. Change from baseline QTcF at different time points using the categories <20ms, 20-40ms and >40ms in patients who went on to develop an increase in their QTcF from baseline of ≥60ms and those that did not.

Time point	Change from baseline	Total assessed (n=274)	Prolongation ≥60ms (n=91)	P-value
4 Hour	<20ms	185	44 (23.8%)	<0.001
	20-40ms	75	37 (49.3%)	
	>=40ms	14	10 (71.4%)	
Week 1	<20ms	160	37 (23.1%)	<0.001
	20-40ms	88	40 (45.5%)	
	>=40ms	26	15 (57.7%)	
Week 2	<20ms	150	35 (23.3%)	<0.001
	20-40ms	93	37 (39.8%)	
	>=40ms	31	20 (64.5%)	
Week 3	<20ms	144	27 (18.8%)	<0.001
	20-40ms	97	43 (44.3%)	
	>=40ms	31	21 (67.7%)	
Week 4	<20ms	142	28 (19.7%)	<0.001
	20-40ms	87	31 (35.6%)	
	>=40ms	42	32 (76.2%)	

Table A11. Performance of QTcF cut-off values 410-450ms at the 4-hour time point amongst all Long regimen participants

Cut-off	Sensitivity	Specificity	PPV	NPV	Distance
410	62.50%	62.02%	9.26%	96.39%	0.5338
415	62.50%	69.77%	11.36%	96.77%	0.4817
420	62.50%	80.62%	16.67%	97.20%	0.4221
425	25.00%	89.92%	13.33%	95.08%	0.7567
430	25.00%	96.90%	33.33%	95.42%	0.7506
435	25.00%	96.90%	33.33%	95.42%	0.7506
440	12.50%	98.45%	33.33%	94.78%	0.8751
445	12.50%	98.45%	33.33%	94.78%	0.8751
450	12.50%	100.00%	100.00%	94.85%	0.875

Table A12. Performance of QTcF cut-off values 410-450ms at the week 3 time point amongst all Long regimen participants

Cut-off	Sensitivity	Specificity	PPV	NPV	Distance
410	66.67%	56.03%	7.27%	97.01%	0.5517
415	66.67%	67.24%	9.52%	97.50%	0.4674
420	66.67%	80.17%	14.81%	97.89%	0.3878
425	66.67%	85.34%	19.05%	98.02%	0.3641
430	66.67%	89.66%	25.00%	98.11%	0.349
435	66.67%	93.97%	36.36%	98.20%	0.3388
440	66.67%	97.41%	57.14%	98.26%	0.3343
445	66.67%	98.28%	66.67%	98.28%	0.3338
450	66.67%	100.00%	100.00%	98.31%	0.3333

Chapter 3: Clinical management of QT prolongation in the STREAM Stage 1 trial and effect on patient outcome

Background:

This chapter aims to provide a descriptive analysis of how patients were managed when they developed a QT or QTc of ≥500ms on either a 12-lead ECG or Holter monitor. It will also assess whether development of a QT or QTc of ≥500ms affected patient outcome.

Methods:

All patients from both regimens that developed a QT or QTcF ≥500ms on either a 12-lead ECG or Holter monitor were identified. They were then divided into four groups. Group A and B were Short regimen patients that first reached the 500ms threshold on a 12-lead ECG or Holter respectively. Group C and D were Long regimen patients that first reached the 500ms threshold on a 12 lead ECG or Holter respectively. The clinical management of the patients in each group were then recorded. The outcome for patients on the Short regimen was assessed using the primary outcome definition in STREAM Stage 1 with patients divided into those who developed QT or QTcF ≥500ms and those who did not.

Results:

In total, 64 patients were identified as having reached a maximum QT/QTcF of ≥500ms during 52 weeks of follow up (54 Short regimen; 10 Long regimen). Of the

54 Short regimen participants, 29 were first identified as reaching the 500ms threshold on a 12 lead ECG and 25 first identified on a Holter recording. Of the 10 Long regimen patients, seven were first identified on a 12 lead ECG and three first identified on Holter. In total, 72% (39/54) of Short regimen participants had their treatment interrupted, modified, or changed after they reached 500ms, compared with only 20% (2/10) on the Long regimen. This occurred in the intensive phase for 69% (27/39) of participants on the Short regimen.

Comparison of outcome status between Short regimen patients showed a favourable outcome for 43/54 (79.6%) of those who developed QT or QTcF ≥500ms on either a 12-lead ECG or Holter monitor compared with 167/228 (73.2%), p = 0.333 for those who did not. Comparison of status in Long regimen patients showed a favourable outcome in 6/10 (60%) of those who reached a QT or QTcF ≥500ms on either test compared with 102/132 (77.3%), p=0.217 of those who did not.

Conclusion:

Inclusion of Holter recordings almost doubled the number of participants in STREAM Stage 1 who were identified as having reached a maximum QT/QTcF of ≥500ms. Most participants underwent treatment interruption, modification or change after having reached the 500ms threshold with most doing so in the intensive phase. Outcome did not appear to be affected in participants who reached the 500ms threshold.

Background

Monitoring for adverse events related to drugs and managing them appropriately when they occur forms an important part of tuberculosis treatment programmes. Drug-resistant TB (DR-TB) treatment can lead to adverse drug reactions not experienced in drug sensitive treatment due to the difference in regimens. As some of the drugs and regimens are relatively new, there has been less time to develop a standardised approach for monitoring and managing some of these adverse events. In the latest WHO guidelines for DR-TB, drugs are divided into three categories (A-C) in order of recommendation (Table 3.1). [(1)] As discussed in my introduction, these recommendations were largely based on an individual patient data meta-analysis that looked at treatment success and reduced mortality with regimens that included these drugs but did not assess adverse events.[(2)] Click or tap here to enter text.Of the seven drugs in Category A and B, four of them (moxifloxacin, levofloxacin, bedaquiline and clofazimine) are known to prolong the QT interval.

Table 3.1. Grouping of medicines recommended for use in drug-resistant TB regimens (taken from the WHO 2020 consolidated guidelines on TB. [(1)]

Groups and steps	Medicine	Abbreviation
Group A: Include all three medicines	Levofloxacin *or* moxifloxacin	Lfx Mfx
	Bedaquiline[b,c]	Bdq
	Linezolid[d]	Lzd
Group B: Add one or both medicines	Clofazimine	Cfz
	Cycloserine *or* terizidone	Cs Trd
Group C: Add to complete the regimen and when medicines from Groups A and B cannot be used	Ethambutol	E
	Delamanid[e]	Dlm
	Pyrazinamide[f]	Z
	Imipenem–cilastatin *or* meropenem[g]	Ipm–Cln Mpm
	Amikacin (*or* streptomycin)[h]	Am (S)
	Ethionamide *or* prothionamide[i]	Eto Pto
	P-aminosalicylic acid[j]	PAS

Given most patients with QT prolongation are asymptomatic but this can progress to tachyarrhythmia and sudden death in some patients, close monitoring is warranted. Currently there is no clear universally accepted approach for managing QT prolongation in patients with DR-TB, who need a minimum of 6 - 9 months treatment.[3] A cross-sectional survey to clinicians treating patients with DR-TB in the WHO Europe region, showed that that whilst ECG monitoring was common, there was heterogeneity in terms of ECG frequency, as well as the clinical management of adverse cardiac events.[4] Guidelines exist in other areas for management of acquired QT prolongation, for example many psychiatric medications can cause QT prolongation.[5] Most anti-psychotic medications have similar efficacy allowing drug changes to be made if QT prolongation develops. A key limitation of this approach in the treatment of DR-TB is that many of the most efficacious drugs can cause QT prolongation meaning a switch may lead to an inferior regimen.

It is conceivable that patients who need interruptions, dose reduction or changes to their treatment may have poorer outcomes due to the development of further drug resistance, failure to culture convert or relapse. A large meta-analysis including 5346 patients treated for MDR-TB found 2602 (57.3%) experienced at least 1 kind of adverse drug reaction (ADR).[6] A subgroup analysis of studies, which included data on the impact of adverse drug reactions on treatment within this same meta-analysis, found 1147 of 1519 (76%) patients required change of treatment including temporary interruption, change of medications or dose, or permanent discontinuation. Poorer outcomes have been seen in MDR-TB patients with both short and long sporadic treatment interruptions[7-8] and severity of adverse events in XDR-TB has been shown to negatively affect sputum culture conversion.[9] Patients experiencing cardiac toxicity due to their MDR-TB regimen may have poorer outcomes due to

frequent interruptions or changes in their original regimen (which could reduce efficacy). However, this has yet to be proven and indeed a case-control study looking at clinical impact of drug-induced hepatotoxicity with anti-tuberculous drugs did not find a statistically significant difference in outcome.[10] Patient adherence has been shown to be adversely affected by side-effects from medication[11,12] and length of waiting time at a healthcare facility.[11,12] Both these factors could be relevant to patients experiencing QT prolongation. Frequent visits to a healthcare facility for close monitoring of the QT interval and being aware of the significance of QT prolongation, once it has developed, may impact adherence.

Treatment outcome status for TB is divided into favourable (successful) or unfavourable (unsuccessful) and differences exist in the definition for what constitutes favourable status and what constitutes unfavourable status.

Previous work has explored several different primary outcome definitions using the STREAM Stage 1 dataset, with similar overall results.[13] In addition to the outcome definitions described in the Phillips et al paper [13] the WHO proposed new treatment outcome definitions in April 2021[14] to recognise the changes to RR-TB and MDR-TB treatment regimens and duration since the 2013 framework.[15] The different unfavourable outcome definitions are detailed in Table 3.2.

As severe adverse events such as QT prolongation may be linked to unfavourable outcome status (due to treatment failure or drug resistance) this was investigated using the STREAM primary outcome definition with some of the definitions in Table 3.2 also explored.

Table 3.2. Summary of unfavourable treatment outcome definitions for RR-TB/MDR-TB

	Unfavourable Outcome – Treatment failed
STREAM Primary outcome [16]	1. Starting ≥2 new drugs, or 2. Treatment extension beyond the permitted duration, or 3. A positive culture from one of the two last specimens
WHO end of treatment outcomes [14]	Permanent stop or change of ≥2 drugs because of: 1. No culture conversion by IP end, or 2. culture reversion in CP after conversion, or 3. Acquired resistance to FQ or Second Line Injectable, or 4. ADR
WHO update (2014) [15]	As above with addition of post-treatment relapse
TBNET [17]	Positive culture status ≥6 months after treatment initiation or relapse ≤1 year after treatment completion.

Modified WHO outcome for Short course [18]	Treatment termination or need for permanent regimen change of ≥2 drugs due to: ≥1 positive culture after ≥6 months of treatment ≥2 consecutive grade ≥2+ sputum smears after 6 months of treatment (if cultures are not available)
End of follow-up Week 132 [13]	Culture positive at 132 weeks after randomisation or culture positive when last seen <132 weeks.

Of these various outcome definitions, some such as the original STREAM Stage 1 primary outcome, WHO 2013 and WHO 2021 include permanent changes due to adverse drug reactions as unfavourable. STREAM also included treatment extension (which may be due to an interruption for adverse reaction) as unfavourable. Other outcome definitions such as TBNET and End of follow-up (Week 132) do not mention drug changes or treatment extension and focus on a microbiological measure of treatment failure with positive culture. From a QT prolongation perspective and a patient perspective, an extension of treatment by ≥8 weeks or the change of ≥2 drugs from the original regimen, may not be considered treatment failure if microbiologically and clinically they are cured.

Chapter one has shown how QT prolongation evolves over time on treatment along with predictive factors for prolongation, which were country (Mongolia) and baseline

QT interval. Chapter two has shown that QTc values early in treatment can be used to identify which patients go on to develop QT or QTc of ≥500ms. This chapter aims to provide a descriptive analysis of how patients were managed when they did develop a QT or QTc of ≥500ms on either a 12-lead ECG or Holter monitor. It will also assess whether QT prolongation affected patient outcome and whether this differs for different outcome definitions.

The aim, hypothesis and objectives for this chapter are outlined below.

Aim

Determine how participants in STREAM Stage 1 were managed when they reached a QT or QTc ≥500ms and assess whether it affected their outcome status.

Hypothesis

1. Patients who developed QT prolongation were more likely to have an unfavourable outcome than those who did not.

Objectives

1. Describe how patients were managed from a clinical perspective when they developed a QT or QTcF ≥500ms.
2. Assess whether those who developed QT/QTcF prolongation ≥500ms were more likely to have an unfavourable outcome compared to those who didn't.

Methods

A retrospective review of patient records was undertaken from the STREAM 1 trial database. Patients with a QT or QTcF ≥500ms on a 12 lead ECG during 52 weeks of follow-up from randomisation were identified from both regimens. A separate log was kept of Holter recordings from which patients reaching a QTcF ≥500ms could be identified. Due to uncertainty and concern about cardiac safety, all participants with a baseline QT/QTcF ≥450ms underwent a 24-hour Holter monitor and any participants below this threshold at baseline who later reached 450ms on treatment also underwent a Holter. As the trial progressed, the Holter requirement was later dropped so participants with recordings were mainly the earliest to be recruited. Prolongation in the Holter recordings was defined as ≥2 hourly QTcF mean values ≥500ms within a 24-hour period. The values shown are taken from the highest of these hourly mean values.

Patients were divided into four groups. Groups A and B were limited to Short regimen patients. Group A comprised patients first identified as reaching a QT or QTcF ≥500ms on a 12-lead ECG at one of the time points within 52 weeks of follow-up. Group B included patients first identified as reaching a QTcF ≥500ms on a 24-hour Holter monitor. Long regimen patients were also included, with Group C made up of those first identified as reaching a QT or QTcF ≥500ms on a 12-lead ECG and Group D those first identified as reaching a QTcF ≥500ms on a 24-hour Holter monitor.

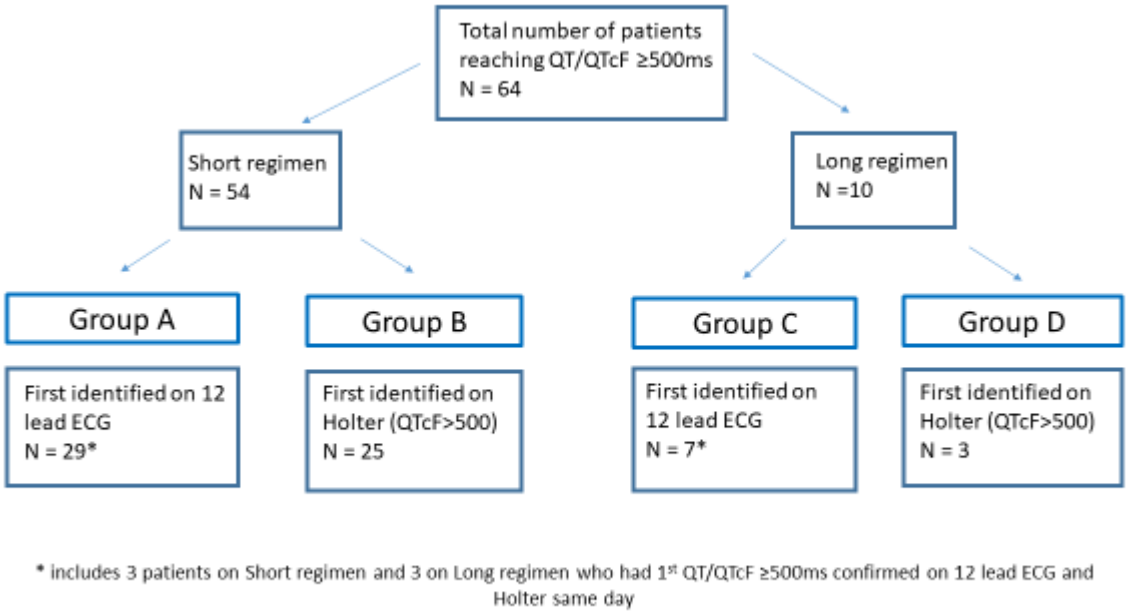
Patients reaching a QT or QTcF ≥500ms on both a 12 lead ECG and a Holter monitor during follow-up were categorised based on their first event.

Outcome was assessed using the STREAM Primary outcome definition whilst other outcome definitions, listed in Table 3.2, were also explored. To begin with all 424 participants were assessed with a comparison made across regimens. This was followed by an assessment of Short regimen participants only and dependent on whether they were in Group A or B having reached the 500ms threshold or not.

A chi square test was performed to compare favourable outcome status between those on the Short regimen who reached 500ms (groups A and B) and those who did not as well as those on the Long regimen who reached 500ms (groups C and D) and those who did not.

Results

Figure 3.1. Flow diagram showing how patients were identified from both regimens having reached a QT or QTcF of ≥500ms



64 patients were identified in total as having reached a maximum QT/QTcF of ≥500ms during 52 weeks of follow up (54 Short regimen; 10 Long regimen). Of the 54 Short regimen participants, 29 were first identified as reaching the 500ms threshold on a 12 lead ECG and 25 first identified on a Holter recording. Of the 10 Long regimen patients, seven were first identified on a 12 lead ECG and three first identified on Holter. In addition to the 64 patients mentioned above there were six patients (5 Short regimen; 1 Long regimen) who never recorded a QT or QTcF ≥500ms on a 12 lead ECG or 24-hour Holter but did have treatment modification due to their QTcB interval being ≥500ms on either an ECG or Holter. In total, 135/424 participants in STREAM Stage 1 underwent a Holter monitor with 36 of these showing ≥2 hourly QTcF mean values ≥500ms within a 24-hour period. Some participants underwent more than 1 Holter.

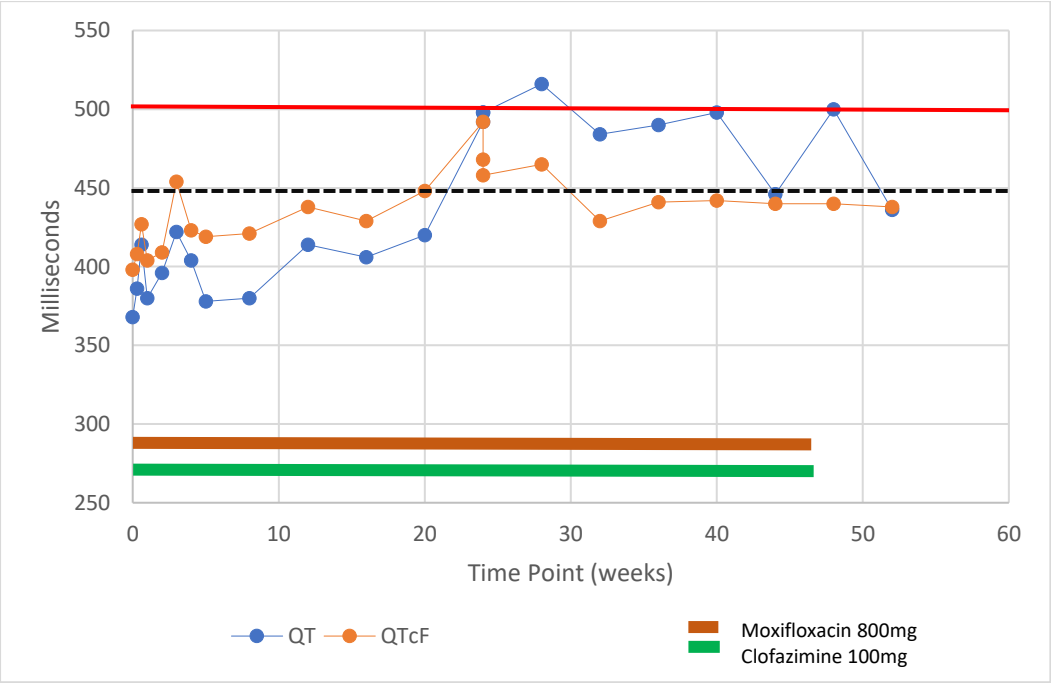
Clinical management

Group A – Short regimen participants with QT or QTcF ≥500ms first identified on a 12-lead ECG

Twenty-nine patients on the Short regimen were first identified as having a QT/QTcF ≥500ms on a 12-lead ECG.

Seven continued their treatment after reaching the 500ms threshold at a median of 28 weeks (24, 40) with a median QT 512 (494, 518) and median QTcF 500ms (491, 510). All patients had single episodes only ≥500ms, apart from one patient (detailed below in Figure 3.2) who continued to fluctuate around the 500ms threshold until completing treatment at week 44.

Figure 3.2. Patient from a South-African site in Group A that continued treatment with no changes after reaching a QT of ≥500ms



Three patients had a single dose reduction of moxifloxacin to 400mg after they reached a QT/QTcF ≥500ms. All three patients had single episodes ≥500ms at 24, 28 and 36 weeks of follow-up, respectively.

Two patients had a single interruption of moxifloxacin (≤ 7 days) and clofazimine (≤ 11 days) after reaching a QTcF of 512 and 538ms respectively, after three weeks of treatment. Both had single episodes ≥500ms.

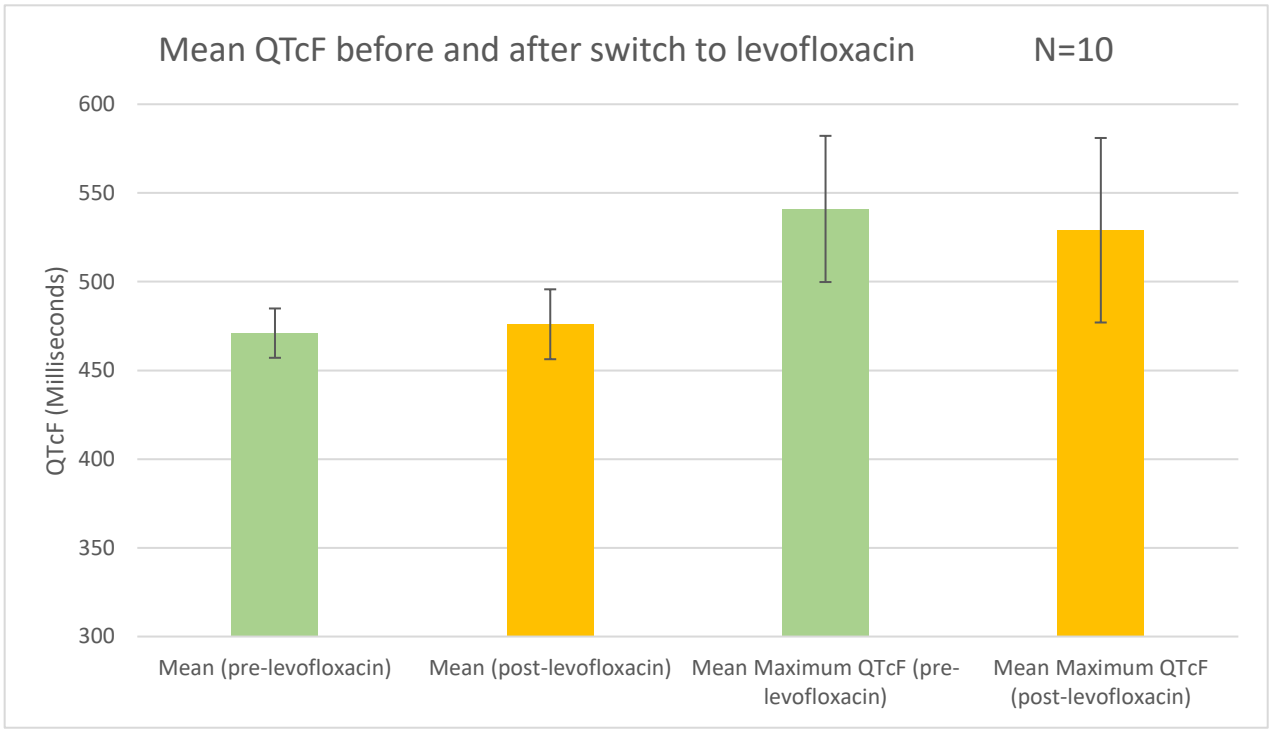
A large heterogeneous group of seventeen patients had more than one modification. Levofloxacin replaced moxifloxacin in ten of the patients after other modifications such as treatment interruption or dose reduction had been unsuccessful. The change to levofloxacin occurred at a median of 25 weeks (11, 34). Table 3.3 and Figure 3.3 show the impact on QTcF for these patients following the switch from moxifloxacin to levofloxacin. The error bars in Figure 3.3 represent the standard deviation of each measure for the ten patients. Though the mean QTcF increased slightly after switching to levofloxacin, the mean maximum QTcF value was lower after the change in fluoroquinolone. Three of the ten patients changed to levofloxacin on the same day as stopping moxifloxacin, the remaining seven changed after period of between 3 to 28 days between drugs.

An additional patient was switched to levofloxacin early in treatment for non-QT related reasons but then later developed QT prolongation >500ms necessitating further modification. Four of the eleven patients who switched to levofloxacin also had further changes made to their clofazimine. This was a dose reduction to 50mg in two patients, one of whom later discontinued clofazimine permanently along with two other patients.

Table 3.3. Comparison of QTcF before and after change from moxifloxacin to levofloxacin

	Mean (SD) QTcF (n=10)
Mean (pre-levofloxacin)	471 (13.9)
Mean (post-levofloxacin)	476 (19.7)
Mean Maximum QTcF (pre-levofloxacin)	541 (41.2)
Mean Maximum QTcF (post-levofloxacin)	529 (52.0)

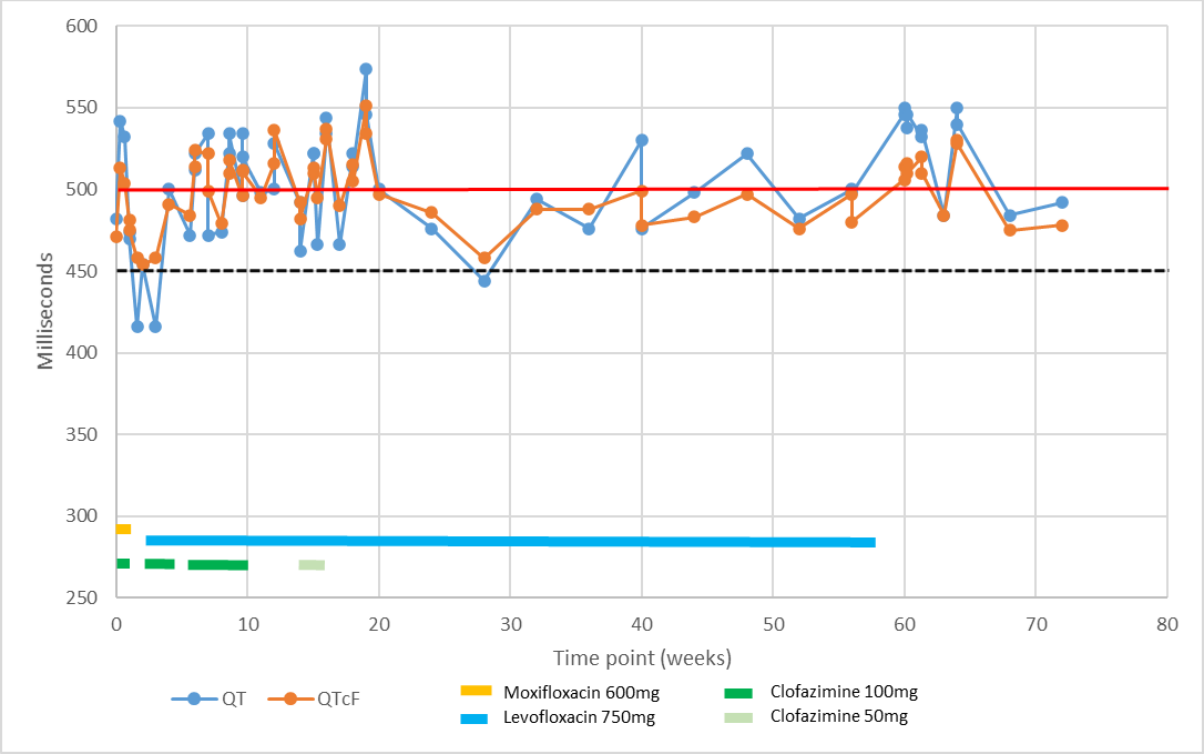
Figure 3.3. Impact on QTcF for Group A patients that changed from moxifloxacin to levofloxacin



Only one patient continued to have further episodes of QT/QTcF prolongation ≥500ms after levofloxacin replaced moxifloxacin and changes were made to clofazimine (Figure 3.4). The patient was from the Mongolian site and had a baseline QTcF of 471ms that increased to 513ms on an ECG performed two hours after their

first dose. Treatment was suspended for a few days before moxifloxacin was changed to levofloxacin at week one. Further treatment interruptions of clofazimine and a dose reduction to 50mg were unsuccessful at maintaining a QT/QTcF <500ms so it was discontinued permanently at the end of the intensive phase. Despite these changes, the patient went on to have several further episodes of QT/QTcF prolongation ≥500ms before completing an extended treatment course at week 58. The graph in Figure 3.4 shows that after completing treatment the patient went on to have further episodes ≥500ms for another six weeks.

Figure 3.4. Patient from the Mongolian site in Group A that continued to have ongoing episodes of QT/QTcF ≥500ms despite multiple interventions



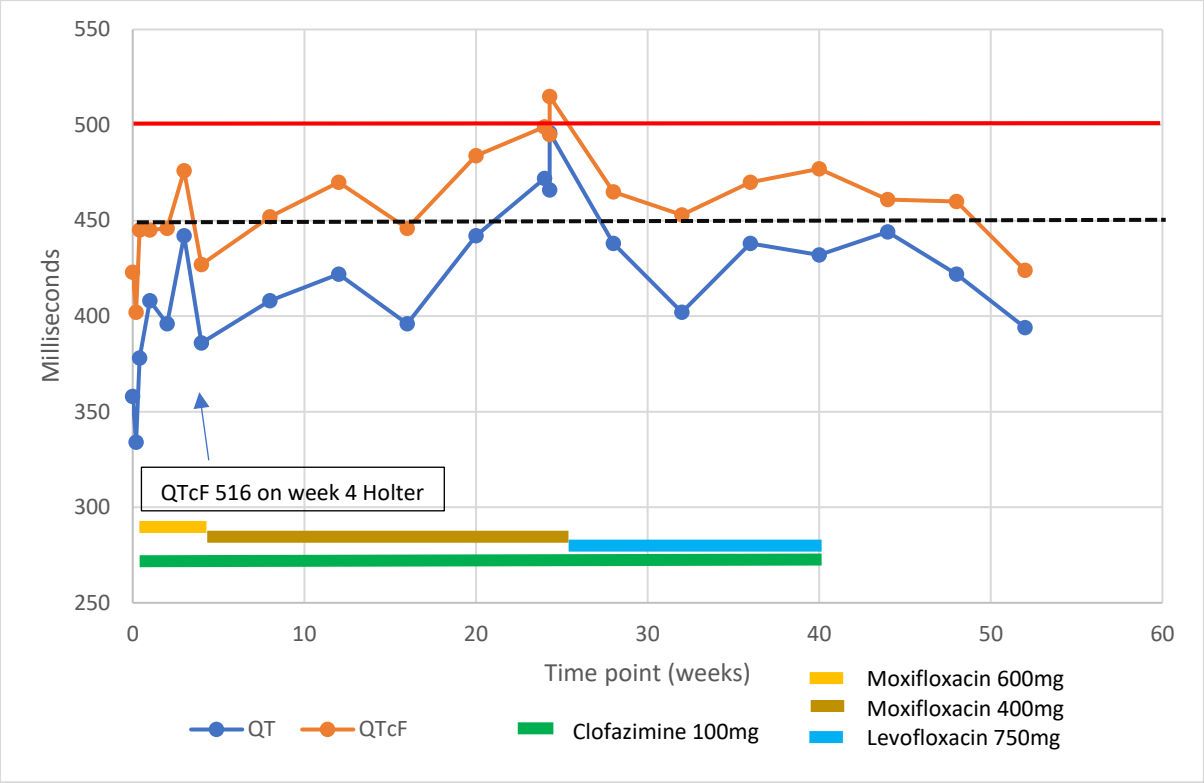
All twenty-nine of the patients in Group A underwent a 24-hour Holter monitor at least once in their treatment course, though only five recorded a QTcF ≥500ms. All five patients had a QTcF ≥500ms on a Holter and 12-lead ECG at the same time point.

Group B - Short regimen participants with QT or QTcF ≥500ms first identified on a Holter

Twenty-five patients on the Short regimen were first identified as having a QTcF ≥500ms on a 24-hour Holter ECG at a median of 9 weeks (2, 20). Only four of the patients ever reached a QT/QTcF ≥500ms on a 12-lead ECG, all at a time point after their Holter reading and all had treatment modified.

Eight of the patients continued treatment after reaching the 500ms threshold. Ten of the patients had a permanent moxifloxacin dose reduction after their Holter recordings showed a QTcF ≥500ms. Only one of these patients reached a QT/QTcF ≥500ms on a 12-lead ECG after the moxifloxacin dose reduction, though no further changes were needed. Two patients had a temporary moxifloxacin dose reduction. Five patients had a combination of treatment interruptions and/or moxifloxacin dose reduction. Only one patient from Group B had to change from moxifloxacin to levofloxacin. This patient from the Vietnamese site (Figure 3.5), had a dose reduction of moxifloxacin at week 4 after their Holter recording showed a QTcF ≥500ms. A 12-lead ECG at week 24 showed a QTcF of 515ms at which point levofloxacin replaced moxifloxacin and no further episodes ≥500ms were recorded.

Figure 3.5. Patient from the Vietnamese site in Group B who developed further episodes of QTcF prolongation after moxifloxacin dose reduction, required switch to levofloxacin



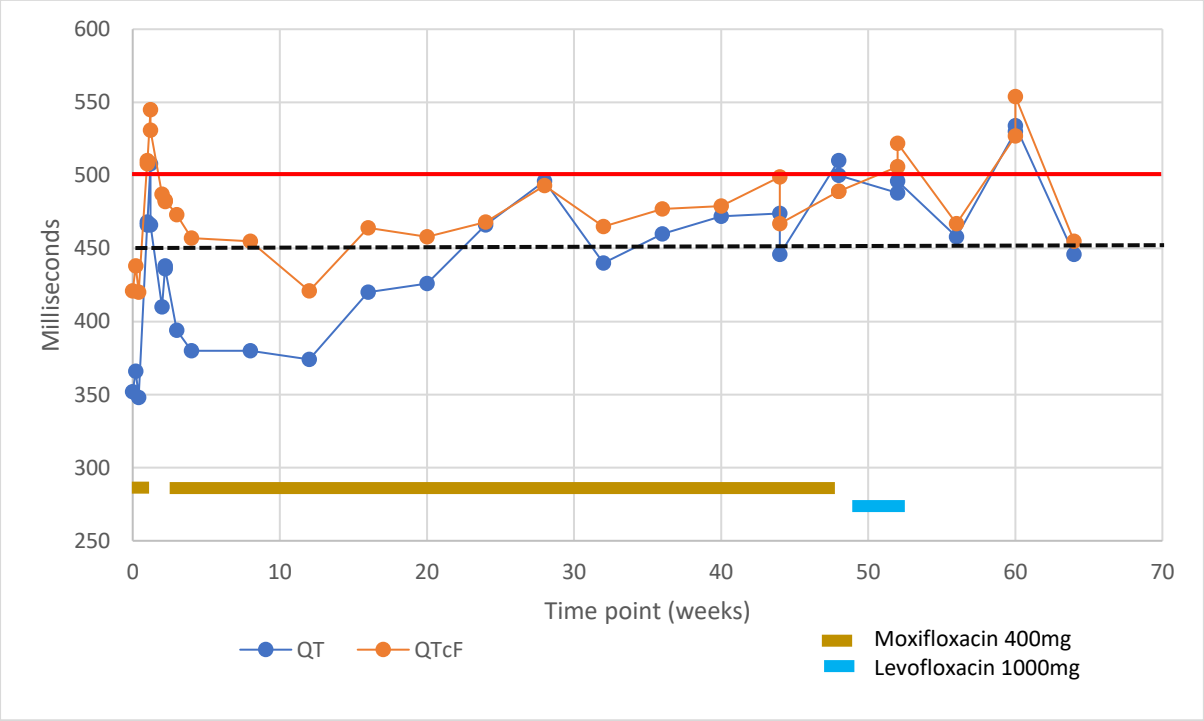
Group C - Long regimen participants with QT or QTcF ≥500ms first identified on a 12-lead ECG

Seven patients on the Long regimen were first identified as having a QT/QTcF ≥500ms on a 12-lead ECG at a median of 3 weeks (2, 40). Five continued their treatment after reaching the 500ms threshold. The two patients who had a treatment change started on standard dose moxifloxacin (400mg) as per the protocol. Both had a treatment interruption and later changed to levofloxacin; this was a permanent change in one patient shown in Figure 3.6. This patient, from a site in South Africa, developed QT/QTcF prolongation at week 1 of treatment that initially responded to a

treatment interruption. However, later episodes of prolongation >500ms required a switch to levofloxacin which was also eventually stopped at week 52 due to ongoing episodes ≥500ms and PAS was added for the remainder of treatment.

Five of the patients also had a Holter recording available, with a QTcF ≥500ms confirmed in three of them at the same time point as the 12-lead ECG.

Figure 3.6. Patient from a South-African site in Group C who had multiple episodes of QT/QTcF prolongation >500ms that required >1 intervention.



Group D - Long regimen participants with QT or QTcF ≥500ms first identified on a Holter

Three patients on the Long regimen were first identified as having a QT/QTcF ≥500ms on a 24-hour Holter ECG. Only one of these patients ever had a 12-lead ECG also showing a QT/QTcF >500ms. All continued treatment after reaching the 500ms threshold.

Summary of clinical management by regimen

Of the patients taking the Short regimen, 72% (39/54) had their treatment interrupted, modified, or changed after they reached a QT/QTcF ≥500ms on either a 12-lead ECG or 24-hour Holter (Groups A and B). Of the patients taking the Long regimen, only 20% (2/10) had their treatment interrupted after they reached a QT/QTcF ≥500ms from either a 12-lead ECG or 24-hour Holter (Group C and D). One of these two patients also had a permanent change from moxifloxacin to levofloxacin following the treatment interruption.

For patients first identified with a QT/QTcF ≥500ms on a 12-lead ECG (Group A and C), 24% (8/34) also reached a QTcF ≥500ms on a 24-hour Holter when it had been performed. Conversely, 18% (5/28) of patients who reached a QTcF ≥500ms on a Holter (Group B and D) had a QT/QTcF ≥500ms on a 12-lead ECG during follow-up. Most patients on the Short regimen underwent treatment interruption, dose reduction or drug change during the intensive phase of treatment i.e., from randomisation up to week 16 (Figures 3.7 and 3.8). There were two patients who reached the 500ms threshold at 2-hours and 4-hours post first dose respectively, who are identified at week 0 in figure 7.

Fig 3.7. Timing of treatment change in Group A

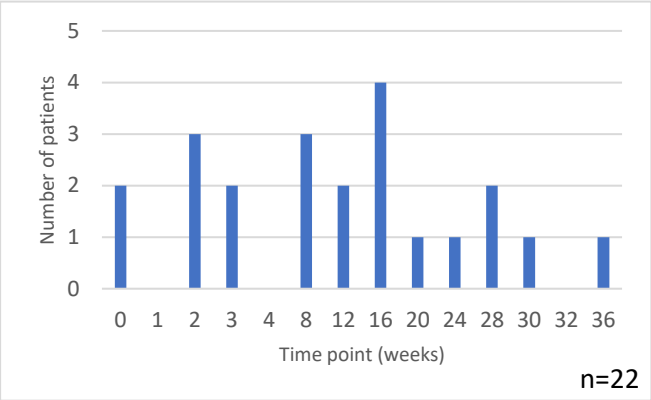
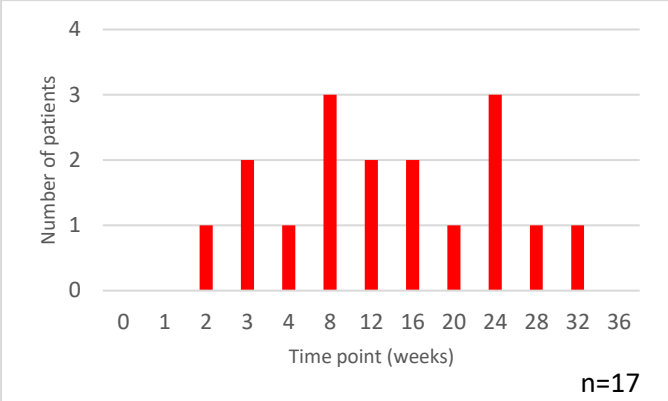


Fig 3.8. Timing of treatment change in Group B



In addition to the 64 patients already described, six patients had treatment modified based on a QTcB ≥500ms, all within the first 12 months of the trial (before a change in working practice), and all but one was taking the Short regimen. One of the patients was taking the Long regimen and reached a QTcB of 507ms at week 12 resulting in a 7-day interruption of moxifloxacin. Of the other five patients, all taking the Short regimen, three had a treatment interruption of moxifloxacin and two had a dose reduction of moxifloxacin all in the intensive phase.

Outcome for participants with clinically-relevant QT prolongation

The outcome for all 424 randomised patients in STREAM Stage 1 is shown in Table 3.4, divided into Short and Long regimen. As previously reported, non-inferiority was demonstrated in favourable outcome status between Long (79.8%) and Short (78.8%) regimen participants.(18)

Table 3.4. Outcome for all patients on both Short and Long regimens irrespective of QT interval based on STREAM primary outcome in Table 3.2

	Short regimen		Long regimen	
Outcome	Frequency	Percentage	Frequency	Percentage
Favourable	210	74.47	108	76.06
Unfavourable (Extended Treatment)	8	2.84	0	0.00
Unfavourable (Started ≥2 drugs)	28	9.92	9	6.34
Unfavourable (Died during treatment or follow-up)	17	6.03	8	5.63
Unfavourable (No culture at or after 76 weeks)	11	3.90	9	6.34
Unfavourable (>1 of last 2 cultures is positive)	0	0.00	2	1.41
Unfavourable (Not assessable_Reinfection)	7	2.48	1	0.70
Unfavourable (Not assessable_lost >76 weeks)	1	0.35	5	3.52
Total	282	100%	142	100%

The data in the following table show the STREAM primary outcome (described in Table 3.2) for Short regimen patients who developed QT/QTcF ≥500ms on either a 12-lead ECG or Holter monitor and those who did not (Table 3.5).

Outcome for participants on the Short regimen

Table 3.5. Outcome for Short regimen patients who reached a QT/QTcF ≥500ms on either 12-lead ECG or Holter monitor and those who did not, based on the STREAM primary outcome

	QT/QTcF ≥500ms		**QT/QTcF <500ms**		**P value**
Outcome	**Frequency**	**%**	**Frequency**	**%**	
Favourable	43	79.6	167	73.2	0.33
Unfavourable (Extended Treatment)	3	5.6	5	2.2	
Unfavourable (Started ≥2 drugs)	2	3.7	26	11.4	
Unfavourable (Died during treatment or follow-up)	4	7.4	13	5.7	
Unfavourable (No culture at or after 76 weeks)	1	1.9	10	4.4	
Unfavourable (Not assessable Reinfection)	1	1.9	6	2.6	
Unfavourable (Not assessable_lost >76 weeks)	0	0	1	0.4	
Total	54	100 %	228	100 %	

For the 54 patients in Group A and B who developed QT/QTcF prolongation ≥500ms on either a 12-lead ECG or Holter (Table 3.5), 79.6% (43) recorded a favourable outcome, compared to 73.2% (167) (p=0.333), who did not develop QT/QTcF prolongation ≥500ms.

Outcome for participants on the Long regimen

Table 3.6. Outcome for Long regimen patients who reached a QT/QTcF ≥500ms on either 12-lead ECG or Holter monitor and those who did not, based on the STREAM primary outcome

	QT/QTcF ≥500ms		**QT/QTcF <500ms**		**P value**
Outcome	**Frequency**	**%**	**Frequency**	**%**	
Favourable	6	60	102	77.3	0.217
Unfavourable (Extended Treatment)	0	0	0	0	
Unfavourable (Started ≥2 drugs)	2	20	7	5.3	
Unfavourable (Died during treatment or follow-up)	2	20	6	4.5	
Unfavourable (No culture at or after 76 weeks)	0	0	9	6.8	
Unfavourable (>1 of last 2 cultures is positive)	0	0	2	1.5	
Unfavourable (Not assessable Reinfection)	0	0	1	0.8	
Unfavourable (Not assessable lost >76 weeks)	0	0	5	3.8	
Total	10	100%	132	100%	

For the 10 patients who reached a QT/QTcF ≥500ms either 12-lead ECG (group C) or Holter (group D), 60% (6/10) recorded a favourable outcome, compared to 77.3% (102/132), p=0.217 of the patients who did not reach this threshold.

If the WHO 2013 and 2021 outcome definitions were applied (detailed in Table 3.2), there would be little change in these figures with 3/54 (5.6%) more patients in the prolongation groups having had a favourable outcome versus 5/228 (2.2%) in the no prolongation group. This would be due to a favourable microbiological outcome not being affected by whether a patients treatment duration was extended. Though this does not consider whether treatment extensions were due to drug interruptions rather than ongoing positive cultures. If the TBNET[16] or End of follow-up (Week 132) outcomes were applied then a higher number of patients in Table 3.4 would have a favourable outcome but there would also be many patients in Table 3.5 that would move to favourable outcome status, if having started ≥2 new drugs did not result in unfavourable outcome status provided cultures at the end of treatment were negative. Though again, this analysis did not look at how many of the drug changes were due to adverse reactions and how many were to treatment failure or relapse, which would be important when interpreting whether adverse events affected outcome status.

Discussion

This Chapter has shown that many patients were found to have developed QT or QTcF prolongation ≥500ms on a 24-hour Holter monitor in addition to those already known to have developed prolongation ≥500ms on a 12-lead ECG within 52 weeks of follow-up. In keeping with the findings in Chapter 1, a higher proportion of patients experienced QT or QTcF prolongation on the Short regimen compared with the Long regimen and as expected more patients had treatment modified on the Short regimen compared with the Long because of the prolongation. As expected from previous analysis, fewer patients on the Long regimen were shown to reach a QT/QTcF ≥500ms on either a 12-lead ECG or Holter. Only two of the ten patients required treatment modification, and this was based on a 12-lead ECG reading rather than a Holter.

Though the division of patients into the four different groups was somewhat arbitrary, it does allow a comparison across regimens and allows an inclusion and comparison of the large amount of ECG Holter data, which until now, had not been analysed. Few of the patients in Group A, who were first identified as having prolongation on a 12 lead ECG, also had evidence of prolongation ≥500ms on a 24-hour Holter monitor, though the five who did have evidence on both tests, had both investigations performed at the same time point. This suggests that if the remaining 24 patients in Group A had gone on to have their Holter monitor on the same day as the 12-lead ECG, the number with evidence of prolongation ≥500ms on both could have been higher. Most patients in Group B, who were first identified as having prolongation ≥500ms on a Holter monitor, did not have evidence on a 12-lead ECG. Only four patients in this group reached the 500ms threshold on a 12-lead ECG in addition to the Holter. All patients in Group B had their highest 12-lead ECG

QT/QTcF reading after the Holter. It is unclear whether the 12 lead ECG or the 24-hour Holter monitor is more able to detect clinically relevant QT or QTcF prolongation. Continuous cardiac monitoring over a 24-hour period should in some ways be better at detecting prolongation as it may be higher at certain times of day or after medication, which may be missed when a 12-lead ECG is performed at a single point in time. It could be argued that prolongation ≥500ms detected at a single point in time on a 12-lead ECG may increase the likelihood of prolongation at other points in time that may not be captured without continuous monitoring. How meaningful QTcF prolongation is on a Holter is also not entirely clear, especially when it is not detected on regular 12-lead ECG monitoring. Some patients were only identified as reaching 500ms on a Holter and not a 12 lead ECG, though treatment modification may have affected this. As the equipment is expensive and requires cardiology expertise for interpretation its usefulness in monitoring for national treatment programmes is far from certain. It may though have a role in patients who have ongoing issues with their QT interval after treatment modification to gauge how frequently they are crossing the 500ms threshold during a 24-72 hour period and whether they have developed any concerning runs of arrhythmia during the Holter monitoring.

Prolongation of either a QT or QTcF interval ≥500ms almost doubled in the Short regimen patients when Holter recordings were also included with the 12-lead ECG results, with 19.1% (54/282) of patients randomised to the Short regimen reaching the threshold. This compared with 11% using 12-lead ECG monitoring up to week 52 of follow-up alone. For patients taking the Long regimen, there was little difference,

with 7% (10/142) of patients having recorded either a QT or QTcF interval ≥500ms on a 12-lead ECG or Holter compared with 6.4% using 12-lead ECG readings alone.

The impact of the different types of treatment modification was difficult to assess as there was a lot of heterogeneity. Patients were affected at different time points; their treatment interruption was often of different durations and may also have included temporary or permanent dose reduction of either moxifloxacin and/or clofazimine with some also changing drugs. The management of QT or QTcF prolongation ≥500ms in STREAM Stage 1 was based on discussions between the trial team and the cardiologist advisor as no guideline existed at the time. All sites were advised to suspend the drugs most likely to be contributing first, which was high-dose moxifloxacin and clofazimine for the Short regimen and either standard dose moxifloxacin or levofloxacin in the Long regimen. If repeat ECGs showed that the interval had reduced, then these drugs were restarted, sometimes with a temporary dose reduction. Moxifloxacin was often stopped before clofazimine as it has a shorter half-life of a few hours so a response in QT interval would be seen more promptly. For similar reasons it was often restarted before clofazimine. If prolongation ≥500ms continued to occur, then moxifloxacin was switched to levofloxacin. For Short regimen patients with ongoing episodes of prolongation despite these measures, clofazimine was discontinued. There is clearly a lot of heterogeneity as the above steps did not have the same effect on all patients. This analysis was able to look at the impact of a moxifloxacin to levofloxacin drug change for patients on the Short regimen in Group A (Table 3.3 and Figure 3.3). In all but one of the eleven patients who had a switch to levofloxacin and/or clofazimine dose reduction or discontinuation, only one continued to have episodes of QT/QTcF

prolongation ≥500ms. For the patients described in Table 3.3 and Figure 3.3, 70% had lower maximum QTcF values after the change to levofloxacin. This suggests that even though the mean QTcF increased slightly after levofloxacin was started, overall, it did appear to reduce further episodes of clinically relevant prolongation. This may be partly explained by a “washout” period between stopping moxifloxacin and starting levofloxacin in 7 of the 10 patients affected. This ranged between 3 to 28 days, so the QT interval was likely to have been lower immediately prior to starting levofloxacin as most of this group were not on a fluoroquinolone.

A balance exists between drug efficacy and adverse effect. Moxifloxacin is often considered marginally more effective than levofloxacin in treating TB though similar 3-month culture conversion rates have been seen with both drugs. [19] Levofloxacin is felt to have a better safety profile than moxifloxacin in respect to QT prolongation. [20]

The timing of the treatment interruption, dose change or drug change is potentially quite important. If patients reach a QT or QTcF ≥500ms in the first few weeks of treatment during the intensive phase, then a treatment modification when the tuberculous bacilli are rapidly dividing could be more likely to lead to drug resistance and treatment failure as opposed to the continuation phase when there are more persister cells. The histograms in Figures 3.7 and 3.8 show that most patients on the Short regimen had their first treatment modification during the intensive phase in relation to a prolonged QT or QTcF interval. This is important as it was hypothesised that patients in need of treatment modification because of their QT/QTcF interval, would be more likely to experience an unfavourable outcome. As most patients had treatment modification in the intensive phase this would further support a hypothesis

that an unfavourable outcome would be more likely. This was not the case, however. When outcome was examined in those patients who reached the 500ms threshold and those who did not, as seen in Tables 3.5 and 3.6, a higher proportion of Short regimen participants in Group A and B who reached a QT/QTcF ≥500ms achieved a favourable outcome than those who did not. This was unexpected, particularly given 72% of Short regimen patients in Groups A and B had treatment modification at some point in response to a prolonged QT or QTcF interval and that for the majority it was at some point during the intensive phase. The original primary outcome definition from STREAM Stage 1 was used for this comparison as described in Table 3.2. As alluded to earlier, this may not be the most appropriate outcome definition for deciding whether patients that developed QT prolongation had poorer outcomes or not. The inclusion of treatment extension beyond 48 weeks for an unfavourable outcome affected two of the patients in Group A. The first had treatment extension to week 59 following several episodes of treatment interruption and modification for a prolonged QT/QTcF interval and is shown in Figure 3.4. The second had treatment extension to week 52 for the same reason. Both these patients had their first interruption in the intensive phase, but culture converted by week 4 and remained culture negative to the end of follow up at week 132. It is possible that a longer duration of treatment may improve a patient's chance of a favourable outcome status but as treatment was extended to make up missed doses, this seems less applicable in these two patients. The inclusion of ≥2 drug changes to an unfavourable outcome status is more complex. If the drug changes are because of treatment failure or relapse and the patient then culture converts and stays negative for the remainder of follow-up, then it is likely the new regimen contributed to this. Though there will be patients who have drug changes due to adverse events such as QT prolongation,

rather than treatment failure or relapse, so declaring these types of patients unfavourable is perhaps less appropriate if they remain culture negative. There were two patients in Group A who had an unfavourable outcome status due to a change of ≥2 drugs. The first was for treatment relapse and the development of pre-XDR TB. They started a modified salvage regimen which included linezolid and PAS. The second was for treatment failure, with a change to a salvage regimen that included linezolid and thioridazine. Both these patients were culture negative at week 132 and at least one earlier time point.

If the ≥2 drug changes are due to treatment failure or relapse, then categorising the patients as unfavourable seems logical but if the patient remained culture negative then developed an adverse event requiring drug changes then it could be argued that these should still count as favourable if the patient remains culture negative.

Using the STREAM Stage 1 outcome definition, a slightly higher proportion of participants who reached a QT/QTcF 500ms threshold on either 12-lead ECG (group A) or Holter (Group B) were deemed to have favourable outcome status than those who did not. It therefore didn't seem necessary to formally assess this further using different outcomes definitions such as TBNET and End of follow-up (week 132), as they would have clearly improved the favourable outcome status for both groups.

Based on the above findings it is difficult to recommend a universal management approach for all patients who develop QT prolongation with a high degree of certainty, but the stepwise approach described allowed the majority of patients to safely complete their treatment without adversely affecting their outcome status and only 3 patients needed to discontinue clofazimine permanently. The heterogeneity

seen was probably a reflection of several factors affecting the QT interval and patients respond differently to treatment modifications.

This Chapter has several limitations. First, it is only able to provide a largely descriptive analysis of the clinical management for patients who developed QT/QTcF prolongation. There was a large amount of heterogeneity in terms of when and what changes occurred and for how long and in what combination. This made it difficult to provide a meaningful comparison of which treatment modifications were most effective. Second, there were many potential confounding factors such as electrolyte disturbance or details on concomitant medications, which made interpretation difficult. Third, the Holter recordings were analysed for evidence of prolongation and the patients assessed to see whether any treatment modification occurred. This analysis does not provide details on other areas that may have been useful such as whether ventricular bigeminy cycles occurred in these patients or whether there were brief runs of VT. Fourth, I did not have data on adherence or pharmacokinetics/pharmacodynamics for the patients analysed in this thesis. This clearly limits the understanding of whether they contributed to the “success” of treatment modifications. Future work would need to include these measures prospectively to make an assessment of their affect on QT prolongation and treatment modifications.

There are several strengths to this Chapter. Firstly, it is to my knowledge the largest analysis of Holter recordings on patients being treated for drug-resistant TB which included high dose moxifloxacin and clofazimine. Secondly, it provides useful information about how patients were managed once they did develop QT/QTcF prolongation and gives case examples of how the interval responded after treatment was modified over a full treatment course. Thirdly, it provides important information

about the impact of QT/QTcF prolongation and treatment modification and how this affects patient outcome.

Conclusion

A larger proportion of patients taking the Short regimen were found to have reached a QT or QTcF ≥500ms compared with the Long regimen. Almost twice as many patients on the Short regimen were found to have reached a QTcF ≥500ms when Holter recordings were analysed, though few had prolongation ≥500ms on a 12-lead ECG as well. Treatment modification at some point in response to a prolonged QT or QTcF interval occurred in 72% of Short regimen patients in Groups A and B, with the majority of these prior to week 16. Most patients on the Short regimen who experienced QT or QTcF prolongation ≥500ms on either a 12-lead ECG or Holter, had their first treatment modification in the intensive phase, though this did not appear to worsen outcome status.

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Chapter 4: Validation of QT measurements from automated machine readings in STREAM Stage 1

Background:

Patients in the STREAM Stage 1 trial underwent regular ECG monitoring. Automated machine readings were utilised to identify QT or corrected QT (QTc) prolongation. This chapter aimed to investigate whether the machine QT intervals could be relied upon in all circumstances when compared to the gold standard manual reading.

Methods:

After a period of training (described separately in appendix) by a cardiologist specialising in rhythm disorders, blinded ECGs were selected from three different time points (baseline, early and late) during the first year of treatment for 200 participants. All 600 ECGs were read manually, blind to subject, regimen and timepoint. The readings were then unblinded and analysed according to allocated regimen and whether or not the participant was classified as high-risk (defined as a QT or QTcF ≥500ms or a QTcF ≥60ms increase from baseline at any point during monitoring) or low-risk (defined as never reaching the high-risk thresholds) based on machine readings. Bland-Altman plots were created to visually evaluate the difference between machine minus manual QT interval readings for the different regimens and time-points. T-tests (paired or unpaired as appropriate) were performed to assess any differences observed between the machine and manual QT interval reads.

Results:

For the Short regimen, the mean absolute difference of the machine minus the manual QT interval varied between 19ms (baseline), 23.47ms (early time point) and 24.25ms (late time point). For the Long regimen the difference varied between 19.39ms (baseline), 25.59ms (early time point) and 23.56ms (late time point).

The mean (SE) difference increased at the early time point on treatment 2.1ms (1.9), p=0.27, (95% CI -5.87 to 1.64) and whilst lower at the late time point it was still above the baseline difference 0.69ms (2.3), p=0.76 (95% CI -3.79 to 5.16).

Comparison between early and late time points for each regimen showed no significant difference. This was a mean (SE) difference of 0.55ms (1.44), p = 0.35 (95% CI -3.40 to 2.30) for the Short regimen suggesting a decline in accuracy with time. The Long regimen showed an improvement with accuracy between early and late time points though again was not significant, 2.87 (2.17) p = 0.90 (95% CI -1.51 to 7.25).

Variance of the discrepancies increased on treatment but was unrelated to length of QT interval. Of the 10 measurements outside the limits of agreement (LOA), all but two ECGs had evidence of T wave morphology abnormality.

Conclusion:

Compared with manual QT readings the ECG machines overestimated the QT interval by a mean of between 19 and 26ms depending on regimen and time-point but could still be considered reliable. The less reliable readings, defined as being outside the LOA, occurred infrequently and may be affected by abnormal T wave morphology. These ECGs might benefit from a manual QT measurement and cardiologist input.

Background

Automated machine readings are commonly used to assess patients' QT interval unless they are under the care of a cardiologist. In STREAM Stage 1 patients underwent regular ECG monitoring for QT prolongation using the machine output, though it was recognised that in some circumstances the machine reading might not always be an accurate assessment of the QT interval. Patients who were noted to have a QT or QTc ≥500ms often had their ECGs reviewed by a cardiologist.

The aim of this chapter is to investigate whether and in what circumstances the machine readings for patients on MDR-TB treatment can be relied upon for QT monitoring, using data from the STREAM Stage 1 trial.

Importance of Electrocardiograms (ECGs)

ECGs allow clinicians to monitor for and diagnose several important heart conditions. Some of these conditions, particularly ones affecting the electrical conduction pathway, may produce few symptoms in patients. Therefore, relying on patient reported symptoms alone is insufficient. As QT interval prolongation can be caused by a few anti-tuberculous medications (including moxifloxacin and clofazimine) and lead to serious and sometimes fatal cardiac arrythmia if left undetected, it is important to try and identify it early. ECG machines not only record the electrical tracing of the cardiac cycle but can also calculate a number of important measures such as the heart rate and QT interval that are given on the print-out.

Advantages versus disadvantages of automated machine readings

Automated ECG analysis was first developed in the 1950s and has improved a great deal in the decades that followed.[1–3] They have now replaced manual readings for common measures such as heart rate and QT interval in most patients. Whilst machine automated QT readings have several advantages over manual readings such as speed, low skill set, reduced inter and intra-reader variability [4] difficulties can be encountered. The QT interval relies on determining the end of the T wave. This can be problematic when either the isoelectric line is noisy or the T wave morphology is abnormal i.e., flat, notched, biphasic or overlapping on a U wave.(5) Expert consensus is that manual measurement of QT interval is preferred to machine automated reading [6], though in many clinical settings this may not be practical.

Manual versus machine estimate of the QT interval

Automated machine readings can overestimate the QT interval compared with manual readings.[7- 9] In most cases small differences in the QT interval may not be clinically relevant but larger differences between methods, particularly if they affect patient management, could be an issue. A large study by St George's University (London) examined the precision of QT measurements by GE Healthcare (Milwaukee, WI, USA) ECG machines with manual measurements using two different 12SL ECG algorithms based on a median beat from 12 simultaneous leads.[10] They looked at two sets of ECGs – Set A was of good quality (15,194 ECGs) and Set B of poorer quality (29,866 ECGs) with more noise pollution. They compared two different versions of the 12SL algorithm in which 95.9% (new

algorithm) and 76.6% (old algorithm) of ECGs were within 10ms of the manual measurement for Set A. The larger Set B with worse quality ECGs performed less well and showed 83.9% (new algorithm) and 59.5% (old algorithm) of automated QT readings were within 10ms of the manual read.

Another study compared manual and machine readings for healthy patients versus those with hypertrophic cardiomyopathy.[9] ECGs with U waves, flat T waves or noise were excluded from the analysis. There was a 20ms difference between machine and manual mean QT interval in the 339 ECGs from healthy subjects, suggesting an overestimation by the machine. The machine also overestimated the QT interval in the 258 cardiomyopathy patients with a mean difference of 7ms from the manual read.

A comparison of two automated methods and a computer-assisted manual method showed the fully automated methods overestimated the QTcF interval.[7] A sample of twenty-three healthy subjects took part in a single blind, placebo controlled randomised trial to assess the effect of moxifloxacin 400mg on the QTcF using different methods for ECG analyses. They provided 1907 ECGs for the analysis in which intrasubject QTcF variance was the key outcome of precision. The computer-assisted manual method was found to be more precise but also gave lower change from baseline values than the fully automated method.

Finally, a study that looked at three thorough QT studies and compared manual versus two different automated machine methods for calculating the QTcF interval, found in one of the studies the proportion of QTcF measurements above 451ms was higher with the 12SL algorithm compared with the Hewlett Packard Analysis Program (HPAP algorithm which uses the median QT interval from 8 stable leads)

and manual methods.[10] The authors noted this finding was expected as the 12SL algorithm generated higher baseline values. They did note in another two studies comparing automated HPAP with manual technique that the manual method generated a higher proportion of recordings above 450ms, this was also reflected in the baseline values.

The reasons for machines often overestimating the QT interval are due to differences in where the end of the QT interval is measured by the computer algorithm (Figure 4.1) and factors affecting this measurement such as noise, wandering baseline or abnormal T wave morphology e.g., flat T waves (Figure 4).

Inter-reader variability

There is no agreed limit of acceptable inter-reader variability. A study found that using the tangent method inter-observer variability for QT interval measurement ranged between −10 ± 16 ms.[11] Another found 95% limits of agreement of between +/- 20ms amongst experienced cardiologists analysing the QT interval in a cohort of congenital long QT patients and controls.[12] Finally, Long QT syndrome (LQTS) experts demonstrated QTc variability of up to 44ms when analysing ECGs from healthy patients and up to 70ms when analysing ECGs from patients with LQTS.(13)

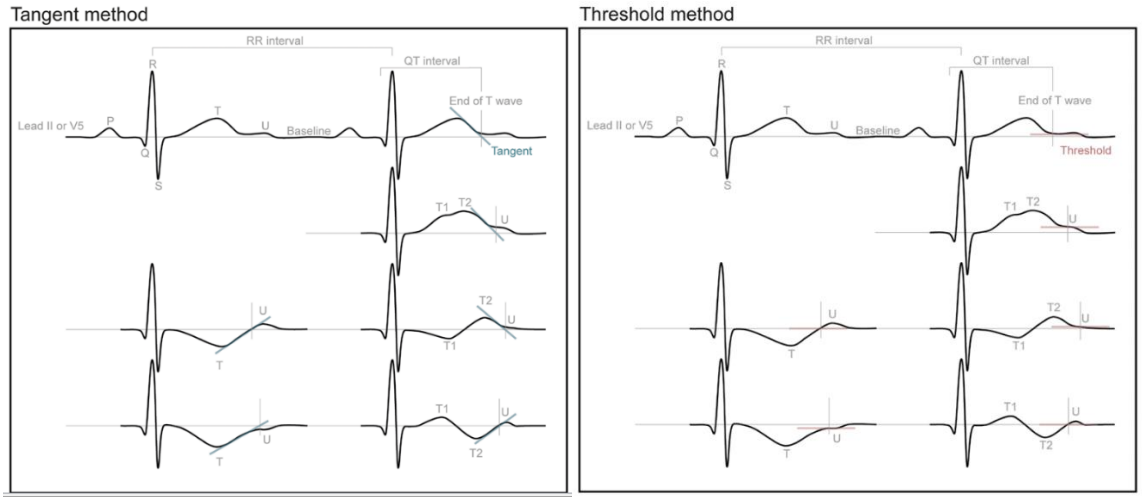
Gold standard of QT interval measurement

Manual calculation of the QT interval is considered the gold standard compared with machine measurement but there is discord in how this manual measurement is calculated. Both the FDA (14) and EMA [15] provide guidance on manual measurement of the QT interval for safety management in early trials and who should read the ECG but do not specify the preferred “gold standard” method to measure the QT interval. Inter and intra reader variability is one of the concerns with QT measurement. The EMA recommend limiting the number of readers analysing the QT interval and that three or more cycles should be averaged to determine the interval duration when a single lead is used. Whilst there is no clear definition of who a reader should be, the EMA mention they support a technician reading with a cardiologist over-read. The FDA mention that the gold standard for collection and assessment of 12-lead ECGs in a trial can vary depending on the level of precision needed. A “thorough QT/QTc study” will use a different standard to safety monitoring in a clinical trial for example. The FDA guidance for a “thorough QT study” is that a few skilled readers +/- with computer assistance will operate from a centralised ECG laboratory in reading the ECGs and should be blinded to time, treatment and subject identifier with one of the readers reviewing all ECGs from a given subject. Variability can then be assessed by having the assessors reread a selection of the data (normal and abnormal) under blinded conditions. This may not be necessary in later clinical trials where ECG reading by a machine has a role. There is no standard agreement of which lead(s) to use when measuring the QT interval and how many QT measurements should be taken from a single ECG. Whilst lead II is often used [10,16] other studies have used different leads. For example, lead V5 was used in analysis of Framingham Heart Study data [17] and some studies have used a

combination of leads (18). Deciding how many complexes to analyse is also not standardised.

Several different methods have been used to measure the QT interval in “thorough QT/QTc studies”. The two most used are the Tangent method and the Threshold method, illustrated in Figure 4.1.

Figure 4.1. Illustration of the Tangent and Threshold method for calculation of the QT interval [12]



The tangent method involves drawing a line from the peak of a T wave to the steepest point of the descending line of the T wave. The point at which this line then crosses the isoelectric baseline is considered the end of the T wave.[12] An alternative to the tangent method is the threshold method where the point at which the first part of the descending line of the T wave merges with the isoelectric line determines the end. The tangent method, first described by Eugene Lepeschkin and Borys Surawicz in 1952 [19], outlined how abnormal T wave morphology and overlapping enlarged U waves could lead to confusion and overestimation of the QT interval in cases such as electrolyte abnormalities. A study found that whilst inter-

observer difference increased as the QT interval reached >450ms using a non-tangent method this was not the case when the tangent method was used in which inter-observer difference remained within +/- 20ms regardless of the QT interval.[20] Measurements using the tangent method often produce a shorter QT interval than the threshold method. However, there is no universal consensus over which should be used. The cardiological adviser for STREAM suggested using the tangent method for the work in this chapter which is illustrated in figure 1. This method allowed us to manually calculate the QT interval and also exclude U waves from the measurement.

Previous studies comparing machine and manual QT interval readings have described 20ms as a relatively narrow limit of agreement (LOA). A study comparing manual calculation of QT interval versus two different automated methods in three thorough QT studies (TQT), found a mean absolute QT value of between -19.0 to 7.2ms with LOA between 17.7 and 31.3ms respectively.[10] Another similar study compared five TQT studies and found mean difference ranging from 7.3ms in one study to -19.2ms in another with LOA of 21.2ms and 28.5ms respectively.[21]

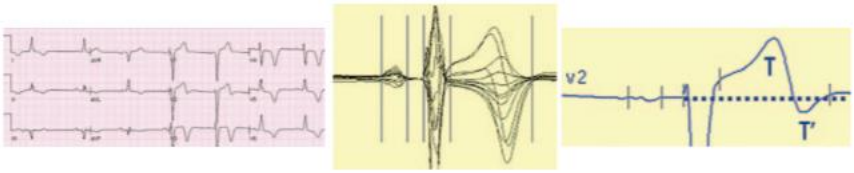
Methods

Measuring QT interval in STREAM Stage 1

QT monitoring in STREAM Stage 1 was based on the automated reports from the ECG machines provided for the trial. All sites in the study used a MAC 800 (GE Healthcare) machine to record their ECGs using a paper speed of 25mm/s and voltage gain of 10mm/mV. All machines were calibrated at baseline with the 12SL measurement and interpretation algorithm used to calculate the QT interval. This

uses a waveform complex taken from all 12 leads to produce a median superimposed complex with measurements taken from global fiducial points in all 12 simultaneous leads.

Figure 4.2. 12SL QT measurement algorithm used in GE Healthcare ECG devices [22]



Protocol relevant to ECG readings

QT and QTc intervals were read from the ECG report and recorded on the Case Report Form (CRF) by the site staff. The protocol for STREAM Stage 1 mandated that all patients should have a screening ECG. Patients with a QT or corrected QT ≥500ms at screening were excluded from the trial.

The corrected QT interval in STREAM Stage 1 used Fredericia's formula (QT interval divided by the cube root of the R-R interval).

Patients deemed eligible for randomisation had ECGs performed for monitoring at the following time points: baseline (pre-dose), 4 hours (post first dose), weeks 1-4, 12, 24 and 36. The QT and QTc interval produced by the ECG machine was then recorded on a CRF and sent to the MRC CTU. Of the 424 patients randomised not all had ECG recordings at every time point for reasons such as missed visits, withdrawal from the trial or death. Sites were asked to send a copy of the baseline ECG for all patients to the MRC CTU. Early versions of the Working Practice Document (WPD) requested sites to send ECGs for all patients at all the time points. As sites became more comfortable performing ECGs and managing patients

appropriately, the need for MRC CTU to review all ECGs diminished and later versions of the WPD requested sites only send ECGs beyond baseline if there were concerns about QT prolongation. This meant ECGs were not available for all patients in the trial at all time points, even if there was a QT or QTc reading on the database.

Patient population

Participants in the STREAM Stage 1 trial were included in this validation study based on whether they had ECGs available to review and whether their automated machine QT or QTcF interval readings showed severe prolongation or not.

Out of 282 patients randomised to the Short regimen, 94 (33.33%) patients had clinically relevant QT prolongation on at least one ECG (31 QT/QTcF of ≥500ms and 92 an increase of ≥60ms in their QTcF from baseline) within 52-weeks of follow-up. Two patients reached a QT/QTcF ≥500ms whilst not reaching an increase of ≥60ms from baseline value. Of 142 patients randomised to the Long regimen, 14 (9.85%) patients had clinically relevant QT prolongation on at least one ECG (8 QT/QTcF of ≥500ms and 12 an increase of ≥60ms in their QTcF from baseline) within 52 weeks of follow up. Two patients reached a QT/QTcF ≥500ms whilst not reaching an increase of ≥60ms from baseline value. The 108 patients who developed clinically relevant QT prolongation were deemed “high-risk”.

As not all participants were available at each time point for an ECG due to reasons such as missed visit, death or withdrawal from the trial, the total number of participants available at each time point differs but the second row of table 4.1 shows the total number who were eligible at that time point for an ECG. A check was made to see how many of these patients had ECGs available at each of the time points

that could be viewed. This is shown in the bottom half of the table and indicates the total number who had recordings on the database and an ECG to view at that time point.

In total, 96.93% of baseline ECGs were available for patients who had recordings performed. This reduced to 81.62% for 4-hours and 54.21% for week 36. By combining early time points (weeks 1-4) and late time points (weeks 12-36) it was possible to increase the number of available ECGs to 80.58% and 72.39% respectively by allowing patients to be included in the study if they had a baseline ECG and an available ECG at least once in the early time window and at least once in the late time window.

Table 4.1. Breakdown of patients with available ECG at each time point

	Baseline	4 hours	Week 1	Week 2	Week 3	Week 4	Week 12	Week 24	Week 36	Week 1,2,3 or 4	%	Week 12, 24 or 36	%
Participants	424	419	415	412	411	411	398	391	380				
ECG readings													
AHRI	55	54	54	54	53	53	50	52	52	54		52	
St Peters	71	71	71	71	71	71	69	67	65	71		69	
NCCD	33	31	31	31	31	32	31	31	30	32		31	
PNTH	100	100	99	98	99	99	97	94	92	99		97	
Durban	90	90	87	87	88	88	88	84	81	88		88	
Doris Goodwin	14	14	14	14	14	14	13	13	14	14		14	
Sizwe	61	59	59	57	55	54	50	50	46	59		51	
Total	424	419	415	412	411	411	398	391	380	417		402	
Available ECGs													
AHRI	52	49	40	39	38	36	31	27	24	42	**77.78**	36	**69.23**
St Peters	70	56	58	57	56	56	48	46	38	58	**81.69**	50	**72.46**
NCCD	33	30	23	23	26	27	22	22	14	29	**90.63**	27	**87.10**
PNTH	100	82	83	79	75	74	63	58	52	84	**84.85**	72	**74.23**
Durban	83	66	65	66	67	63	60	53	52	68	**77.27**	66	**75.00**
Doris Goodwin	14	6	5	5	5	3	0	0	0	5	**35.71**	0	**0.00**
Sizwe	59	53	50	46	44	43	37	30	26	50	**84.75**	40	**78.43**
Total	411	342	324	315	311	302	261	236	206	336		291	
%	**96.93**	**81.62**	**78.07**	**76.46**	**75.67**	**73.48**	**65.58**	**60.36**	**54.21**		**80.58**		**72.39**

The rationale for having early and late time points was that the pharmacokinetics (PK) and subsequent adverse effects of moxifloxacin, should be seen in the first few days-weeks of treatment whereas for clofazimine this will more likely occur later in treatment at 3-months and beyond. The three time points allowed analysis of whether the accuracy of the machine QT interval measurement is affected by time on treatment compared to the manual read. As there was a possibility of there being more than one ECG available at the early and late time points for many patients, the ECG that was available with the highest QTcF reading was included to allow me to address the hypothesis and objectives.

Of the 108 high-risk patients, 89 had a QT and QTcF interval recorded on the database at baseline and at least once amongst early time points and at least once amongst later time points. Seven of the 89 patients did not have an ECG available to view at baseline and at least one of the early and late time points, leaving 82 high-risk patients.

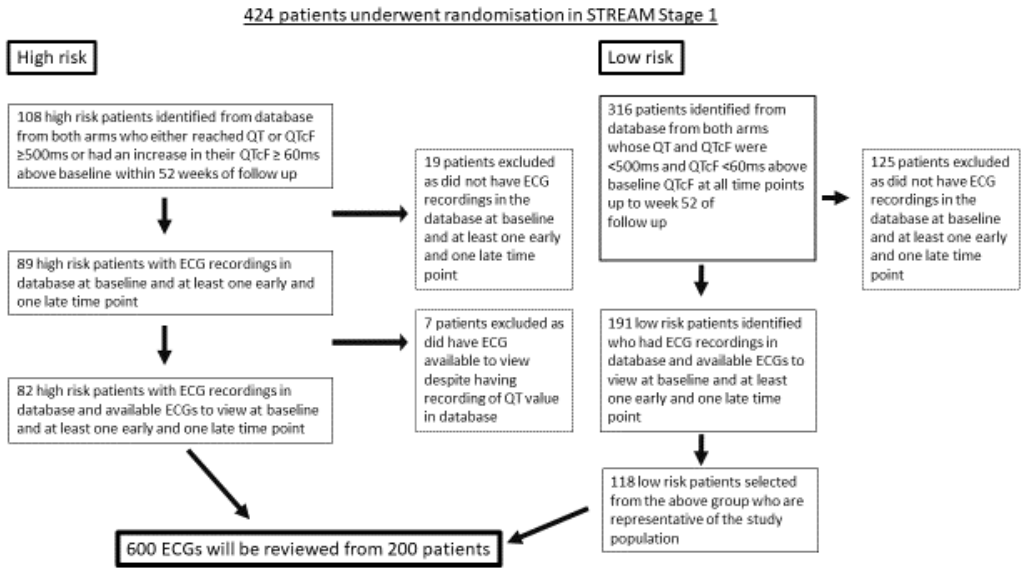
A random selection of 118 low-risk patients were included as a comparison. This comprised patients from both regimens who never experienced clinically relevant QT prolongation i.e., all readings QT/QTcF <500ms and <60ms above baseline QTcF.

In total, 200 participants were selected for this study made up of 82 high-risk and 118 low-risk participants with available ECGs. This number was slightly arbitrary but a balance of fulfilling the study objectives whilst not creating unnecessary work of limited additional value. All 82 participants who developed clinically relevant QT/QTcF prolongation, with an available ECG at each of the three time points were included along with 118/191 “low-risk” participants who did not develop clinically

relevant QT/QTcF prolongation and had available ECGs at each time point. The selection of low-risk patients was representative of the entire population (424 patients) in respect to country, age and gender. A representative sample of low-risk patients was used to allow investigation of differences between groups. The figure of 200 participants and 600 ECGs was deemed sufficient to address the studies aims and objectives.

In some way this study was a case-control design, but it was not possible to completely match the low-risk participants with high-risk as at some sites e.g. Mongolia there was a disproportionate number of high-risk patients in a relatively small group so was not a 1:1 match. To illustrate this difficulty, of the 33 participants who were randomised from Mongolia, 18 met the definition of high-risk and had available ECGs, but of the remaining pool of 15 low-risk participants, only 6 had available ECGs. Also, country was prioritised over age and gender with the matching and other factors such as BMI and weight which may be important were not included.

Figure 4.3. Flow diagram of how patients and their ECGs were selected for analysis



The rationale for including high and low risk patients was to allow investigation of the following study aim, hypotheses and objectives.

Aim

Determine whether and in what circumstances machine measurements of QT can or cannot be relied upon for monitoring and management of patients in STREAM Stage 1.

Hypotheses

1. Given severe QT prolongation affected a higher proportion of patients on the Short regimen compared to the Long regimen; the accuracy, defined as the magnitude of difference between the machine and manual QT interval, is lower for patients on the Short regimen.
2. The accuracy of automated QT interval measurements declines the longer patients are on treatment.

3. The longer the QT interval, the poorer the agreement between the manual and machine reading.

Objective 1.

To assess what impact treatment regimen has on the accuracy of machine QT interval measurements in STREAM Stage 1.

Objective 2.

To assess what impact treatment duration has on the accuracy of machine QT interval measurements.

Objective 3.

To determine whether agreement between machine and manual QT readings worsens with QT interval prolongation.

Training methods and results

Before independently reviewing the QT interval of the full set of 600 ECGs, a period of training was undertaken with a cardiologist to ensure competency and to provide quality assurance. This involved us both independently reviewing a sample of thirty blinded ECGs to allow a comparison of our manual QT interval calculation. Once our readings were within a pre-defined threshold of +/-20ms, I went on to review the full set. This limit is consistent with inter-reader variability described in other studies.[23–25]

The description of the training methods and results are shown separately in the appendix.

Main study methods

Study procedures

A statistician identified patients from the high and low risk groups who had ECGs available at the three time points and generated a spreadsheet with a separate list of patients for each group. The ECGs for these patients were identified and saved into a folder. The ECGs were labelled according to patient study number and time point. The folder was then duplicated leaving the original ECGs in one and the ECGs to be blinded in the other. The study number, time point, automated machine QT value, date and time of each ECG were recorded on a spreadsheet by someone not involved in the reading of the ECGs. That person also redacted the identifiable information on the ECG and assigned a new identification number that had been generated from Stata by the statistician. The statistician and the individual editing the ECGs kept a list along with the spreadsheet so that the redacted ECGs could

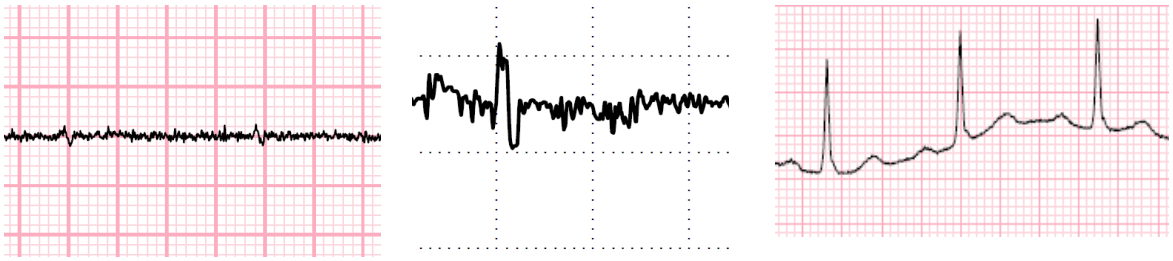
later be identified when unblinding occurred and any errors in the process could be identified from the spreadsheet.

The reader was blinded to the patient's original study number, treatment arm, time point and automated machine QT interval when reviewing the ECGs and manually calculating the QT interval. Following review of all the ECGs the data was sent back to the statistician and the ECGs unblinded so that analysis could take place.

The QT interval was calculated using the Tangent method.[12] Digital callipers were used for all measurements (Cardio Calipers version 3.3 *Iconico, New York*). Three consecutive complexes were examined in lead II and V5 after which an average was taken. In situations where only two complexes were available, such as bradycardia, an average was taken. Any ECGs with a single assessable complex in either lead was excluded from the analysis. If the waveform in lead II or V5 was of poor quality, defined as noise, low amplitude T wave complex or wandering baseline (Figure 4.4), then an adjacent lead was used.

Figure 4.4. Examples of wave complexes and T waves of poor quality that may affect accuracy of QT assessment

a. Low amplitude complex b. Noise c. Wandering baseline



Further information included R-R interval (to allow calculation of a corrected QT interval), PR interval, QRS duration and evidence of right or left bundle branch block was also recorded as this can affect the QT interval.

Statistical Methods

Scatter plots were created displaying manual versus machine generated QT interval for each ECG. Histograms were also produced to show the difference between manual and machine generated QT intervals and the frequency of these differences. Uncorrected QT intervals were compared for both machine and manual readings as per previous studies.[8-10]

To assess the agreement between the manual and machine generated QT interval for each ECG the Bland-Altman method was used.[10,26,27] This method calculates the difference between the manual and machine QT interval and plots this against the mean value of the two readings for each ECG. The mean difference between all readings is then displayed with limits of agreement (LOA) either side, defined as +/-1.96 multiplied by the standard deviation. A difference of +/-20ms was considered acceptable between manual and machine readings.

The analysis used all ECGs deemed to be of good quality defined as normal amplitude T wave (>1mm), minimal to no noise and no baseline wandering (>1mm).

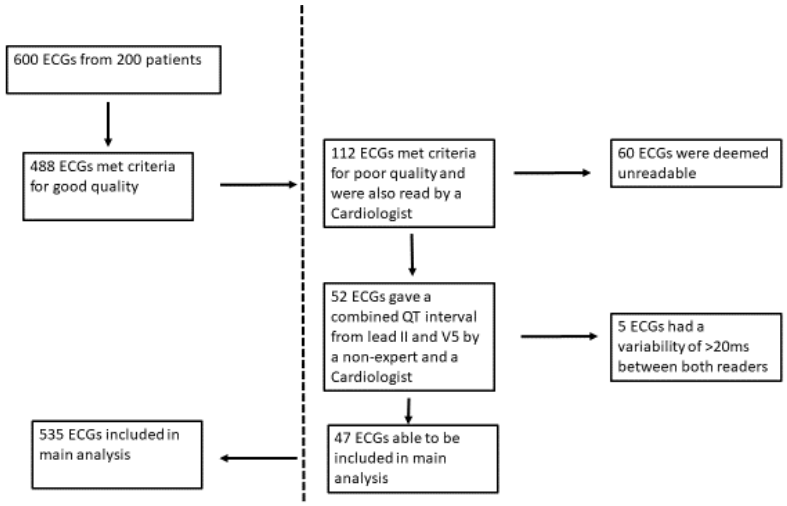
Any ECGs with leads of poor quality (Figure 4.4) were also reviewed by a cardiologist and if the difference between the QT interval in each read was <20ms, these ECGs were also included in the main analysis.

Independent t-tests were performed to assess for difference between regimens and paired t-tests were performed to assess for difference between time-points within the same regimens.

Results

After reviewing 600 ECGs from 200 patients, the majority were able to be included in the main analysis without over-read by a cardiologist. Figure 4.5 shows how many of the original ECGs were included without a second read, how many were included after a second read and how many were excluded entirely from the main analysis.

Figure 4.5. CONSORT diagram of ECGs included in the study and those excluded from analysis



In total, 488 ECGs were deemed of good quality. Lead II was able to be used in 86.3% (421/488) and V5 was able to be used in 83.4% (407/488) to manually calculate the QT interval. In 73.2% (357/488) of the ECGs both lead II and V5 could be used to calculate the combined mean QT interval. We were able to include 47/112 poor quality ECGs in the final analysis after a cardiologist review. In contrast to the 488 good quality ECGs in which alternate leads could be used if needed, only Lead II and V5 were used to calculate a combined mean QT interval in the 47 poor quality ECGs.

Accuracy of machine QT interval measurements at baseline

In order to determine whether and in what circumstances the machine measurement of the QT interval can be relied upon it was first important to establish whether the readings were accurate at baseline compared with the gold standard manual read i.e. independent of treatment regimen and duration of treatment.

Figures 4.6 and 4.7 show the association between the QT intervals calculated by the MAC 800 (GE Healthcare) machine using the 12 SL algorithm and those manually calculated by a clinician using the tangent method. Figure 4.6 represents patients on the Long regimen and Figure 4.7 the Short regimen. The red dotted line indicates a perfect correlation. Readings above the line indicate the machine overestimated the QT interval and readings below the line indicate the machine underestimated the QT interval, when compared to the reference manual QT read.

Figure 4.6. Association between machine and manual QT interval at baseline

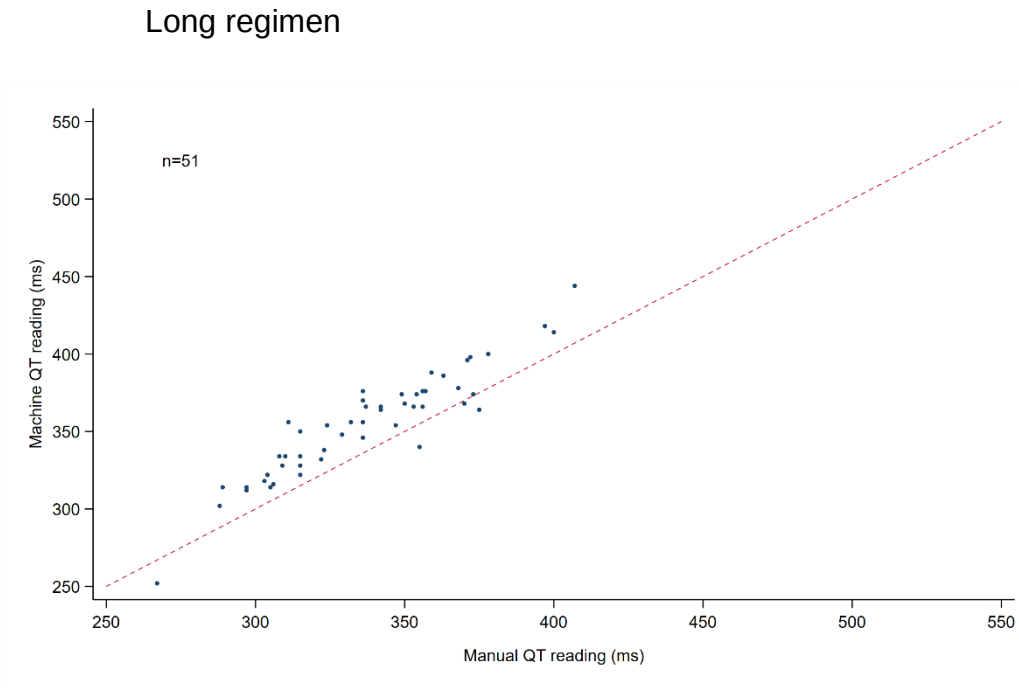
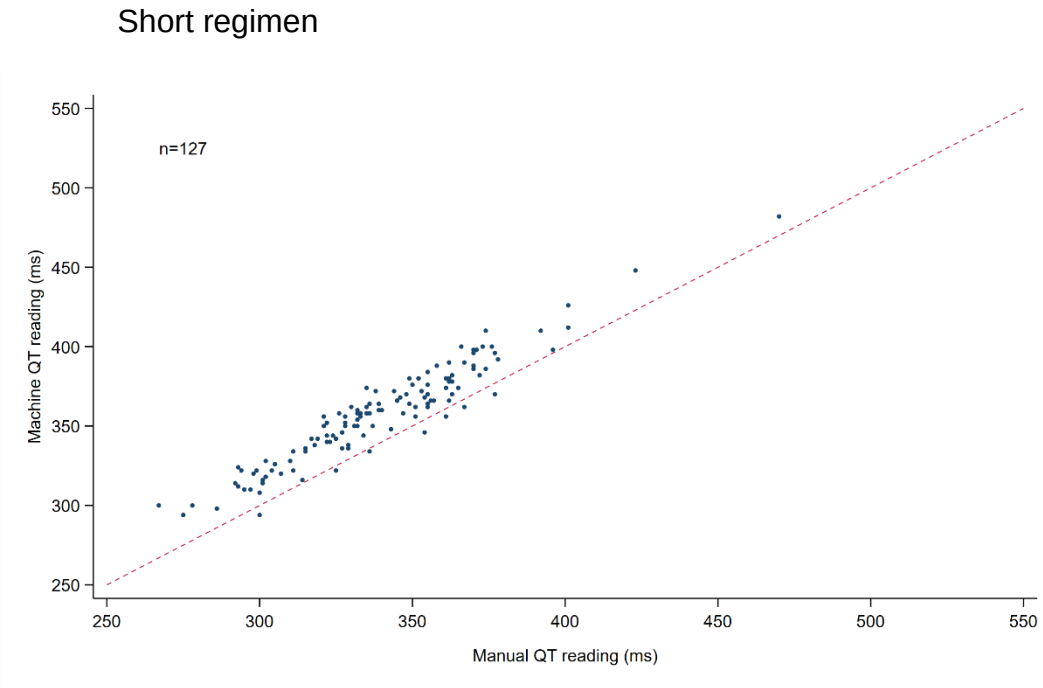


Figure 4.7. Association between machine and manual QT interval at baseline



The majority of readings are located above the line indicating the machine overestimated the baseline QT interval for patients on both regimens.

The histograms displayed in Figures 8 and 9 show the frequency of the absolute difference between the machine and the manual QT interval calculation. The mean (SD) difference on the Long regimen was -17.71ms (12.19) with a minimum difference of -45ms and a maximum difference of 15ms. The mean (SD) difference on the Short regimen at baseline was similar at -18.43 (9.58) with a minimum difference of -39ms and a maximum difference of 8ms.

The red broken line indicates the point at which no difference occurred between machine and manual QT interval reads. A negative difference to the left of the line indicates the machine reading was higher than the manual read and therefore an overestimate. A positive difference to the right of the line indicates the machine reading was lower than the manual read and gave an underestimate of the true QT interval.

Figure 4.8. Histogram for Long regimen patients at baseline

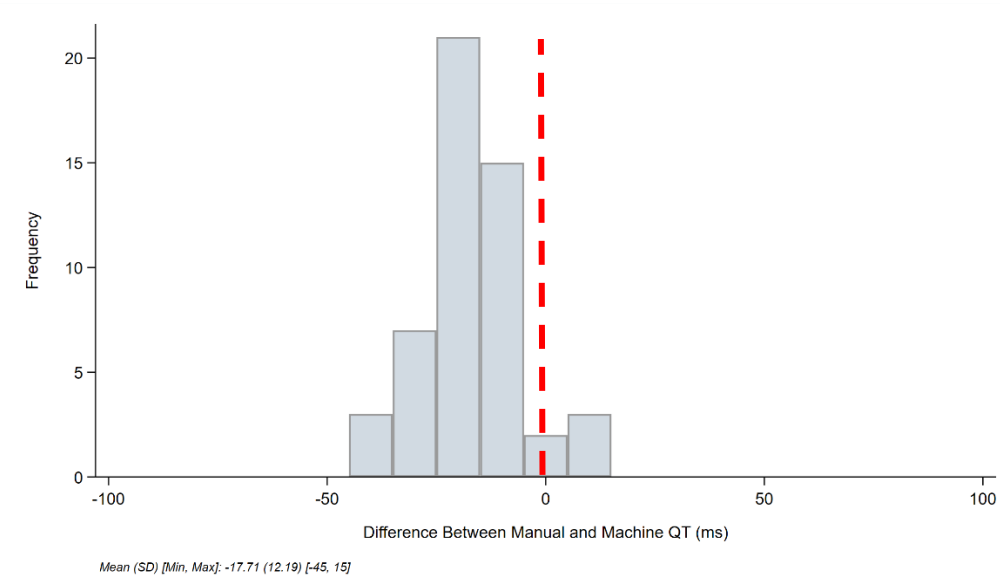
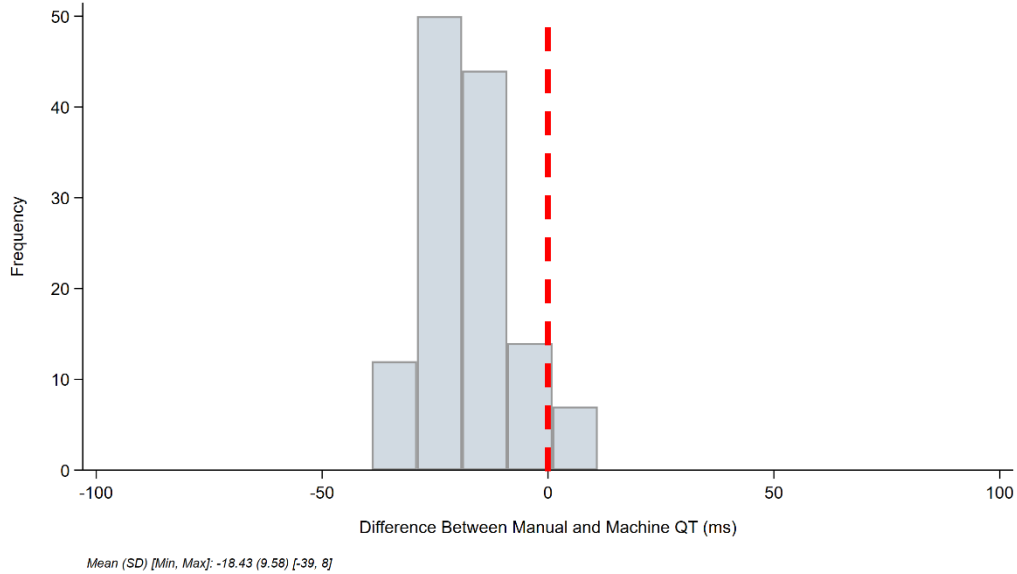


Figure 4.9. Histogram for Short regimen patients at baseline



To measure agreement between the machine and manual QT interval, Bland-Altman (BA) plots were used, comparing the difference between manual and machine QT interval against the mean reading of both. Figures 4.10 and 4.11 shows Bland-Altman plot for all baseline readings using both methods. The middle line indicates the mean difference for all readings and the limits of agreement (LOA) above and below are defined as +/- 1.96 x SD of the mean. Readings above the mean indicate the machine underestimated the true QT interval and readings below indicate the machine overestimated the true QT interval. The BA plots display the mean difference between machine and manual readings (irrespective of whether it was positive or negative) and the

table below displays the mean absolute difference. The mean absolute difference ignores whether the value is positive or negative as the magnitude in either direction could cancel out any difference seen and affect the T-tests.

Figure 4.10. Long regimen at baseline

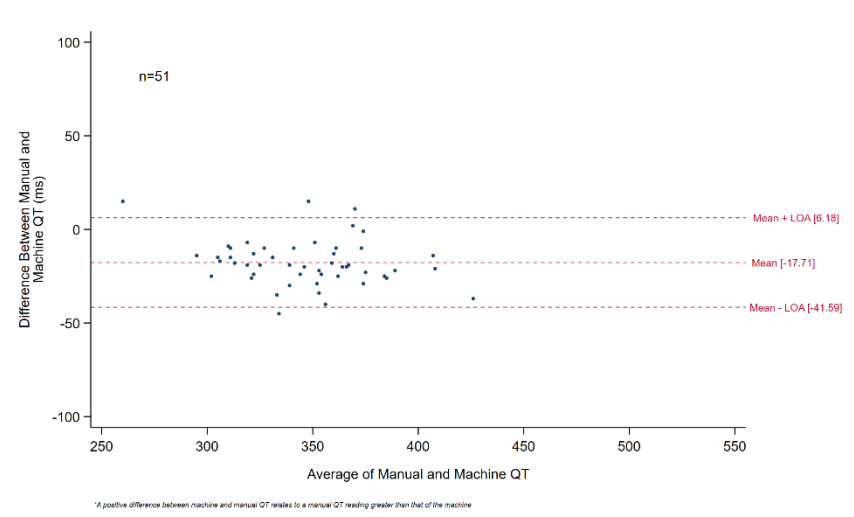
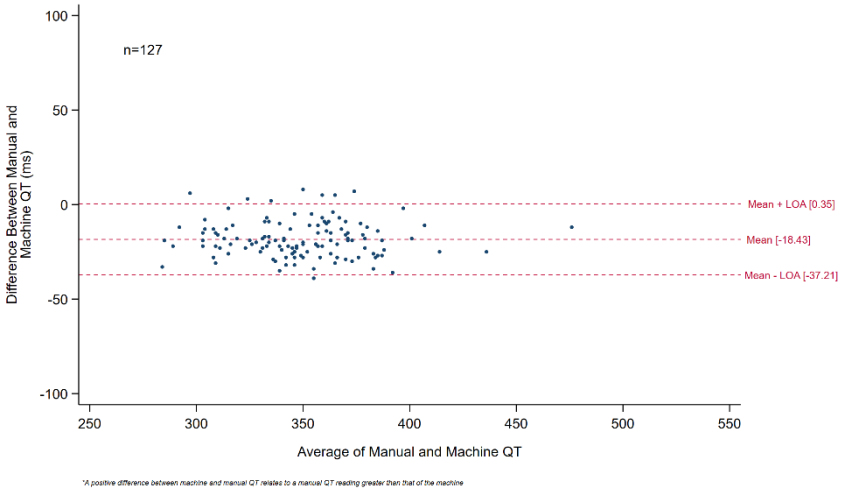


Figure 4.11. Short regimen at baseline



As expected, there was little difference in accuracy between regimens at baseline

Effect of regimen on accuracy (Objective 1)

Early time point

The effect of regimen on accuracy for the machine readings within the first month of treatment was compared. The scatter plots in Figure 4.12 and Figure 4.13 show the association between machine and manual QT readings for patients on the Long and Short regimen respectively.

Figure 4.12. Long regimen at early time point.

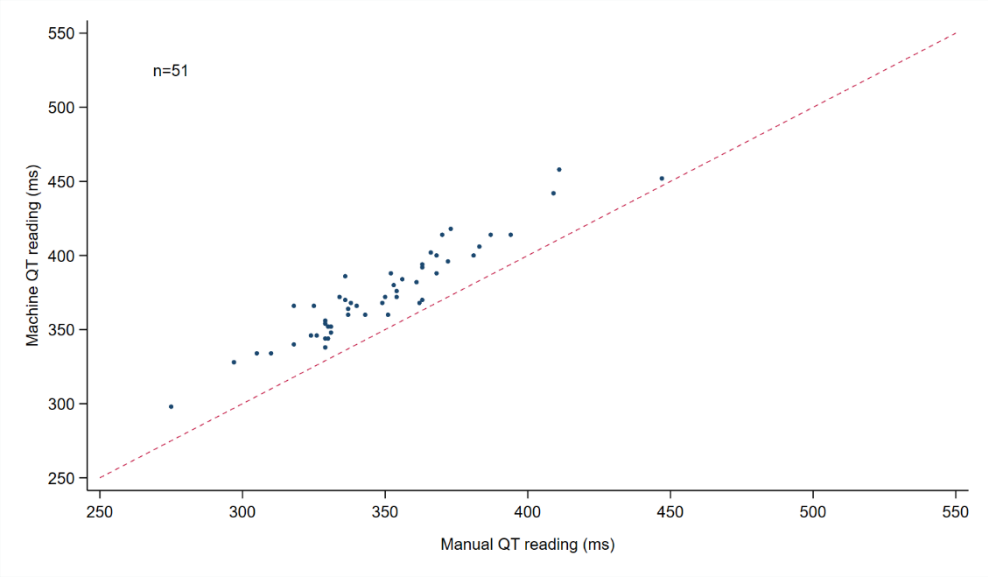
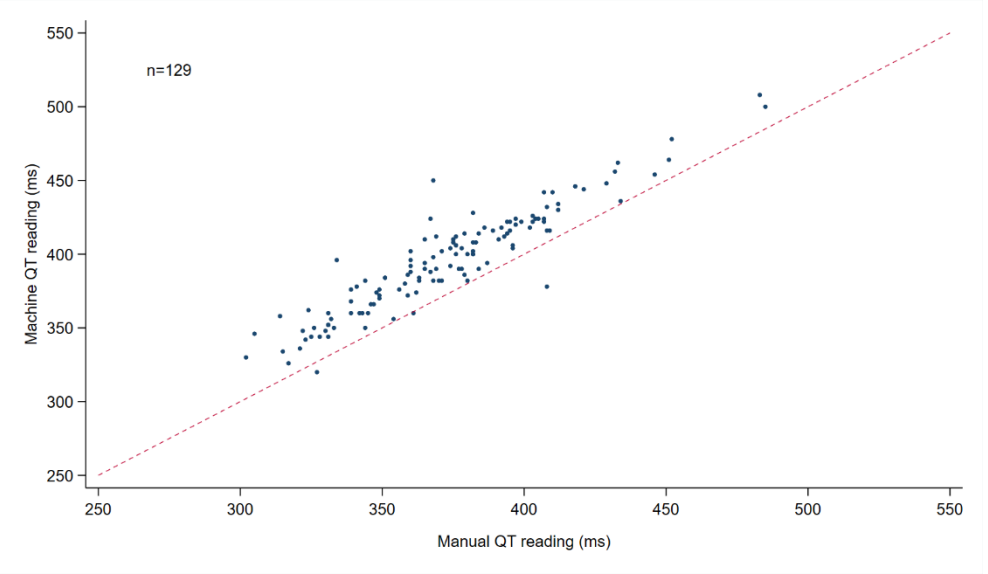


Figure 4.13. Short regimen at early time point.



The majority of readings are above the line indicating the machine overestimated the QT interval. Compared with the baseline readings the points appear less concentrated.

The histograms in Figures 4.14 and 4.15 show the frequency of the differences between the machine and the manual QT interval calculation at the early time point. The mean (SD) difference on the Long regimen was -25.59ms (10.61) with a minimum difference of -50ms and a maximum difference of -5ms. The mean (SD) difference on the Short regimen at baseline was -22.88ms (12.94) with a minimum difference of -82ms and a maximum difference of 30ms. Compared with baseline readings the mean difference has increased across both regimens and is still slightly larger on the Long regimen. The range between minimum and maximum difference has increased from baseline and is larger on the Short regimen compared with the Long regimen.

Figure 4.14. Histogram for Long regimen patients at early time point

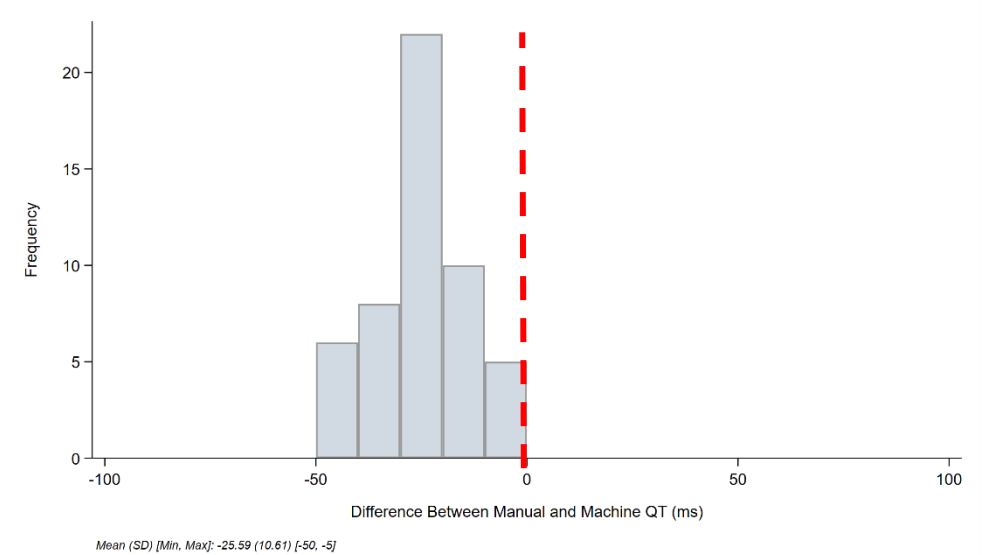
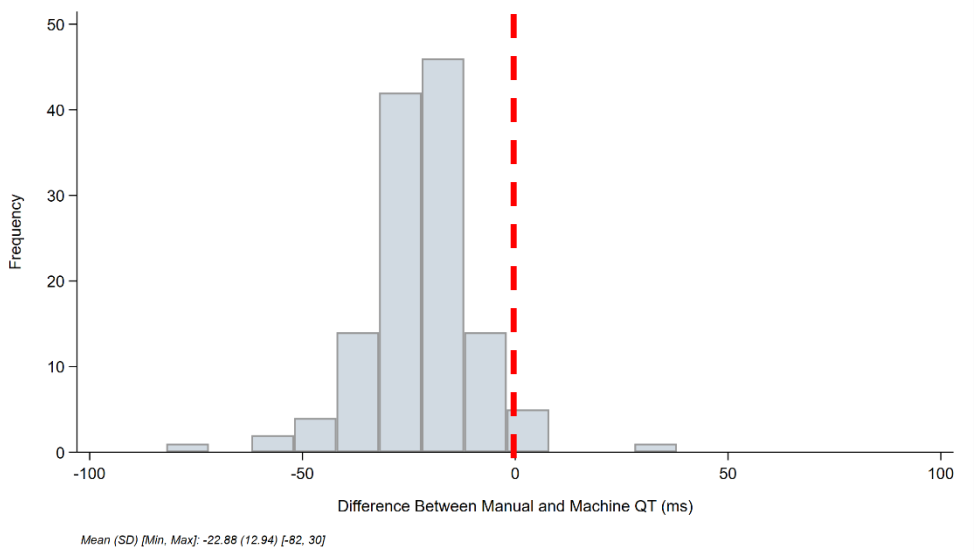


Figure 4.15. Histogram for Short regimen patients at early time point



Bland-Altman plots comparing the difference between machine and manual reads for both the Long regimen (Figure 4.16) and Short regimen (Figure 4.17) at the early time points are shown below.

Figure 4.16. Long regimen early time point

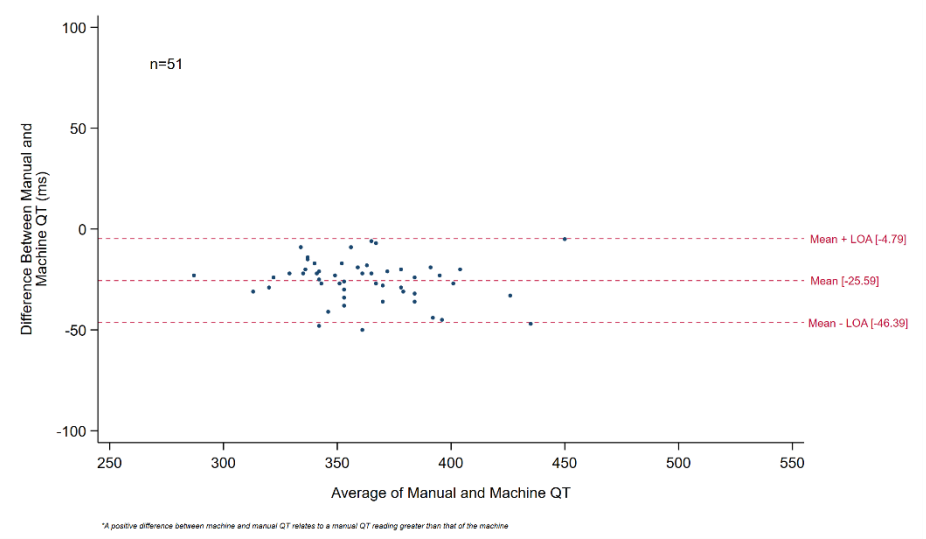
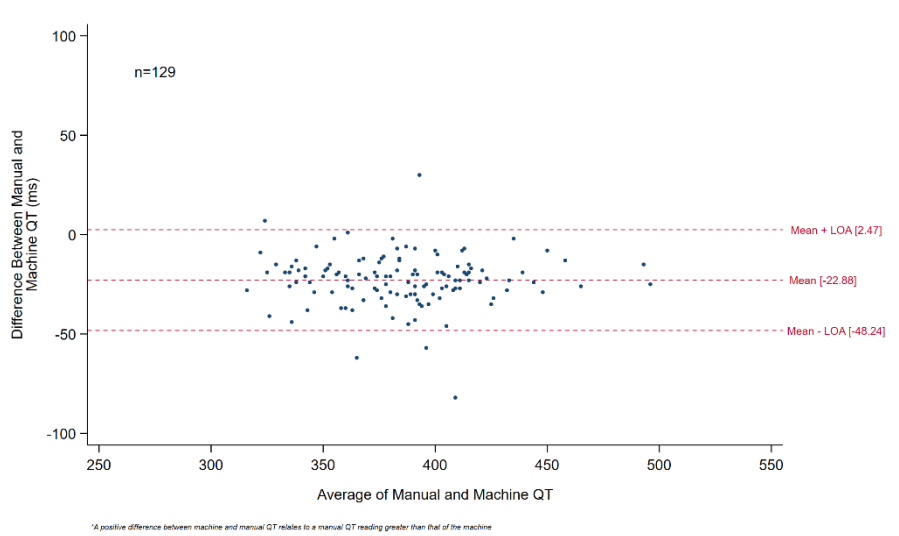


Figure 4.17. Short regimen early time point



Whilst the mean difference between regimens was larger at the early time point compared with baseline, there was no evidence of a significant difference in accuracy with a mean (SE) difference between regimens of -2.12 (1.90), p = 0.27 (95% CI -5.87 to 1.64).

Table 4.2 Independent t-test comparing Short and Long regimen mean difference of manual and machine QT readings at early time point

Regimen	**No. of ECGs (n= 180 total)**	**Mean difference (ms)**	**Standard Error**	**SD**	**95% CI**		**P-value**
Short	129	23.47	1.04	11.82	21.41	25.53	
Long	51	25.59	1.49	10.61	22.60	28.57	
		-2.12	1.90		-5.87	1.64	0.2675

Late time point

The effect of regimen on accuracy at the late time point of ≥3 months into treatment was also investigated. The scatter plots in Figure 4.18 and Figure 4.19 show the association between manual and machine QT calculations.

Figure 4.18. Long regimen at late time point

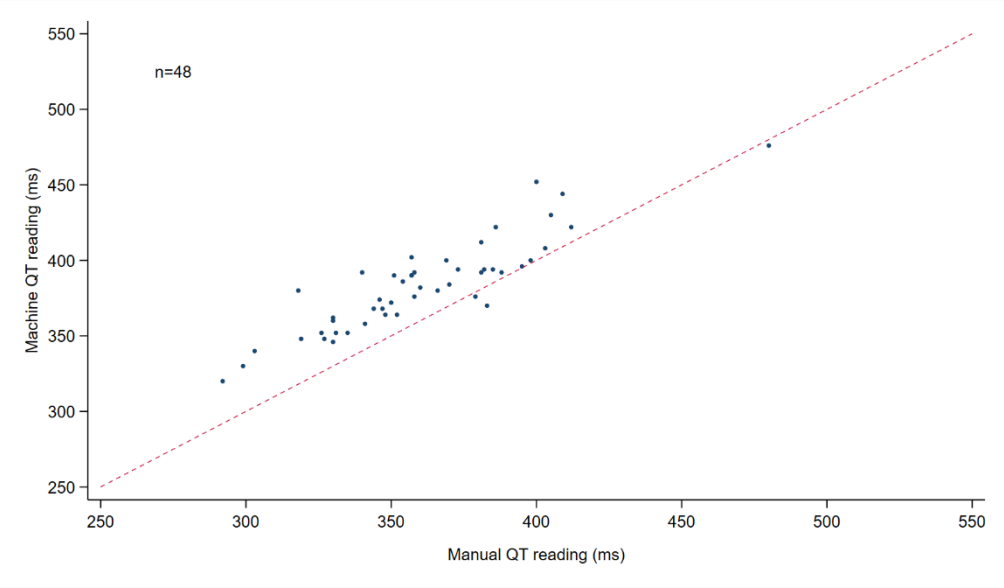
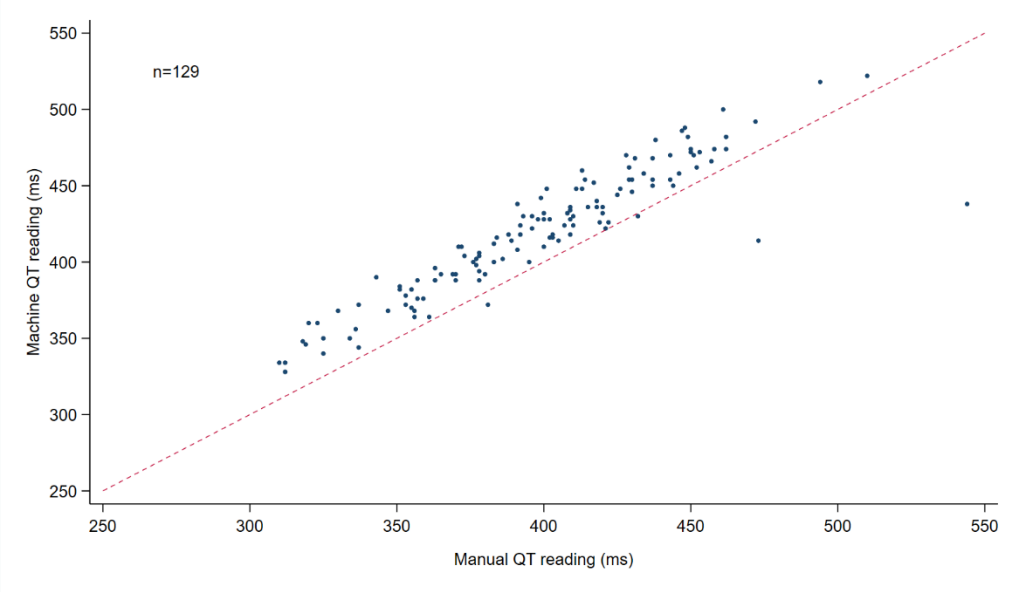


Figure 4.19. Short regimen at late time point



As with the baseline and early time point ECGs, the same pattern was seen for late time point ECGs with the machine, in most cases, overestimating the QT interval compared to the manual measurements. The two QT outliers below the red line indicate the machine underestimated the QT interval.

The histograms in Figure 4.20 and Figure 4.21 show the spread of difference data at the late time points. The mean (SD) difference on the Long regimen was -22.73ms (15.10) with a minimum difference of -62ms and a maximum difference of 13ms. The mean (SD) difference on the Short regimen at baseline was -21.52ms (17.39) with a minimum difference of -47ms and a maximum difference of 106ms. Compared with early readings the mean difference has decreased slightly across both regimens and is still slightly larger on the Long regimen. Other than the two outliers the spread of data does not appear to have increased from the early time point on the Short regimen but does appear to have increased on the Long regimen.

Figure 4.20. Long regimen at late time point

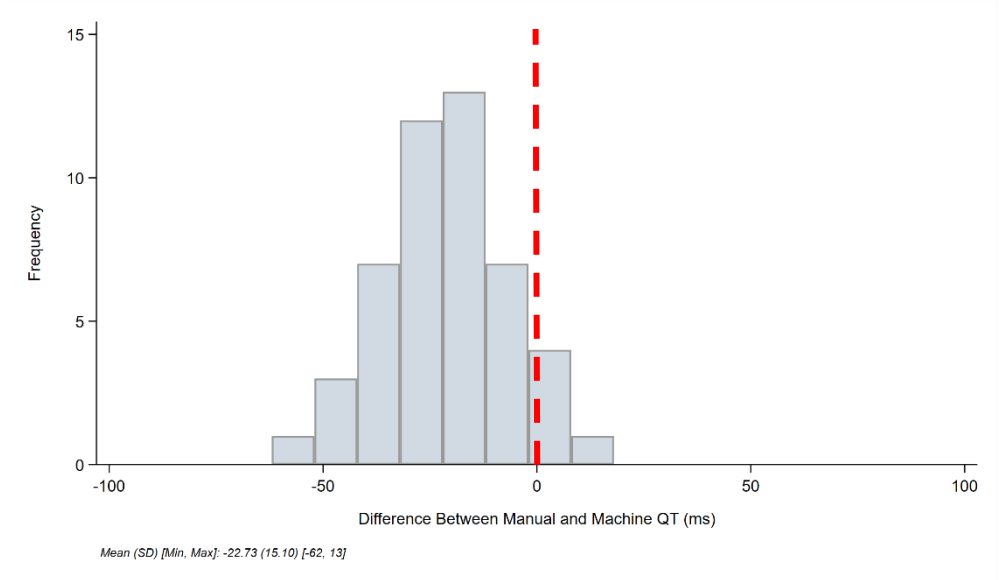
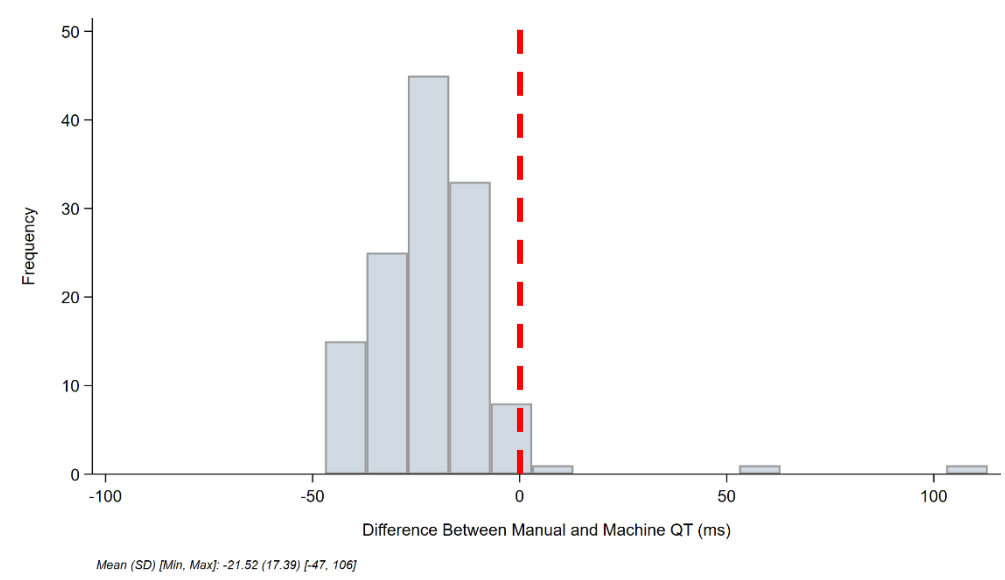


Figure 4.21. Short regimen at late time point



Bland-Altman plots comparing the difference between machine and manual reads for both the Long regimen (Figure 4.22) and Short regimen (Figure 4.23) at the late time point are shown below.

Figure 4.22. Long regimen at late time point

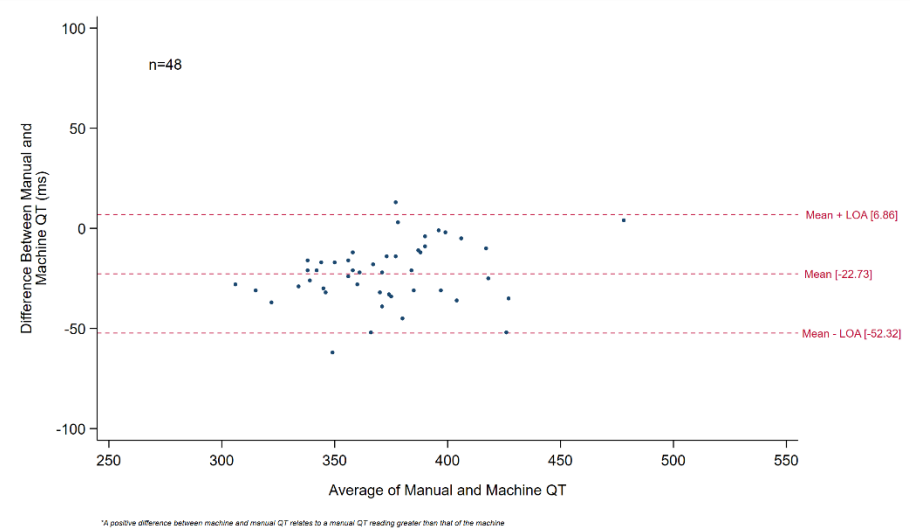
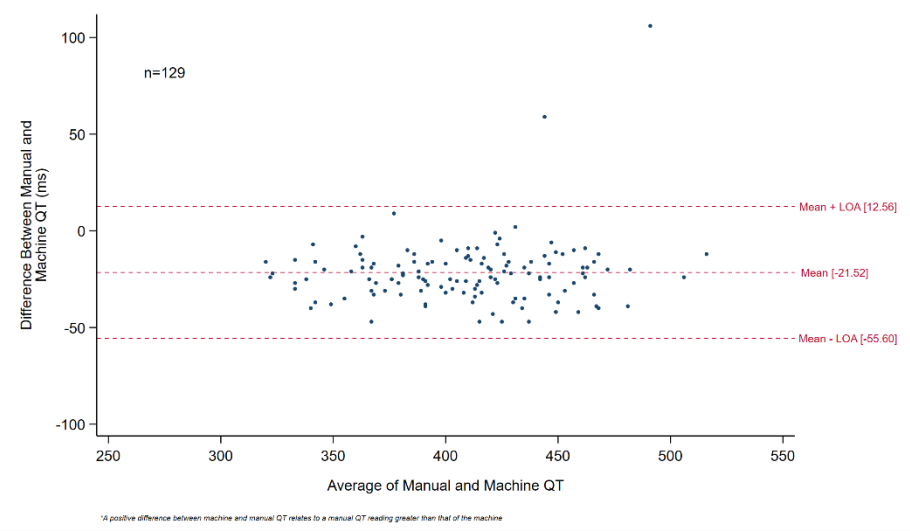


Figure 4.23. Short regimen at late time point



There was no evidence of a significant difference in accuracy between regimens at the late time point with a mean (SE) difference between regimens of 0.69ms (2.27), p = 0.7627 (95% CI -3.79 to 5.16).

Table 4.3. Independent t-test comparing Short and Long regimen mean difference of manual and machine QT readings at late time point

Regimen	**No. of ECGs (n= total)**	**Mean difference (ms)**	**Standard Error**	**SD**	**95% CI**		**P-value**
Short	129	24.25	1.17	13.29	21.93	26.56	
Long	48	23.56	1.98	13.73	19.58	27.55	
		0.69	2.27		-3.79	5.16	0.7627

Effect of duration on treatment on accuracy (Objective 2)

The following tables show the results of the paired t-test for effect of time on treatment for the accuracy of the machine readings. A negative value for the mean difference between time points indicates poorer agreement later on whereas a positive value indicates improved agreement. Table 4.4 shows the combined results for both regimens, displaying little difference between early and late time points with a mean SE of 0.37ms (1.21) which was not significant, p = 0.6206 (95% CI -2.01 to 2.75). Table 4.5 shows the paired results for early and late time points focusing on the Short regimen only. Whilst there was a small deterioration in the mean (SE) difference from early to late of -0.55ms (1.44), this again was not significant, p = 0.3518 (95% CI -3.40 to 2.30). The difference

between early and late time points for the Long regimen patients is shown in Table 4.6. There was no evidence of a deterioration in accuracy. The mean (SE) decreased to 2.87 (2.17), showing improved accuracy with time, though this was unexpected it was also not significant p = 0.9030 (95% CI -1.51 to 7.25).

Table 4.4. Paired results for all regimens comparing early and late readings

Regimen	**No. of ECGs (n= total)**	**Mean difference (ms)**	**Standard Error**	**SD**	**95% CI**		**P-value**
Early	167	24.41	0.88	11.37	22.68	26.15	
Late	167	24.04	1.02	13.22	22.02	26.06	
		0.37	1.21	15.59	-2.01	2.75	0.6206

Table 4.5. Paired results for Short regimen only comparing early and late readings

Regimen	No. of ECGs (n= total)	Mean difference (ms)	Standard Error	SD	95% CI		P-value
Early	122	23.91	1.08	11.88	21.78	26.04	
Late	122	24.46	1.20	13.24	22.09	26.83	
		-0.55	1.44	15.91	-3.40	2.30	0.3518

Table 4.6. Paired results for Long regimen only comparing early and late readings

Regimen	No. of ECGs (n= total)	Mean difference (ms)	Standard Error	SD	95% CI		P-value
Early	45	25.78	1.47	9.87	22.81	28.74	
Late	45	22.91	1.98	13.26	18.93	26.90	
		2.87	2.17	14.58	-1.51	7.25	0.9030

Relationship between length of QT interval and agreement between manual and machine readings (Objective 3)

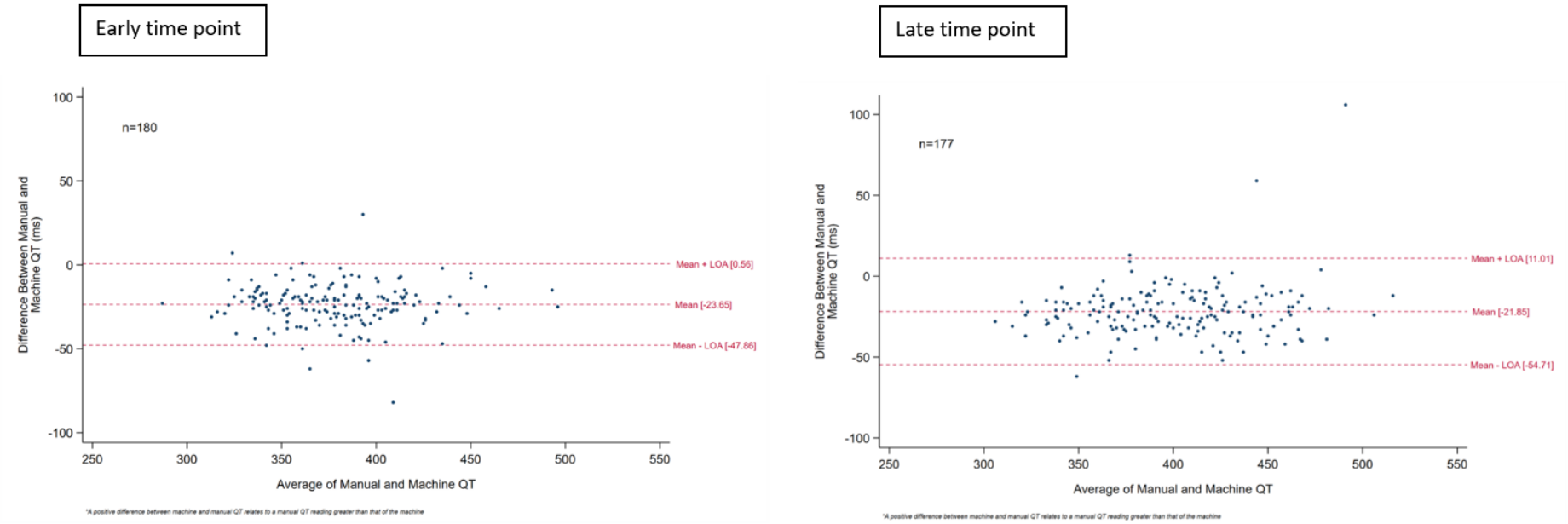
The scatter plots in Figures 4.6-7,4.12-13 and 4.18-19 and the Bland-Altman plots in Figures 4.10-11, 4.16-17 and 4.22-23 show the relationship between the length of the QT interval and agreement between the manual and machine readings. With the exception of the two outliers in Figures 4.19 and 4.23, showing the effect of the Short regimen on accuracy of the machine at the late time point, there did not appear to be any clear evidence of worsening agreement with increasing QT interval.

Bland-Altman plots show that the more extreme outliers (indicating poor agreement) occurred at later time points, though overall agreement for both regimens changed little between time points with a mean (SD) of 24.41 (0.88) at the early time point and 24.04 (1.02) at the late time point.

Chapter 1 has already demonstrated that QT prolongation is more likely to occur late in treatment than early for patients taking the Short regimen. This is again demonstrated here in Figures 4.22 and 4.23 with a higher number of QT readings >450ms at the late time points compared with the early time points (Figures 4.16 and 4.17).

Outliers

Figure 4.24. Participants from both regimens at each time point to identify outliers



To assess whether there were any patterns amongst the outlier readings a review of the individual ECGs was undertaken. Figure 4.24 shows participants from both regimens combined at the early and late time points. At the early time points there were two readings outside the limit of agreement (LOA) in which the machine underestimated the true QT interval and four readings outside the LOA in which the machine overestimated the true QT interval.

At the late time point three readings outside the LOA were underestimated by the machine and one was overestimated. These ten QT readings in total were from ECGs in ten different individual participants. The regimen, time-point and T wave morphology are summarised in Table 4.7.

T wave morphology for outlier ECGs.

There were few common themes for the QT readings showing the largest discrepancy. Though most were from patients taking the Short regimen, there were twice as many patients taking this regimen.

Table 4.7 summarises the outliers in figure 4.24 which were outside the LOA. Each participant was different i.e., the ECGs with large discrepancies at the early time point were from ECGs in different participants at the late time point, though treatment changes may have taken place.

There was some evidence suggestive of abnormal T wave morphology for both the largest positive and negative discrepancies on the Short regimen, with notched/bifid, asymmetric, flat wave, flat peak and broad T waves being present at least once. This will be covered in greater detail in Chapter 5.

Table 4.7. T wave morphology for participants with the largest positive and negative discrepancies between machine and manual QT readings.

Patient no.	Regimen	Time point	QT discrepancy (ms)	Notched	Asymmetric	Flat wave	Flat peak	Broad
1	Short	Week 1	82	0	0	0	0	4
2	Short	Week 2	62	0	0	0	0	0
3	Long	Week 24	62	0	2	0	0	0
4	Short	Week 2	57	0	2	0	0	0
5	Long	Week 3	50	0	0	2	0	0
6	Short	Week 4	-7	0	0	0	0	2
7	Long	Week 12	-13	0	0	0	0	0
8	Short	Week 3	-30	0	0	0	2	0
9	Short	Week 12	-59	3	0	0	0	2
10	Short	Week 36	-106	4	0	2	0	3

Columns 5-9 indicate number of ≥2 adjacent ECG leads displaying that particular T wave abnormality at the time point shown. Blue rows indicate no T wave abnormalities present on that particular ECG and beige rows that at least one of the abnormalities were present

Breakdown of Unreadable ECGs

A total of 60 ECGs were deemed unreadable by both readers as shown in Figure 4.5. This is summarised in Table 4.8 showing details of regimen, time point and site. There was a roughly equal split between the two regimens (Long 21: Short 39) allowing for the 2:1 randomisation used in the STREAM Stage 1 trial. There was also no real difference between time points for these ECGs with 20 coming from baseline ECGs, 19 an early time point and 21 a late time point. The mean (SD) of these machine QT readings was 384 (49.95). A noticeable difference was observed for the trial sites with 40 (66.7%) of the ECGs coming from participants at the Sizwe site in South Africa, this was 43% of all the ECGs looked at in the analysis from that site. This included 21 ECGs from the seven patients who had unreadable ECGs at all three time points.

Table 4.8. Summary of unreadable ECGs by regimen, time point and site

	Regimen		Time point									Site		
	Short	Long	Baseline	Early	Late	AHRI	St Peters	NCCD	PNTH	Sizwe	King Dinuzulu			
Frequency	39 ECGs/27 patients	21 ECGs/13 patients	20	19	21	1	2	6	6	40	5			
%	65/67.5	35/32.5	33.3	31.6	35	1.7	3.3	10.0	10.0	66.7	8.3			

Seven (11.6%) of the unreadable ECGs gave a machine QT ≥450ms with one >500ms. The single participant >500ms had no clinical management changes as their clofazimine had already been suspended and moxifloxacin changed to levofloxacin based on prolongation that occurred earlier in treatment.

For the 39 unreadable ECGs shown in Table 4.8, these were from 27 participants taking the Short regimen with 21 unreadable ECGs for 13 participants taking the Long regimen.

Discussion

While it has previously been observed that machine readings using the 12SL algorithm can overestimate the true QT interval compared with the gold standard manual read in certain settings [7-10], these studies have not looked within a population being treated for TB with drugs known to be associated with QT prolongation. The STREAM data additionally provided the opportunity to examine duration of treatment.

QT versus QTc

This secondary analysis of the STREAM data compared the QT interval rather than the QTc interval between machine and manual reads. Although the QTc is more relevant clinically, it is derived from the QT interval and is dependent on the R-R interval which is itself dependent on a patient's heart rate. The machine calculation of the R-R interval is less likely to be affected by the quality of the ECG or the morphology compared with the QT interval which is why the focus of the analysis was not on the corrected QT interval. Also, any difference in accuracy between machine and manual QT interval would also be seen in the QTcF interval. For an ECG in which the QT interval is 420ms the QTcF will also be 420ms for a heart rate of 60 beats per minute (bpm). An overestimation by the machine of 20ms will not change that patient's individual risk of arrythmia and treatment can continue with no modification needed. For the same patient, a bradycardia of 55 bpm would give a QTcF of 408ms which again would not need treatment modification. If the patient had a tachycardia of 100 bpm the QTcF would be 498ms which would be close to

meeting the definition of severe QT prolongation with an increased risk of arrythmia that would likely require treatment modification. An overestimation of the machine QT reading by 20ms in the tachycardia example may result in overdiagnosis of QT prolongation and treatment modification that may not be needed. It is likely that the discrepancy observed would be exaggerated in most cases if the QTcF was used, as with the exception of bradycardic ECGs, the reading will be higher than the absolute QT interval.

Assessing accuracy

A difficulty encountered in this work is that there is no clear standard definition of accuracy when comparing machine QT readings with a manual read. Some studies have described a mean QT difference of around -19ms with limits of agreement between 20 and 30ms.(10, 21) For the purposes of this chapter, accuracy was defined as the magnitude of difference between machine and manual absolute QT readings with a mean difference of 20ms considered reasonably accurate. The mean absolute QT difference of readings was within 20ms for both regimens at baseline so the machine could be considered fairly accurate before treatment began. There was no significant difference between the two regimens which was expected given the patients were randomised and yet to start treatment when these readings were taken. The mean difference between machine and manual reads of -19ms on the Short regimen and -19.4ms on the Long regimen suggests the machine overestimated the true QT interval in the majority of reads which is well illustrated in the scatter plots (Fig. 4.6 and 4.7) and Bland-Altman plots (Fig. 4.10 and 4.11).

The baseline accuracy might not be maintained once treatment started for two main reasons. Firstly, it was expected that the QT interval would increase, which one study had found was related to a decline in accuracy.[10] Secondly, the effect of the drugs could cause the T wave morphology to change and for U waves to develop which could affect the accuracy of the automated machine method for calculating the QT interval. The accuracy between regimens was also expected to change once treatment had started as the above reasons were more likely to be exacerbated on the Short regimen which contained high dose moxifloxacin and clofazimine which are known to affect the *hERG* potassium channel. A comparison of accuracy between regimens was investigated at each of the two time points on treatment, early and late. As suggested in the hypothesis, the accuracy diminished once treatment had started with an increase in the mean absolute QT difference for both regimens at the early time point; Short 23.47ms and Long 25.59ms. The higher mean value for the Long regimen and lower mean value for the Short regimen was unexpected and might suggest that accuracy was marginally poorer for the Long regimen patients at the early time point, though the difference between regimens of -2.12ms was not significant (p=0.268). The difference between regimens at the late time point was in keeping with the hypothesis in that the accuracy was poorer for the Short regimen (24.25ms) compared with the Long regimen (23.56ms) giving a mean difference of 0.69ms which again was not significant (p=0.763). Though there was a difference between regimens on treatment at both the early and late time points it was small and also not significant using the independent t-test.

A comparison of time points on treatment for each regimen was also undertaken using a paired t-test. The Short regimen showed a non-significant slight deterioration in accuracy between early and late time points of -0.55ms (p=0.352). The Long

regimen also showed a slight difference of 2.87ms between early and late time points but in the opposite direction which suggested an improvement in accuracy as time on treatment increased, though again this was not significant (p=0.903).

It had been expected that the accuracy of the machine readings would continue to worsen the further patients were into their treatment course. Whilst this was true for the Short regimen, the difference between time points was relatively small. It was unexpected that accuracy would improve between early and late time points on the Long regimen but again the difference was small. The majority of treatment changes occurred at week 12 and beyond for Short regimen participants who reached a QT/QTcF> 500ms, so most would've had treatment modified after the early time point. Only 2 Long regimen participants had treatment modified and both were at the early time point. This may partly explain the differences seen between the early and late time points.

The PK of clofazimine may also partially explain why the accuracy worsened between time points on the Short regimen as it has a long half-life (approximately 25-34 days), reaching a steady plasma state after 18 to 21 weeks.(28-29) As the late time point ECGs were from either week 12, 24 or 36 of treatment it was likely that any effect of clofazimine on machine QT interval accuracy would only have been evident at the late time point. In contrast, the high dose of oral moxifloxacin on the Short regimen would have been rapidly absorbed, reaching a maximum serum concentration (T_{max}) in 0.75–3.5 hours with a half-life of 6-12 hours.[30] All the early time point ECGs were from the first month of treatment (weeks 1, 2, 3 or 4) so the effect of moxifloxacin would have been seen at this point. It is unclear why accuracy improved slightly on the Long regimen between early and late time points but may have been a chance finding.

The majority of machine QT readings were an overestimate compared with the gold standard manual read, with an average of 22.5ms across the three time-points. This meant that there might have been some increased anxiety around high QT readings that may have in fact been lower if they had been manually calculated and in some cases treatment may have been modified unnecessarily. At the same time, it is reassuring that there were few cases of the machine underestimating the QT interval as this could have resulted in patients potentially coming to harm. An overdiagnosis of severe QT prolongation can result in unnecessary anxiety for both patients and clinicians. Treatment may be modified unnecessarily resulting in extension of the regimen duration and possibly increased risk of resistance, treatment failure and non-adherence. Conversely, given the potentially life-threatening risk from arrythmias that may develop with underdiagnosed severe QT prolongation in patients that remain on treatment, on balance it is probably better to over diagnose a few cases than underdiagnose.

Outliers

The limits of agreement (LOA) for the Bland Altman plots are defined as 2 SD from the mean so 5% of readings will be outside these limits. These LOA change depending on the population in each analysis. Though there were few common themes for the outliers in each analysis there did appear to be a possible link with abnormal T wave morphology for these readings. This would be expected as the accuracy of the QT interval machine readings depends on where the machine algorithm decides the end of the T wave is. Abnormal morphology will affect this

measurement and is one of the reasons that manual readings are felt to be more accurate.

The largest discrepancies, defined as being outside the LOA, between machine and manual QT reads are shown in Table 9. There were three ECGs in which the machine underestimated the QT interval by more than 20ms, all were in Short regimen patients and on treatment. The biggest underestimate of 106ms was for a Vietnamese patient at their week 36 ECG. The machine reading of 438ms was 544ms when measured manually. This patient also had a QT of 525ms and QTcF of 499ms on a Holter reading on a different date. The ECG at week 36 did have notched, broad T waves which may have affected the machine accuracy. No treatment modifications were made for this patient as this was guided by the machine reading. Fortunately, the patient was close to completing their 9 months of treatment and came to no harm. The other two patients with underestimates of 59 and 30ms also had abnormal T wave morphology on their ECG with notched T waves again prevalent. Both patients had treatment changes in relation to their QT interval but not on the basis of the ECG reviewed at the time point showing the discrepancy.

Though the ten ECGs described in table 9 were from ten different participants, it didn't take into account whether treatment changes had been made which may have explain why those with abnormalities at the early time point were not the same as at the late time point.

Unreadable ECGs

In total, 10% of the 600 ECGs reviewed in this analysis were deemed unreadable by both readers based on pre-defined reasons i.e., the waveform was of poor quality,

defined as noise, low amplitude T wave complex or wandering baseline. Despite this, the machine calculated a QT interval in all 60 of these ECGs. Though there appeared to be no particular pattern in terms of time point or regimen, a large number came from the same site at Sizwe in South Africa. This was 40 (66.7%) of the total unreadable ECGs, including 21 ECGs from seven participants who had unreadable ECGs at all three time points. Though the analysis of unreadable ECGs was not weighted by total number of participants at each site, there were 61 randomised at Sizwe which suggests the difference was unlikely to be due to a larger number of participants and ECGs performed. This may suggest there was a training issue with how ECGs were performed at this particular site.

Guidance for sites and clinicians from this work

One of the key areas of research impact from this chapter would be in providing clinicians and healthcare workers with some guidance on which ECGs to be cautious of when interpreting the automated machine QT reading in a drug-resistant TB setting. Overall, the majority of readings from the 200 patients selected for this analysis were fairly reliable. In most cases overestimation of the machine QT interval of between 19ms and 26ms probably will not make much difference in terms of decisions about patient safety and management. However, there were some cases in which the machine reading was unreliable. The largest positive and negative discrepancies for both regimens were at different time points and in different patients with no real common themes, though abnormal T wave morphology was prevalent in ECGs in which the most extreme discrepancies between machine and manual reads occurred.

Most treatment programmes are likely to now use the Short regimen with the possible addition of bedaquiline in place of kanamycin for an all-oral regimen. The principles would apply to similar regimens, but the findings may not apply directly to patients taking bedaquiline.

The following guidance may help clinicians involved in the management of drug-resistant TB patients to decide which machine QT readings to be cautious of. There would need to be a period of training as undertaken in this chapter prior to the clinicians manually calculating the QT interval themselves. This could easily be done remotely as was the case for this work.

1. If there is evidence of noise, flat T wave complexes (<1mm amplitude) or wandering baseline (>1mm) as illustrated in Figure 4, then the machine reading may be unreliable. The ECG leads and electrodes should all be checked for correct placement and connection before repeating the ECG at the same visit or the next available opportunity.

2. If there is evidence of abnormal T wave morphology, particularly notched, asymmetric, broad or flat T waves, then manual calculation of the QT interval and specialist cardiology input should be sought.

3. The manual calculation of the QT interval should use the combined mean of lead II and V5 after measuring the QT interval in at least two complexes using the tangent method as described in this chapter.

4. Tachycardia will increase the corrected QT interval so it may also be worth repeating ECGs with a QTc close to the 500ms threshold. A cardiology over-read may also be appropriate if the QTc remains high.

Limitations

This chapter has limitations.

First, ECGs were selected from just under half of all patients in STREAM Stage 1, though included all participants with clinically relevant QT prolongation that had available ECGs at each time point and 118/191 who did not develop severe QT prolongation, representative of the study population based on country, age and gender. Many ECGs for each participant were not used as this would have involved considerably more time and work but also ECGs were not available from the same time points for all patients. It was felt more appropriate to try and standardise the ECGs used by identifying one available from an early time point (weeks 1 to 4) and one available from a late time point (weeks 12, 24 or 36). Though it may have been better to pick exactly the same ECG visit at each time point this would have resulted in a reduction in the available number of participants for the analysis as the ECGs available varied at each time point. By grouping visits into early and late and allowing any of the ECGs in that window to be used it allowed an optimisation of the total number of participants.

Some of the patients who did develop severe QT prolongation were not included in this analysis as they did not have ECGs available at the required time points. Other patients who developed severe QT prolongation were included but the highest machine reading could not be used as it did not fall on one of the above time points. For patients who reached a machine QT/QTcF ≥500ms, 62.5% (5/8) of Long regimen patients and 80.6% (25/31) of Short regimen patients were included in this analysis. No ECGs from one of the South African sites were used as there were no patients with ECGs available at all the required time points, though this site only recruited just over 3% (14/424) of all patients randomised in STREAM Stage 1.

Second, there was a difference in method between the manual and machine calculation of the QT interval which may have contributed to the overestimation of the QT interval by the machine. For STREAM Stage 1, MAC 800 (GE Healthcare) machines were used at all sites to record ECGs. All machines were calibrated at baseline with the 12SL measurement and interpretation algorithm used to calculate the QT interval. As this uses a median superimposed complex from all leads it will be affected by poor quality tracings that may occur in some of the leads. In contrast, the manual QT interval used a mean QT interval from three complexes in lead II and three complexes in lead V5 as this was advised by the cardiologist supporting this work to be more representative of the true QT interval. Though some studies have tried to use a similar method to compare manual and machine reads [8-9] others have not. (7, 10) The studies by Barbery et al and Darpo et al both used manual QT measurements from a single lead and compared these with an automated method that used all 12 leads.

Third, this analysis did not take treatment modification into account for each patient. It is possible that as treatment affected the accuracy of the machine readings, interruption, dose change or drug change for any reason could also have affected the validity of the results, though as the majority of patients on both regimens did not require treatment modification this would not have affected many of the ECGs. Focussing on the 10 patients in table 4.7 with the largest discrepancies between machine versus manual calculation, only 3 had treatment modifications based on their machine calculated QT interval. It could therefore be argued that even larger differences did not affect patient management, particularly if the machine calculation was an overestimate in most cases, though there was one patient who the machine had underestimated their QT interval by 106ms and no treatment changes had been

made putting them at risk of cardiac arrythmia. This would be an important consideration in future work which could retrospectively look at treatment modifications in a population with MDR-TB based on a machine calculated QT interval.

Strengths

This chapter has a number of strengths.

First, prior to the main sample of 600 ECGs being reviewed, a period of training was undertaken with a cardiologist specialising in electrophysiology (appendix). This allowed a quality assurance exercise to be completed on a smaller sample of ECGs which ensured a non-cardiologist clinician was able to manually calculate the QT interval to within +/- 20ms of a cardiologist. The protocol also allowed 112 of the 600 ECGs in the main sample to be over-read by a cardiologist due to meeting pre-defined criteria (noise, low amplitude complexes of <1mm or wandering baseline >1mm). Both readers agreed on sixty ECGs being unreadable and only five of the fifty-two better quality ECGs showed a variability greater than +/-20ms between readers. These steps add further weight to the validity of the results.

Second, this was a large dataset of 600 ECGs from 200 patients and included a mix of Short and Long regimens patients from a range of different geographic and ethnic backgrounds. Patients with severe QT prolongation were selected along with those who did not develop severe QT prolongation. These lower risk patients were representative of the study population with respect to country, age and gender.

Third, a longitudinal analysis was performed on the same patients, followed at three different time points up to 9 months of treatment so the analysis was not reliant on a

single ECG reading and took into account time on treatment to look for any differences.

Fourth, the tangent method is known to provide a shorter QT interval than the threshold method.[12] In this analysis, both the manual and machine 12SL algorithm used the tangent method to determine the end of the T wave so this would have limited the effect on difference in mean absolute QT intervals between manual and machine reads.

Fifth, as T wave morphology was assessed at the same time, on the same sample of ECGs for the analysis in chapter 5, it was possible to look for reasons in which there was a large discrepancy between manual and machine QT interval calculations.

Conclusion

This work has shown that a clinician involved in managing TB patients with no prior specialist cardiology experience can be trained to a reasonable level to manually calculate the QT interval and identify when ECGs are of poor quality and the machine reading should not be relied upon. Overall, there was reasonably good agreement between manual and machine calculations of the QT interval. The machine overestimated the mean absolute QT interval by 22.5ms on average and can be relied upon in most cases. Less reliable readings (a large discrepancy outside LOA) occurred infrequently and may be affected by abnormal T wave morphology. These ECGs might benefit from a manual QT measurement and cardiologist input. This could have implications for the monitoring of patients in programmatic settings.

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Appendix 4 – Training and Quality Assurance

One of the aims from this study was to inform clinicians treating patients for drug resistant tuberculosis about the reliability of machine readings of the QT interval. In general, the doctors responsible for managing TB will not be cardiologists so it was important to demonstrate that a non-cardiologist clinician could be adequately trained to manually read the QT interval and that this process was validated.

The validation process and results are described below.

Training methods

Before independently reviewing the QT interval of the full set of ECGs, a period of training was undertaken to ensure competency and to provide a quality control. A number of ECGs were reviewed and discussed on a video call with a cardiology electrophysiology (EP) specialist, allowing an opportunity for a manual calculation of the QT interval to be taught using the tangent method. Thirty ECGs were then selected from ten patients in the study deemed low-risk and twenty deemed high-risk as defined above. The ECGs selected were from time points not included in the later analysis and the automated readings blinded. The specialist and trained non-specialist then independently calculated the QT interval for each of the ECGs. The mean QT interval of lead II and V5 was used to assess inter-reader variability.

Following discussion with the EP specialist, a limit of 10ms was deemed reasonable to ensure I was close to the cardiology specialist and give more validity to my independent QT interval reads.

Training and reliability of readings

A breakdown of inter-reader variability during training for the thirty ECGs is shown in Table A1. Four of the ECGs were deemed non-assessable by either of the two readers and were not included in the comparison.

Table A1. Inter-reader variability following first period of training comparing the difference in manual QT interval calculation between cardiologist and non-cardiologist readers.

Percentiles		Smallest		
1%	1	1		
5%	4	4		
10%	5	5	Obs	26
25%	9	7	Sum of Wgt.	26
50%	20		Mean	21
		Largest	Std. Dev.	13.75209
75%	27	33		
90%	45	45	Variance	189.12
95%	50	50	Skewness	.6544198
99%	51	51	Kurtosis	2.734419

The mean difference (SD) was 21ms (13.75). As this was felt not to be sufficiently accurate, the following steps were taken.

First, a review of the ECGs with the highest variability showed either an abnormally flat T wave making accurate assessment of the QT interval difficult or a noisy recording, again making assessment difficult. A period of further training was undertaken on a video call using the tangent method.

Second, a review of another second set of ECGs was performed using the same process as outlined above. A sample of ten ECGs from high and low risk patients were reviewed independently by each reader.

Third, the 10ms threshold was widened to 20ms which was consistent with inter-observer variability seen in previous studies.(11-13)

Table A2. Inter-reader variability following second period of training comparing the difference in manual QT interval calculation between cardiologist and non-cardiologist readers.

Percentiles		Smallest		
1%	0	0		
5%	0	1		
10%	.5	1	Obs	10
25%	1	2	Sum of Wgt.	10
50%	2.5		Mean	3.2
		Largest	Std. Dev.	2.529822
75%	5	4		
90%	7	5	Variance	6.4
95%	8	6	Skewness	.5885417
99%	8	8	Kurtosis	2.287905

A summary of ECGs reviewed during the second period of training is shown in Table A2. An improved mean difference (SD) of 3.2ms (2.53) was seen. The variability was less than 10ms for all ten of the ECGs. I then then went on to review the larger set of 600 ECGs independently with the following caveats.

Pre-defined rules for second reader

There are three specific scenarios in which the measurement of the QT interval can be problematic increasing the possibility of inaccurate measurements.

The first is when the T wave is “flat” leading to problems assessing where it terminates when calculating the QT interval. If the T wave amplitude is <1mm [31] or <1/8th amplitude of the R wave [32] in the chosen lead, then a second reader should also review those ECGs and calculate the QT interval so that a comparison can be made.

The second scenario is that “noise” can lead to problems determining the end of the T wave to calculate the QT interval. The noise can be generated by muscular contractions (electromyographic), electronic devices, poor electrode contact, or the base-line drift seen with respiration.[33] In situations where there is a lot of noise on the ECG a second reader should also review those ECGs and calculate the QT interval.

The third is when the baseline is “wandering”. ECGs in which the baseline of the chosen lead is wandering >1mm will be read by a second reader.

If the difference in the QT interval between the two readers is <20ms then these ECGs will be included in the main analysis. The QT interval from the non-cardiologist reader will be chosen for consistency.

Pre-defined rules for third reader

I had originally planned that for ECGs in which there is ≥20ms difference in the combined QT interval (mean lead II and V5) between the non-specialist and specialist reader, a third reader would be used. This would have allowed a comparison between two cardiology specialists. Had the difference for the combined QT interval reading between the two specialists been <20ms, this would have suggested the accuracy of the non-specialist may be at fault. Had the difference

between the two specialists been ≥20ms then this would have suggested making an accurate assessment of the QT interval is troublesome even amongst cardiology specialists for that particular ECG. As <1% of the total ECGs were affected by the above scenario, this step did not take place and the five ECGs were deemed unreadable and not used in the main analysis.

Results after cardiology over-read from main sample

488 of the 600 ECGs were deemed of good quality after review by the trained non-cardiologist clinician. Of the 112 poorer quality ECGs, 60 were excluded from the analysis as both readers were unable to calculate a combined mean QT interval from lead II and V5. This was due to either flat T wave complex, noise or wandering baseline. Of the remaining 52 ECGs that had a cardiologist read a comparison was performed to assess agreement between the two readers (Table A3).

Table A3. Agreement between trained non-cardiologist and cardiologist.

Difference

Percentiles		Smallest		
1%	-42	-42		
5%	-19	-22		
10%	-16	-19	Obs	52
25%	-11.5	-18	Sum of wgt.	52
50%	-2		Mean	-2.160256
		Largest	Std. dev.	13.06286
75%	6	14.33333		
90%	12	22	Variance	170.6383
95%	22	25	Skewness	.0902562
99%	35	35	Kurtosis	4.186411

A median magnitude of variation of 2ms was observed between readers with a mean (SD) of -2.16ms (13.06). Only five of the 52 readings had a variability greater than +/-20ms and these were excluded from the main analysis. As the number was small, it

was not felt necessary to get a second cardiologist to read these five ECGs and they were excluded from the main analysis.

In summary, the initial period of training on a sample of thirty ECGs demonstrated a median variation of 20ms, which improved to 2ms after further training on a sample of ten ECGs of similar patient type, demonstrating sufficient training had occurred. This quality check met the pre-defined criteria for an independent review of the larger set of 600 ECGs by a non-cardiologist clinician on the basis that those with low amplitude T wave complex (<1mm), noise or wandering baseline (>1mm) would get an over-read. Of the 600 ECGs, 112 met the criteria for needing a cardiology over-read of which sixty were deemed unreadable and five had variability of greater than +/- 20ms. This left 535 ECGs remaining for the full analysis. The over-read by the cardiologist of 112 ECGs showed agreement for 95.54% (107/112) of the ECGs either meeting the unreadable criteria or having a QT difference of <20ms between readers. This again demonstrates sufficient training of the non-cardiologist and adds further weight to the validity of the main results.

Chapter 5: T wave morphology abnormalities from ECG monitoring in STREAM Stage 1

Background

T wave morphology abnormalities have been shown to identify patients with congenital Long QT syndrome. Similar abnormalities have been demonstrated in healthy patients when exposed to a limited duration of drugs that can disrupt the voltage gated potassium channels. Patients in STREAM Stage 1 took a minimum of 9 months treatment for the Short regimen and 20+ months for the Long regimen. Both contained a fluoroquinolone, with clofazimine also present in the Short regimen. These drugs are known to disrupt the *hERG* potassium channels and as a result cause QT prolongation. It is not clear what effect they have on T wave morphology over several months of treatment, whether certain groups are more likely to display T wave abnormalities and whether they could be used to identify patients at risk of severe QT prolongation.

Method

After a period of training with a cardiologist specialising in rhythm disorders, blinded ECGs were selected from three different time points (baseline, early and late) during the first year of treatment for 200 patients; including 142 on the Short regimen and 58 on the Long regimen. All 82 participants who developed clinically relevant QT/QTcF prolongation (≥500ms or increase in ≥60ms from baseline) and had available ECGs were included along with 118/191 who did not, representative of the study population based on country, gender and age.

A manual review was then performed of the T wave morphology in each of the 12 leads for each of the 600 ECGs (7200 leads in total). A retrospective review was

performed, and T-wave morphology categorised as normal or abnormal (notched, asymmetric, flat wave, flat peak or broad). Classification of abnormalities required that they be present in ≥2 adjacent leads in the same ECG. Differences between groups were assessed using Chi Squared test (paired/unpaired, as appropriate) and logistic regression was used to assess differences over time.

Results

At baseline 22.5% (45/200) of participants displayed ≥1 abnormality, increasing to 45% (90/200, p<0.001) at the late time point. A significant increased frequency between baseline and late time-points was observed for notched (1.5% vs 11%, p<0.001); flat peak (0% vs 7%, p<0.001) and broad (2% vs 13%, p<0.001) categories. T-wave abnormalities on treatment were more common in Short regimen ECGs 37.6% (88/234) compared to Long regimen 21.1% (16/76), p = 0.008 and ECGs from participants in the high-risk group 43.8% (56/128) compared with those who were not 26.4% (48/182), p = <0.001. T wave abnormalities occurred prior to the development of a QT/QTcF ≥500ms in 53% (16/30) of participants and prior to the development of a QTcF increase in ≥60ms above baseline in 40% (31/78) of participants.

Conclusion

This chapter demonstrates T-wave morphology abnormalities occurred in nearly half the participants and that differences were observed between regimen, time point and risk group as well as between the frequency of each abnormality seen. The abnormalities observed may allow early detection of patients at risk of clinically relevant QT prolongation.

Background

The STREAM Stage 1 trial found a higher proportion of participants on the Short (study) regimen experienced severe QT prolongation (QT/QTcF ≥500ms or ≥60ms increase from baseline) compared to the Long (control) regimen. The findings from chapter 4 demonstrated that the accuracy of machine readings of the QT interval can be affected by abnormal T wave morphology. Severe QT prolongation can have serious implications for patient safety due to increased risk of tachyarrhythmia such as Torsades de Pointes which can lead to sudden cardiac death in some patients. The diagnosis of acquired Long QT Syndrome (LQTS), caused by drug toxicity, usually relies on measurement of the QT interval alone. In contrast diagnosing congenital LQTS relies on both the QT interval and specific T wave morphology abnormalities that can be indicative of repolarisation pathology. Indeed, some cardiologists feel visual inspection of the T wave is of equal or even greater importance than the QT interval in diagnosing congenital LQTS.[1] Patients carrying genes for congenital LQTS can display normal QT intervals [2,3] making assessment of T wave abnormalities a useful diagnostic tool in helping to prevent development of life-threatening arrhythmias. If T wave morphology abnormalities can be utilised in the same way for predicting which apparently healthy patients are likely to have repolarisation abnormalities during treatment, they could be a very useful tool in the management of acquired LQTS.

There are a number of T wave morphology abnormalities such as notching, asymmetry and flatness that can be used as a biomarker for *hERG* potassium channel blockade [4 - 7] which can lead to QT prolongation and Torsades de Pointes. T wave inversion [8] and biphasic t waves [9] have been associated with congenital LQTS, though are less specific. Presence of U waves can also be a biomarker for

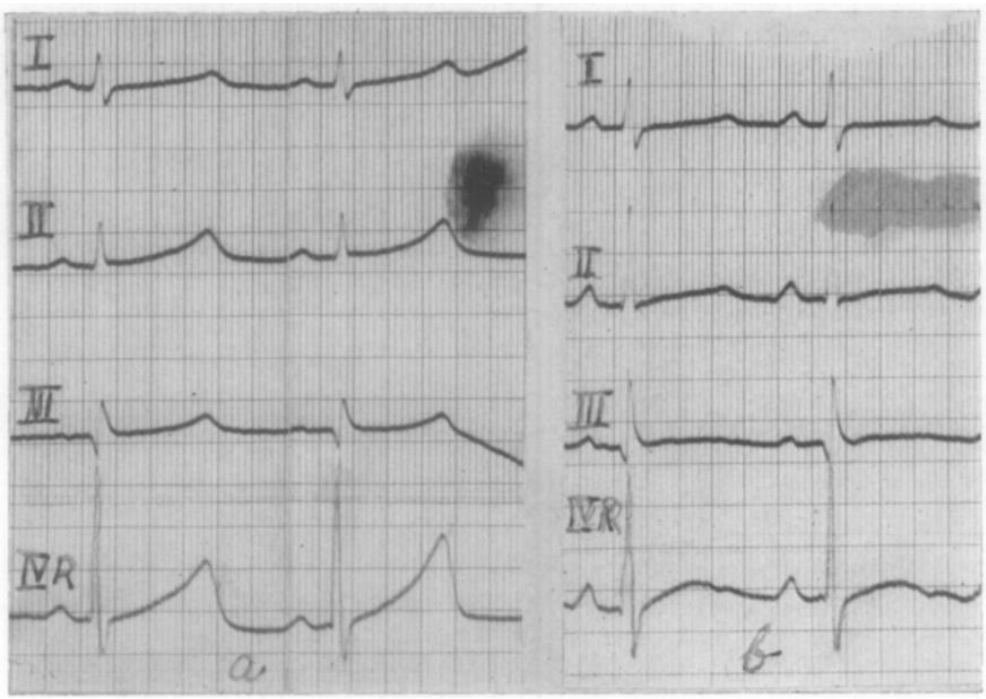
QT prolongation and Torsades de Pointes (10-11) but again is non-specific. If patients at risk of severe QT prolongation can be identified early on treatment from abnormalities in the ECG T wave morphology, then this could have important implications for patient safety.

History of T wave morphology changes (congenital v acquired)

The first description of congenital long QT syndrome was in children from a Norwegian family that included a number of T wave abnormalities prior to their significance being known. In 1957, the Norwegian physicians Drs Anton Jervell and Fred Lange-Nielsen, described deafness and “functional heart disease” in four children from the same family with three of them having died suddenly.(12) With hindsight, a review of their ECGs shows a number of T wave abnormalities associated with disruption of the voltage gated potassium channels including flattening, asymmetry and notching. At the time the significance of the QT interval and abnormal T wave morphology was not appreciated and the link between sudden cardiac death had not been made.

Figure 5.1. Anton Jervell [13] (left) and Fred Lange-Nielsen [14] (right)

Figure 5.2. Abnormal T wave morphology from an ECG 3 months before one of the girls in the family died of sudden cardiac death(12)



Some of the earliest descriptions of T wave morphology changes associated with medications known to prolong the QT interval were of the phenothiazine agent thioridazine. Thioridazine was first developed shortly after chlorpromazine in the 1950's as a typical anti-psychotic agent that had less extra-pyramidal side effects.(15) Whilst it was effective at treating schizophrenic patients there were several adverse effects associated with its use including cardiac arrhythmias leading to sudden death in some cases.(16) Early descriptions were of ECG abnormalities in psychiatric patients taking the drug and included a broadening and flattening of the T wave with reduced amplitude and/or a "double-hump" or notching of the T wave.(17-18) The drug later fell out of favour in psychiatry due its predisposition to cause cardiac arrhythmias when other equally effective anti-psychotic treatments became available and the drug was withdrawn in 2005.(19)

A recently published large review of 270, 039 individuals investigated T wave morphology and risk of mortality.(20) The study demonstrated a higher adjusted mortality hazard ratio for patients with greater abnormal morphology compared to those with less abnormal morphology. The study showed T wave morphology can provide important prognostic information independent of various factors such as QT prolongation.

T wave morphology abnormalities and their relevance to tuberculosis treatment

Around the same time thioridazine was withdrawn (due to availability of alternatives with less cardiac risk) it was being repurposed for the treatment of Extensively Drug-Resistant (XDR) TB when few efficacious drugs were available. In-vitro activity of thioridazine against *Mycobacterium tuberculosis* was demonstrated around a decade before its market withdrawal for the treatment of schizophrenia.(21) Its ability to target TB within macrophages following phagocytosis, independent of the level of multi-drug resistance exhibited, made it an exciting prospect.(22) Prior to the introduction of bedaquiline, delamanid and pretomanid for the treatment of Multi-Drug Resistant (MDR) and XDR-TB, there were calls for clinical trials to evaluate thioridazine.(23) Thioridazine was mentioned under the group 5 drugs in the WHO 2011 MDR-TB guidelines albeit that the evidence was inconclusive, and it was not recommended as part of standard therapy.(24) A small retrospective study of seventeen adult patients with XDR-TB in a single centre in Argentina found 58% (7/12) met the cured definition on a regimen that included thioridazine, linezolid and moxifloxacin with the majority having culture converted after 12 weeks.(25) None of the patients studied developed QT prolongation or adverse cardiac effects with close

monitoring, though information on T wave morphology changes was not available. A review article listing the pros and cons of repurposed drugs for MDR, and XDR-TB highlighted that whilst thioridazine could inhibit growth, kill phagocytosed bacilli within macrophages (due to a concentration effect) and be an adjuvant to other drugs, it was not useful in treating pulmonary cavitatory disease as the concentration needed outside macrophages would not be achieved with standard dosing. The review also highlighted the increased risk of arrhythmias as a limiting factor.(26)

The WHO 2019 guidelines for treatment of drug resistant TB did not mention thioridazine, likely due to a number of newer and more efficacious drugs.(27) Drugs are grouped from A to C in decreasing order of preference. Four of the seven drugs in the first two groups are known to prolong the QT interval (moxifloxacin, levofloxacin, bedaquiline and clofazimine), though less is known about their effect on T wave morphology. Moxifloxacin has been shown to induce T wave changes consistent with disruption of the *hERG* potassium channel. A thorough QT study using moxifloxacin 400mg found the incidence of abnormal T wave morphology increased from 5% after placebo to 9.9% across all time points.(28) Another study was able to show through a computerized ECG technique that subtle T wave morphological changes outperformed QTc measurements in predicting which patients received moxifloxacin in a cohort of healthy individuals.(29) In a cohort of healthy individuals, moxifloxacin was shown to cause subtle changes in cardiac repolarisation that could be detected using a T wave morphology score.(30) Though the above studies suggest moxifloxacin induced T wave morphology changes occur in healthy individuals, not all studies have found conclusive evidence. One study looking at a cohort of healthy volunteers given placebo, moxifloxacin and nalmefene

found that whilst moxifloxacin led to an expected increase in the mean QTc, there was no evidence of an increased likelihood in T wave morphology changes.(31)

T wave morphology abnormalities as a predictive tool and their relevance to STREAM

Whilst many studies use a morphology combination score to evaluate abnormalities of T wave morphology via software such as QT Guard Plus (GE Healthcare), this requires digitalised ECGs which were not available from STREAM Stage 1. Some studies looking at congenital LQTS and T wave morphology have instead relied on manual measurement of T wave pattern, duration and amplitude for each ECG.(33) Others have categorised changes into groups based on visual inspection alone.(33–36) Khositseth et al (36) looked at the effect of adrenaline in inducing T wave morphology abnormalities in patients with known congenital LQTS matched to controls using a single blinded reader. Notched patterns were more common in patients with LQTS-2 after low dose adrenaline (25%) compared with baseline (14%), though an increase was also seen in healthy controls (9%) compared to none at baseline.(36) Chorin et al (35) also used a visual inspection of T wave morphology to measure the effect of “QT stretching” on 100 patients with congenital LQTS and 100 matched controls. Two cardiologists, who were blinded to participant group, classified T wave morphology by consensus with numbers and percentage reported. For LQTS patients the percentage displaying normal T wave morphology decreased from 47% at baseline to 27% at maximum QT stretching. This contrasted with normal T wave morphology in >80% of control patients at baseline that was largely unchanged after the test. (35) Certain specific abnormalities such as notched or

broad T waves, associated with disruption of the *hERG* potassium channel, are rare in the healthy population. Chorin et al [35] described only 1% of healthy controls had these present at baseline and Khositseth et al [36] found no evidence of notching at baseline in their healthy control population.

As the STREAM Stage 1 trial used high dose moxifloxacin, combined with clofazimine over a 9–11-month period in the Short regimen, and patients received regular ECG monitoring over 52 weeks, this is a unique data set to investigate the effects of these drugs on T wave morphology. In addition to being a biomarker for QT prolongation, changes in T wave morphology could also lead to inaccurate calculation of the QT interval based on automated measurements. Measurement of the QT interval relies on determining the end of the T wave which can be problematic when either the isoelectric line is noisy or the shape of the T wave is abnormal, e.g., flat, notched, biphasic or overlapping on a U wave.[37] These are all features of *hERG* potassium channel blockade. In the literature, the term notched, or bifid T waves describe the same type of morphology abnormality and are often used interchangeably. If changes are seen then it will be useful to know whether they occurred before, at the same time or after QT/QTcF prolongation. If found to occur before then they could be a useful predictor of which patients are likely to experience severe QT/QTcF prolongation ≥500ms at a later point. If severe QT/QTcF prolongation is found to occur at the same time as the appearance of T wave abnormalities then the accuracy of automated measurements may be affected as shown in Chapter 4.

The aim of the study described in this chapter is to assess whether T wave morphology abnormalities occurred in STREAM Stage 1 and if so to explore whether

they could be a useful tool to identify patients who later developed severe QT prolongation.

Aims, Hypotheses and objectives

The following aims, hypotheses, and objectives were explored.

Aims

Determine whether any of the T wave morphology abnormalities consistent with hERG potassium channel disruption occurred in STREAM Stage 1 and if so to explore whether there was a relationship between regimen, time on treatment and risk of severe QT prolongation.

Hypotheses

1. T wave morphology abnormalities consistent with hERG potassium channel blockade such as notching, asymmetry, flatness and broadening occurred in STREAM Stage 1

2. These abnormalities were observed more frequently in participants taking the Short regimen compared to the Long regimen and in the high-risk group compared to the low-risk group

3. T wave morphology abnormalities present at baseline were predictive of which participants were more likely to develop clinically-relevant QT prolongation (QT/QTcF of ≥500ms and/or an increase of ≥60ms in QTcF from baseline) at a later point.

4. T wave morphology abnormalities occurred more frequently at the late time point ECGs compared with early or baseline ECGs.

5. When T wave abnormalities occurred, these were prior to the development of *clinically-relevant* QT prolongation in most participants

Objective 1

To demonstrate that T wave morphology abnormalities consistent with hERG potassium channel disruption such as notching, asymmetry, flatness and broadening occurred in STREAM Stage 1.

Objective 2

To assess whether there was any difference in T wave morphology abnormalities between regimen and risk group. Chi-square tests were used to compare groups.

Objective 3

To determine whether T wave morphology abnormalities were present at baseline and if so, can they predict which participants subsequently developed clinically-relevant QT prolongation? Chi-squared tests (paired) were used for this comparison.

Objective 4

To assess whether T wave morphology abnormalities occurred more frequently at the late time point compared with the early or baseline readings. Here, logistic regression was used to assess differences over time. Robust standard errors were calculated accounting for the longitudinal nature of the data.

Objective 5

To determine whether T wave morphology abnormalities present at the early or late time point on treatment, occurred before, at the same time or after the development of clinically-relevant QT prolongation. This was a descriptive analysis only.

Methods

Patient population and selection process for ECGs

All participants in STREAM Stage 1 had regular ECG monitoring up to week 52 of follow-up, with a machine reading of the QT interval being the key cardiac safety measure. Some participants did not have ECGs available at all time points due to reasons such as missed visits, withdrawal from the study or death. Sites were initially asked to send copies of all participants' ECGs to the MRC CTU via email. When it was judged that sites were able to consistently record ECGs of acceptable quality sites were asked to send all baseline ECGs and then targeted ECGs for participants who developed clinically relevant QT prolongation defined as a QT/QTcF of ≥500ms or an increase of ≥60ms in QTcF from baseline.

The patient population and ECGs used are the same as those studied for the validation of machine-reported QT intervals described in chapter 4. As described in chapter 4, all high-risk participants, defined as those that developed clinically relevant QT prolongation, from both regimens with available ECGs at baseline, at least one early time point (weeks 1-4) and at least one late time point (weeks 12, 24 or 36) were included, giving 82 participants. In total, 118/191 low-risk participants (QT/QTcF <500ms and QTcF <60ms above baseline during follow-up to week 52) were selected randomly from both regimens if they had available ECGs at baseline, at least one early time point and at least one late time point, so a comparison could

be made. These low-risk participants were representative of the study population in respect to country, gender and age.

The rationale for choosing the early and late time points described is as follows. Early abnormalities to T wave morphology may be more useful than late abnormalities in terms of a predictive tool. Given the pharmacokinetic properties of moxifloxacin with a T_{max} of 0.75–3.5 hours, it may be possible to see T wave morphology abnormalities appear as early as week 1. Late time points from week 12 and above will allow an investigation of the impact clofazimine has on T wave morphology, as it has a long half-life (approximately 25-34 days), reaching a steady plasma state after 18-21 weeks.

Sample size

The sample of participants and ECGs used for the study described in this chapter has previously been described in the work for chapter 4. The only difference being that all 12 leads in all 600 ECGs were reviewed.

Study procedures

A spreadsheet was created after manually reviewing all 424 participants ECG folders to identify those with available ECGs to review at each time point. A statistician then identified participants from the high and low risk groups who had at least one ECG available at each of the following three time points; baseline, early (weeks 1,2,3 or 4) and late (weeks 12, 24 or 36) and a spreadsheet was generated with a separate list of participants for each group; 82 high-risk and 118 low-risk.

The ECGs were saved into a folder. Information including date, time, trial number and automated machine QT value was redacted by a doctoral research student not involved in the reading of the ECGs. They were then renamed with a new identification number.

The reader was blinded to the participant's original study number, treatment arm, time point and automated QT interval when the ECGs were reviewed, and T wave morphology assessed. Following review of all the ECGs the data was sent back to the statistician and the ECGs unblinded, so that analyses of the data could take place.

A review of ECGs from different time points for each participant allowed an assessment of whether T wave morphology abnormalities occurred early or late in the treatment course. The QT value used to identify high and low risk groups was from the automated machine reading.

As with the analysis for chapter 4, a period of training was undertaken with a cardiologist specialising in electrophysiology before the full set of ECGs were reviewed independently for T wave abnormalities.

The method and results are described separately in the appendix. For quality assurance of the non-cardiologist reader's assessment, a sample of thirty ECGs were read by both readers and compared before an independent assessment of T wave morphology for all 600 ECGs was undertaken. Results (in the appendix) showed 83% agreement with a kappa value of 0.56 between readers for identifying any abnormality.

The method and results of the training are described separately in appendix 5. For quality assurance of the non-cardiologist reader's assessment, a sample of thirty ECGs were read by both readers and compared before an independent assessment of T wave morphology for all 600 ECGs was undertaken. Results (in appendix 5), showed 83% agreement with a kappa value of 0.56 between readers for identifying any abnormality.

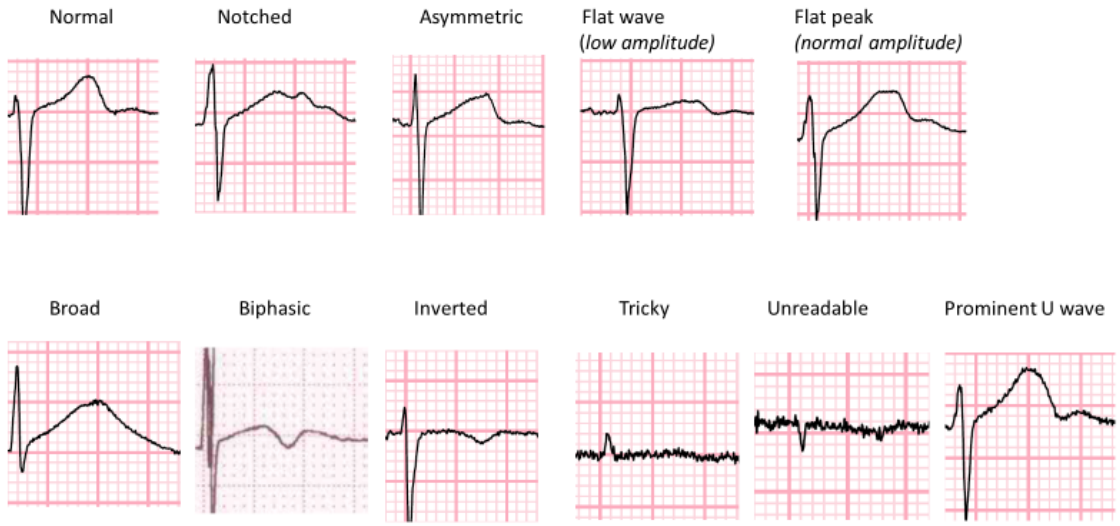
The following T wave abnormalities were assessed for in the full set of 600 ECGs.

1. Normal
2. Notched/Bifid
3. Asymmetric
4. Flat wave (<1mm amplitude)
5. Flat peak (>1mm amplitude)
6. Broad (symmetric with broad base)
7. Biphasic
8. Inverted
9. Tricky (T wave height <1mm, noisy, isoelectric line wandering i.e., >1mm from baseline)
10. Unreadable (low amplitude throughout lead i.e., <1mm QRS complex)

An additional note was made of the presence of any prominent U waves in each of the 12 leads which was defined as >1-2mm or 25% of the T wave amplitude.

On the advice of the cardiologist, it was deemed that notched, asymmetric, flat wave, flat peak and broad T waves would be the most informative categories. These were therefore the focus of the analyses covered in the results.

Figure 3. Examples of the different T wave morphology that were categorised from participants in the STREAM Stage 1 trial



The categorisation of T wave morphology for each lead was reflective of the majority of the complexes. For example, if there were three T wave complexes in each lead but only one was deemed abnormal then the lead was categorised as normal. If two of the three T waves were abnormal and shared the same morphology but one was normal then the lead was categorised as abnormal. Leads in which there were only two T wave complexes were included in the analysis if the morphology was the same.

Following discussion with the cardiologist, an abnormality was only deemed to have been present in the ECG at each time point if the same abnormality was also seen in at least one other adjacent lead as it was felt isolated abnormalities were likely to be of less clinical interest.

After I had reviewed and classified the abnormalities in all 600 ECGs the data was sent to the statistician and unblinded.

The number and percentage (%) of abnormalities present was recorded for all participants at each of the three time points but had to be present in at least ≥2 adjacent leads in the same ECG to be counted. Few ECGs had either entirely normal or entirely abnormal (of the same category) T waves in each of the 12 leads, so some ECGs could be included in more than one of the categories.

Participants were then divided by Short regimen versus Long regimen and high-risk versus low-risk and the number of abnormalities were then compared. This was repeated for each of the three time points.

The analyses differed for each of the five objectives.

Objective 1 - To demonstrate that T wave morphology abnormalities consistent with hERG potassium channel disruption such as notching, asymmetry, flatness and broadening occurred in STREAM Stage 1.

All participants and ECG readings from each of the three time points were included to demonstrate whether the specific T wave abnormalities discussed earlier were present or not. For the purposes of the analyses ECGs with leads that displayed biphasic, inverted, tricky, unreadable T waves or had U waves present were deemed "normal" if they did not display notched, asymmetric, flat wave, flat peak or broad T waves.

Objective 2 - To assess whether there was any difference in T wave morphology abnormalities between regimen and risk group.

The analysis for objective 2 excluded all baseline readings as participants were not on treatment so any differences observed at that time point would have been due to chance. ECGs were removed from all time points if any of the participants had that particular abnormality present at baseline e.g., for the analysis looking at notched T waves, this was restricted to participants who didn't have these present at baseline, so the denominator differs between the analysis of each abnormality. A comparison of the total number of ECGs on treatment in the analysis would mean the observations were not independent i.e., a patient could provide two abnormal ECGs on treatment (early and late). To overcome this a patient comparison was performed as opposed to comparison of ECGs only. Differences between participants displaying T wave abnormalities at early and/or late time point ECGs were compared between regimen and risk groups.

Objective 3 - To determine whether T wave morphology abnormalities were present at baseline and if so, can they predict which participants subsequently developed clinically relevant QT prolongation?

Only baseline ECGs were included in the analysis to predict whether presence of abnormalities before treatment had started could predict later risk of developing *clinically relevant* QT prolongation.

Objective 4 - To assess whether T wave morphology abnormalities occurred more frequently at the late time point compared with the early or baseline readings.

Analyses included readings from all three time points. Each participant was assessed as to which (if any) of the T wave morphology abnormalities were present at each time point to see how these changed over time and whether the abnormalities observed at each time point were for the same or different participants.

Objective 5 - To determine whether T wave morphology abnormalities present at the early or late time point on treatment, occurred before or after the development of clinically relevant QT prolongation.

The descriptive analysis for objective five was restricted to high-risk participants from both regimens comparing the timing of their severe QT prolongation and the timing of any T wave morphology abnormality that may have occurred.

Results

In total, 600 ECGs were reviewed at the three time points for the same 200 participants; the 200 participants included 118 in the low-risk group and 82 in the high-risk group. They were further grouped by regimen with 58 having taken the Long regimen and 142 the Short regimen. Of the 82 high-risk participants, 30 developed QT or QTcF prolongation ≥500ms (25 Short regimen: 5 Long regimen) and 78 developed a ≥60ms increase in their QTcF from baseline (72 Short regimen: 6 Long regimen).

Objective 1:

To demonstrate that T wave morphology abnormalities consistent with hERG potassium channel disruption such as notching, asymmetry, flatness and broadening occurred in STREAM Stage 1.

Table 5.1. Summary of T wave morphology categories for all ECGs reviewed.

	Normal	Any abnormality	Notched	Asymmetric	Flat wave	Flat peak	Broad	Total ECGs
N (%)	405 (67.5)	195 (32.5)	29 (4.8)	73 (12.2)	70 (11.6)	19 (3.2)	42 (7)	600

Abnormalities consistent with voltage gated potassium channel disruption were demonstrated to have occurred in some participants at least once in one of the three time points. Of the 600 ECGs reviewed, 32.5% (n=195) had evidence of at least one

of the five abnormalities in at least ≥2 adjacent leads in the same ECG (Table 5.1 and Figure 5.4).

Figure 5.4. Summary of T wave morphology categories for all ECGs reviewed.

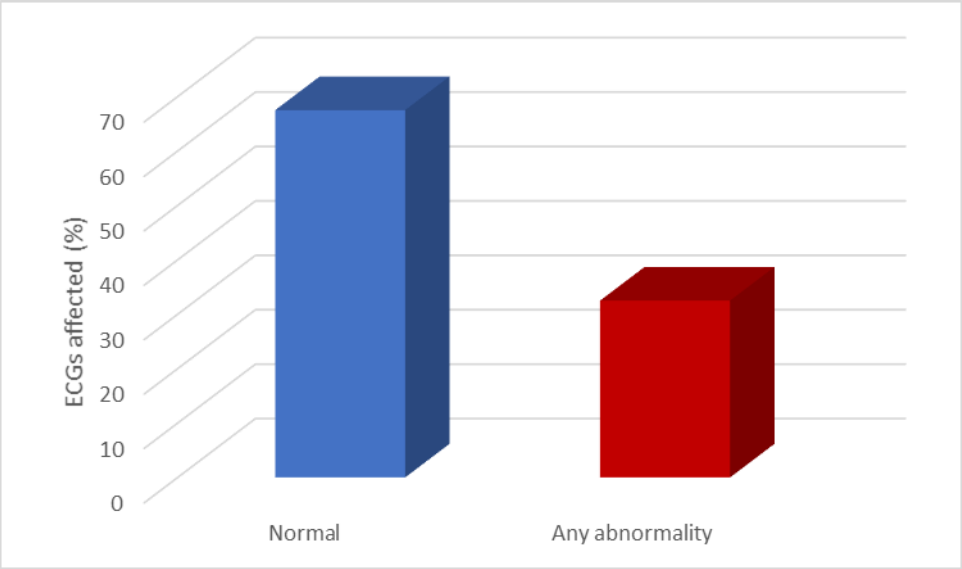
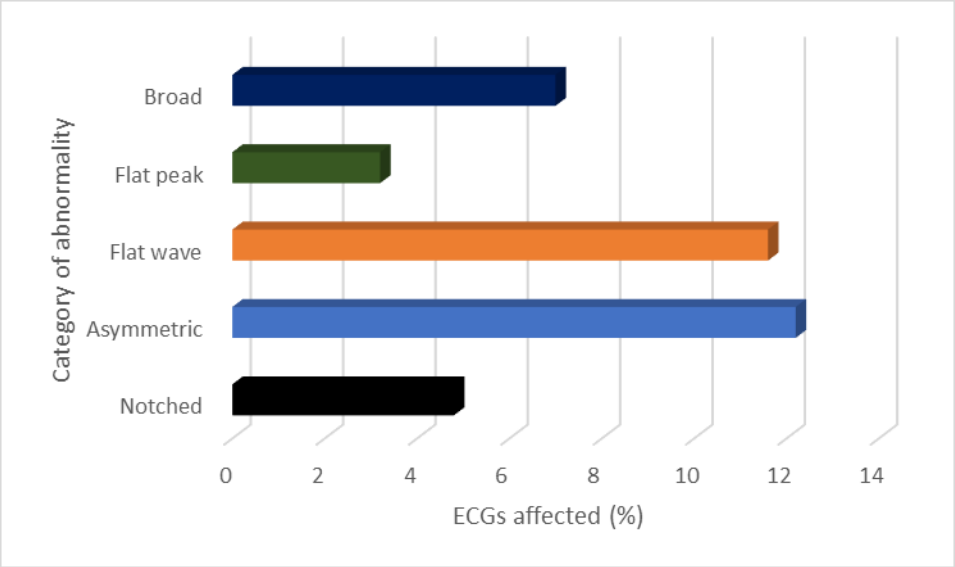


Figure 5.5 summarises these 195 abnormalities by category. Asymmetric T waves were the most frequent abnormality with 12.2% (73/600) of ECGs affected, followed by flat waves with 11.6% (70/600) of ECGs affected. Flat peaked T waves were the least frequent category with 3.2% (19/600) of ECGs having shown evidence of these at any of the time points. As some ECGs had more than one abnormal category the total number and % when individual categories are added together exceeds that of any abnormality as some ECGs could be counted more than once.

Figure 5.5. Summary of abnormal T wave morphology by categories for all ECGs reviewed.



Objective 2

Investigate whether there was any difference in T wave morphology abnormalities between regimen and risk groups

Regimen group

Table 5.2. Comparison of T wave abnormalities in regimen groups

	Regimen		P
	N=38 patients	N=117 patients	
	Long (N=76 ECG)	Short (N=234)	
Normal	60 (78.9%)	146 (62.4%)	
Any abnormal	16 (21.1%)	88 (37.6%)	
Patients with at least 1 abnormality (%*)	14 (36.8%)	75 (64%)	0.003
	Long (N=112 ECG)	Short (N=282 ECG)	
	N=56	N=141	
Normal or non-notched abnormality	107 (95.5%)	262 (92.9%)	
Notched	5 (4.5%)	20 (7.1%)	
Patients with at least 1 notched (%*)	5 (8.9%)	20 (14.8%)	0.317
	N=47	N=130	
	Long (N=94)	Short (N=260)	
Normal or non-asymmetric abnormality	89 (94.7%)	227 (87.3%)	
Asymmetric	5 (5.3%)	33 (12.7%)	
Patients with at least 1 asymmetric (%*)	5 (10.6%)	31 (28.9%)	0.054
	N=52	N=130	
	Long (N=104)	Short (N=260)	
Normal or non-flat wave abnormality	97 (93.3%)	232 (89.2%)	
Flat wave	7 (6.7%)	28 (10.8%)	
Patients with at least 1 flat wave (%*)	6 (11.5%)	26 (20.0%)	0.176

	N=58	N=142	
	Long (N=116)	Short (N=284)	
Normal or non-flat peak abnormality	115 (99.1%)	266 (93.7%)	
Flat peak	1 (0.9%)	18 (6.3%)	
Patients with at least 1 flat peak (%*)	1 (2%)	18 (13%)	0.017
	N=56	N=140	
	Long (N=112)	Short (N=280)	
Normal or non-broad abnormality	106 (94.6%)	249 (88.9%)	
Broad	6 (5.4%)	31 (11.1%)	0.121
Patients with at least 1 broad (%*)	6 (10.7%)	28 (20.0%)	

*Percentage of N patients per group (column)

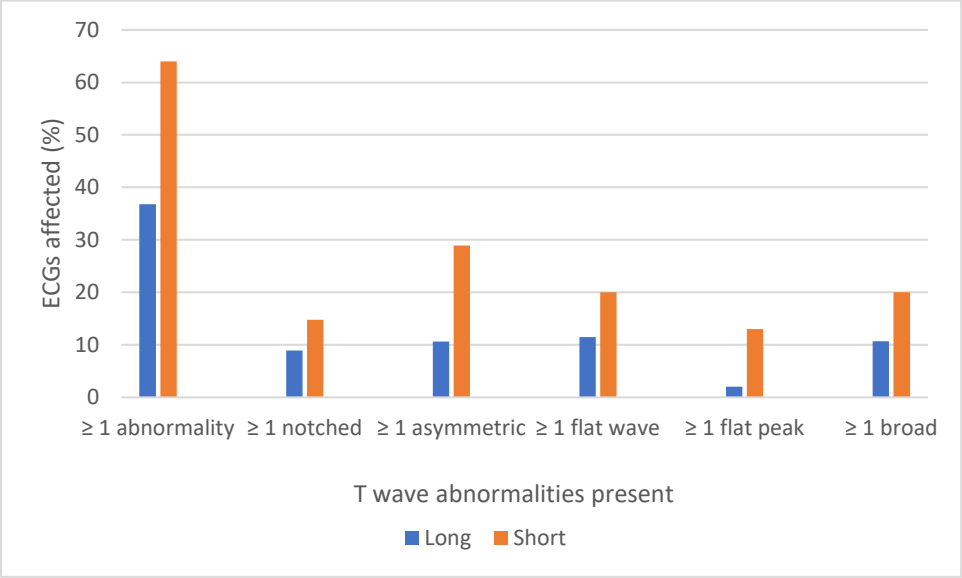
A comparison of abnormalities between participants taking each regimen is shown in table 5.2. The first two rows in each section indicate the numbers of ECGs and the third row indicates the number of patients with at least one of the relevant abnormalities. To begin, a comparison of any of the five abnormalities being present was undertaken, followed by a breakdown of each abnormality individually.

For the population that included those with any abnormality that occurred on treatment, a difference between regimens was observed with 36.8% (14/38) of Long regimen participants having demonstrated at least one of the five abnormalities in ≥2 adjacent leads in the same ECG compared with 64% (75/117), p = 0.003 of Short regimen participants.

When each of the specific abnormalities was analysed individually, only the asymmetric and flat peak T waves showed a significant difference between regimens. There were 10.6% (5/47) of Long regimen participants that developed

asymmetric T waves on treatment compared with 28.9% (31/130), p = 0.054 on the Short regimen. For the flat peak category, 2% (1/58) of Long regimen participants developed the abnormality compared with 13% (18/142), p = 0.017 of Short regimen participants. Figure 5.6 illustrates the differences seen between regimen groups.

Figure. 5.6 Comparison of T wave abnormalities in regimen groups



A significant difference was also observed between risk groups with 47.3% (43/91) low-risk participants having demonstrated T wave abnormalities versus 74% (46/64), p = 0.002 of high-risk participants (Table 5.3).

When the specific abnormalities were compared between risk groups, a significant difference was seen for notched and broad categories. Notched T waves were seen in 7.8% (9/115) of participants in the low-risk group compared with 19.8% (16/81), p = 0.013 of participants in the high-risk group. Broad T waves were seen in 12.1% (14/116) of participants in the low-risk group compared to 25% (20/80), p = 0.019 in the high-risk group. These findings are illustrated in Figure 5.7.

Risk group

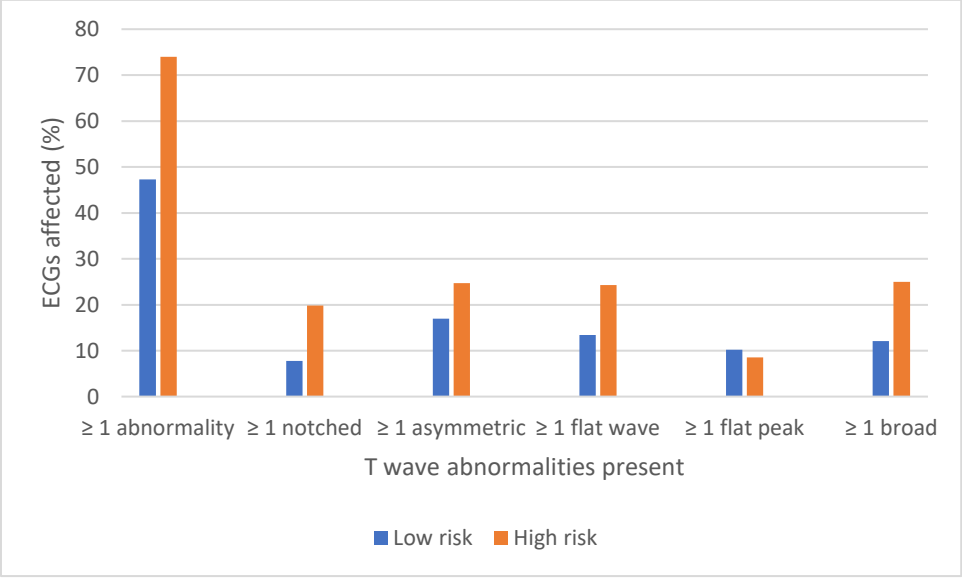
Table 5.3. Comparison of T wave abnormalities by risk groups

	Risk		P
	N= 91 patients	N=64 patients	
	Low (N=182)	High (N=128)	
Normal	134 (73.6%)	72 (56.3%)	
Any abnormality	48 (26.4%)	56 (43.8%)	
Patients with at least 1 abnormality (%*)	43 (47.3%)	46 (74%)	**0.002**
	N=115	N=81	
	Low (N=232)	High (N=162)	
Normal or non-notched abnormality	223 (96.1%)	146 (90.1%)	
Notched	9 (3.9%)	16 (9.9%)	
Patients with at least 1 notched (%*)	9 (7.8%)	16 (19.8%)	**0.013**
	N=100	N=77	
	Low (N=200)	High (N=154)	
Normal or non-asymmetric abnormality	181 (90.5%)	135 (87.7%)	
Asymmetric	19 (9.5%)	19 (12.3%)	
Patients with at least 1 asymmetric (%*)	17 (17.0%)	19 (24.7%)	0.209
	N=112	N=70	
	Low (N=224)	High (N=140)	
Normal or non-flat wave abnormality	208 (92.9%)	121 (86.4%)	
Flat wave	16 (7.1%)	19 (13.6%)	
Patients with at least 1 flat wave (%*)	15 (13.4%)	17 (24.3%)	0.06
	N=118	N=82	
	Low (N=236)	High (N=164)	

Normal or non-flat peak abnormality	224 (94.9%)	157 (95.7%)	
Flat peak	12 (5.1%)	7 (4.3%)	
Patients with at least 1 flat peak (%*)	12 (10.2%)	7 (8.54%)	0.698
	N=116	N=80	
	Low (N=232)	High (N=160)	
Normal or non-broad abnormality	218 (94.0%)	137 (85.6%)	
Broad	14 (6.0%)	23 (14.4%)	**0.019**
Patients with at least 1 broad (%*)	14 (12.1%)	20 (25%)	

*Percentage of N patients per group (column)

Figure. 5.7 Comparison of T wave abnormalities in risk groups



Objective 3 - To determine whether T wave morphology abnormalities were present at baseline and if so, can they predict which participants subsequently developed clinically relevant QT prolongation?

Table 5.4. Abnormalities present in baseline ECGs for all participants by risk group

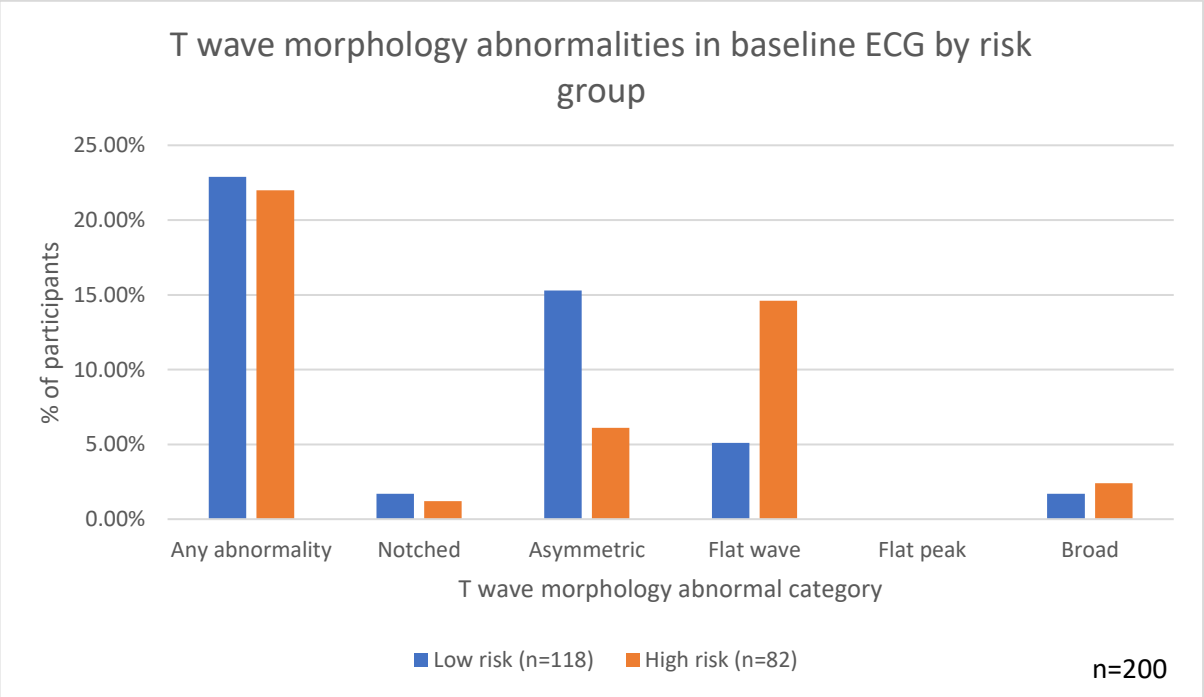
	Baseline ECG		
	Low risk (n=118)	High risk (n=82)	P value
Normal	91 (77.1%)	64 (78%)	
Any abnormality	27 (22.9%)	18 (22%)	0.877
Normal	116 (98.3%)	81 (98.8%)	
Notched	2 (1.7%)	1 (1.2%)	0.786
Normal	100 (84.7%)	77 (93.9%)	
Asymmetric	18 (15.3%)	5 (6.1%)	**0.046**
Normal	112 (94.9%)	70 (85.4%)	
Flat wave	6 (5.1%)	12 (14.6%)	**0.020**
Normal	118 (100%)	82 (100%)	
Flat peak	0 (0%)	0 (0%)	n/a
Normal	116 (98.3%)	80 (97.6%)	
Broad	2 (1.7%)	2 (2.4%)	0.712

In total, 22.9% (27/118) of low-risk participants had ≥1 of the five abnormalities at baseline versus 21.95% (18/82) p=0.877 of high-risk participants. Few low or high-

risk participants had evidence of notched or broad T waves at baseline and no participant had evidence of a flat T wave peak with normal wave amplitude at baseline (Table 5.4, Figure 5.8).

For asymmetric T waves, 15.3% (18/118) of low-risk participants had these present at baseline versus 6.1% (5/82) p=0.046 for high-risk participants. Flat T waves of low amplitude were present in 5.1% (6/118) of the low-risk group compared with 14.6% (12/82) p=0.020 for the high-risk group.

Figure 5.8. T wave morphology abnormalities present in baseline ECGs divided by low and high-risk group



Objective 4

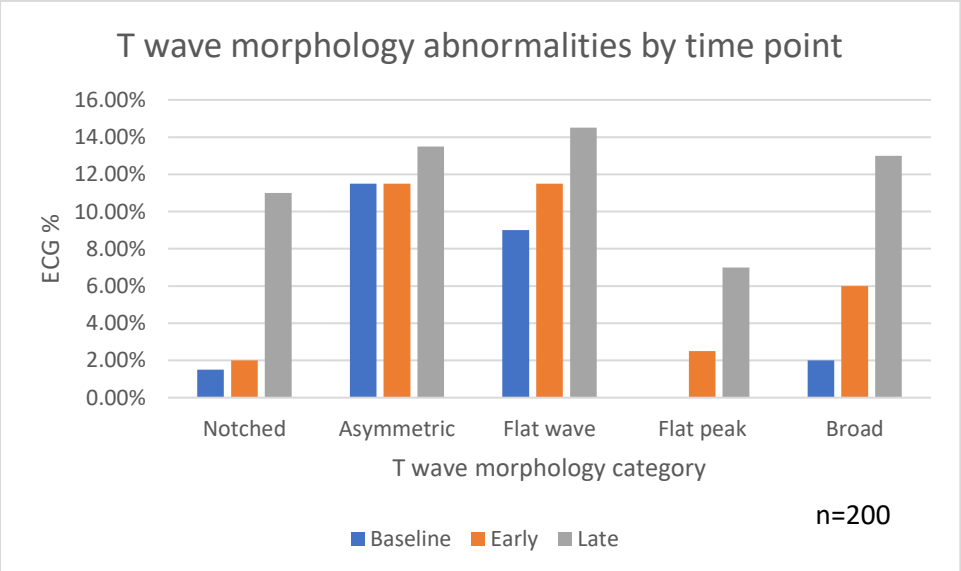
To assess whether T wave morphology abnormalities occurred more frequently at the late time point compared with the early or baseline readings

Table 5.5. Summary of T wave morphology abnormalities at each of the three time-points

	ECG time point			P value
	Base (n=200)	Early (n=200)	Late (n=200)	
Normal	155 (77.5%)	140 (70.0%)	110 (55.0%)	**<0.001**
Any abnormal	45 (22.5%)	60 (30.0%)	90 (45.0%)	
Normal or non-notched abnormality	197 (98.5%)	196 (98.0%)	178 (89.0%)	**<0.001**
Notched	3 (1.5%)	4 (2.0%)	22 (11.0%)	
Normal or non-asymmetric abnormality	177 (88.5%)	177 (88.5%)	173 (86.5%)	0.779
Asymmetric	23 (11.5%)	23 (11.5%)	27 (13.5%)	
Normal or non-flat wave abnormality	182 (91.0%)	177 (88.5%)	171 (85.5%)	0.230
Flat wave	18 (9.0%)	23 (11.5%)	29 (14.5%)	
Normal or non-flat peak abnormality	200 (100.0%)	195 (97.5%)	186 (93.0%)	**<0.001**
Flat peak	0 (0.0%)	5 (2.5%)	14 (7.0%)	
Normal or non-broad abnormality	196 (98.0%)	188 (94.0%)	174 (87.0%)	**<0.001**
Broad	4 (2.0%)	12 (6.0%)	26 (13.0%)	

Table 5.5 shows that at baseline 22.5% (45/200) of participants had at least one of the five abnormalities present in ≥2 adjacent leads in the same ECG, which increased to 30% (60/200) at the early time point and 45% (90/200), p<0.001 at the late time point. This pattern of increase with time on treatment was observed in all of the abnormality categories with the exception of the asymmetric T waves which were most prevalent on late ECGs but present in equal numbers on baseline and early ECGs. The notched pattern increased from 1.5% (3/200) at baseline to 11% (22/200), p<0.001 at the late timepoint. The flat peaked (0% to 7% (14/200), p<0.001) and the broad category T waves (2% (4/200) to 13% (26/200), p<0.001) also demonstrated a significant increase between baseline and late time point ECGs. A non-significant increase between baseline and late time points was seen for asymmetric (11.5% (23/200) to 13.5% (27/200), p = 0.779) and flat T wave with low amplitude (9.0% (18/200) to 14.5% (29/200), p = 0.230).

Figure 5.9. T wave morphology abnormality by time point showing percentage of ECGs affected



The graph in Figure 5.9 illustrates how the notched, flat wave, flat peak and broad T wave abnormalities increased in frequency during time on treatment, being most

likely to be present at the late time point ECG. The asymmetric T wave abnormality showed less of a difference between time points, though was still present in more ECGs at the late time point compared to the early and baseline points.

Table 5.6. T wave abnormalities present across the three time points

	Any	Notched	Asymmetric	Flat wave	Flat peak	Broad
Type	Count (n=134)	Count (n=28)	Count (n=59)	Count (n=50)	Count (n=19)	Count (n=38)
Baseline only	14 (10.4%)	2 (7.1%)	12 (20.3%)	5 (10.0%)	0 (0.0%)	3 (7.9%)
Early only	26 (19.4%)	4 (14.3%)	14 (23.7%)	13 (26.0%)	5 (26.3%)	9 (23.7%)
Late only	48 (35.8%)	21 (75.0%)	20 (33.9%)	16 (32.0%)	14 (73.7%)	22 (57.9%)
Baseline and early	4 (3.0%)	0 (0.0%)	6 (10.2%)	3 (6.0%)	0 (0.0%)	0 (0.0%)
Baseline and late	12 (9.0%)	1 (3.6%)	4 (6.8%)	6 (12.0%)	0 (0.0%)	1 (2.6%)
Early and late	15 (11.2%)	0 (0.0%)	2 (3.4%)	3 (6.0%)	0 (0.0%)	3 (7.9%)
All timepoints	15 (11.2%)	0 (0.0%)	1 (1.7%)	4 (8.0%)	0 (0.0%)	0 (0.0%)

In contrast to table 5.5, which showed the total number of T wave abnormalities at each time point, table 5.6 summarises each participant across the three time points to show whether they displayed the same abnormality at each of the three time points, two of the three time points or one of them and whether it was present at baseline, the early or the late ECG. The first column indicates whether any of the five abnormalities were present rather than the same type which is shown in the remaining columns.

Looking at whether any of the abnormalities were present, 66% (88/134) of participants had evidence of an abnormality at a single reading (either baseline, early

or late) with only 23% (31/134) of participants having abnormalities across multiple time points and 11% (15/134) across all three.

A similar pattern was seen when broken down by individual T wave morphology abnormality, with higher numbers having the abnormality on a single ECG (most frequently the late time-point ECG) and fewer participants displaying them across multiple time points or all three ECGs.

For the notched T wave category, 75% (21/28) of participants had evidence of these on the late ECG only with only one participant displaying them on more than one time point and none across all three.

In the asymmetric and flat T wave categories, most participants displayed them on ECGs at single time points with more present on the late time point.

For asymmetric T waves, 20% (12/59) of participants displayed these across multiple time points, with only one participant displaying them across all three. In the Flat T wave category, 26% (13/50) of participants had these present across multiple time points, and in all three time points in four participants.

Flat peak T waves were the least frequently seen category and only present at early or late time points in five and fourteen participants respectively. They were not seen across multiple time points for any participant.

In common with other categories, broad T waves were seen more frequently at the late time point. Only four participants had these present across multiple time points and none across all three.

Objective 5 - To determine whether T wave morphology abnormalities present at the early or late time point on treatment, occurred before or after the development of clinically relevant QT prolongation.

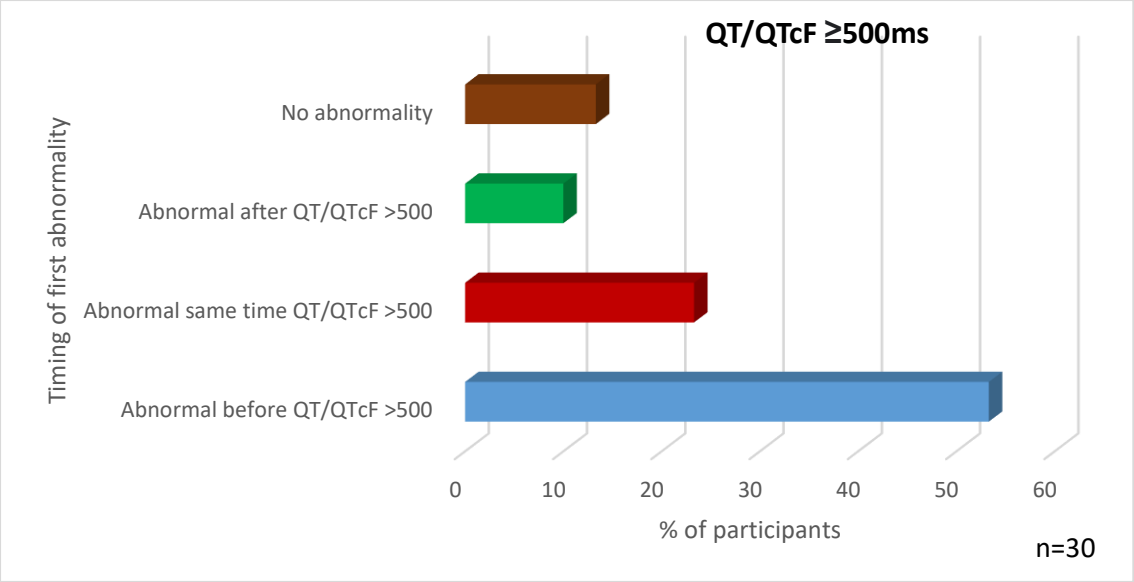
QT/QTcF ≥500ms

Table 5.7. Timing of T wave morphology abnormality in relation to development of QT/QTcF prolongation ≥500ms.

	Total participants (n=30)	
Timing	Participants	%
Abnormal before QT/QTcF >500	16	53.3
Abnormal same time QT/QTcF >500	7	23.3
Abnormal after QT/QTcF >500	3	10
No abnormality	4	13.3

In total, 53% (16/30) of participants who reached a QT/QTcF ≥500ms had evidence of T wave morphology abnormalities prior to reaching this threshold (Table 5.7 and Figure 5.10). The second highest proportion, 23% (7/30) were those who developed T wave morphology abnormalities at the same time they recorded their first episode of QT/QTcF prolongation ≥500ms. A minority had already reached a 500ms threshold by the time they had evidence of abnormal T wave morphology and a similar proportion had recorded a QT/QTcF ≥500ms with no evidence of T wave morphology abnormality on the ECGs that were reviewed.

Figure 5.10. Timing of T wave morphology abnormality in relation to development of a QT/QTcF ≥500ms.



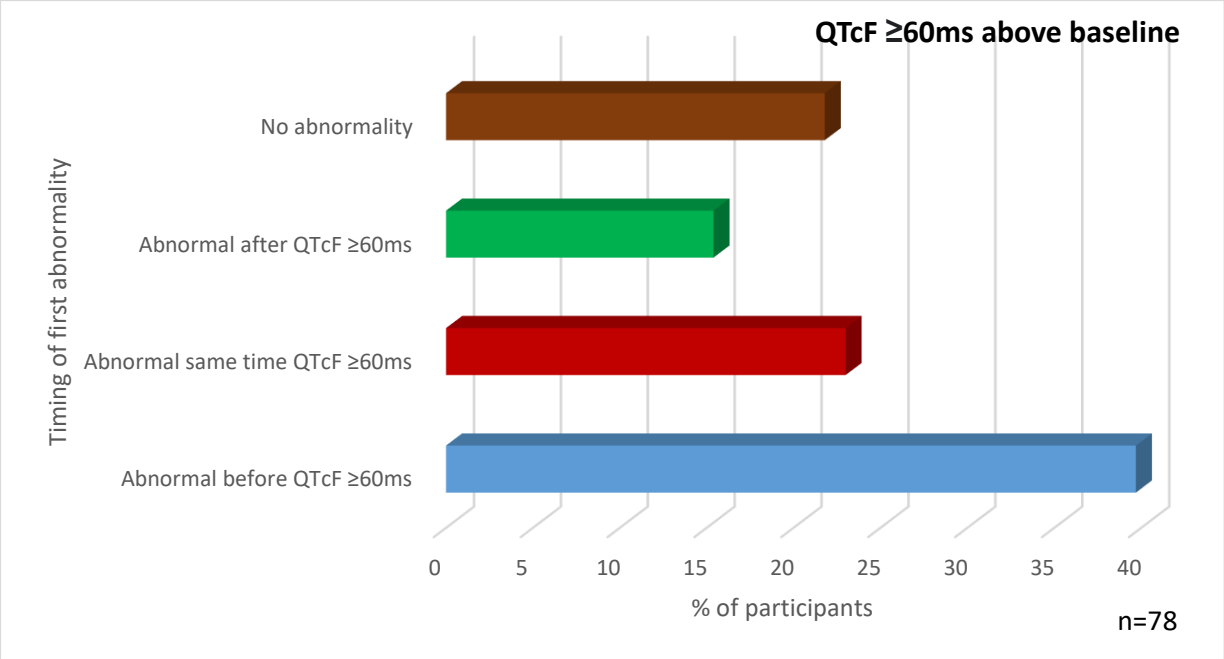
≥60ms above baseline

In the analysis looking at timing of T wave abnormalities in relation to development of a QTcF ≥60ms above baseline, the largest proportion of participants (39.7% (31/78) developed T wave abnormalities prior to reaching the QTcF threshold. The second largest proportion (23% (18/78), developed T wave abnormalities at the same time they reached the QTcF threshold, and the smallest proportion were those who developed the abnormalities after reaching the threshold. A number of participants (21.8% (17/78) had no evidence of T wave morphology abnormalities despite reaching a QTcF ≥60ms above baseline.

Table 5.8. Timing of T wave morphology abnormality in relation to development of a QTcF ≥60ms above baseline.

	Total participants (n=78)	
Timing	Participants	%
Abnormal before QTcF ≥60ms	31	39.7
Abnormal same time QTcF ≥60ms	18	23
Abnormal after QTcF ≥60ms	12	15.4
No abnormality	17	21.8

Figure 5.11. Timing of T wave morphology abnormality in relation to development of a QTcF ≥60ms above baseline.



Discussion

Objective 1

To demonstrate that T wave morphology abnormalities consistent with hERG potassium channel disruption such as notching, asymmetry, flatness and broadening occurred in STREAM Stage 1.

The association between certain T wave morphology abnormalities (e.g., notched, asymmetric and flat) is well established in congenital long QT syndrome.(7, 12, 36) The same abnormalities have also been established in acquired long QT syndrome, albeit to a lesser extent.[29-30] To the best of my knowledge nobody has demonstrated these abnormalities in a population with multi-drug resistant tuberculosis who received continuous treatment for a minimum of 9-months.

The abnormalities of interest were found in a third of ECGs reviewed, though some occurred more frequently than others e.g., asymmetric and flat T waves, both of which are commonly used in the morphology combination score to assess abnormal T waves in congenital and acquired QT prolongation.[12, 29, 30, 36]

Objective 2

To assess whether there was any difference in T wave morphology abnormalities between regimen and risk group.

After demonstrating that the abnormalities specific to voltage gated potassium channel disruption had occurred, I wanted to investigate whether there were any significant differences between participants. Firstly, were there any differences in T wave morphology abnormalities in participants taking the Short regimen compared

with those taking the Long regimen? Secondly, were there any differences between participants in the high-risk group, who we know went on to develop clinically relevant QT/QTcF prolongation compared with those in the low-risk group, who we know didn't?

A significantly higher proportion of participants on the Short regimen developed T wave abnormalities on treatment compared with those participants on the Long regimen. The majority of participants from both regimens had no evidence of the specific T wave abnormalities. Whilst all of the five specific T wave abnormalities occurred more frequently in Short regimen participants compared with Long regimen participants, there was a 2:1 randomisation in the trial in favour of the Short regimen which also had a higher proportion of participants that developed QT/QTcF prolongation. This meant there were a higher number of participants and ECGs included for this analysis who had taken the Short regimen compared with the Long regimen. Despite this, there was still a significantly higher proportion of participants taking the Short regimen who developed asymmetric and flat peak T waves compared with Long regimen participants which may indicate these changes are specifically related to clofazimine and higher dose moxifloxacin contained in the Short regimen.

Focusing on the risk groups, a significantly larger proportion of participants in the high-risk group had evidence of T wave abnormalities compared to the low-risk group. There were differences in the specific types of abnormality with a significantly larger proportion of participants ECGs in the high-risk group having notched and broad T waves compared with participants in the low-risk group.

The analysis showed that many ECGs from those in the high-risk group had no evidence of T wave abnormalities on treatment which suggests not everyone who develops QT/QTcF prolongation >500ms or >60 ms above baseline will have these. This is important as it may limit the usefulness of monitoring T waves in patients on treatment and lead to false reassurance that they are not at risk if the T waves are “normal”. The analysis also showed a number of participants in the low-risk group had evidence of abnormal T waves which could again limit the usefulness of monitoring T waves as some patients will undergo unnecessary monitoring which could be a burden on resources and cause undue anxiety.

As notched and broad T waves occurred significantly more frequently in high-risk participants than low-risk but not in Short regimen versus Long regimen participants, this suggests these abnormalities are more specific to identify clinically relevant QT/QTcF prolongation irrespective of regimen and may be due to the fluoroquinolone present in each regimen rather than clofazimine and could also possibly indicate some element of congenital long QT syndrome.

The asymmetric and flat-peak T waves appeared to be more specific to the Short regimen rather than those developing clinically relevant QT/QTcF prolongation given there was a significant difference between regimen but not risk-group and may be related to the high dose fluoroquinolone in combination with clofazimine that the Short regimen participants received.

Objective 3

To determine whether T wave morphology abnormalities were present at baseline and if so, can they predict which participants subsequently developed clinically relevant QT prolongation?

If abnormalities in T wave morphology are present in baseline ECGs of high-risk participants prior to development of clinically relevant QT prolongation, this may imply a pre-existing or congenital abnormality in the voltage gated potassium channels. This would be useful to know in advance of starting treatment as these patients could be identified early and receive more frequent monitoring or adjusted treatment regimens. Abnormalities in T waves at baseline could also confound any differences seen between risk groups later on.

The number of abnormalities at baseline was less frequent compared with later time-points as described in analyses for some of the other objectives. Nearly a quarter of all ECGs across both risk groups had at least one of the abnormalities present in ≥2 adjacent leads in their baseline ECG.

Comparison between the two risk groups showed no significant difference between the frequency of abnormalities at baseline when any of them were present.

Asymmetric T waves were significantly more common in low-risk participants than high-risk participants at baseline, which was unexpected. Flat T waves were significantly more common in the high-risk versus low-risk participants. Few baseline ECGs from either group showed notched or broad T waves and there was no significant difference between groups. Flat peak T waves were not found on any of the 200 baseline ECGs, which suggests these may only occur on treatment.

Objective 4

To assess whether T wave morphology abnormalities occurred more frequently at the late time point compared with the early or baseline readings.

Analysis of T wave abnormalities across time points, confirmed the hypotheses that they would be more frequent on the late time point ECGs compared with the early or baseline ECGs. There were significant differences between some of the specific abnormalities with notched, flat peak and broad T waves having occurred more frequently on the late ECGs compared to baseline ECGs.

When the ECGs for each individual participant were analysed across time points, the highest proportion with any of the abnormalities was at the late time point only. The notched, flat peak and broad T waves were most frequently present at the late time point only. A minority of participants had the abnormalities present on all 3 of their ECGs across time points and few had the abnormalities present on their baseline ECG only.

Both sets of analyses showed that abnormalities did seem to evolve on treatment which suggests the longer patients were on treatment the greater the disruption of their voltage gated channels.

Objective 5

To determine whether T wave morphology abnormalities present at the early or late time point on treatment, occurred before, at the same time or after the development of clinically relevant QT prolongation.

Of all the objectives, the final was perhaps the most important. If T wave morphology abnormalities occurred in the ECG after a participant had already developed clinically relevant QT prolongation then its use in monitoring would be fairly limited. However, if it could be demonstrated that these abnormalities occurred early in follow-up prior to the development of QT prolongation above the thresholds described, then it could be a very useful finding.

Of the 30 participants that reached a QT/QTcF ≥500ms, 53% (16/30) had evidence of T wave abnormalities in an ECG before they reached the 500ms threshold. This suggests the abnormalities, indicative of disruption to the voltage gated potassium channels, may allow identification of most patients at risk, early in their treatment course at which point increased monitoring or adjustments to their regimen could be made before they reach a point where they are at increased risk of cardiac arrythmia.

The second largest proportion of participants (23%) developed T wave abnormalities on the same ECG in which a QT/QTcF ≥500ms had been reached for the first time.

It is possible that the 14 participants who did not develop early T wave abnormalities had been missed due to the selection of only 3 ECGs and perhaps if more had been reviewed there would have been evidence that these abnormalities had occurred.

For the 78 participants that developed an increase of ≥60ms in their QTcF from baseline reading, the largest proportion (40%) had evidence of abnormal T waves before they reached this threshold. Although the risk of cardiac arrythmias is considered lower in those reaching the 60ms threshold compared to 500ms, the analyses showed that a sizeable proportion of participants still had evidence that the

medications were likely causing disruption to the voltage gated channels before they had reached a 60ms increase in their QTcF.

As the number of high-risk participants on the Long regimen was small in comparison to the Short regimen, analyses between regimens were limited.

Guidance for sites and clinicians from this work

This work has shown that T wave morphology abnormalities may be a useful tool in monitoring cardiac safety for patients on MDR-TB treatment. As the majority of clinicians involved in managing TB patients will have a background in respiratory medicine or infectious diseases, they are likely to be aware of some abnormalities like biphasic and inverted T waves but may not have come across some of the specific abnormalities linked to voltage gated channel disruption which are described in this work. Therefore, they would need to liaise with a cardiologist who has an interest in rhythm disorders and is local to their site of practice, so they could undergo training before it could be taken any further.

As a clinician with experience of managing TB but with no specific cardiology background, I have demonstrated that it is possible (with around 3 sessions of 15 hours in total) to be trained up to identify the T wave morphology abnormalities described using a simple visual manual method. Advanced computer programs (if available) may also be an option if trained appropriately to use them.

It was clear that in some patients the specific abnormalities occurred early in treatment prior to the development of clinically relevant QT prolongation. If clinicians see these abnormalities they should increase the monitoring frequency of their patients and check for electrolyte (potassium, magnesium, calcium) imbalance and

thyroid function tests which may be exacerbating the problem. If these are all normal but the problem persists and QT prolongation worsens, treatment modification is likely to be needed, with interruption, dose reduction and drug changes all potentially needed as described in chapter 3. As alluded to in chapter 4, T wave morphology can affect the accuracy of the machine's ability to calculate the QT interval and lead to an overestimation. The guidance from this chapter would be that clinicians pay close to attention to the T wave morphology categories described, particularly when the machine reading produces a high QT interval close to 500ms or greater, at which point manual calculation would be preferable using the methods described in chapter 4. Early liaison with a cardiologist for review of ECGs and advice would also be sensible.

Limitations

This study had limitations.

First, machine readings of the QT interval were used to decide high and low risk participants. As noted in chapter 4, manual calculations of the QT interval were more reliable than machine readings. As ECGs for both chapters were analysed at the same time, it was not possible to wait until the validation work had been completed before re reviewing all the ECGs for T wave abnormalities. It is possible that there may have been fewer high-risk participants and more low-risk participants in these analyses but as the overestimation was between a mean of 19-25ms this shouldn't have affected many participants.

Second, the categorisation of T wave morphology relied on manual visual interpretation rather than computer software program like QT Guard Plus (GE Healthcare). However, this could also be considered a strength as many doctors in low middle income countries in programmatic settings would also probably have to rely on visual interpretation rather than advanced computer programs.

Third, not all ECGs on treatment were reviewed. Only one from the early time point and one from the late time point were chosen. It is possible that there were more morphology abnormalities that were not detected due to only two ECGs on treatment being reviewed per participant.

Fourth, information on treatment changes and other factors that could have affected the morphology such as electrolyte deficiencies were not routinely collected.

Fifth, ECGs which had tricky or unreadable leads and none of the specific five abnormalities described earlier were categorised as “normal” rather than abnormal. It is possible that had the leads and T waves been clearer for some of these ECGs then this may have affected the analyses had the specific abnormalities been seen.

Sixth, and perhaps most importantly, post treatment ECGs were unavailable for all participants. This work was therefore unable to demonstrate that T wave abnormalities had reverted to normal after completion of treatment and prove they were treatment-associated changes. This would also have allowed an assumption that baseline pre-treatment abnormalities were caused by TB which had been treated. It is possible that the abnormalities described in this chapter relate to permanent changes in the voltage gated channels that need further investigation, though this is not in keeping with our understanding of other types of a-LQTS which are temporary. Even if all 200 patients included here had ECGs available from

weeks 40-52, investigation of post-treatment T wave morphology would have been difficult as the Long regimen patients were on treatment for nearly 2-years and some Short regimen patients extended treatment beyond week 52 due to treatment interruptions, Though not possible retrospectively this would form an important part of a prospective study investigating the cardiac effects of DR-TB treatment.

Strengths

This study had a number of strengths.

First, the participants included were a mix from both regimens and included those we know developed clinically relevant QT prolongation and those we know who didn't. Multiple different ethnic groups were also included, and the low-risk group were representative of the study population with respect to age, gender and site as well as being randomly chosen.

Second, as noted in chapter 4 a large number of participants and ECGs were reviewed, just under half of the entire study population.

Third, ECGs were picked from 3 separate time points allowing an assessment of pre-existing abnormalities at baseline and then whether there was a difference between the first months in treatment and the later on from month three onwards. Due to the pharmacokinetics of the drugs, this would have allowed a distinction between the effects of the fluoroquinolone at the early time point and clofazimine and fluoroquinolone at the late time point for the Short regimen participants.

Fourth, prior to me having undertaken the review and categorisation of ECGs, a period of training took place with a cardiologist specialising in electrophysiology disorders which included independent assessments of 30 ECGs followed by a comparison of categorisations to provide a quality check.

Fifth, all the ECGs reviewed were blinded to study number, machine QT interval, regimen and risk group. This reduced the chance of bias and confounding and adds strength to the validity of the findings.

Conclusions

The study described in this chapter has shown for the first time that patients on MDR-TB therapy develop T wave abnormalities specific to pathology in the voltage gated potassium channels of the myocardium. The analyses also showed that there were significantly more T wave abnormalities in participants on the Short versus the Long regimen and those that developed clinically relevant QT prolongation (high-risk) versus those that did not (low-risk). Baseline T wave abnormalities did not appear to be predictive of whether patients developed clinically relevant QT prolongation or not, but a significantly higher proportion of T wave abnormalities occurred at the late time point compared to baseline. Finally, there was clear evidence that the abnormalities occurred prior to the development of clinically relevant QT prolongation which may be useful for monitoring purposes.

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Appendix - 5

Training and Quality Control check

Before reviewing the T wave morphology of the full set of 600 ECGs, a period of training was undertaken to ensure competency and also to provide a quality control. A number of abnormal ECG patterns were reviewed and discussed on a video call with a cardiology electrophysiology (EP) specialist to highlight what abnormalities associated with voltage gated potassium channel disruption may be seen. Thirty ECGs were then selected to review; ten from patients in STREAM Stage 1 deemed low-risk on the basis of machine QT interval monitoring and twenty deemed high-risk as defined earlier. The ECGs selected were from time points not included in the later analysis and the automated machine QT interval readings were blinded. The T wave morphology for each of the 12-leads was visually inspected and categorised as one of the following:

1. Normal
2. Bifid or notched
3. Asymmetric
4. Flat (<1mm amplitude)
5. Biphasic
6. Inverted
7. Prominent U waves (>1-2mm or 25% of the T wave amplitude)
8. Unreadable (too noisy or QRS complex of low amplitude)

Independent reviews of all 30 ECGs were undertaken by the cardiologist and me. We reviewed each of the 12 leads in each ECG and as described in the main methods, the predominant T wave morphology in each lead was recorded based on the categories above. The data was then compared by measure of agreement between each reader.

As the data used categorical variables e.g., normal v abnormal, the analysis used kappa co-efficient values to address inter-reader variability and a threshold of 0.6 was deemed sufficient before the non-cardiology reader could continue analysing the full set of 600 ECGs. Previous studies have used a kappa value of 0.8 to represent excellent agreement with values below 0.4 to represent poor agreement.(39-40)

The data in Table A2 shows the frequency and percentage of each of the abnormalities. There were 360 leads to review for the 30 ECGs. The % column for agreement represents the number of leads each reader agreed on divided by the total number of leads that either reader deemed met that morphology.

One of the ECGs was of poor quality, with both readers having deemed each of the leads unreadable. Of the 360 leads reviewed, there was agreement between readers for 251 of the leads (69.72%). As the same lead could be interpreted differently for each reader, the disagreement column counts most of the leads twice. There was good agreement between readers for some of the typical abnormalities associated with *hERG* channel disruption such as bifid/notched and asymmetric T waves, whereas other abnormalities showed poorer agreement.

Table A2. Agreement for each abnormality expressed as frequency and percentage

		Agree	Disagree	Total	
1	Normal	161	62	223	72.2%
2	Notched/Bifid	17	10	27	63.0%
3	Asymmetric	5	5	10	50.0%
4	Flat	24	45	69	34.8%
5	Biphasic	2	12	14	14.3%
6	Inverted	7	33	40	17.5%
7	Prominent U waves	5	6	11	45.5%
8	Unreadable	30	32	61	49.1%

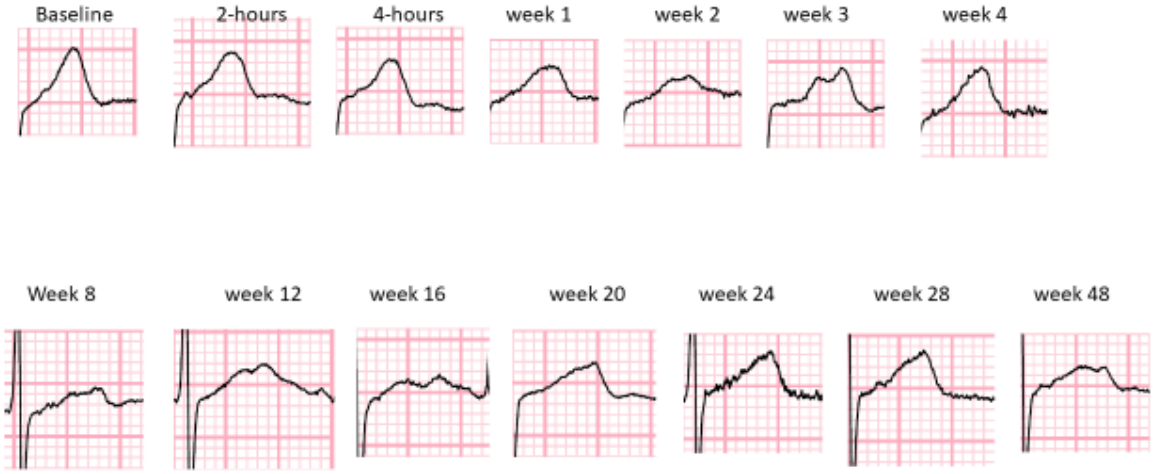
Following the training and quality check of 30 ECGs between the specialist cardiologist reader and myself as a non-specialist, a kappa value of 0.57 was calculated. This compared agreement between us in identifying normal T waves and abnormal T waves (notched, asymmetric, flat, biphasic and inverted). Some abnormal T wave categories (notched, asymmetric and flat) are more specific to the pathology of interest and a kappa value of 0.6 was reached for agreement between readers using these categories alone. This met the threshold of ≥0.6 for me as a non-specialist to go on and review the full set of 600 ECGs independently.

After further discussion the categories were modified slightly for the full review of all 600 ECGs.

1. Normal
2. Notched/Bifid
3. Asymmetric
4. Flat (<1mm amplitude)
5. Flat (>1mm amplitude with flat peak)
6. Broad (symmetric with broad base)
7. Biphasic
8. Inverted
9. Tricky (T wave height <1mm, noisy, isoelectric lane wandering i.e., >1mm from baseline)
10. Unreadable (low amplitude throughout lead i.e., <1mm QRS complex)

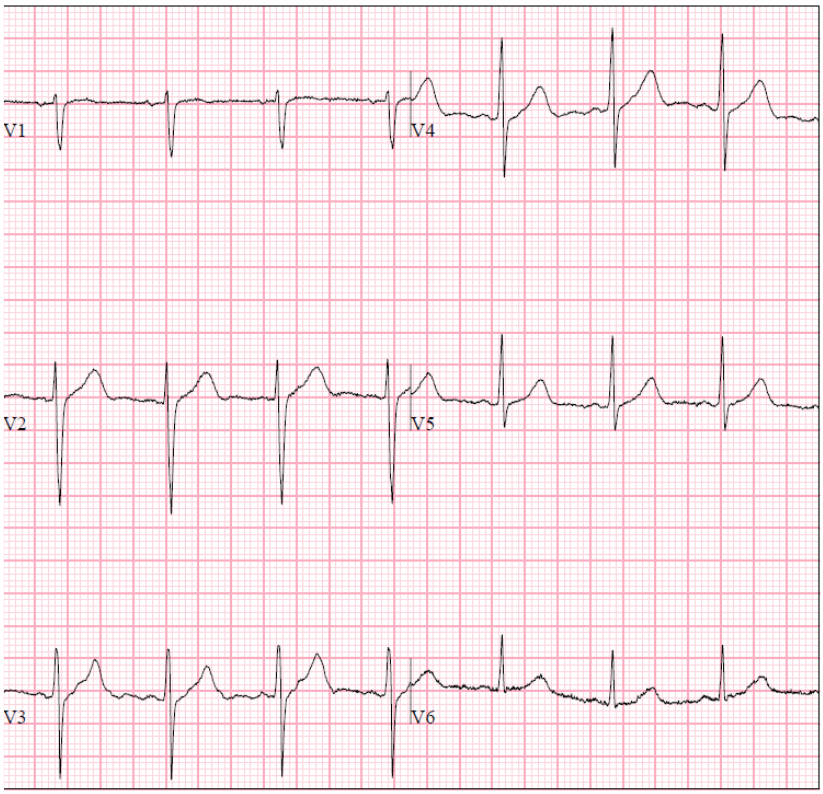
An additional note was made of the presence of any prominent U waves in each of the 12 leads which will be defined as >1-2mm or 25% of the T wave amplitude.

Fig A1. Example of progressive T wave morphology changes in a 41-year-old female Mongolian participant.



The ECGs shown in Figures A1, A2i and A2ii are from a participant who received the Short regimen and developed QTcF prolongation >500ms. Images of Lead V4 from baseline up to week 48 of follow-up are shown.

Figure A2. T wave morphology from precordial leads in the 41-year-old Mongolian patient described in A1 from baseline (i) and week 2 (ii) of follow-up.



i.

ii.

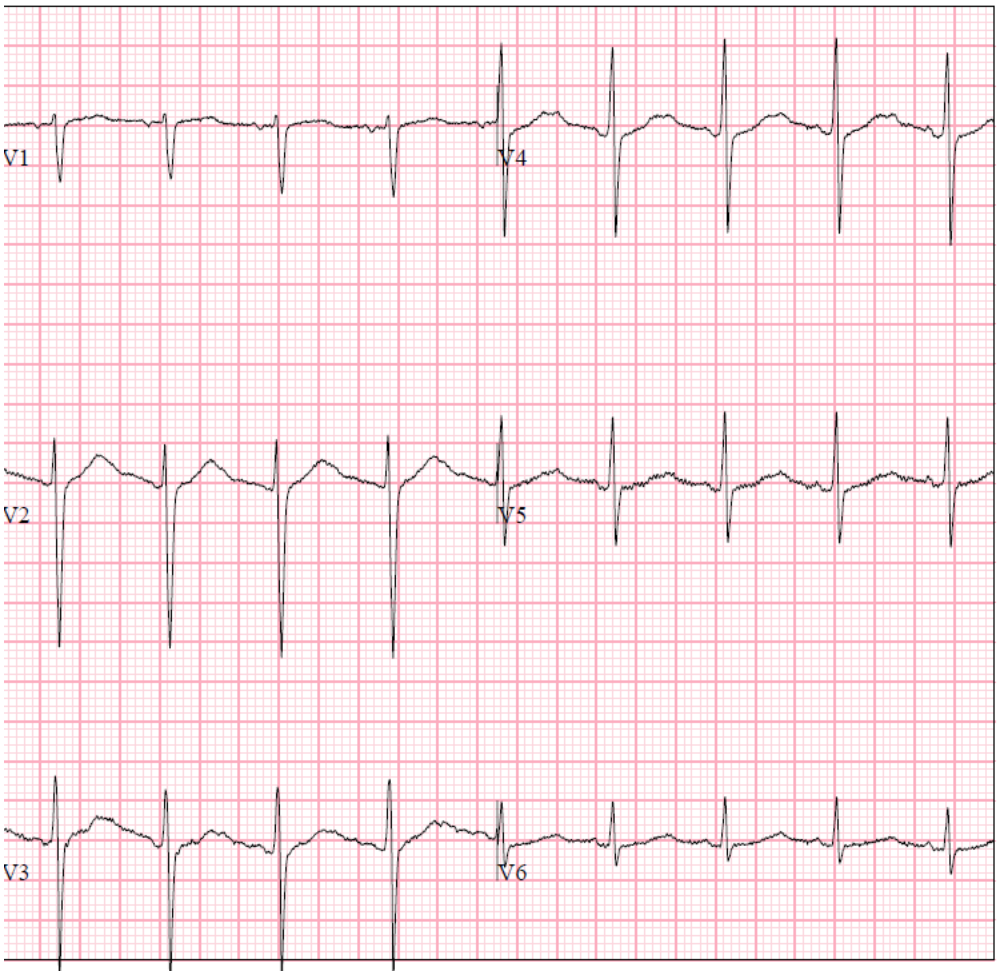
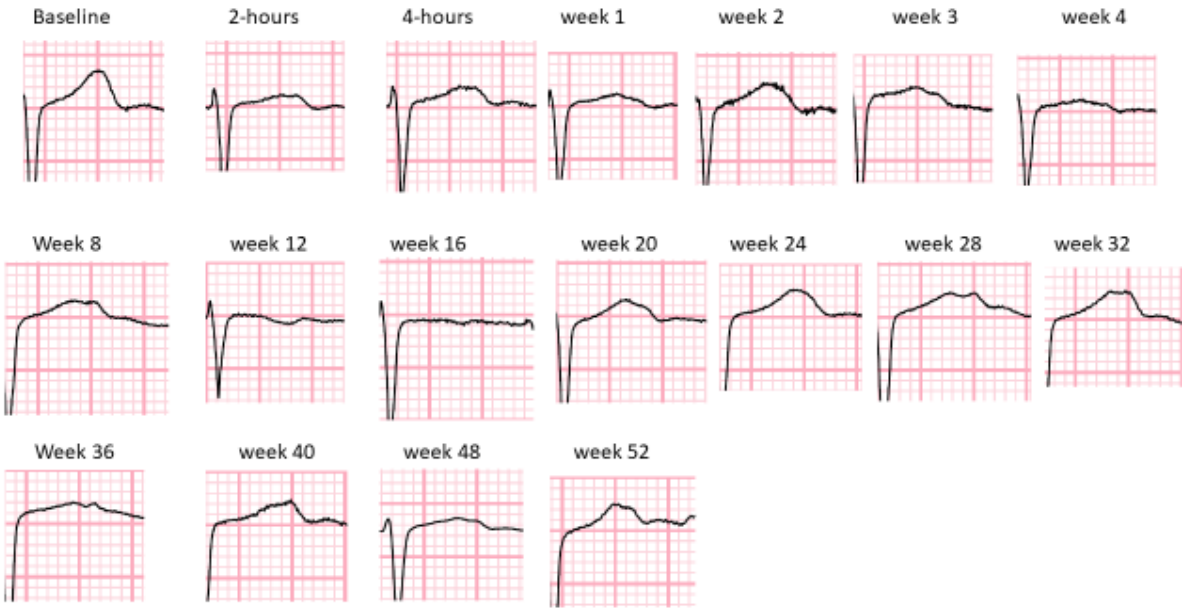


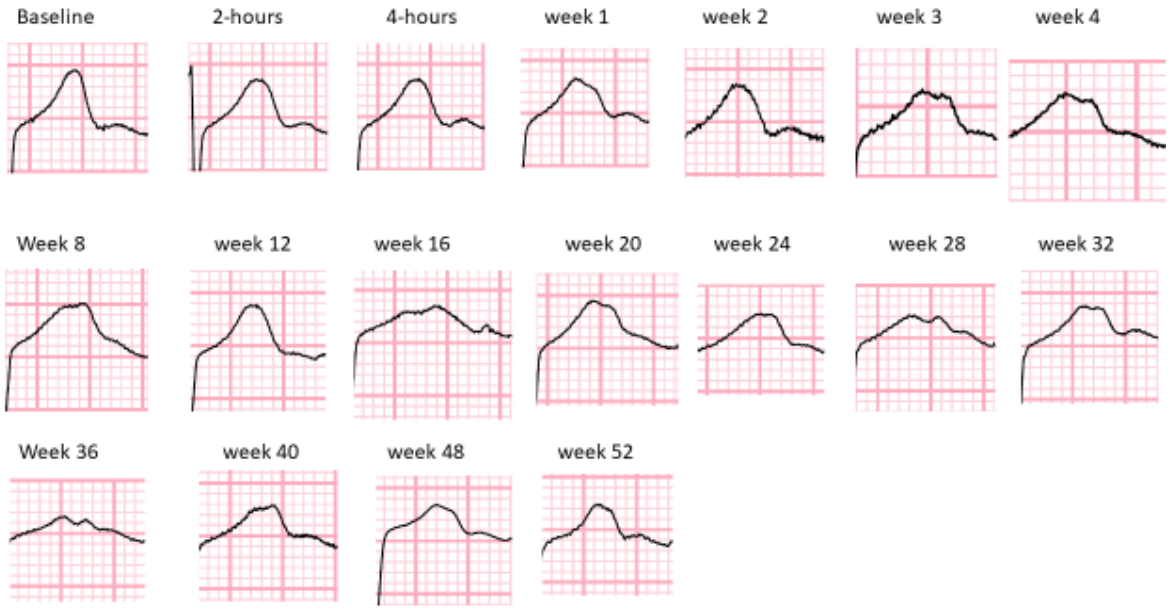
Figure A3. Example of progressive T wave morphology changes in a 58-year-old male Vietnamese patient.

i

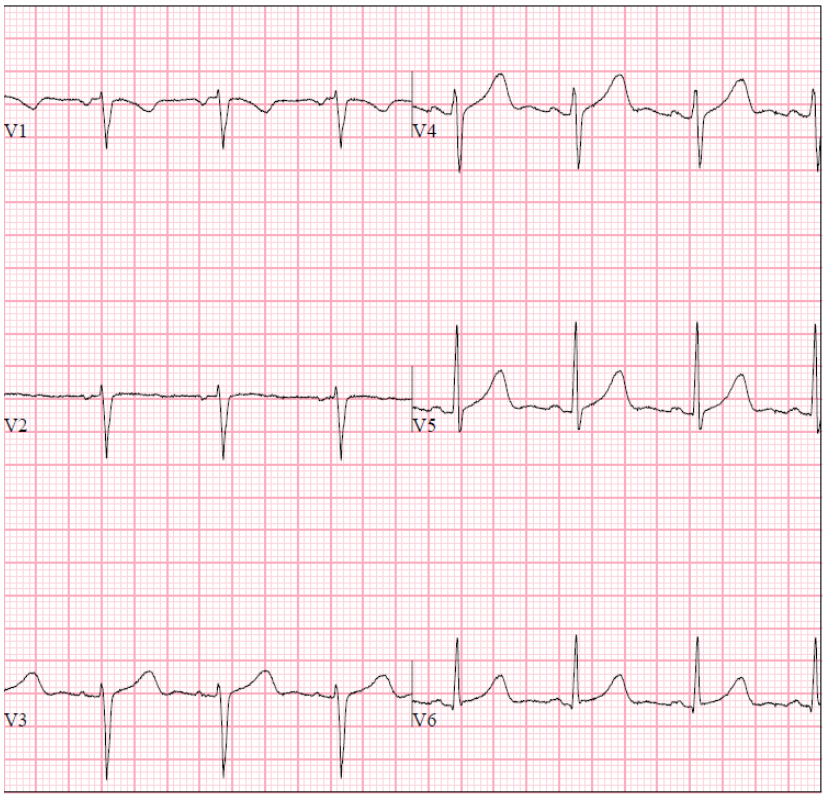


The ECGs shown in Figures A3, A3i and A3ii are from a participant who received the Short regimen and developed QTcF prolongation >500ms. Images of Lead V3 (A3i) and Lead V4 (A3ii) from baseline up to week 52 of follow-up are shown.

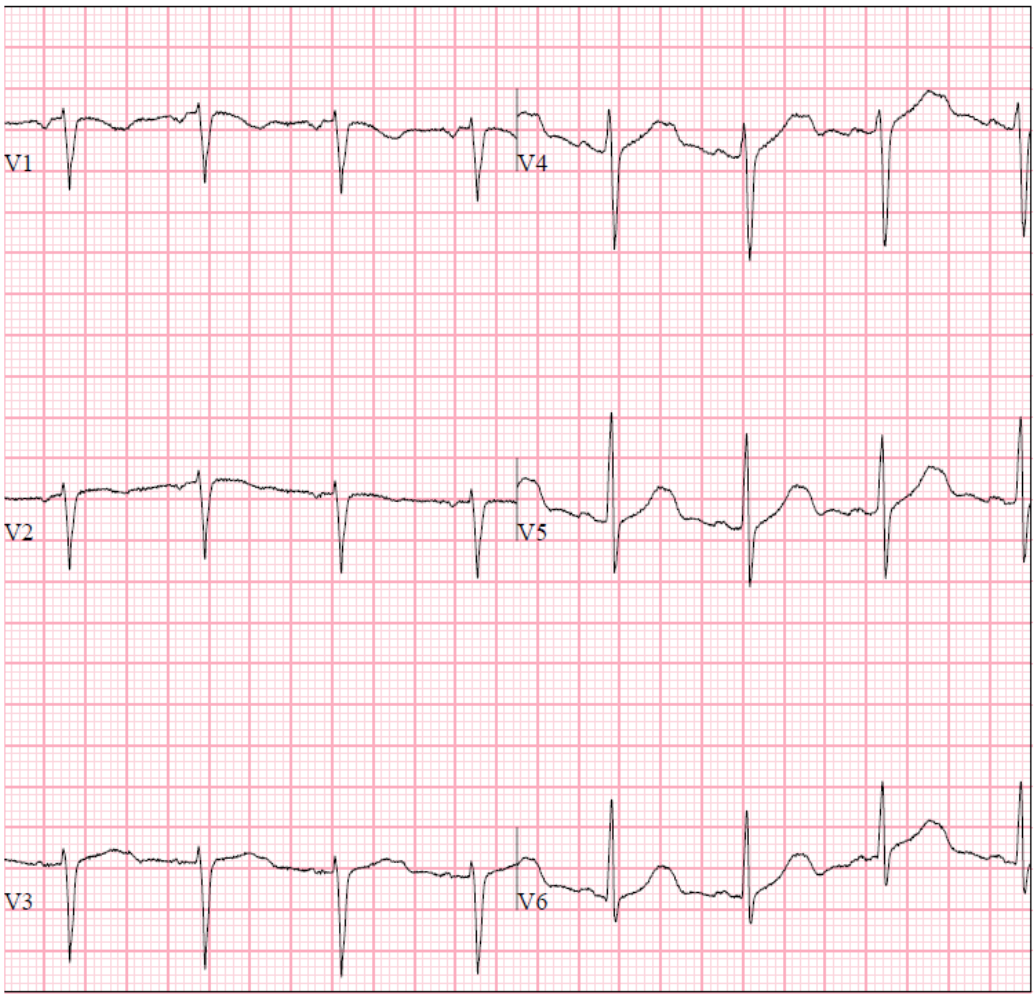
ii



A4. T wave morphology from precordial leads in the 58-year-old Vietnamese patient described in A3 from baseline (i) and week 3 (ii) of follow-up.



i.



ii.

Applying results to practice

Patients

As part of the pre-treatment counselling patients receive before starting DR-TB treatment, adverse effects and how they will be monitored for should be discussed. This will include regular blood tests to exclude liver toxicity for several drugs, hearing tests for aminoglycosides and ECGs for QT interval prolongation (leading to cardiac arrythmia in some patients) amongst other investigations. Though pre-treatment counselling is aimed at preparing and informing patients it may also lead to a degree of anxiety about some adverse effects such as cardiac arrythmia with QT interval prolongation. My work has provided a better understanding of the adverse cardiac effects of some DR-TB drugs which may help to alleviate some patient anxiety as well as showing the potential to reduce cardiac monitoring in some patients based on their QT interval readings in the first few weeks of treatment.

Clinicians

This work has informed clinicians which patients appear to be at higher risk of developing QT interval prolongation, who benefits from closer monitoring and who could receive less frequent monitoring, how patients can be managed when they develop QT prolongation whilst providing reassurance that these patients do not appear to experience worse outcomes. The validation of machine readings will provide reassurance that in most cases the automated QT interval can be relied upon but gives clinicians some guidance on which readings to question and what to do when they encounter these. Finally, the work on T wave morphology

abnormalities is exciting and may allow clinicians to identify patients at risk before they develop clinically relevant QT prolongation which could put them at risk of arrythmias. It is however, still in its infancy and would ideally need a computer program to identify and quantify these abnormalities when they occur.

Policy makers

When designing and updating national TB treatment programmes, patient safety is clearly an important component of this. My work has provided policy makers with new information on at risk patients and the how monitoring can be targeted to make better use of scarce resources in low- and middle-income countries which suffer a disproportionately high burden of TB. The validity of machine readings will provide reassurance as trying to manually monitor the QT interval in all patients within a treatment programme would be a logistical nightmare. The findings on T wave morphology needs further work but may allow policy makers to factor in these abnormalities when advising treatment centres which patients to be more concerned about and allow early intervention to reduce the development of QT interval prolongation which can put patients at risk of life threatening arrythmias.

Future directions

The work for my thesis used data from the STREAM Stage 1 trial. The STREAM Stage 2 trial involved many of the same drugs but with the addition of bedaquiline to two of the regimens and has now completed. As bedaquiline is known to prolong the QT interval and has unusual pharmacokinetics, it will be important to investigate these further and the methods from my thesis could be applied to Stage 2 data. The Short regimen in Stage 1 of the trial formed the control regimen in Stage 2 and was compared against the bedaquiline containing regimens.

The control regimen in Stage 1 contained a fluoroquinolone at standard dose which explains some of the findings from my thesis such as in Chapter 1, where I showed the mean QTcF was elevated in the Long regimen and above baseline at 52 weeks when patients would still have been taking treatment, though to a lesser extent than the Short regimen that contained high dose moxifloxacin and clofazimine. Comparison of the Short regimen in Stage 1 with a DS-TB population taking standard treatment with rifampicin, isoniazid, pyrazinamide and ethambutol would likely show a bigger difference between the two groups over the first 6 months as none of these drugs are known to prolong the QT interval.

There are several ongoing trials for patients with DR-TB and DS-TB that involve drugs known to prolong the QT interval, so many of the principles for my thesis could be explored further in these populations.

There was clear evidence in my thesis of ethnic differences in how well patients tolerated the Short regimen in terms of their QT interval. It is possible that patients in Mongolia are to an extent genetically isolated and some of the genes known to cause congenital long QT syndrome such as KCNQ1, KCNH2 and SCN5A may be

more prevalent in this population. To the best of my knowledge no previous studies have investigated this in the Mongolian population, though populations in other East Asian countries have been investigated before.

It is likely that other ethnic groups around the world may also experience different degrees of adverse effects from some of the drugs within DR-TB regimens such as QT prolongation. Isolated communities may be at risk of adverse cardiac events due to genetic differences. A study in Papua New Guinea (1) in a small cohort of 26 patients with DR-TB showed an almost identical frequency of QTcF prolongation (11%) as seen in STREAM Stage 1. Though very different geographically and ethnically to the Mongolian population in STREAM, the relative genetic isolation of this community may explain some similarities in the degree of QT prolongation.

In non-tuberculous mycobacteria, particularly *M.abscessus*, treatment relies on a combination of drugs which can include moxifloxacin and clofazimine taken for several months. The populations affected by this often include those with cystic fibrosis. Investigating how these patients tolerate the drugs from a cardiac perspective would be an important future direction of the work from my thesis. As clofazimine is also contained in the regimen for paucibacillary and multibacillary leprosy at a dose of 300mg monthly and 50mg daily for between 6 and 12 months respectively, this would be another non-tuberculous mycobacterial population worth exploring given the QT interval prolongation associated with clofazimine use.

Fluoroquinolones are often used in treatment of complex bone and joint infections given their good bioavailability and anti-microbial spectrum. Patients are usually treated for a minimum of 6-12 weeks so are at risk of QT interval prolongation whilst at home on treatment. I have already been approached by a clinician in one of the

UKs largest outpatient parenteral antibiotics (OPAT) service to provide some guidance on how to monitor and manage these patients from a QT interval perspective.

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Division of labour/Author contribution statement

All the work presented in this thesis is my own. I conceived, designed, and managed the research for each of the five chapters.

I received some help from a statistician to perform the analyses for chapters 1, 2, 4 and 5. A cardiologist provided training for manual QT calculation in chapter 4 and interpretation of T wave morphology in chapter 5 before I independently reviewed all the ECGs in the main data set.

Appendices

Appendix 1 - Abstracts

1. Predictive factors of QT prolongation in the STREAM 1 trial. Poster presentation – 50th Union World Conference on Lung Health, Hyderabad, India, October 2019
2. QT prolongation and its evolution over time in the STREAM 1 trial. Oral presentation - 50th Union World Conference on Lung Health, Hyderabad, India, October 2019
3. Predictive analysis of QT prolongation from ECG monitoring in STREAM Stage 1 – Oral presentation. 51st Union Conference on Lung Health, virtual conference October 2020
4. Validation of machine readings of QT interval during monitoring in the STREAM Stage 1 trial. Oral presentation, 53rd Union Conference on Lung Health, virtual conference, November 2022
5. Can T-wave morphology abnormalities identify patients at risk of clinically-relevant QT prolongation during drug-resistant tuberculosis treatment? Oral presentation, Union Conference on Lung Health, virtual conference, November 2022

VOLUME 23
NUMBER 10
NOVEMBER 2019
SUPPLEMENT 1

PAGES S1-S648
ISSN 1027 3719

The International Journal of Tuberculosis and Lung Disease

The Official Journal of the International Union Against Tuberculosis and Lung Disease

ABSTRACT BOOK

50th World Conference on Lung Health of the International Union Against Tuberculosis and Lung Disease (The Union)

HYDERABAD • INDIA
30 OCTOBER – 02 NOVEMBER 2019

PS -18-697-01 Predictive factors of QT prolongation in the STREAM 1 trial

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Background: STREAM 1 was a randomised phase 3 trial in rifampicin-resistant participants comparing a 9-11 month "short regimen" to a "long regimen" following WHO 2011 guidelines. Severe QT prolongation was more frequent on the short regimen, defined as QT or QTcF ≥500 ms (11% v 6.4% p=014) OR an increase ≥60ms above baseline (64% v 41%). These differences may be due to high dose moxifloxacin and clofazimine in the short regimen. We explored additional predictive factors for development of severe QT prolongation.

Methods: Trial participants had 4-weekly ECG monitoring for the year after randomisation. The 282 participants randomised to the short regimen were analysed to identify factors associated with severe QT prolongation using Pearson's chi-squared test and a two-sample t test.

Results: Of the 282 participants in the short regimen, 182 had severe QT prolongation on at least one ECG; 31 had QT/QTcF of ≥500ms and 181 an increase of ≥60ms from baseline. Risk factors included a baseline QT/QTcF >450ms and recruitment from the Mongolia site. There was no significant association with age, gender, BMI, hypokalaemia, diabetes or HIV infection. The median time to severe QT prolongation was 20 weeks (IQR 8.0 to 28 weeks). The median change was 104ms (IQR 83 to 137) in those with QT/QTcF ≥500ms and 67ms (IQR 63-77) in those with ≥ 60ms increase from baseline.

Conclusions: Whilst the baseline QT/QTcF level may be expected to influence whether it later increased, the reason for increased risk of patients from Mongolia was unclear, possibly due to genetic or environmental differences affecting pharmacodynamics of the trial medications. Although 2 patients developed a QTcF >500ms within 4 hours of their 1st dose of medication the median time of 20 weeks suggests the change takes several weeks, indeed 80% occurred at 8 weeks or later.

OA-28-496-02 QT prolongation and its evolution over time in the STREAM 1 trial

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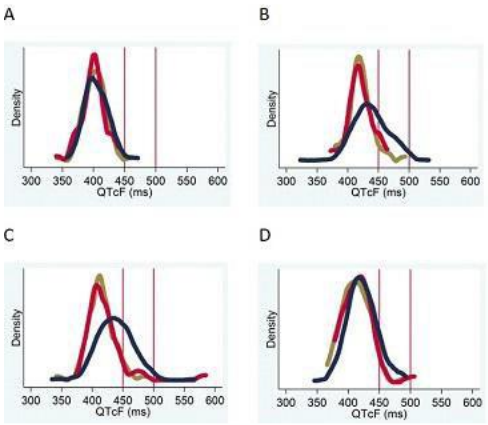
Background: STREAM-1 was a randomised phase 3 trial in rifampicin-resistant TB comparing a 9-11 month "short regimen" to a "long regimen" following WHO 2011 guidelines. ECG monitoring was undertaken for the first year after randomisation. QT prolongation (QT or QTcF ≥500 ms) was more frequent on the short regimen (11% vs. 6.4%, p=0.14), likely due to moxifloxacin (high-dose) and clofazamine in the short regimen. The long regimen included standard-dose moxifloxacin or levofloxacin. We examined the evolution of QTcF in trial participants over time.

Methods: Participants were analysed according to allocated regimen; those on the long regimen were subdivided by fluoroquinolone used. Mean QTcF and mean QTcF change from randomisation were calculated for each visit. Univariate kernel density plots were fitted to ECG data at randomisation (pre-dose), Week 16 (end of intensive phase on short regimen), Weeks 40 and 52.

Results: Mean QTcF and mean QTcF changes were greater on the short than long regimen, maximum difference in means between regimens was 24ms for QTcF at week 28 and 19ms for QTcF change at week 32. Both mean QTcF had returned to within 10ms of baseline at week 52. QTcF distributions by regimen (Fig 1) illustrate no difference at baseline, but a broadening of the distribution and a shift to the right in the short regimen apparent at weeks 16 and 40 (images B and C).

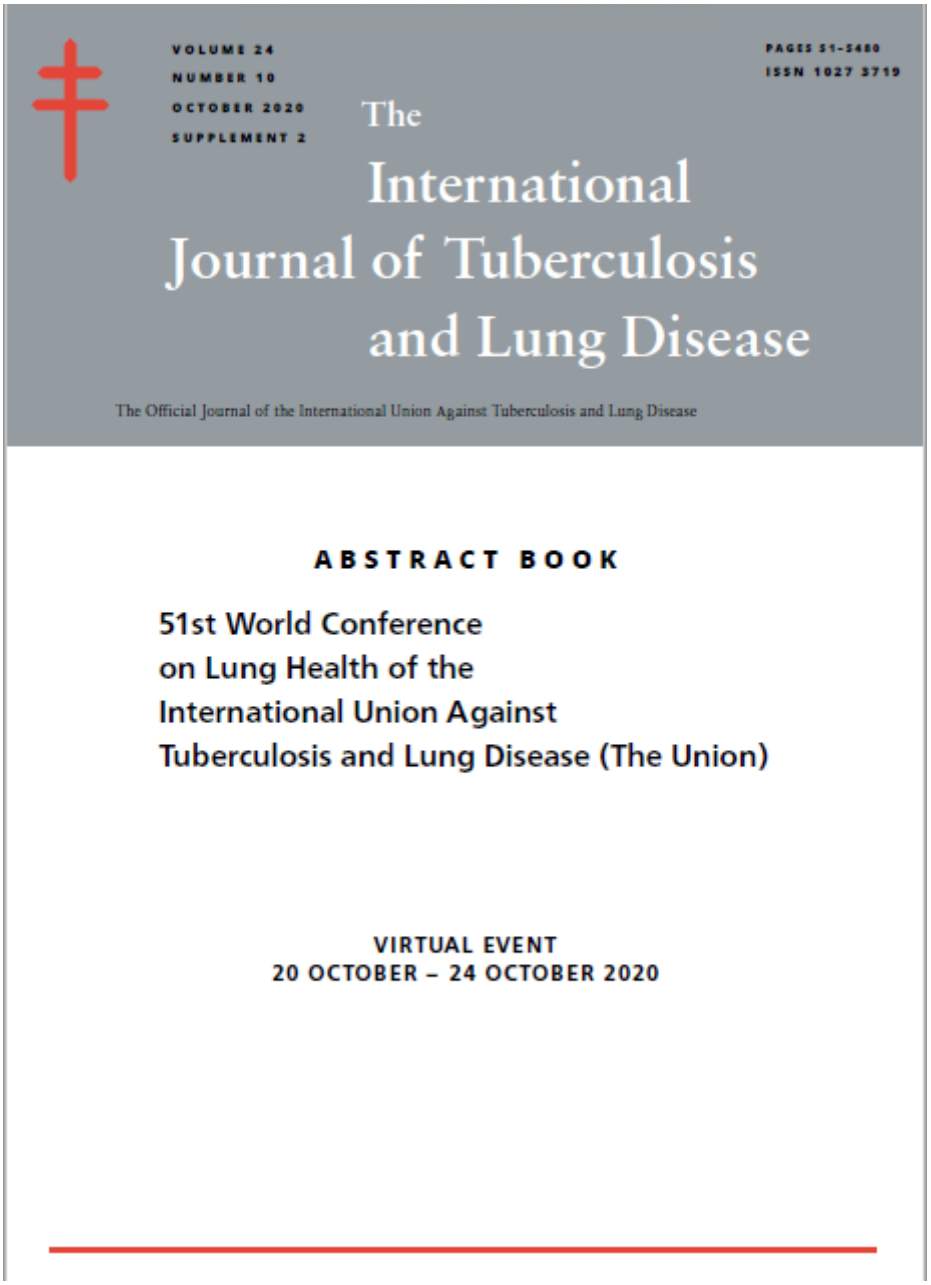


[Figure 1. Univariate kernel density plots of QTcF interval at baseline (A), 16 (B), 40 (C) and 52 (D) weeks reflecting pre-treatment, end of intensive phase, end of continuation phase and 1 year post randomisation. The lines correspond to the short regimen (blue), the long regimen with moxifloxacin (red) and the long regimen with levofloxacin (brown)]

On the long regimen changes in QTcF were greater in patients receiving moxifloxacin than those receiving levofloxacin.

Conclusions:

QTcF patterns suggest the short regimen, including clofazimine and high-dose moxifloxacin, alters the population QTcF distribution, which is resolved by week 52, when most patients had completed treatment. Patients on the long regimen continued treatment for at least 18 months; any effect of treatment on QTcF would still be present at week 52.



OA-17-608-22 Predictive analyses of QT prolongation from ECG monitoring in STREAM Stage 1

G. Hughes,[1] H. Bern,[1] S. Meredith,[1 1]Medical Research Council, Institute of Clinical Trials & Methodology, London, United Kingdom of Great Britain and Northern Ireland. e-mail: gareth.hughes@ucl.ac.uk

Background: STREAM Stage 1 was a randomised noninferiority phase 3 trial comparing a 9-month "short" regimen versus standard of care for MDR-TB patients. The primary safety outcome was occurrence of a ≥Grade 3 adverse event; this included a corrected QT interval (QTc) ≥500 milliseconds (ms) (associated with cardiac arrhythmia). More QTc prolongation events occurred in the short regimen, likely due to high-dose moxifloxacin and clofazimine. We explored whether there was a cut-off value that could allow prioritisation of ECG monitoring, potentially useful in programmatic settings with limited resources.

Design/Methods: Trial participants on the short regimen who developed QT/QTcF ≥500ms on treatment were identified. QT/QTcF measurements at baseline, 4 hours post first dose, weeks 1-4 and 12 were investigated to determine whether patients who developed QT/QTcF ≥500ms could be predicted. Fisher's exact test was used to compare the proportion of patients developing QT/QTcF ≥500ms to those that did not.

Results:
31 of 275 patients on the short regimen developed QT/QTcF ≥500ms. No significant relationship between QT/QTcF ≥500ms and QT/QTcF changes from baseline was identified. Absolute QT/QTcF values and development of QT/QTcF ≥500ms was found to be significant ($p<0.05$) for the majority of cut-off levels explored at baseline, 4 hours, weeks 1-4 and 12. Selection of the optimal values involved a trade-off between sensitivity and specificity; 425ms at 4 hours combined with 430ms at week 3 appeared most promising (Table 1). 5/30 patients were missed based on the 4 hours cut-off alone, but all 5 patients would have been captured at week 3 and could then receive more frequent monitoring.

Time point post randomisation	QTcF	Prolongation =500ms (n=30)	Total	Sensitivity	Specificity
4 hours	<415ms	3(2.5%)	120	90%	47.95%
	>=415ms	27(17.5%)	154		
	<425ms	5(3.0%)	169	**83.33%**	**67.21%**
	>=425ms	25(23.8%)	105		
	<430ms	9(4.68%)	192	70%	75%
	>=430ms	21(25.61%)	82		
	<435ms	13(5.9%)	220	56.67%	84.84%
	>=435ms	17(31.5%)	54		
	<445ms	18(7.2%)	249	40.00%	94.67%
	>=445ms	12(48.0%)	25		
Time point post randomisation	**QTcF**	**Prolongation =500ms (n=6)**	**Total**	**Sensitivity**	**Specificity**
Week 3	<415ms	1(1.12%)	89	83.33%	54.32%
	>=415ms	5(6.33%)	79		
	<425ms	1(0.85%)	118	83.33%	72.22%
	>=425ms	5(10%)	50		
	<430ms	1(0.78%)	129	**83.33%**	**79.01%**
	>=430ms	5(12.8%)	39		
	<435ms	2(1.4%)	141	66.67%	85.80%
	>=435ms	4(14.8%)	27		
	<445ms	4(2.52%)	159	33.33%	95.68%
	>=445ms	2(22.2%)	9		
Combined cut-offs				**96.77%**	**52.89%**

Table 1. QTcF at 4 hours post first dose and week 3 in patients who developed severe QT/QTcF prolongation, and those who did not, using different QTcF cut-off values. The proposed cut-offs were significant at the 5% level at both time points. One patient in the prolongation group at 4 hours and three at week 3 in the no prolongation group had missing data so are not included in each analyses.

Conclusions: Our analysis suggests that using a combination of cut-offs with 425ms at 4 hours and 430ms at week 3 might permit a reduction in ECG monitoring frequency in 47% of patients on the short regimen. These results need further validation.

Union Conference 2022 – Abstract

Validation of machine readings of QT interval during monitoring in the STREAM Stage 1 trial
Oral Abstract session (OA)
OA-10– Clinical trials and operational research for new treatment for TB (adults and Children)
2022-11-08
16:30-17:50 Central European Time (CET)
OA10-272-08

Validation of machine readings of QT interval during monitoring in the STREAM Stage 1 trial

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Background

STREAM Stage 1 was a randomised-controlled trial in rifampicin-resistant TB, comparing a Short versus Long regimen. Participants received regular ECG monitoring using machine-generated readings to identify QT prolongation, which was more frequent in Short regimen participants. This sub-study compares machine with gold-standard manual readings to investigate whether they are reliable, accurate and/or affected by treatment.

Design/Methods

All participants with available ECGs at baseline, an early (weeks 1,2,3 or 4) and a late (weeks 12,24 or 36) time-point on treatment were identified. A representative sample (by country, age and gender) of 118/191 participants was selected, enriched by all who developed QT/QTcF ≥500ms or ≥60ms increase from baseline (n=82). There were 142 Short and 58 Long regimen participants.

ECGs were blinded to regimen and time-point; QT intervals were calculated by Tangent method and compared to automated readings. A difference of +/-20ms is commonly considered acceptable in cardiological practice. Machine/manual differences were assessed using Bland-Altman plots by regimen and time-point.

Results

Mean (SD) differences between machine and manual QT measurements varied with time-point, but not regimen: +19ms (8.4) and +19.4ms (9.2) at baseline, 23.47ms (11.82) and 25.59 (10.61) at the early time-point and +24.3ms (13.3) and +23.6ms (13.7) at the late time-point on the Short and Long regimens, respectively.

Variance of the discrepancies increased on treatment but was unrelated to length of QT interval. Of the 10 measurements outside the limits of agreement (LOA), all but two ECGs had evidence of T wave morphology abnormality.

Conclusions

Compared with manual readings, machine readings overestimated the QT interval by 22.5ms on average but were otherwise reasonably reliable. Less reliable readings (a large discrepancy outside LOA) occurred infrequently and may be affected by abnormal T wave morphology. These ECGs might benefit from a manual QT measurement and cardiologist input.

Union Conference 2022 – Abstract

Title of your abstract	*Can T-wave morphology abnormalities identify patients at risk of clinically-relevant QT prolongation during drug-resistant tuberculosis treatment?*
Type of session	*Oral Abstract session (OA)*
Title of session	*OA-08– Causes and effects of DR-TB*
Session date	*2022-11-08*
Session time	*15:00-16:20 Central European Time (CET)*
Abstract reference number	*OA08-260-08*

Title: Can T-wave morphology abnormalities identify patients at risk of clinically-relevant QT prolongation during drug-resistant tuberculosis treatment?

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Background:

STREAM Stage 1 was a randomised controlled trial for patients with rifampicin resistant TB, comparing a Short versus Long treatment regimen. Clinically-relevant QT/QTcF prolongation (≥500ms or increase in ≥60ms from baseline) was more common amongst Short regimen participants, who received high dose moxifloxacin and clofazimine. T-wave morphology abnormalities may be a useful early biomarker for QT prolongation that increases the risk of malignant ventricular arrhythmia.

Design/Methods:

ECGs were selected from baseline, early and late time-points on treatment in 200 participants (Short 142; Long 58) with available ECGs.

The sample included all 82 participants with available ECGs who developed clinically-relevant QT prolongation and 118/191 who did not, representative of the study population based on country, gender and age.

ECGs were blinded to regimen, time-point and QT interval. T-wave morphology was categorised as normal or abnormal (notched, asymmetric, flat wave, flat peak or broad). Differences between groups were assessed using Chi Square tests (paired/unpaired, as appropriate).

Results:

At baseline, 23% (45/200) of participants displayed ≥1 abnormal category, increasing to 45% (90/200, p<0.001) at the late time point. An increased frequency between baseline and late time-points was observed for notched 2% (3/200) vs 11% (22/200), p<0.001; flat peak 0% vs 7% (14/200), p<0.001 and broad 2% (4/200) vs 13% (26/200), p<0.001, categories.

T-wave abnormalities were more common in Short regimen participants ECGs 38% (88/234, p = 0.008) compared to Long regimen 21% (16/76), and ECGs in participants who developed clinically-relevant QT prolongation, 44% (56/128, p = <0.001). T-wave abnormalities occurred prior to a QT/QTcF ≥500ms in 53% of participants (Long 2/5; Short 14/25).

Conclusions:

T-wave morphology abnormalities occurred in Stream Stage 1. Differences existed between regimen, time-point and whether or not clinically-relevant QT prolongation occurred. The abnormalities observed may allow early detection of patients at risk of QT prolongation.

Appendix 2 - Publications

1. **G Hughes**, H Bern, C-Y Chiang, RL Goodall, AJ Nunn, ID Rusen, SK Meredith. QT prolongation in the STREAM Stage 1 Trial. *Int J Tuberc Lung Dis 2022;* 26(4) April: 334-340. doi: 10.5588/ijtld.21.0403. PMID: 35351238; PMCID: PMC8982645.
2. **G Hughes**, H Bern, C-Y Chiang, RL Goodall, AJ Nunn, ID Rusen, SK Meredith. ECG monitoring in STREAM Stage 1: Can we identify those at increased risk of QT prolongation? *Int J Tuberc Lung Dis* – accepted and in press May 2022