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Olivier Meyer

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Ecole doctorale des Sciences de la Vie et de la Santé de Strasbourg

Institut de Pharmacologie Clinique – F. Hoffmann-La Roche

Thèse

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**Application des méthodes automatiques de mesure
électrocardiographique continues pour l'évaluation des
risques torsadogènes lors des essais cliniques :
Une alternative fiable aux méthodes conventionnelles ?**

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Résumé

Introduction

1. Introduction générale

Les médicaments provoquant un allongement de la repolarisation cardiaque, mesuré sur l'électrocardiogramme par la prolongation de l'intervalle QT, ont été associés à une augmentation du risque pro-arythmique, et plus particulièrement à la survenue de Torsades de pointes (TdP), une tachycardie ventriculaire polymorphe potentiellement mortelle. Au cours des dernières décennies, plus d'une dizaine de molécules ont été retirées du marché, ou ont vu leurs notices d'utilisation modifiées car elles entraînaient une prolongation de l'intervalle QT. En 2005, les autorités réglementaires des Etats-Unis, d'Europe, et du Japon ont adopté les recommandations de la Conférence Internationale sur l'Harmonisation (ICH) qui proposait des méthodes standardisées pour l'évaluation des effets des médicaments sur la prolongation du QT lors des études précliniques (ICH S7B) et cliniques (ICH E14). La directive E14 prescrit aux laboratoires pharmaceutiques d'évaluer le potentiel effet pro-arythmique de la plupart des molécules biodisponibles (à l'exception de la majorité des anticorps monoclonaux) en développement lors d'une étude standardisée appelée « Thorough QT study » (TQT). L'étude TQT est généralement réalisée sur des sujets sains et inclut un contrôle positif et négatif. Les tracés ECG sont enregistrés pendant 24 heures sur des Holters 12-dérivations. On extrait de ces enregistrements des portions correspondant à des temps de mesure établis dans le protocole auxquelles correspondent des mesures de la concentration plasmatique du composé testé. Le nombre de sujets inclus et d'ECG analysés dans ces études, ainsi que les méthodes statistiques associées doivent être rigoureusement établis pour exclure avec certitude un effet pharmacologique seuil d'environ 5 millisecondes sur le QT corrigé (QTc ; QT ajusté à la fréquence cardiaque) à chacun des temps de mesure. Au moment de son implémentation, les auteurs de la directive E14 ont exprimé leur manque de confiance vis-à-vis de la fiabilité des méthodes automatiques de mesure de l'intervalle QT, qui, malgré leurs avantages en matière de reproductibilité et de rapidité, ont généralement été associées à des erreurs ou inexactitudes de mesure électrocardiographiques, surtout en présence de formes d'ondes T anormales, de faible

amplitude, ou de bruit. Par conséquent, il est aujourd'hui devenu habituel que les entreprises pharmaceutiques sous-traitent l'analyse des ECG enregistrées lors des études TQT par des laboratoires spécialisés dans les mesures électrocardiographiques réalisées par des cardiologues à l'aide de logiciels de mesure semi-automatique (SA).

Les analyses réalisées par ces laboratoires spécialisés se restreignent à l'extraction de quelques complexes ECG aux temps de mesure spécifiés dans le protocole (12 au maximum sur 24 heures). Cette pratique se traduit par l'exclusion de la majorité des données enregistrées, une faible résolution temporelle, des coûts et besoins élevés en termes de temps et de main d'œuvre, une dérivation sous-optimale des facteurs de correction du QT, et une potentielle variabilité inter-observateurs. En revanche, l'inclusion de tous les battements enregistrés sur 24h et mesurés par des méthodes automatiques de mesure ECG a le potentiel de résoudre ces inconvénients. Par conséquent, au vu de l'évolution rapide et continue des méthodes automatiques de mesure ECG, nous avons émis l'hypothèse que les pratiques actuelles pouvaient être améliorée par l'utilisation conjointe de logiciels automatiques de mesures ECG et de méthodes avancées d'analyses continues qui ne sont pas applicables lorsque l'analyse des données ECG est restreinte à des temps de mesure espacés.

Le but de cette thèse est d'évaluer, comparer, améliorer, et si possible, valider l'applicabilité des méthodes automatiques de mesure ECG et l'utilisation conjointe d'une méthode de correction du QT basée sur la distribution des données enregistrées lors d'études QT standardisées (TQT ou premières administrations à l'Homme). Ces études, réalisées à l'institut de pharmacologie clinique Roche, avaient été préalablement analysées par des laboratoires spécialisés dans les mesures électrocardiographiques, permettant ainsi la comparaison directe des résultats obtenus par ces méthodes automatiques à des résultats jugés fiables par les autorités de régulations.

2. Le syndrome du QT long et torsades de pointes

Le syndrome du QT long est caractérisé par une repolarisation ventriculaire retardée qui crée un environnement électrophysiologique propice au développement d'arythmies, notamment de torsades de pointes (TdP), une arythmie ventriculaire polymorphe qui évolue généralement vers une rémission spontanée, mais qui peut, rarement, dégénérer en fibrillation ventriculaire et mort subite. Les deux principales manifestations du syndrome du QT long sont des syncopes (parfois

seulement des étourdissements dus à l'altération de la circulation cérébrale) et des anomalies électrocardiographiques qui se produisent habituellement dans des conditions de stress physique ou émotionnel. La repolarisation ventriculaire retardée peut être d'origine congénitale ou acquise (médicamenteuse) et se traduit sur l'électrocardiogramme de surface par le prolongement de l'intervalle QT, mesuré du début du complexe QRS (début de la dépolarisation ventriculaire), jusqu'à la fin de l'onde T (fin de la repolarisation).

2.1. Syndrome du QT long acquis

Le syndrome du QT long acquis est généralement provoqué par des agents pharmacologiques, mais peut aussi résulter de cardiomyopathies (bloc cardiaque, infarctus du myocarde, bradycardie) ou de déficits ioniques (hypokaliémie, hypocalcémie, hypomagnésémie). Toutefois, les agents pharmacologiques demeurent la cause la plus fréquente avec plus de 150 médicaments actuellement référencés pour avoir le potentiel d'allonger l'intervalle QT. Bien que la plupart des cas reconnus de syndrome du QT long induit par les médicaments aient été rapportés lors de traitements par des antiarythmiques de classe III, plusieurs autres classes de médicaments ont été associées à un retard de la repolarisation cardiaque et un risque accru de torsades de pointes. Par conséquent, la question du syndrome du QT long d'origine médicamenteuse a attiré l'attention des autorités de réglementation du médicament.

On croyait auparavant que plus un médicament avait d'affinité pour bloquer le courant hERG (I_{Kr}), plus l'intervalle QT était prolongé, et plus accru était le risque d'événements arythmiques. Toutefois, des avancées dans la compréhension de la physiopathologie des torsades de pointes médicamenteuses ont démontré que la prolongation de l'intervalle QT et son étendue ne sont pas les seuls déterminants impliqués dans la pathogenèse des torsades de pointes. Bien que pratiquement tous les médicaments induisant des torsades de pointes et prolongeant le QT aient été associés à une inhibition des courants K^+ (I_{Kr} et I_{Ks}), il est maintenant bien établi que l'induction de torsades de pointes ne résulte pas uniquement d'un blocage des courants potassiques. En effet, les torsades de pointes ne peuvent seulement se produire lorsqu'une combinaison de plusieurs facteurs de risque crée une fenêtre de vulnérabilité favorisant le développement d'événements arythmiques susceptibles de déclencher des torsades de pointes.

3. Étude approfondie (« thorough ») du QT

Depuis la mise en place des directives ICH E14 en mai 2005, presque tous les nouveaux médicaments en développement doivent démontrer une sécurité relative pour le risque d'induction de torsades de pointes (TdP), évalué par la prolongation du QT / QTc (le biomarqueur accepté par les autorités de régulation pour évaluer le risque de torsades de pointes), mesurée au cours d'une étude "thorough" QT (TQT). Cette étude de référence, qui ressemble beaucoup à l'étude "gold standard" de télémétrie chez le chien en termes de design et de conditions, vise à quantifier la prolongation de la repolarisation cardiaque (mesurée par l'allongement de l'intervalle QT) afin d'identifier des médicaments qui ont le potentiel d'entraîner des arythmies mortelles. En d'autres termes, le but de l'étude TQT n'est pas la quantification directe du risque de torsades de pointes, mais la détection qualitative d'un effet pharmacologique seuil de prolongation de l'intervalle QT d'environ ~ 5 ms, qui a été défini empiriquement par les experts au cours de la préparation de la directive E14. Le but de ces recommandations ICH était d'assurer la sécurité du public en proposant une méthodologie uniforme pour l'évaluation clinique des effets des nouveaux traitements sur la repolarisation cardiaque, au cours d'une étude TQT bien standardisée. Toutefois, les experts ont volontairement donné des recommandations "ouvertes" pour permettre l'utilisation futures de méthodes de mesure et d'analyse du QT lorsque celles-ci deviendraient disponibles. Les études TQT sont généralement réalisées chez des sujets sains, incluent à la fois un contrôle positif (généralement la moxifloxacin) et un contrôle négatif (placebo), des enregistrements ECG 12 dérivations en continu (dispositifs Holter), des temps de mesures spécifiques établis dans le protocole de l'étude (pour les niveaux plasmatiques du médicament et les extractions ECG), et utilise une population d'étude appropriée (~ 40-80 sujets pour une étude en cross-over) ainsi que des modèles statistiques (généralement des modèles à effets mixtes) afin d'exclure de manière fiable un effet pharmacologique seuil sur la repolarisation cardiaque à chaque temps de mesure. Une étude TQT est caractérisée négative lorsque l'effet d'allongement de l'intervalle QT / QTc exclut un effet moyen d'environ 5 ms, établis lorsque les bornes supérieures des intervalles de confiance à 95% excluent un allongement de 10 ms à tous les temps de mesure prévus dans le protocole.

Dans le cas où au moins un temps de mesure démontre un intervalle de confiance > 10 ms, l'étude TQT est caractérisée positive et nécessite alors presque toujours une évaluation étendue des risques d'arythmie au cours de la phase II et III du développement du médicament. Cette

évaluation ECG étendue n'est généralement pas nécessaire pour les médicaments aillant démontré une étude TQT négative, pour lesquels une surveillance ECG standard est suffisante. De plus, pour établir la sensibilité de l'étude, le contrôle positif (moxifloxacine) doit démontrer une borne inférieure de l'intervalle de confiance à 95% > 5 ms à un ou plusieurs temps de mesure autour du T_{max} (généralement 1 à 4 heures après l'administration de moxifloxacine).

L'étude TQT est rapidement devenue bien standardisée et la plupart des promoteurs ont généralement choisi d'externaliser l'analyse des données ECG à des laboratoires spécialisés dans les mesures ECG semi-automatisées (SA) réalisées par des cardiologues. Bien que la lecture centralisée par des laboratoires spécialisés ait longtemps été estimée être le meilleur choix possible pour réduire la variabilité associée à la mesure et à l'analyse du QT, il reste néanmoins à déterminer si cette pratique est toujours actuellement la méthode optimale, en particulier en ce qui concerne l'évolution rapide des techniques de mesure d'ECG.

4. L'intervalle QT

4.1. Biomarqueur

L'utilisation de la prolongation de l'intervalle QT comme biomarqueur de risque de torsades de pointes a été largement critiquée (Bednar, Harrigan et al 2001; Sager 2008). Les TdP ont été associées à un retard de la repolarisation cardiaque du fait que l'apparition de torsades de pointes est toujours associée à une prolongation de l'intervalle QT, et que presque tous les médicaments qui ont produit des torsades de pointes ont démontré la capacité de bloquer le courant I_{Kr} (hERG). Pour ces raisons, la prolongation de l'intervalle QT a été acceptée comme le principal biomarqueur de risque de torsades de pointes (Bednar, Harrigan et al 2001; Joshi, Dimino et al 2004). Le principal problème de cette conceptualisation réside dans le fait que même si tous les médicaments qui induisent des torsades de pointes prolongent l'intervalle QT, l'inverse (c'est à dire que tous les médicaments qui prolongent l'intervalle QT induisent des torsades de pointes) n'est pas vrai (Antzelevitch et Shimizu 2002; Belardinelli, Antzelevitch et al, 2003; Yap et Camm 2003). De plus, il n'existe pas de corrélation claire entre l'ampleur de la prolongation de l'intervalle QT et le risque de torsades de pointes. En effet, de nombreux exemples existent, tels que le ziprasidone et l'halopéridol. Alors que les deux médicaments sont associés à une augmentation de l'intervalle QTc, le ziprasidone a montré une augmentation de l'intervalle QTc plus grande par rapport à l'halopéridol, mais n'a pas été associé à des torsades

de pointes tandis que l'halopéridol l'a été (Sager 2008). D'autres exemples tels que la moxifloxacine, l'amiodarone, ou le vérapamil (Roden 2004; Morganroth, Dimarco et al 2005), sont des composés associés à un allongement du QT qui n'induit pas de torsades de pointes. D'autre part, la terfénaire ne provoque qu'une légère prolongation de l'intervalle QT, mais est associée à un risque important de torsades de pointes (Fossa, DePasquale et al 2002; Ando, Hombo et al 2005).

Ces exemples montrent que l'intervalle QT est un mauvais indicateur du potentiel torsadogène. Toutefois, un concept important est que, malgré le fait que la prolongation de l'intervalle QT est un mauvais biomarqueur du risque torsadogène, l'absence de prolongation du QT reste un bon biomarqueur de sécurité relative, du fait qu'un médicament ne prolongeant pas le QT est très probablement dénué de potentiel pro-arythmique. Ainsi, en l'absence d'un test standard pour détecter les risques proarythmiques, les autorités de régulation ont fait le choix de détecter tous les médicaments qui ne prolongent pas l'intervalle QT (et donc, sont susceptibles d'être sûrs) et de classer tous les composés qui prolongent l'intervalle QT comme "potentiellement dangereux" jusqu'à preuve du contraire. En d'autres termes, il est préférable d'écarter les faux positifs (rejeter des médicaments sûrs) que de prendre le risque d'accepter des faux négatifs (autoriser les drogues dangereuses). Bien que ce concept ait démontré une certaine efficacité pour empêcher des médicaments potentiellement nocifs d'atteindre le marché, il a aussi probablement donné lieu à l'arrêt du développement de nombreux médicaments sûrs et à fort potentiels.

Ces dernières années, la mise en places des réglementations concernant le potentiel proarythmique des médicaments a entraîné des retraits, ré-étiquetage, et restrictions d'accès à des médicaments déjà mis sur le marché, et a été la cause la plus fréquente de la cessation de développement de médicaments en phases précliniques (Shah 2002).

Par conséquent, la détermination de l'approche optimale et des meilleurs indicateurs de risque de torsades de pointes restent un besoin aujourd'hui non satisfait qui ne pourra être répondu qu'une fois les mécanismes de torsades de pointes complètement compris. Il est probable que dans un avenir proche, l'analyse complémentaire d'autres biomarqueurs que le QT, comme la TDR, la variabilité à court terme (STV) (Hinterseer, Thomsen et al 2008; Hinterseer, Beckmann et al 2010), la morphologie de l'onde T (Zabel et Malik 2002; Couderc 2009), et les analyses de restitution (Fossa, Wisialowski et autres, 2007) jouera un rôle important dans la différenciation entre les molécules torsadogènes et les molécules non-torsadogènes. À l'heure actuelle,

l'évaluation de la prolongation de l'intervalle QT seul ne permet pas de différencier entre un médicament proarythmique et non-proarythmique, et, tant qu'un meilleur biomarqueur ne sera pas validé et accepté, l'évaluation de la prolongation de l'intervalle QT lors des études TQT restera sans doute la seule norme réglementaire pour déterminer le risque torsadogène d'un médicament en développement (Fenichel 2004 Malik et al; Pugsley, Authier et al 2008).

4.2. Mesure de l'intervalle QT à partir de l'ECG de surface

La mesure juste et reproductible de l'intervalle QT est essentielle pour l'évaluation du risque proarythmique d'un médicament en développement. Cependant, plusieurs facteurs physiologiques, techniques et cliniques ont le potentiel d'influencer la variabilité de la mesure du QT. Dans les conditions physiologiques, l'intervalle QT est modulé par la fréquence cardiaque, à la fois en termes de durée (dépendance du rythme cardiaque) et d'adaptation (hystérèse), et par le système nerveux autonome (indépendant du rythme cardiaque). D'autres influences telles que le sexe, l'âge, le rythme circadien et les variations génétiques ont été associées à des différences de l'intervalle QT et contribuent à la nature variable de la repolarisation cardiaque. En outre, la détermination (manuelle et automatique) de la fin de l'onde T est toujours associée à une certaine incertitude due à une compréhension incomplète du processus de repolarisation et de sa projection sur la surface du corps (Kautzner 2002). Des facteurs techniques tels que la résolution de l'appareil d'enregistrement d'ECG, le bruit électrique, les faibles amplitudes, les ondes T-U fusionnées, la dérive de la ligne de base et l'expérience de l'opérateur et / ou la performance de l'algorithme automatisé peuvent aussi rendre difficile la mesure juste et reproductible de l'intervalle QT. L'administration de composés pharmacologiques qui affectent les morphologies de forme d'onde, ou modifient d'autres propriétés physiologiques du cœur, peut aboutir à des formes d'onde anormales qui peuvent être très difficile à mesurer, tant pour les lecteurs humains que pour les algorithmes automatisés. Collectivement, l'interaction de ces multiples sources de variabilité fait de la mesure fiable de l'intervalle QT un véritable défi.

4.3. Relation QT-RR

La repolarisation ventriculaire est dépendante et varie proportionnellement à la durée du cycle cardiaque. Cela signifie que lorsque l'intervalle RR augmente, l'intervalle QT augmente aussi. La dépendance au rythme de l'intervalle QT est reconnue depuis longtemps (Bazett 1920) et est le

résultat de la cinétique des canaux ioniques des cardiomyocytes qui varient à différentes longueurs de cycle et / ou à travers la modulation du système nerveux autonome (Ahnve et Vallin 1982; Browne, Prystowsky et al. 1983). Afin de comparer les intervalles QT à différentes périodes et entre sujets, une formule de correction est nécessaire. En 1920, Bazett (Bazett 1920) a établi une première formule de correction «universelle» (fixe). Cette formule «universelle» a été utilisée pendant de nombreuses années et est encore parfois utilisé en dépit de ses inconvénients bien connus. En effet, il est aujourd'hui largement admis que la relation QT-RR est propre à un individu (Batchvarov et Malik 2002), ce qui en d'autres termes, signifie que chaque individu a sa propre formule de correction optimisée (variabilité inter-individuelle), et que cette formule ne varie pas beaucoup (stabilité intra-individuelle), sauf en présence de pathologies ou d'effets induits par les médicaments. Il est probable que les différences inter-individuelles soient la manifestation de distributions individuelles spécifiques de canaux ioniques dans les tissus cardiaques qui varient en fonction de chaque génotype individuel (Malik, Hnátková et al. 2008). La relation QT-RR suit également des modèles circadiens où la pente de la relation est généralement plus forte lors de la journée par rapport aux périodes nocturnes. Ces effets sont dus à la modulation circadienne de l'équilibre sympatho-vagal, où le tonus sympathique est plus prononcé au cours de la journée, et le tonus vagal plus prononcé pendant la nuit (Smetana, Batchvarov et al. 2003). Par conséquent, utiliser une formule de correction fixe est intrinsèquement associé à de potentielles erreurs de mesure et la modélisation d'une relation QT-RR individuelle pour chaque sujet est maintenant accepté comme la méthode de correction la plus appropriée. Récemment, de nouvelles études sur les facteurs de correction individuels ont démontré que les courbures QT-RR sont elles aussi spécifiques à chaque individu, ce qui signifie que l'utilisation d'un modèle de régression individuel spécifique (linéaire, log-linéaire...) est important afin de réduire les erreurs de mesure en présence de médicaments présentant d'importants effets sur le rythme cardiaque (Malik, Hnátková et al. 2012).

Cependant, d'autres approches ont également été proposées pour éviter la nécessité de corriger le QT. Ces méthodes, telles que la méthode des "bins", les études de restitution, la dispersion transmurale de la repolarisation, et la variabilité à court terme, visent à éviter la nécessité d'utiliser des techniques de correction du rythme qui sont généralement complexes.

4.4. Hystérèse du QT

Une autre propriété physiologique fondamentale du cœur est le retard à l'adaptation de l'intervalle QT à un changement brusque de RR. Ce retard dans l'adaptation de l'intervalle QT

est appelé hystérèse et a déjà été évalué chez l'homme (~ 2,5 min) lors d'études utilisant des fréquences de stimulation constantes (Lau, Freedman et al. 1988). D'autres études utilisant des enregistrements ECG à long terme sans fréquences de stimulation fixes ont rapporté que les différences dans le profil d'hystérèse QT-RR pourrait stratifier le risque d'arythmie chez les survivants d'un infarctus du myocarde (Pueyo, Smetana et al 2004; Smetana, Pueyo et al 2004). Plus récemment, les profils d'hystérèse QT-RR ont été étudiés chez des sujets sains et ont démontré que les profils d'hystérèse QT-RR étaient eux aussi individuels et que la combinaison de la correction individuelle de l'hystérèse et la correction individuelle du QT pourraient conduire à des améliorations dans les études sur la repolarisation cardiaque (Malik, Hnátková et al . 2008). En outre, il a été récemment rapporté que l'hystérèse QT-RR est également médiée par les effets du système nerveux autonome (Pelchovitz, Ng et al. 2012). Par conséquent, les effets de l'hystérèse QT-RR sont assez complexes et plusieurs méthodes ont été mises en place pour modéliser (à travers des formules mathématiques) ou éviter (en sélectionnant des périodes de stabilité du rythme) ces effets.

4.5. Influences indépendantes du rythme cardiaque

Bien que la fréquence cardiaque soit le principal moteur de la durée de la repolarisation cardiaque, d'autres variables indépendantes du rythme ont été identifiées pour influencer l'intervalle QT (Magnano, Holleran et al. 2002). Le système nerveux autonome, et plus précisément, l'équilibre entre le système sympathique et vagal, a été associé à des changements dans la durée de l'intervalle QT. En règle générale, l'augmentation du tonus sympathique raccourci l'intervalle et une augmentation du tonus vagal augmente l'intervalle QT. Ces effets sont d'autant plus évidents au cours de l'exercice (Davidowski et Wolf 1984) ou des périodes de sommeil (Browne, Prystowsky et al. 1983) et repas (Nagy, DEMEERSMAN et al. 1997).

4.6. Méthodes de correction du QT – Facteurs de correction

En l'absence de directives réglementaires spécifiques, les sponsors ont été libres d'utiliser plusieurs modèles de repolarisation *in vivo* et ont généralement utilisé des corrections du QT basées sur des précédents historiques plutôt que sur des observations scientifiques objectives et pertinentes.

De nombreux modèles mathématiques ont été développés pour décrire la relation QT-RR. Les formules de correction les plus simples et les plus couramment utilisées sont les régressions log-linéaires établies à partir de ~ 35 et ~ 50 sujets en 1920 par Bazett (QTcB) (Bazett 1920) et Fridericia (QTcF) (Fridericia 1920), respectivement. Les formules sont $QTcB = QT / RR^{1/2}$ et $QTcF = QT / RR^{1/3}$. Ces formules de correction du rythme ont été calculées pour être universellement appliquées pour tous les individus. D'autres modèles mathématiques, comme les régressions linéaires (par exemple la formule de Framingham: $QTcFr = QT - 0,154(1-RR)$ ou Hodges $QTcHodges = QT - 1,75(HR-60)$) ont été étudiées dans le but de potentiellement obtenir de meilleures formules de correction. Au total, plus de 70 formules fixes ont été établies et comparées (Kawataki, Kashima et al 1984; Ahnve 1985; Sagie, Larson et al 1992; Funck-Brentano et Jaillon 1993), mais aucune d'entre elles n'a été en mesure de démontrer une supériorité constante par rapport aux autres. Avec le temps, il est devenu évident qu'aucun de ces modèles ne pourrait jamais décrire universellement la relation entre les intervalles QT et RR, comme il a été démontré par la suite que chaque individu est mieux caractérisée par une formule de correction qui lui est spécifique ($QTcB = QT / RR^{\beta}$).

4.7. Laboratoires spécialisés dans les mesures ECG

Depuis la mise en place de la directive ICH-E14 qui a déclaré (en 2005) que « l'étude « thorough » QT / QTc nécessite une attention particulière pour la mesure des intervalles de l'ECG. À l'heure actuelle, cela implique généralement la mesure des intervalles par quelques lecteurs qualifiés (assistés ou non par ordinateur) opérant de manière centralisée dans un laboratoire spécialisé en mesure ECG », les entreprises pharmaceutiques confient généralement l'analyse des données des études TQT à laboratoires spécialisés dans les annotations d'intervalles semi-automatisées (SA) réalisées par des cardiologues bien formés. Les analyses effectuées par ces laboratoires sont bien standardisées et se composent généralement d'annotations semi-automatiques de 3-9 battements obtenus lors des périodes de stabilité de la fréquence cardiaque ($bpm \pm 2$) extraits des temps de mesures définis dans le protocole de l'étude (Malik, Hnátková et al. 2008). Bien que cette méthode fournit des mesures fiables de l'intervalle QT pendant les périodes spécifiées, il existe encore, à ce jour, de nombreuses limitations, telles que l'exclusion de la majorité des données enregistrées, une résolution temporelle insuffisante, de fortes exigences en matière de temps et de ressources (main-d'œuvre, coût), la variabilité inter-observateur et de possibles erreurs de mesure lorsque seulement quelques intervalles QT sont sélectionnés pour un intervalle RR spécifique (Fossa 2004; Extramiana, Badilini et al 2007; Strachan, Hughes et al 2009; Holzgrefe, Ferber et al

2012). Surtout, les laboratoires spécialisés doivent compter sur des corrections du rythme sous-optimales telles que les corrections fixes ou d'étude du fait que le nombre réduit de données QT-RR obtenues avec l'analyse SA empêche le calcul et l'utilisation de facteurs de correction individuels. Par conséquent, bien que les laboratoires spécialisés fournissent généralement une analyse de données fiable, il reste encore possible d'améliorer substantiellement l'analyse du QT.

4.8. Analyse basée sur la distribution

Les principaux avantages de l'analyse basée sur la distribution sont l'inclusion de presque tous les complexes ECG enregistrés (> 70000 battements par 24 h) et l'intégration de la nature probabiliste de l'intervalle QT dans l'analyse. La propriété de la variabilité du QT indépendante du rythme cardiaque précise que de multiples valeurs de QT existent pour chaque intervalle RR. Pour chaque intervalle RR, l'intervalle QT existe en tant que valeur probabiliste qui ne peut être estimée de façon fiable qu'en utilisant la moyenne d'un nombre de complexes ECG associé suffisamment grand pour chaque valeur RR. Cependant, lorsque les valeurs de QT probabilistes sont substituées aux mesures ponctuelles du QT dans un modèle de régression QT-RR, les coefficients de correction du QT résultant permettent de dissocier les effets de la fréquence cardiaque de l'intervalle QT avec plus de précision. Bien que cette constatation ait été confirmée indépendamment par plusieurs chercheurs (Champeroux-Pascal 2009; Champeroux, Ouille et al 2010 ; Honda, Komatsu et al 2010; Komatsu, Honda et al 2010), les méthodologies antérieures n'ont pas intégré la propriété de la variabilité du QT indépendante du rythme cardiaque dans les modèles de régression QT-RR, entraînant ainsi une augmentation de la variabilité intra- et inter-étude.

Pour chaque jeu de données de 24 h, le calcul d'une correction individuelle est effectué en attribuant d'abord la valeur de chaque intervalle QT à un « bin » de RR de 10 ms, puis en appliquant un modèle de régression log linéaire ($\log(QT) = \beta \cdot \log(RR) + \alpha$) pour calculer les paramètres de la relation QT-RR à partir des moyennes des intervalles QT et RR de chaque « bin ». La valeur de la pente (β) provenant de l'ensemble des données sans traitement est ensuite appliquée dans la formule $QT_{ca} = QT / RR^{\beta}$ pour chaque valeur de QT enregistrée (Holzgreffe, Cavero et al. 2007). La grande quantité de données générées est ensuite condensée en des segments de temps appropriés (de 10 ou 5 minutes) pour décrire les effets

dépendants du temps des médicaments analysés. Cette méthode, d'abord évaluée chez le chien et le singe cynomolgus, a démontré des résultats fiables et une bonne applicabilité, indépendamment des espèces analysées.

4.9. Méthode des « bins »

La méthode des « bins » a été conçue pour éviter la nécessité de corriger le QT en comparant l'intervalle QT non corrigé avec et sans traitement à des fréquences cardiaques similaires. Pour une période de temps donnée (habituellement une période autour du C_{max}) tous les complexes ECG individuels sont regroupés dans un « bin » de RR spécifique en fonction de leur valeur de RR associée (Badilini, Maison-Blanche et al. 1999). Tous les complexes précédés par une stabilité de la fréquence cardiaque présents dans un « bin » de RR spécifique sont utilisés pour calculer un battement médian. Un battement médian se compose d'une seule forme d'onde représentative de tous les complexes associés. Il est généralement bien défini (en raison de la moyenne) et permet l'utilisation de mesures hautement automatisés, suivi par l'examen de l'opérateur. Cette méthode des « bins » permet ainsi la comparaison de l'intervalle QT non corrigé à un RR normalisé (généralement 1000 ms), qui est particulièrement adapté pour évaluer l'intervalle QT en présence d'un effet sur la fréquence cardiaque induit par le médicament (Extramiana, Maison-Blanche et al. 2006). Le principal problème lié à la méthode des « bins » est le fait que la période d'intérêt doit être présélectionnée, entraînant ainsi la perte des effets dépendants du temps. Cependant, une variante, appelée « time-binning » a été développée et permet le calcul des battements médians de complexes obtenus sur des périodes de temps au lieu de « bins » de RR (Extramiana, Badilini et al. 2007).

Objectifs

Aujourd'hui, en raison des diverses influences qui peuvent avoir un impact sur la mesure juste et reproductible de l'intervalle QT, l'une des principales questions encore sans réponse est de savoir si l'analyse des études TQT telle qu'elle est actuellement évaluée avec les méthodes semi-automatiques, est optimale, et dans le cas contraire, si elle peut être optimisée?

Les objectifs du travail actuel sont d'évaluer l'applicabilité des technologies de mesure automatique de l'ECG en combinaison avec une méthodologie d'analyse continue du QT / QTc afin d'améliorer l'évaluation de la repolarisation cardiaque au cours des essais cliniques et de

produire des résultats comparables ou supérieurs à ceux actuellement obtenus avec des méthodes semi-automatiques plus laborieuses.

Dans les parties I et II, les analyses ECG continues utilisant différentes algorithmes de mesure de l'ECG sont comparés à l'analyse semi-automatique réalisée par des laboratoires spécialisés. Des comparaisons parallèles sont effectuées après traitement avec placebo, moxifloxacine (l'agent de référence des études TQT), et saquinavir boosté par ritonavir (un médicament caractérisé par des modifications de la morphologie de l'onde T).

La confirmation de la justesse et reproductibilité de l'analyse ECG en continu employant plusieurs méthodes automatiques avancées apporterait de nouvelles méthodes puissantes caractérisées par:

- Amélioration de la puissance et de la résolution temporelle due à l'inclusion de tous les complexes ECG enregistrés
- Mesures plus détaillées et fiables des effets possibles du médicament au cours du temps
- Diminution de la variabilité due à l'utilisation de méthodes de correction du QT plus adaptées
- Diminution des exigences en temps et ressources (main-d'œuvre, coût)

En raison de l'actuelle absence de standardisation pour l'analyse ECG continue, la partie III évalue diverses influences physiologiques, techniques et pharmacologiques ainsi que leur impact sur la reproductibilité et la justesse des évaluations de la repolarisation cardiaque. L'objectif de ces évaluations est de fournir des recommandations pour une utilisation optimale des méthodes d'évaluation ECG en continu.

Résultats

1. Partie I: Validation d'une méthode de reconnaissance de forme pour les mesures ECG en continu

1.1. Justification

L'analyse continue des tracés ECG exige que des mesures ECG soient effectuées automatiquement. Toutefois, la mesure automatique des intervalles ECG reste un défi car

diverses influences techniques, physiologiques et cliniques peuvent affecter la mesure juste et reproductible réalisée par des logiciels de mesure automatisés. La première étape nécessaire afin d'effectuer une évaluation précise des effets induits par les médicaments sur la repolarisation cardiaque est de s'assurer que tous les intervalles ECG sont évalués de manière appropriée. Les logiciels de mesure ECG entièrement automatisés dépourvus de fonction de révision ont généralement été associés à des erreurs de mesure importantes en présence de morphologies de forme d'onde atypiques, ce qui a limité l'utilisation à grande échelle et l'acceptation des méthodes d'analyse d'ECG en continu pour l'évaluation des risques de torsades de pointes (McLaughlin, Campbell et al 1996; Malik et Camm 2001). Récemment, un module de reconnaissance de forme (PRO) a été développé et permet la mesure de milliers de battements en se basant sur quelques complexes de référence sélectionnés et ajustés manuellement. Ainsi, nous avons émis l'hypothèse que l'analyse réalisées avec la reconnaissance des formes pouvait fournir des mesures de l'intervalle QT plus fiables, même en présence de médicaments induisant des changements morphologiques de l'onde T. Par conséquent, le critère d'évaluation principal est d'évaluer les performances et les améliorations potentielles associées à l'analyse de reconnaissance de forme (PRO) par rapport à une méthode entièrement automatisée (ALG) et à la méthode semi-automatique (SA) conventionnelle dans trois études différentes qui comprenaient des médicaments avec et sans effets sur les morphologies de forme d'onde. En tant que critère d'évaluation secondaire, toutes les analyses ont été effectuées en utilisant une analyse basée sur la distribution (Holzgreve, Ferber et al. 2012) afin de valider davantage cette méthode comme une pratique appropriée pour les évaluations ECG en continu des risques torsadogènes dans les études cliniques.

1.2. Résumé de publication

Actuellement, l'évaluation clinique de la prolongation de la repolarisation cardiaque induite par les médicaments reste une préoccupation majeure pour les organismes de réglementation et tout nouveau médicament en développement est tenu de démontrer une sécurité relative pour le risque d'induire des torsades de pointes (TdP), indirectement évalué par l'estimation de leur potentiel d'allongement de l'intervalle QT. Les compagnies pharmaceutiques sous-traitent généralement l'analyse des données ECG à des laboratoires spécialisés dans les analyses semi-automatisées (SA) réalisées par des cardiologues. Bien que l'analyse effectuée par ces laboratoires soit actuellement reconnue comme la méthode de référence, il existe encore de

nombreuses limites bien connues qui peuvent être résolues grâce à l'utilisation de logiciels de mesure ECG automatiques. Cependant, les logiciels de mesure automatiques qui emploient des algorithmes de détection de l'onde T (ALG) ne sont pas bien acceptés par les organismes de réglementation pour l'analyse principale des études TQT. Nous avons alors émis l'hypothèse que le logiciel de reconnaissance de formes nouvellement développé (PRO) pouvait améliorer cette situation.

La performance des méthodes de mesure automatiques (ALG et PRO) a été évaluée par comparaison directe de l'effet prolongeant l'intervalle QT induit par la moxifloxacin aux temps de mesures analysés par un laboratoire spécialisé lors d'une étude TQT.

Comparé à ALG, PRO réduit la fréquence des erreurs de mesure de la fin de l'onde T (5,6% vs 0,1%), réduit la variabilité intra-individuelle du QT ($12,6 \pm 5,9$ vs $4,9 \pm 1,1$ ms) et a permis la récupération de 3/58 sujets qui présentaient un R^2 inacceptable ($<0,9$).

La méthode PRO a le potentiel d'accélérer le processus de développement du médicament en réduisant les besoins en personnel, les frais d'étude liés aux mesures semi-automatiques, et le nombre de volontaires sains à risque, tout en améliorant l'évaluation des risques de prolongation de la repolarisation. Les résultats actuels démontrent que l'utilisation de méthodes de reconnaissance de forme en conjonction avec la méthode basée sur la distribution représente une alternative de choix aux analyses semi-automatisées réalisées par des laboratoires spécialisés.

Tableau 1. Comparaison entre ALG et PRO

	ALG	PRO
Erreurs de mesure	5.6%	0.1%
Valeur des exposants de correction individuelle (β)	0.303 ± 0.06	0.294 ± 0.05
Coefficient de détermination (R^2)	0.963 ± 0.05	0.981 ± 0.01
Sujets exclus ($R^2 < 0.9$)	3	0
Mesure de référence (Jour -1) - écart-type intra-individuel intra-temps de mesure	12.6 ± 5.9	4.9 ± 1.1
Moxifloxacin - écart-type inter-individuel du $\Delta\Delta QT_{ca}$ (ms)	15 ± 4.4	9.1 ± 0.5

1.3. Conclusion

Dans la première partie, il a été démontré que l'utilisation de la méthode de reconnaissance de forme permet d'ajuster efficacement les marquages automatiques ce qui a permis de corriger les erreurs de mesure généralement associées aux mesures de l'algorithme entièrement automatisé. La méthode de reconnaissance de forme a fourni des mesures ECG reproductibles, même pendant les périodes de repas, prises de sang et activités physiques caractérisées par une grande variabilité avec ALG. En outre, l'application de la méthode basée sur la distribution a permis le calcul précis des facteurs de correction individuels entièrement dissociés de la fréquence cardiaque au cours des 24h d'enregistrements ECG en continu. Par rapport à la méthode SA de référence, PRO fournit généralement des résultats équivalents et des conclusions similaires. Par conséquent, PRO, en conjonction avec l'analyse basée sur la distribution offre une alternative fiable à la méthode SA conventionnelle pour l'évaluation du risque de torsades de pointes, tout en réduisant le coût de l'étude et les besoins en main-d'œuvre, et en augmentant la résolution temporelle.

2. Partie II: Comparaison des méthodes

2.1. Justification

Les analyses décrites dans la partie I ont démontré que les erreurs de mesure liées aux algorithmes automatiques pouvaient être corrigées grâce à l'utilisation de la méthode de reconnaissance de forme. Cette comparaison a été réalisée avec un logiciel unique de mesure ECG, Ponemah (ALG et PRO). Bien que la correction des erreurs de mesure ECG avec PRO ait effectivement réduit la variabilité associée à la détection de l'effet du médicament sur l'intervalle QT avec ALG, plusieurs autres méthodes avancées de mesure du QT employant diverses combinaisons d'algorithmes et de fonctionnalités ont été développées (Hnátková, Gang et al 2006; Sarapa, Gussak et al 2009; Strachan, Hughes et al 2009; Tyl, Kabbaj et al 2009; 2011 Couderc, Garnett et al; Green, Kligfield et al 2012; Meyer, Ferber et al. 2012). La plupart de ces nouvelles méthodes ont été comparées à la méthode manuelle et / ou semi-automatique effectuée par des cardiologues et a donné des évaluations du QT équivalentes. Cependant, ces méthodes de mesure ECG n'ont jamais été directement comparées les unes aux autres en utilisant une analyse ECG en continu, sur les mêmes données caractérisées par un allongement du QTc avec et sans changements morphologiques de l'onde T. Nous avons donc émis

l'hypothèse que d'autres plates-formes de mesure ECG utilisant des approches méthodologiques différentes pour mesurer et analyser les tracés ECG pouvaient également fournir une évaluation fiable des risques de torsades de pointes.

Dans la deuxième partie, trois méthodes de mesure automatiques (BioQT, Ponemah PRO, WinAtrec) ont été sélectionnés pour une comparaison parallèle d'une étude TQT qui comprenait un médicament qui induit des changements morphologiques de l'onde T. Bien que conceptuellement similaire (toutes les méthodes fournissent des mesures continues d'intervalle QT et des corrections du QT), chacune de ces applications intègre des fonctionnalités analytiques spécifiques qui lui sont unique. La compréhension globale de ces caractéristiques et leur impact possible sur les évaluations de la repolarisation cardiaque est une étape importante vers la normalisation des méthodes d'analyse ECG en continu.

En bref, BioQT est entièrement automatisé (aucun ajustement de l'opérateur n'est requis) et emploie des transformées en ondelettes et un modèle de Markov caché (HMM) afin de mesurer et d'auto-vérifier les mesures des intervalles QT. Les mesures QT pour chaque battement sont ensuite corrigées individuellement en utilisant un facteur de correction mobile sur le temps (QT_{clc}) (Strachan, Hughes et al. 2009). Ponemah PRO utilise un algorithme de reconnaissance de forme, où l'intervalle QT pour chaque battement est mesuré sur la base de complexes de référence ajustés par un opérateur. L'analyse basée sur la distribution est ensuite appliqué pour obtenir des valeurs de l'intervalle QT corrigées individuellement en se basant sur le facteur de correction obtenu pendant le jour sans traitement (QT_{ca}) (Holzgreffe, Ferber et al 2012; Meyer, Ferber et al 2012). La méthode de « time-binning » de WinAtrec construit et mesure automatiquement des battements médians sur 60 s de la dérivation choisie à partir des données holter. Ces battements médians sont ensuite revus et ajustés par un opérateur. Une correction individuelle est ensuite appliquée à chaque battement médian (QT_{cl_{médian}}) en utilisant un facteur de correction obtenu des données sans traitement (Extramiana, Badilini et al. 2007). Par conséquent, la partie II a consisté à évaluer les performances de trois méthodes de mesure ECG en continu pour l'évaluation de données électrocardiographiques caractérisées par des morphologies de forme d'onde anormales, d'évaluer d'éventuelles différences spécifiques aux méthodes ainsi que leur impact possible sur l'analyse de la repolarisation cardiaque.

2.2. Résumé de publication

Les avancées technologiques récentes permettent maintenant des enregistrements ECG continus de haute résolution qui peuvent être automatiquement annotés par divers logiciels et algorithmes afin d'effectuer des évaluations détaillées et précises de la repolarisation cardiaque. Bien que les applications d'analyse QT entièrement automatisées soient disponibles depuis plusieurs années, ces méthodes d'analyse automatisées évaluant un très grand nombre de données (millions de battements) ne sont pas encore couramment acceptées par les autorités de réglementation pour l'analyse des études TQT où les mesures SA effectuées par les laboratoires spécialisés restent la méthode de référence. Dans la partie II, trois méthodes de mesure automatiques du QT/QTc en continu ont été évaluées:

- BioQT (v. 1.2, OBS medical, Oxford)
- Ponemah avec reconnaissance de forme (v. 5.0, Data Sciences International, ST. Paul, Minnesota)
- WinAtrec (v. 1.2.0, AMPS LLC, New York)

L'objectif de l'étude était d'évaluer l'applicabilité des analyses continues QT / QTc réalisées avec trois méthodes de mesure automatisées du QT afin de reproduire les résultats et les conclusions rapportées par un laboratoire spécialisé pour une étude TQT de référence.

Malgré de légères différences spécifiques aux méthodes de mesure en termes de variabilité de la mesure, d'intervalles de confiance, du nombre de mesures valides, et de quantification de l'effet $\Delta\Delta\text{QTc}$, les trois applications logicielles d'analyse ECG ont démontré une sensibilité de l'étude adéquate et ont reproduit les conclusions obtenues avec la méthode SA de référence, avec, en plus, une forte amélioration en termes de résolution temporelle et une réduction des coûts analytiques associés.

En conclusion, ces méthodes d'analyse en continu offrent une résolution temporelle précédemment indisponibles pour l'évaluation du risque de prolongation de la repolarisation, et permettent l'utilisation d'autres outils d'analyse puissants. Avec plus d'expérience, ces données suggèrent que les méthodes SA actuelles pourraient être efficacement remplacées par une analyse ECG entièrement automatisée.

Tableau 2. Effet moyen du $\Delta\Delta\text{QTc}$ pour chaque traitement et variabilité associée à chacun des temps de mesure.

Traitement	Correction	BioQT *				Ponemah PRO *			
		n	effet moyen	SD	CI	n	effet moyen	SD	CI
Moxi400	QTcF	36.6 ± 5.3	7.9 ± 2.4	7.9 ± 1.0	2.7 ± 0.4	40.8 ± 4.6	7.8 ± 2.3	10.6 ± 1.3	3.4 ± 0.6
	QTcl	36.6 ± 5.3	9.1 ± 2.8	11.1 ± 1.7	3.8 ± 0.6	40.8 ± 4.6	7.9 ± 2.3	11.3 ± 1.3	3.6 ± 0.6
SQR1000	QTcF	31.5 ± 6	9.4 ± 2.2	12.5 ± 2.0	4.7 ± 1.1	37.6 ± 5.3	10.6 ± 2.7	15.2 ± 1.5	5.1 ± 0.7
	QTcl	31.5 ± 6	9.9 ± 3.1	15.3 ± 1.7	5.7 ± 1.1	37.6 ± 5.3	9.0 ± 2.5	15.4 ± 1.5	5.1 ± 0.7
SQR1500	QTcF	32.4 ± 5.9	16.2 ± 2.1	12.1 ± 1.4	4.4 ± 0.9	40.7 ± 4.9	19.5 ± 2.6	17.7 ± 1.4	5.6 ± 0.6
	QTcl	32.4 ± 5.9	16.6 ± 2.9	15.3 ± 2.0	5.6 ± 1.1	40.7 ± 4.9	17.5 ± 2.7	19.0 ± 1.5	6.1 ± 0.7
Traitement	Correction	WinAtrec *				Méthode conventionnelle **			
		n	effet moyen	SD	CI	n	effet moyen	SD	CI
Moxi400	QTcF	47.3 ± 1.9	9.3 ± 2.9	8.6 ± 1	2.5 ± 0.3	53.1 ± 2.1	9.5 ± 2.4	10.2 ± 1.1	2.8 ± 0.3
	QTcl	47.3 ± 1.9	9.5 ± 2.9	8.9 ± 1	2.6 ± 0.3	53.8 ± 2.0	9.1 ± 2.4	10.3 ± 1.0	3.2 ± 0.0
SQR1000	QTcF	45.8 ± 2.4	14.0 ± 3.0	13.3 ± 1.2	4 ± 0.4	54.3 ± 2.3	14.4 ± 3.3	13.3 ± 1.2	3.6 ± 0.4
	QTcl	45.8 ± 2.4	13.5 ± 2.9	13.4 ± 1.2	4 ± 0.4	54.9 ± 2.3	13.9 ± 3.2	13.5 ± 1.5	3.2 ± 0.0
SQR1500	QTcF	47.9 ± 2.8	23.8 ± 3.1	15.2 ± 1.2	4.4 ± 0.3	56.2 ± 2.3	22.8 ± 4.1	14.4 ± 1.5	3.9 ± 0.4
	QTcl	47.9 ± 2.8	23.3 ± 3.1	15.2 ± 1.2	4.4 ± 0.4	56.7 ± 2.2	22.7 ± 4.3	14.3 ± 1.5	3.1 ± 0.0

* moyenne sur 258 temps de mesure continus ** moyenne sur 9 temps de mesure spécifiés dans le protocole.

n = Nombre moyen de sujet à chaque temps de mesure; Effet moyen = Moyenne de l'effet du traitement (ms) sur 24 h; SD = Moyenne d'écart-type inter-sujet de tous les temps de mesure (ms); CI = Moyenne des intervalles de confiance de tous les temps de mesure (ms); QTcF = Correction individuelle; QTcF = Correction de Fridericia

$$* \Delta\text{QTc}_{\text{traitement}} = \text{QTc}_{\text{traitement}} - \text{QTc}_{\text{traitement de référence (jour -1)}}$$

$$\Delta\text{QTc}_{\text{placebo}} = \text{QTc}_{\text{placebo}} - \text{QTc}_{\text{traitement de référence (jour -1)}}$$

$$\Delta\Delta\text{QTc} = \Delta\text{QTc}_{\text{traitement}} - \Delta\text{QTc}_{\text{placebo}}$$

2.3. Conclusion

Les résultats obtenus avec 5 méthodes de mesure ECG (BioQT, ALG, PRO, WinAtrec, SA) lors de 2 études TQT employant un médicament prolongeant l'intervalle QT en induisant des altérations de la morphologie de l'onde T (SQR) et un médicament ne prolongeant pas le QT

(SGLT2i) ont démontré des résultats très proches. Dans les deux études TQT, chaque méthode a effectivement détecté l'effet positif induit par la moxifloxacine et a démontré une sensibilité de l'étude appropriée. Tant l'effet négatif induits par le SGLT2i que l'effet positif induit par le SQR ont été détectés avec précision pour chaque méthode, démontrant ainsi que les méthodes automatiques de mesure ECG sont fiables et peuvent supplanter la méthode SA de référence pour l'analyse des études TQT. Toutes les méthodes évaluées se sont démarquées par plusieurs caractéristiques spécifiques à chaque logiciel. Ces différences spécifiques peuvent influencer sur la variabilité, les intervalles de confiance, le nombre de mesures valides, et l'effet quantitatif du $\Delta\Delta\text{QTc}$. Collectivement, les résultats actuels démontrent que l'analyse ECG en continu en conjonction avec des méthodes d'analyse raffinées sont fiables et permettent d'obtenir une meilleure résolution temporelle qui n'est pas possible avec l'échantillonnage réalisé lors des études SA de référence. La réduction simultanée des coûts associés aux études TQT et des exigences en main-d'œuvre soutient également l'utilisation de l'évaluation ECG continue comme une alternative efficace aux méthodes semi-automatisées.

3. Partie III: Recommandations pour l'analyse ECG continue

3.1. Justification

Dans le cadre des études conformes à la directive E14 pour évaluer les effets des médicaments sur l'intervalle QT de manière continue, quatre possibilités d'amélioration existent:

- Le développement ou l'utilisation d'appareils d'enregistrement ECG plus précis
- L'utilisation de protocoles d'étude plus adaptés
- L'amélioration des méthodes de mesure de l'intervalle QT (manuel ou automatique)
- Le raffinement des méthodes d'analyse.

Ces 4 domaines d'amélioration sont tous interconnectés. Par exemple, l'utilisation d'enregistrement à faible fréquence d'échantillonnage (<500 Hz) peut réduire la performance d'un algorithme automatique pour la mesure précise de l'intervalle QT. De la même façon, un intervalle QT parfaitement mesuré peut fournir des résultats très variables si la méthode d'analyse emploie des facteurs de correction sous-optimaux (par exemple QTcB) ou des résolutions temporelles inappropriées. Il est donc de première importance pour l'industrie et les organismes de réglementation de mesurer le QT et le QTc de manière juste et reproductible. L'obtention de résultats fiables et robustes avec des intervalles de confiance réduits est

nécessaire dans les études TQT car ils réduisent le risque d'obtenir un signal positif potentiellement lié à un temps de mesure isolé caractérisé par une forte variabilité et de larges intervalles de confiance. De nombreux facteurs sont susceptibles d'augmenter ou de diminuer la variabilité associée à l'évaluation de l'effet d'un médicament. La variabilité associée aux mesures QT peut être de deux origines, une partie de la variabilité QT est due aux conditions physiologiques normales, tandis que la partie restante peut être attribuée à des causes artificielles (technique ou méthodologique), comme les imprécisions de mesure du QT, une correction inappropriée du rythme cardiaque ou de l'hystérèse, ou d'autres biais introduit par l'analyste. Dans cette troisième partie, les principales sources de variabilité (physiologiques, techniques et pharmacologiques) ont été examinées afin d'affiner la méthodologie actuelle d'analyse ECG continue en réduisant la variabilité inhérente tout en conservant une haute résolution temporelle afin de fournir des évaluations de la repolarisation cardiaque robustes.

La partie III se concentre sur l'affinage et l'optimisation de la méthode de correction du QT basée sur la distribution des données et des méthodes d'analyses ECG continues de façon générale. L'analyse continue d'enregistrement ECG est à ce jour peu répandue pour plusieurs raisons : sa relative complexité liée au traitement de millions de mesures d'intervalles ECG, une pensée commune aujourd'hui obsolète présentant les méthodes automatiques comme peu fiables, ainsi qu'un manque de standardisation des méthodologies d'analyse au sein de la communauté scientifique. A ce jour, le choix de la méthode de correction de l'intervalle QT ainsi que de nombreux paramètres et filtres associés reste relativement subjectif. Par conséquent, l'évaluation de l'impact de différents paramètres, tels que la relation QT/RR, l'hystérèse, l'utilisation de moyennes, l'âge, le sexe, les variations circadiennes, et divers filtres de données, en termes de variabilité, de justesse, et d'intérêt ont permis d'établir des recommandations pour l'évaluation continue des risques torsadogènes.

3.2. Conclusion

La partie III a démontré que plusieurs caractéristiques techniques, méthodologiques ou analytiques ont le potentiel d'influencer les résultats finaux de l'analyse en continu des études TQT. Les analyses actuelles ont montré des résultats similaires quel que soit le choix de la dérivation analysée, où la seule différence observable consistait à une variabilité accrue lorsqu'un examen attentif et l'ajustement des mesures ECG a été omis. Pour ces raisons, lors

de l'analyse ECG en continu, la dérivation à analyser doit être choisie en fonction de sa capacité à être mesurée de manière reproductible par un algorithme de mesure automatique. En d'autres termes, la dérivation choisie devra démontrer un rapport signal-bruit limité et des ondes de fortes amplitudes et bien inscrites afin de faciliter les mesures automatiques. En présence d'un signal de mauvaise qualité (spécifique au sujet ou à la période), une autre dérivation de bonne qualité (généralement la dérivation II ou V2 à V5) peut être utilisée en remplacement dans la mesure où la dérivation de remplacement est constamment utilisée entre les périodes d'un sujet spécifique. Des conclusions similaires ont été obtenues dans une étude récente qui a démontré que des dérivations alternatives pouvaient être efficacement utilisées lorsque l'intervalle QT ne peut pas être convenablement mesuré dans la dérivation principale (Salvi, Karnad et al. 2012).

Lorsque l'on utilise des méthodes automatiques de mesure ECG en continu, un examen attentif et l'ajustement des mesures doivent toujours être effectués. Malgré le fait que les analyses actuelles ont démontré des résultats acceptables avec les méthodes entièrement automatisées, l'omission de l'étape de revue et d'ajustement des marquages ECG mènera toujours à une augmentation de la variabilité, et, dans certains cas, peut conduire à des erreurs de mesure répétée qui se traduisent par une appréciation erronée de l'effet d'un médicament, en particulier en présence de tracés ECG anormaux.

L'évaluation de la relation QT-RR et d'autres paramètres ECG selon le sexe, l'âge et la période nyctéméral a confirmé certaines des conclusions déjà publiées, comme le fait que l'intervalle QT / QTc est plus long la nuit que le jour. De plus, chez la femme, l'intervalle QTc est plus long (Smetana, Batchvarov et al. 2002), la prolongation induite par les médicaments est plus grande (Benton, Sale et al 2000), et les facteurs de correction individuels sont plus élevés par rapport aux hommes (Extramiana, Maison-Blanche et al 1999; Sredniawa, Musialik-Lydkka et al. 2005). Il a aussi été montré que la diminution de l'écart-type du RR augmente avec l'âge (Tsuji, Venditti et al. 1996). En revanche, les résultats démontrant une diminution de la pente de la relation QT-RR augmentant avec l'âge et au cours de la journée, ou une diminution de la pente de la relation QT-RR la nuit par rapport à la journée n'ont pas été reproduits dans les analyses actuelles (Extramiana, Maison-Blanche et al. 1999), probablement à cause des différences méthodologiques liées à la sélection de complexes ECG précédés par une stabilité du rythme cardiaque comparé à la méthode basée sur la distribution. En dépit du manque de compréhension des mécanismes physiologiques responsables de ces différences, de nombreuses études portant sur l'électrophysiologie cardiaque ont souvent confirmé des disparités entre les sexes (Extramiana, Maison-Blanche et al. 1999; Bonnemeier, Richardt et al.

2003; Sredniawa, Musialik-Lydka et al 2005; Smetana et Malik 2013). Cependant, la signification clinique et l'impact possible de ces différences est encore à déterminer. À la lumière de ces faits, la prolongation du QT induite par les médicaments observée chez les femmes après l'administration de SQR suggère que les études TQT devraient inclure un rapport équilibré entre les hommes et les femmes, car un effectif d'étude uniquement composé de femme ou d'homme pourrait surestimer ou sous-estimer l'effet d'un médicament. Des sujets âgés entre 18 à 60 ans peuvent ainsi être inclus dans les études cliniques du fait que l'analyse actuelle n'a pas démontré d'effets liés à l'âge pouvant influencer considérablement l'évaluation de la prolongation de l'intervalle QT.

Le calcul de la relation QT-RR individuelle basée sur la distribution doit être effectué sur toutes les données QT-RR du jour de l'enregistrement sans traitement. En effet, tandis que les pentes QT-RR mesurées le jour et la nuit étaient généralement similaires, l'inclusion conjointe des données QT-RR du jour et de la nuit pour calculer la relation QT-RR sur 24 heures a été associée à une augmentation artificielle de la pente QT-RR due au mélange, dans les "bins" de RR, de données QT-RR obtenues sous différentes influences du système nerveux autonome.

L'effet d'hystérèse n'a pas démontré d'impact sur les évaluations de l'effet du médicament en continu ($\Delta\Delta\text{QTc}$) du fait que la variabilité de la mesure QTc liée à hystérèse a été éliminée au cours du processus de calcul de la moyenne de milliers de battements lors de la création de segments de temps. Cependant, inclure un certain niveau de calcul de moyenne du RR (sur 60 secondes) dans la formule de mesure du QTc permet de réduire la variabilité du QTc, ce qui fournit encore une amélioration substantielle du fait que l'écart type de l'intervalle QTc est généralement utilisé pour le calcul de la taille des effectifs d'une étude clinique.

La longueur des segments de temps pour représenter l'évolution des effets des paramètres ECG au cours du temps est importante car des segments de temps longs peuvent masquer les effets rapides induits par le médicament, tandis que des temps courts risquent d'augmenter inutilement la variabilité. La présente analyse a démontré que les segments de 5 min fournissent le meilleur équilibre entre information scientifique et variabilité.

De plus, tandis que le travail présenté se concentre principalement sur l'application et le raffinement de l'évaluation de la prolongation du QT par les médicaments de manière continue, l'inclusion d'autres biomarqueurs et / ou l'ajout d'autres méthodologies (méthode des "bins", restitution de l'ECG...) basées sur les mêmes mesures ECG en continu pourraient permettre

une évaluation supérieure du risque de torsades de pointes, comme suggère l'analyse TPE qui a détecté un signal positif avec SQR qui n'a pas été détecté pour la moxifloxacin.

Conclusions

Les objectifs de ce travail étaient d'évaluer si l'utilisation des technologies automatiques de mesure ECG pouvait fournir des analyses fiables de l'intervalle QT / QTc de manière continue, afin de permettre une meilleure évaluation des risques pro-arythmiques lors des études cliniques comparé aux méthodes semi-automatiques traditionnelles.

Les résultats obtenus dans la Partie I ont démontré que:

- Les algorithmes entièrement automatisés sont associés à des erreurs de mesure qui sont amplifiées en présence de modification de la morphologie de l'onde T, et par conséquent, nécessitent la revue et l'ajustement des marquages ECG.
- La mise en œuvre d'une méthode de reconnaissance de forme a permis de corriger efficacement les erreurs de mesure de l'algorithme automatique.
- La méthode PRO a diminué la variabilité des mesures QT pendant le jour sans traitement, en présence de changements de morphologie de l'onde T, et pendant les périodes d'activités physiques non-contrôlées, démontrant ainsi que PRO est bien adapté pour l'analyse des ECG en continu.
- L'application de la méthode basée sur la distribution sur des données continues a permis le calcul précis des facteurs de correction individuels et ainsi de dissocier l'effet de la fréquence cardiaque.
- Par rapport à la méthode semi-automatique de référence, PRO en conjonction avec l'analyse basée sur la distribution a généré des résultats équivalents et des conclusions similaires, mais avec une résolution temporelle considérablement augmentée et des coûts liés à l'analyse réduits.
- En conclusion, la partie I a démontré que les analyses ECG en continu permettent une évaluation plus détaillée de l'intervalle QT/QTc par l'utilisation de méthodes de mesure ECG automatisées (Ponemah PRO) conjointement à une méthode d'analyse appropriée (analyse basée sur la distribution).

Dans la deuxième partie, quatre méthodes différentes de mesure ECG automatisées (BioQT, ALG, PRO, WinAtrec, et SA) ont été évaluées afin de mieux déterminer l'applicabilité de

l'analyse ECG en continu et de découvrir les potentiels impacts et différences liés à chaque technologie et méthode de mesure de l'intervalle QT. Les résultats de ce travail ont démontré que:

- La comparaison en parallèle de toutes les méthodes a démontré des effets induits par la moxifloxacine similaires et une sensibilité de l'étude appropriée.
- Les effets négatifs du SGLT2i et positifs du SQR sur la prolongation du QT ont été détectés avec chaque méthode, ce qui suggère que les méthodes automatisées peuvent supplanter la méthode SA pour l'analyse des études TQT.
- Les fonctionnalités spécifiques à chaque méthode automatisée a donné lieu à des différences de variabilité, d'intervalles de confiance, de nombre de mesures valides et parfois d'effets quantitatifs de l'intervalle QT, en particulier, en présence de modifications de la morphologie de l'onde T. Bien que chaque méthode ait démontré des conclusions finales similaires, certifier de l'exactitude d'une des méthodes évaluées n'est actuellement pas réalisable.
- Le QT corrigé par la formule de Fridericia (QTcF) peut être utilisé de manière fiable tant qu'il n'y a pas d'effets substantiels sur le RR.
- Collectivement, les résultats obtenus avec les trois méthodes de mesure ECG (BioQT, Ponemah PRO, WinAtrec) suggèrent que les analyses ECG en continu sont une alternative fiable pour l'évaluation des risques torsadogènes comparé à la méthode semi-automatisée.

La partie III se focalise sur la formulation de recommandations concernant les aspects techniques, méthodologiques et analytiques de l'évaluation de l'intervalle QT en continu:

- La dérivation à analyser doit être choisie en fonction de sa capacité à être mesurée de façon reproductible par une méthode de mesure automatique et, lorsque la dérivation sélectionnée présente un signal de mauvaise qualité, elle peut être efficacement remplacée par une autre dérivation de bonne qualité (généralement la dérivation II, ou V2 à V5).
- Les méthodes de mesure ECG entièrement automatisées qui omettent les processus de vérification et d'ajustement des marquages ne sont pas recommandées.
- L'analyse des influences du sexe, de l'âge et des périodes nyctémérales sur l'intervalle QT et la relation QT-RR a confirmé les résultats publiés antérieurement. De plus, ces

résultats confirment que l'effectif de l'étude clinique doit inclure à la fois des hommes et des femmes de différents âges.

- La détermination du facteur de correction individuel avec l'analyse basée sur la distribution doit de préférence être effectuée sur des données obtenues en journée car le mélange des données QT-RR obtenues sous différentes influences du système nerveux autonome peut modifier la pente QT-RR.
- L'hystérèse du QT n'a démontré aucune incidence sur l'évaluation de l'effet du médicament en raison de l'inclusion de milliers de battements pour l'analyse ECG en continu. Cependant, l'utilisation de moyennes de RR (60 secondes) dans la formule de correction du QT corrigé permet de réduire la variabilité de la mesure du QTc.
- Une durée de 5 min pour les segments de temps pour l'analyse continue a été démontrée comme optimale pour la représentation de l'évolution des paramètres ECG au cours du temps.
- L'analyse ECG en continu facilite l'évaluation d'autres biomarqueurs qui, avec une meilleure expérience, pourrait fournir une évaluation intégrée et supérieure des risques pro-arythmiques lors du développement des médicaments.

En conclusion, les travaux réalisés démontrent que de nombreux progrès technologiques et méthodologiques ont été réalisés et que de nombreux outils sont maintenant disponibles et permettent d'effectuer efficacement des analyses ECG en continu, qui, représentent aujourd'hui une alternative plus détaillée et fiable par rapport à la méthode semi-automatisée de référence.

Summary

Drugs which induce a delay in cardiac repolarization measured as QT interval prolongation on the electrocardiogram (ECG) have been associated with a potential to increase the risk of arrhythmias, especially Torsades de pointes (TdP), a potentially lethal polymorphic ventricular tachycardia. Over the last decades, a long and growing list of drugs have been subject to withdrawn or relabeled because of drug-induced QT prolongation. In 2005, the regulatory authorities of the United States, Europe, and Japan adopted the International Conference on Harmonization (ICH) guidance which proposes standardized methods for the evaluation of drug-induced QT prolongation in both preclinical (ICH S7B) and clinical studies (ICH E14). The ICH E14 guidance requires that almost all new drugs in clinical development demonstrate relative safety for the risk of arrhythmias, assessed during the well-standardized Thorough QT study (TQT). The TQT study is generally performed in healthy subjects, includes both positive and negative controls, continuous 12-lead ECG recordings (24 h), and protocol-designed time points for plasma drug levels and ECG extractions. Subject number, ECG sampling and the associated statistical methods must be able to reliably exclude a threshold pharmacologic effect of ~5 milliseconds on cardiac repolarization (assessed as a change in the rate-corrected QT interval) at each time point. At the time of its implementation, the authors of the E14 guidance expressed a lack of confidence towards automated ECG measurement methods, which, despite their advantage of being consistent, time-efficient, and reproducible, have generally been associated with waveform measurement errors or inaccuracies in presence of abnormal ECG waveforms, low T wave amplitudes, or noise. Accordingly, outsourcing TQT study data analysis to ECG core laboratories specialized in semi-automated ECG annotations performed by trained cardiologists has become a standard practice among pharmaceutical companies.

Of note, the analyses performed by ECG core laboratories are restricted to the extraction of a few beats at sparse protocol scheduled time points. This practice, by definition, results in the exclusion of the majority of the data, poor temporal resolution, substantial time and resource (manpower, cost) requirements, suboptimal derivation of individual QT-rate correction coefficients, and possibly inter-observer variability. In contrast, the inclusion of all beats measured by computerized methods from continuous 24 h recordings could resolve all of these deficiencies. Accordingly, considering the rapid advances and ongoing evolution of

computerized ECG platforms, it was hypothesized that the current standard practice might be improved by the use of advanced ECG measurement platforms in conjunction with refined continuous analysis methodologies that are not possible when restricting the ECG analysis to sparse scheduled time points.

The purpose of the proposed work is to evaluate, compare, improve, and possibly validate the applicability of computerized ECG measurement methods in conjunction with distribution-based QT rate-correction analysis employing data obtained during well standardized QT studies (TQT, first-in-human) performed at the institute of clinical pharmacology Roche and previously characterized by an ECG core laboratory.

In Part I, the application of a fully-automated ECG measurement method (Ponemah ALG) resulted in a substantial number of measurement errors during baseline periods which were further amplified in presence of saquinavir, a drug associated with T-wave morphology changes. These findings strongly suggest that an automated algorithm should not be applied without the careful review and adjustment (if needed) of an experienced operator. To resolve this issue, a pattern recognition module (Ponemah PRO) recently developed for the Ponemah software was employed during a TQT study characterized by saquinavir-induced T-wave morphological changes. PRO enables the automated marking of millions of ECG complexes based on manually selected and adjusted templates. Compared to ALG, PRO significantly decreased the number of measurement errors, the intra-individual intra-time point standard deviations, and the confidence intervals associated to the $\Delta\Delta\text{QTc}^*$. Moreover, the coefficient of determination (R^2) associated with the individual QT/RR regressions with PRO demonstrated an increased reliability compared to ALG and enabled the inclusion of 3 subjects which would have been excluded with ALG due to ECG measurement errors (Table I). After moxifloxacin administration, PRO yielded similar QTc increases with an associated decrease in variability compared to the traditional semi-automated method. In conclusion, pattern recognition enable the correction of ECG measurement errors associated with the fully-automated ECG measurement methods and reproduces the results obtained by an ECG core laboratory while simultaneously improving temporal resolution and reducing the manpower requirements and study costs.

Table I. Comparison of ALG and PRO

	<i>ALG</i>	<i>PRO</i>
Measurement error	5.6%	0.1%
Coefficient of determination, R ²	0.963±0.05	0.981±0.01
Baseline QT (ms)	376±20	403±22
Baseline intra-individual SD of QT - 24h (ms)	12.6±5.9	4.9±1.1
Baseline intra-individual SD of QT - timepoints (ms)	7.2±1.7	4.1±0.3
Moxifloxacin inter-individual SD of $\Delta\Delta QT_{ca}$ - timepoints (ms)	15±4.4	9.1±0.5

In Part II, two supplementary ECG measurement methods, BioQT and WinAtrec, were evaluated in order to detect possible differences resulting from the application of differing interval measurement technologies. BioQT is fully automated (no operator adjustments are required) and employs wavelet transforms and a Hidden Markov Model (HMM) to measure and self-check the QT intervals. WinAtrec *time-binning* analysis automatically computes and measures 60 s and single lead median beats from the continuous digital recordings which are then reviewed and adjusted by an operator. A detailed comparison demonstrated slight differences in variability, confidence intervals, number of valid measurements, and quantitative QTc effects between different vendor-specific computerized algorithms (BioQT, Ponemah PRO, WinAtrec), which occasionally resulted in algorithm-based measurement errors, especially in presence of abnormal ECG waveforms (Table II). Despite these differences associated with the strengths and weaknesses of each unique ECG measurement method, the three evaluated advanced automated ECG measurement applications were able to replicate the results and conclusions obtained by conventional sparse core laboratory semi-automated analysis, while simultaneously providing more detailed and reliable analysis over the 24 h. Accordingly, with proper experience, the utilization of advanced computerized methodologies is appropriate and reliable for the primary analysis of repolarization risk assessment studies (i.e. TQT studies) and would provide an improved alternative to supplant the standardized, limited evaluation of sparse ECG extractions.

Table II. Mean $\Delta\Delta\text{QTc}$ treatment effect and associated variability from all time points.

Treatment	Correction	BioQT *				Ponemah PRO *			
		n	mean effect	SD	CI	n	mean effect	SD	CI
Moxi400	QTcF	36.6 ± 5.3	7.9 ± 2.4	7.9 ± 1.0	2.7 ± 0.4	40.8 ± 4.6	7.8 ± 2.3	10.6 ± 1.3	3.4 ± 0.6
	QTcI	36.6 ± 5.3	9.1 ± 2.8	11.1 ± 1.7	3.8 ± 0.6	40.8 ± 4.6	7.9 ± 2.3	11.3 ± 1.3	3.6 ± 0.6
SQR1000	QTcF	31.5 ± 6	9.4 ± 2.2	12.5 ± 2.0	4.7 ± 1.1	37.6 ± 5.3	10.6 ± 2.7	15.2 ± 1.5	5.1 ± 0.7
	QTcI	31.5 ± 6	9.9 ± 3.1	15.3 ± 1.7	5.7 ± 1.1	37.6 ± 5.3	9.0 ± 2.5	15.4 ± 1.5	5.1 ± 0.7
SQR1500	QTcF	32.4 ± 5.9	16.2 ± 2.1	12.1 ± 1.4	4.4 ± 0.9	40.7 ± 4.9	19.5 ± 2.6	17.7 ± 1.4	5.6 ± 0.6
	QTcI	32.4 ± 5.9	16.6 ± 2.9	15.3 ± 2.0	5.6 ± 1.1	40.7 ± 4.9	17.5 ± 2.7	19.0 ± 1.5	6.1 ± 0.7
Treatment	Correction	WinAtrec *				ECG core laboratory **			
		n	mean effect	SD	CI	n	mean effect	SD	CI
Moxi400	QTcF	47.3 ± 1.9	9.3 ± 2.9	8.6 ± 1	2.5 ± 0.3	53.1 ± 2.1	9.5 ± 2.4	10.2 ± 1.1	2.8 ± 0.3
	QTcI	47.3 ± 1.9	9.5 ± 2.9	8.9 ± 1	2.6 ± 0.3	53.8 ± 2.0	9.1 ± 2.4	10.3 ± 1.0	3.2 ± 0.0
SQR1000	QTcF	45.8 ± 2.4	14.0 ± 3.0	13.3 ± 1.2	4 ± 0.4	54.3 ± 2.3	14.4 ± 3.3	13.3 ± 1.2	3.6 ± 0.4
	QTcI	45.8 ± 2.4	13.5 ± 2.9	13.4 ± 1.2	4 ± 0.4	54.9 ± 2.3	13.9 ± 3.2	13.5 ± 1.5	3.2 ± 0.0
SQR1500	QTcF	47.9 ± 2.8	23.8 ± 3.1	15.2 ± 1.2	4.4 ± 0.3	56.2 ± 2.3	22.8 ± 4.1	14.4 ± 1.5	3.9 ± 0.4
	QTcI	47.9 ± 2.8	23.3 ± 3.1	15.2 ± 1.2	4.4 ± 0.4	56.7 ± 2.2	22.7 ± 4.3	14.3 ± 1.5	3.1 ± 0.0

* average over 258 continuous time points. ** average over 9 protocol scheduled time points.

n = Average subject number at each time point; Effect = Average drug-induced effect (ms) over 24h; SD = Average of each time point associated between-subject standard deviation (ms); CI = Average of each time point associated confidence intervals (ms); QTcI corresponds to: QTcIc for BioQT, QTca for Ponemah PRO, QTcI_{median} for WinAtrec, QTcS for ECG core laboratory.

Part III focuses on the refinement and optimization of distribution-based analysis and continuous ECG analysis. Continuous ECG analyses are not currently widely used for various reasons including its relative complexity due to the analysis of millions of ECG intervals, an outdated but common belief presenting automated methods as less reliable than manual or semi-automated methods, and the lack of standardization of analysis methodologies in the scientific community. Currently, the choice of the QT correction method, filters, and other various parameters remain subjective. Consequently, the impact of various parameters such as the QT/RR relationship, QT hysteresis, mean values, age, gender, circadian variations, and other data filters were evaluated in terms of variability, applicability, and appropriateness in order to establish recommendations for the continuous evaluation of torsadogenic risks.

In conclusion, the purpose of this work was to demonstrate the pertinence of computerized ECG measurement methods compared to traditional methods, to evaluate the strengths and weaknesses associated with different technologies and methodologies of analysis, and to

provide recommendations for an optimal continuous evaluation of the electrocardiographic data recorded to evaluate the torsadogenic risk of pharmacological compounds.

$$* \Delta QTC_{\text{drug}} = QTC_{\text{drug}} - QTC_{\text{predose baseline}}$$

$$\Delta QTC_{\text{placebo}} = QTC_{\text{placebo}} - QTC_{\text{predose baseline}}$$

$$\Delta\Delta QTC = \Delta QTC_{\text{drug}} - \Delta QTC_{\text{placebo}}$$

Publications, posters and oral communications

Publications

Holzgreffe HH, Ferber G, Morrison R, **Meyer O**, Greiter-Wilke A, Singer T. Characterization of the human QT interval: novel distribution-based assessment of the repolarization effects of moxifloxacin. *J Clin Pharmacol*. Aug 2012;52(8):1222-1239.

Meyer O, Ferber G, Greig G, Holzgreffe HH. Pattern recognition analysis of digital ECGs: decreased QT measurement error and improved precision compared to semi-automated methods. *J Electrocardiol* 2013;46:118.

Meyer O, Greig G, Holzgreffe HH. Comparative TQT analysis with Three Fully-Automated Platforms: Comparison to Core Laboratory Semi-automated Results. *J Electrocardiol* 2013.

Holzgreffe HH, Ferber G, Champeroux P, Gill M, Greiter-Wilke A, Baird T, **Meyer O**, Saulnier M. Preclinical QT Safety Assessment: Cross-Species Comparisons and Human Translation from an Industry Consortium. *J Pharmacol Toxicol Methods* 2013.

Oral communications

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Title: Application of DSI Pattern Recognition Software for Human QT Interval Measurement : Demonstration of Efficacy in 60 Subjects

Author: Olivier Meyer

Posters

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Title: Decreased QT variability with DSI ECG Pattern Recognition: Comparison to Algorithm-Based Analysis in 60 Human Subjects

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Congress: Safety Pharmacology Society Meeting – September 2011– Innsbruck

Title: Efficacy of ECG Pattern Recognition Analysis in Human Subjects with Variable T-wave Morphology: Impact on Study Power

Author: Olivier Meyer, Henry H. Holzgrefe

List of abbreviations

Ach: Acetylcholine

AIDS: Acquired immunodeficiency syndrome

ALG: Algorithm (Ponemah)

ANS: Autonomic nervous system

AP: Action potential

APD: Action potential duration

AV: Atrioventricular

BID: Drug administration twice a day

BP: Blood pressure

Bpm: Beat per minute

BSM: Body surface mapping

cAMP: Cyclic adenosine monophosphate

Ca²⁺: Calcium ion

CHO: Chinese hamster ovary

CI: Confidence interval

Cl⁻: Chloride ion

C_{max}: Maximum concentration

CNS: Central nervous system

CPU: Clinical pharmacology unit

CV: Cardiovascular

DAD: Delayed afterdepolarization

DRL: Differential reinforcement for low rate

DSI: Data Sciences International

EAD: Early afterdepolarization

ECG: Electrocardiogram

ER: Exposure-response

FDA: Food and drug administration

FFT: Fast Fourier transform

HCN: Hyperpolarization-activated Cyclic Nucleotide-gated channels

HEK293: Human embryonic kidney cells

hERG: human ether-a-gogo

hESC-CM: Human embryonic stem cell-derived-cardiomyocyte

HF: High frequencies

HIV: Human immunodeficiency virus

HMM: Hidden Markov Model

HPD: Highest posterior density

HR: Heart rate

HRV: Heart rate variability

I_{cal} : L-type calcium current

I_{CaT} : Transient calcium current

I_{Cl} : Outward chloride current

I_f : Pacemaker or funny current

I_K : Delayed rectifier potassium current

I_{Kf} : Rapid delayed rectifier potassium current

I_{Ks} : Slow delayed rectifier potassium current

I_{K1} : Inward rectifying potassium current

I_{Na} : Sodium current

I_{NCX} : Sodium-calcium exchange current

I_{to} : Transient outward potassium current

ICH: International Conference on Harmonization

IC_{50} : Half maximal inhibitory concentration

Intra-SD: Intra-individual intra-time point standard deviation

IPCR: Institut de pharmacologie clinique Roche

iPSC-CM: Induced pluripotent stem cell-derived-cardiomyocyte

IUT: Intersection-union test

IWT: Implementation working group

JET: Jacketed external telemetry

K^+ : Potassium ion

LF: Low frequencies

LQTS: Long QT syndrome

LTCC: L-type calcium channel

MAD: Multiple ascending dose

MAP: Monophasic action potential

MCMC: Monte Carlo Markov chain

MED: Minimal effective dose

MTD: Maximum tolerated dose

Na⁺: Sodium ion

NCE: New chemical entity

NCX: Sodium-calcium exchanger

NHP: Non-human primate

P-gp: P-glycoprotein

PD: Pharmacodynamics

PI: Protease inhibitor

PK: Pharmacokinetics

PKA: Protein kinase A

PMCA: Plasma-membrane calcium ATPase

PNS: Parasympathetic nervous system

PRO: Pattern Recognition Option (Ponemah)

PVC: Premature ventricular complex

QTbtb: QT beat-to-beat (Fossa)

QTc: Corrected QT

QTca: Distribution-based rate-correction

QTcB: Bazett's rate-correction

QTcF: Fridericia's rate-correction

QTcFr: Framingham's rate-correction

QTcl: Individual-specific rate-correction

QTclc: BioQT rate-correction

QTcl_{median}: WinAtrec median beat-derived rate-correction

QTcS: Study-specific rate-correction

QSAR: Quantitative structure-activity relationship

RTV: Ritonavir

RyR: Ryanodine Receptors

SA: Semi-automated

SA (node): Sinoatrial

SAD: Single Ascending Dose

SD: Standard deviation

SERCA: Sarco/endoplasmic reticulum ATPase

SGLT2: Sodium glucose co-transporter 2

SGLT2i: SGLT2 inhibitor

SNRI: Selective noradrenaline reuptake inhibitors

SNS: Sympathetic nervous system

SQR: Saquinavir boosted ritonavir

SQTS: Short QT syndrome

SQV: Saquinavir

SSA: Steady state activation

SSI: Steady state inactivation

SSRI: Selective serotonin reuptake inhibitors

TdP: Torsades de pointes

TQT: Thorough QT study

TDR: Transmural dispersion of repolarization

TRlaD: Triangulation, Reverse use dependency, electrical Instability of the action potential, and Dispersion

T_{max} : Time of maximum plasma concentration

TRI: Triple reuptake inhibitor

UWT: Undecimated wavelet transform

VBA: Virtual basics for applications

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Introduction

1. Heart physiology

1.1. Heart anatomy

The heart is a muscular organ which pumps blood to all organs and tissues throughout a network of blood vessels, the circulatory system. The heart is located slightly to the left of the center of the chest cavity, between the lungs, and behind the sternum. It generally weighs between 250 and 350 g in healthy humans. The heart is divided into 4 chambers: two superior atria that receive the blood returning from the body, and two inferior ventricles that pump the blood back into the circulatory system. The left and right atria and left and right ventricles are separated by a wall of muscle known as the septum. The septum separates the left arterial heart from the right venous heart. Valves separate the atria from the ventricles and assure a unidirectional blood flow through the heart. Oxygenated blood enters the heart through the left atrium, pass through the left ventricle which contracts and pumps out the blood in the arterial system (through the aorta) to deliver oxygen and nutrients to all organs and tissues. Deoxygenated blood returns through the veins into the right atrium, right ventricle, and is pumped out towards the lungs for reoxygenation (Figure 1).

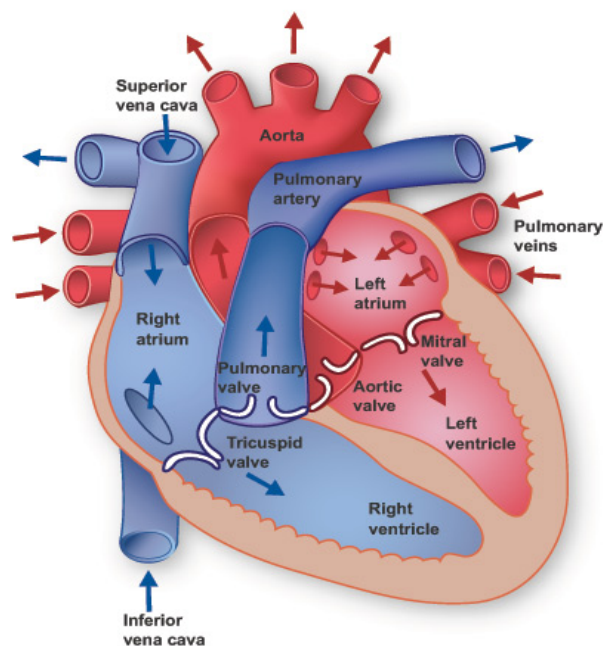


Figure 1. Heart anatomy (picture from Texas Heart Institute)

The cardiac wall is composed of three tissue layers: the endocardium, the myocardium, and the epicardium. The endocardium is the inner and thin layer of epithelial cells. The myocardium is the middle and thickest layer composed mostly of cardiomyocytes, responsible for the heart contraction. The epicardium is the outer layer and each layer is separated by conjunctive tissue while the whole is surrounded by a protective layer known as pericardium.

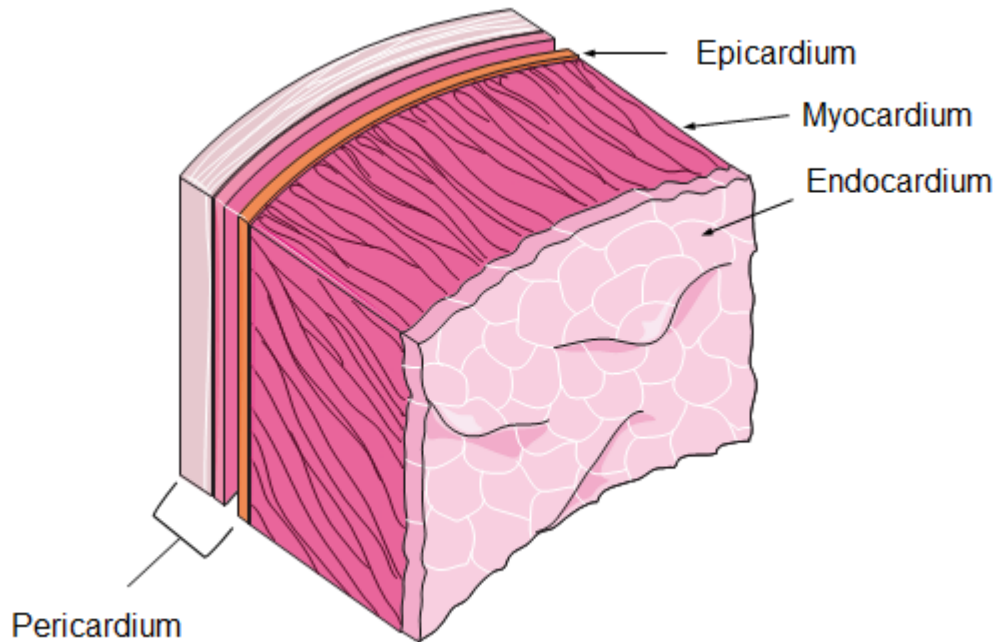


Figure 2. Cardiac wall tissue layers (picture from Servier Medical Art)

1.2. Cardiac cycle

In the healthy heart, the alternation of a contraction (systole) and a relaxation period (diastole) allows the filling and ejection of blood from the atria and ventricles, which together are called a cardiac cycle. A complete cardiac cycle first consists of diastole during which blood passively flow from the atria to the ventricles through the atrioventricular valves. At the end of the diastole, both atria contract (atrial systole) to propel an additional amount of blood into the ventricles. The atrial systole is followed by the ventricular systole during which ventricles contract, both atrioventricular valves close (first heart sound), and aortic and pulmonic (or semilunar) valves open, allowing the blood to be effectively pumped out of the heart. Finally, the ventricles relax and semilunar valves close (second heart sound), preventing blood to re-enter the ventricles while a new cardiac cycle starts.

1.3. Heart cell organization

The myocardium is constituted of two cell types: a majority of contractile cells which enable heart contraction, and autorhythmic cells which are responsible for the generation and propagation of the electrical impulse. Both cell types have the same origin (Cheng, Litchenberg et al. 1999), but differ in their cytoplasmic constituents.

The contractile cardiomyocytes are differentiated cells specifically found in the myocardium. Similar to skeletal muscle cells, cardiomyocytes are striated with narrow dark and light bands, due to the parallel arrangement of actin, myosin, and titin filaments which compose the myofibrils responsible for cell contraction. Cardiomyocytes present a large number of mitochondria (to provide energy as ATP) (Rosca and Hoppel 2010) and cell junctions which concentrate in structures known as intercalated discs. These intercalated discs allow neighboring cells to communicate and work together as a whole organ. Three types of cell junctions physically connect the disk membranes and enable both mechanical and electrical functions. The fascia adherens and macula adherens (or desmosomes) are both responsible for the mechanical anchoring of adjacent cardiomyocytes membranes by binding actin and intermediate filaments. The gap junctions are characterized by connexin channels that enable the cell-to-cell propagation of electrical impulses and chemical signals required for the synchronized activity of the cardiac cells (Balse, Steele et al. 2012).

Autorhythmic cells differ from contractile cells by their lack of striation and their capacity to generate their own rhythmic action potentials independently from the nervous system. These specialized cells are located in the sinoatrial and atrioventricular nodes, His bundle, right and left bundle branches, and Purkinje fibers.

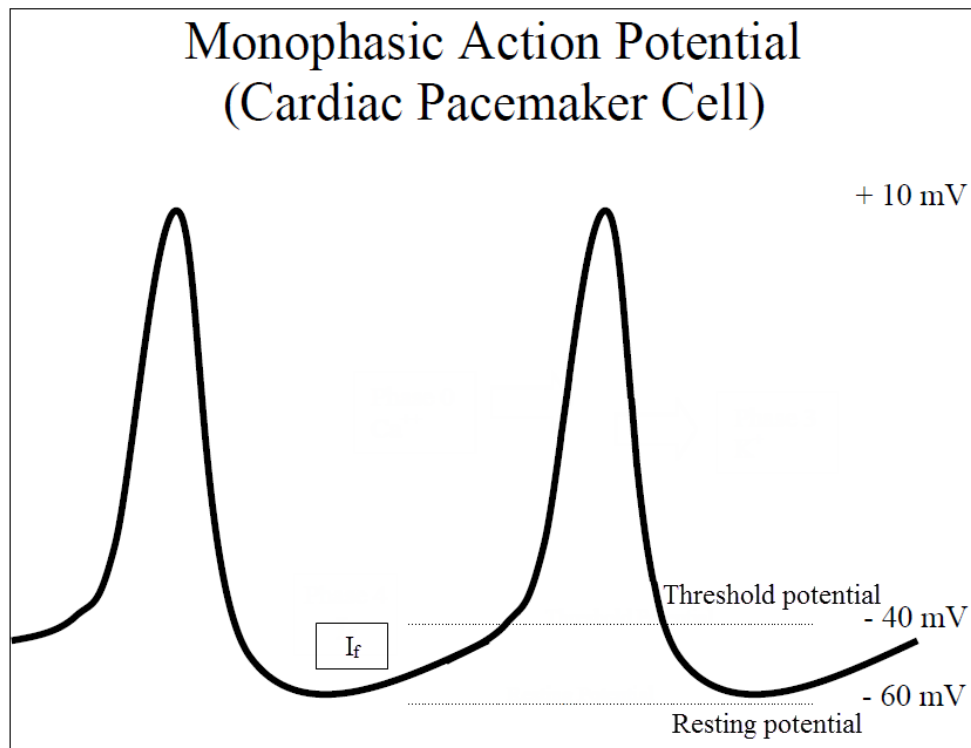
1.4. Heart electrophysiology

1.4.1. Pacemaker (autorhythmic) action potential

In excitable cells, the specific cell membrane permeability and the different ion concentrations between the intra- and extracellular milieu are responsible for the transmembrane potential. In most excitable cells, the transmembrane resting potential remains stable around -80 to -90 mV. However, in autorhythmic cells, the resting potential is around -60 mV and the Hyperpolarization-activated Cyclic Nucleotide-gated channels (HCN) responsible for the funny-current (I_f) slowly depolarize the membrane over time. The slow, spontaneous depolarization due to I_f inward,

mixed Na and K currents continues until it reaches the threshold of depolarization (-40 mV) at which an action potential is automatically generated (Figure 3). The speed at which the spontaneous depolarization reaches the threshold for firing an AP is responsible for the heart rate (DiFrancesco 2010). This phenomenon is called cardiac automaticity and is the result of the absence of a stable resting potential.

Figure 3. Automaticity of cardiac pacemaker cells mediated by the funny current (I_f). The I_f current



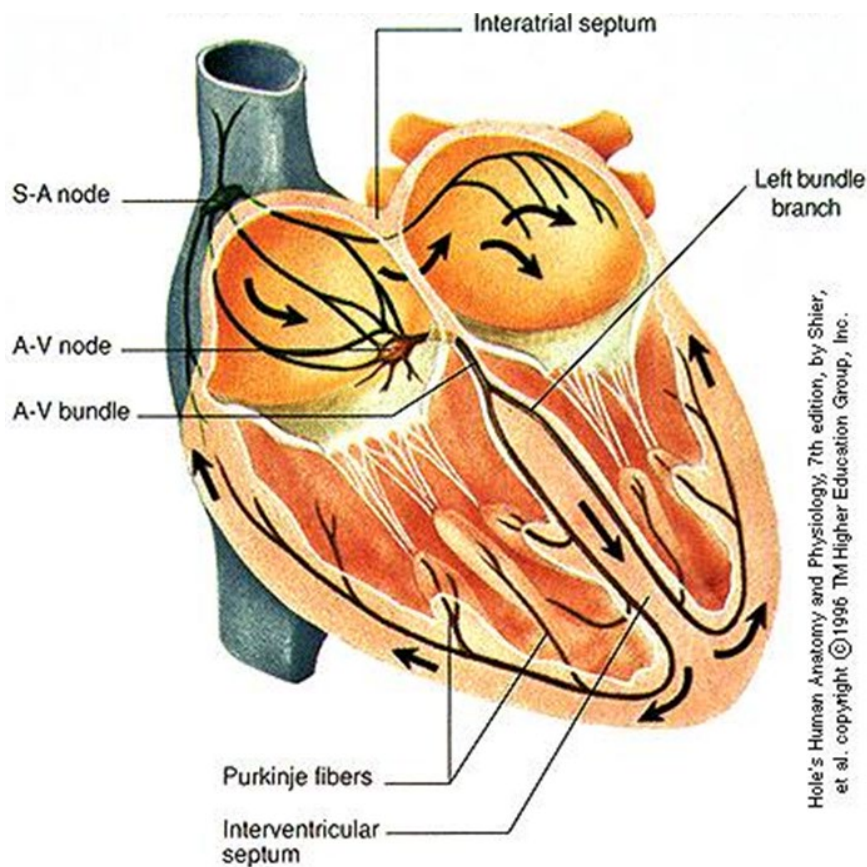
slowly depolarizes the cardiac pacemaker cell until it reaches a threshold at -40 mV which triggers the action potential (Adapted from Homoud, 2008).

1.4.2. Electrical conduction system

In the healthy heart, the heart cycle is modulated by the spontaneous electrical activity of pacemaker cells clustered in the SA node which gives rise to sinus rhythm. The rate of firing of the pacemaker cells can be altered by various influences, such as the autonomic nervous system, hormones, drugs, or pathologies. Once triggered, the action potential propagates from the SA node through three internodal tracts and activates the atria (atrial contraction) until it reaches the atrioventricular (AV) node, located between the atria and the ventricles. The

atrioventricular node relays the electrical signal with a delay (to allow efficient filling of the ventricles), through the bundle of His and Purkinje fibers which stimulates ventricular myocytes (ventricular contraction) (Figure 4).

Figure 4. Cardiac electrical conduction system. The autorhythmic cells present in the SA node trigger action potentials which propagate to the AV node, the bundle branches, and finally, the Purkinje fibers.



1.4.3. Cardiac action potential

Different from pacemaker cells, ventricular myocytes present a stable resting potential (-90 mV) and require the cell-to-cell propagation of the pacemaker-generated electrical impulse through the conduction system to attain the threshold necessary for the generation of their own action potential. The ventricular action potential has 5 phases which are controlled by the sequential opening and closing of many ionic channels (Bednar, Harrigan et al. 2001):

Phase 0 refers to the rapid depolarization phase. This phase is due to the opening of the fast voltage-gated sodium channels which result in the rapid influx of Na^+ ions into the cell. (I_{Na})

Phase 1 represents the early rapid repolarization. The inward Na^+ current (I_{Na}) remains active and the short repolarization phase occurs due to a transient outward current. This current is composed of two components which result in the outward movement of K^+ (I_{to1}) and Cl^- (I_{to2}).

Phase 2 is the plateau phase and consists of a sustained balance between inward Na^+ (I_{Na}) and Ca^{2+} movement through L-type calcium channels (I_{CaL} , I_{CaT}), and outward K^+ movement through the slow delayed rectifier potassium channels (I_{Ks} , I_{Kr} , I_{to}). The Ca^{2+} entry induces the calcium release from the sarcoplasmic reticulum, and triggers the contraction.

Phase 3 represents the rapid repolarization phase which is the result of the progressive inactivation of Ca^{2+} channels while I_{K} channels remain opened. The I_{K} channels consist of rapid and slow delayed rectifier currents, I_{Kr} and I_{Ks} , respectively. This translates into a K^+ positive outward current which results in a negative change of the membrane potential, repolarizing the membrane back to its resting potential of -90 mV . The delayed rectifier K^+ channels close when the resting potential is restored.

Phase 4 refers to the resting phase, and is maintained until depolarization begins. During this phase, I_{K1} channels are opened and contribute to the resting potential.

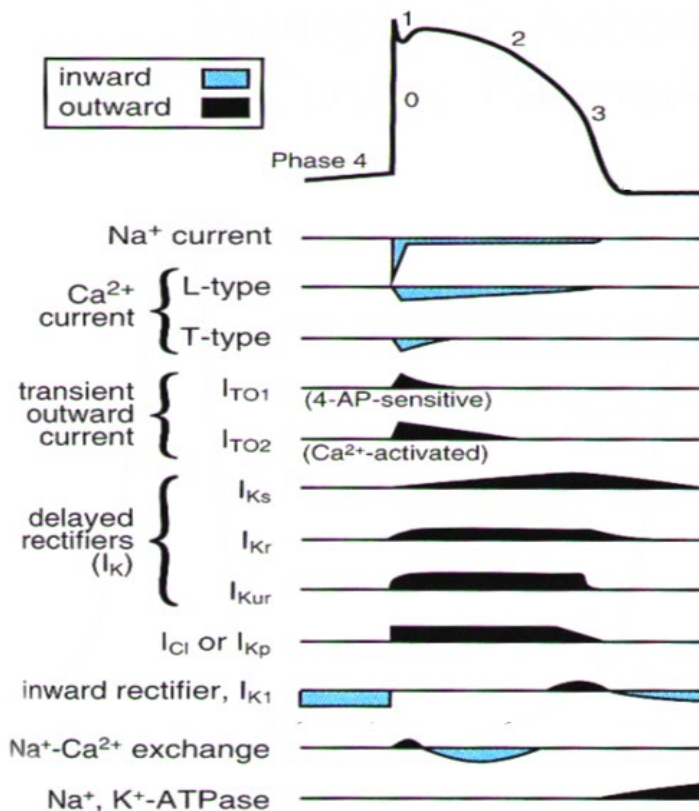


Figure 5. Ionic currents implicated in the phases of the cardiac action potential. (Adapted from Bednar, 2001)

1.5. Excitation-contraction coupling

In the cardiomyocytes, deep invaginations of the sarcolemma (the cardiac muscle cell membrane) called transverse (T)-tubules project into the cell and surround the myofibrils of cardiac cells. When triggered, the action potential is propagated through the conduction system and gap junctions to all cardiomyocytes. The depolarization of the T-tubule results in the opening of voltage-gated L-type calcium channels (ICaL), resulting in inward calcium current which increases the intra-cellular calcium (Ca²⁺) concentration. The small intra-cellular Ca²⁺ increase is detected by ryanodine receptors (RyR) present on the sarcoplasmic reticulum (SR). The activation of RyR quickly releases large Ca²⁺ stores from the SR into the cytoplasm; this mechanism is called calcium-induced calcium release. The resulting high intra-cellular calcium concentration enables Ca²⁺ to bind to the myofilaments (particularly to the troponin C) and modify the conformation of the troponin complex, which allows the myosin head to bind to the actin filament, resulting in the contraction of the cell (Ibrahim, Gorelik et al. 2011).

The intracellular calcium is subsequently pumped back into the SR by the sarco/endoplasmic reticulum ATPase (SERCA) where it is bound by the calsequestrin, or ejected from the cell by the sodium-calcium exchanger (NCX) and/or plasma-membrane calcium ATPase (PMCA), thus reducing the intracellular calcium concentration and ending the contraction (Michalak and Opas 2009) (Figure 6).

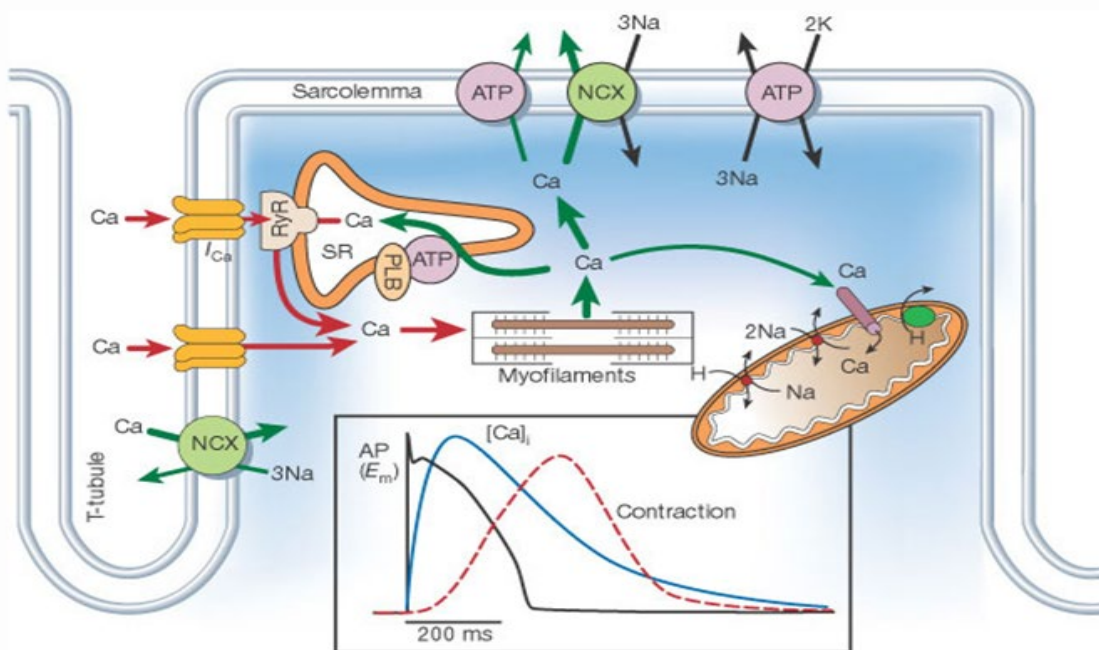


Figure 6. Mechanism of cardiac excitation-contraction coupling (Reproduced from Bers, 2002)

1.6. Autonomic nervous system

The autonomic nervous system (ANS), by definition, is the nervous system which performs and controls involuntary actions such as respiration, vasomotor activity, reflexes, and cardiac regulation. The ANS is divided into two subsystems, namely the sympathetic (SNS) and parasympathetic (PNS) nervous systems which generally act in opposition to each other. Both subsystems consist of a preganglionic neuron which synapses onto a postganglionic neuron that innervates a target organ. The release of neurotransmitters collectively controls the cardiac functions and enables the adaptation of the heart activity to a change in the environment through neurohumoral mechanisms (direct autonomic nerve activity and circulating catecholamines).

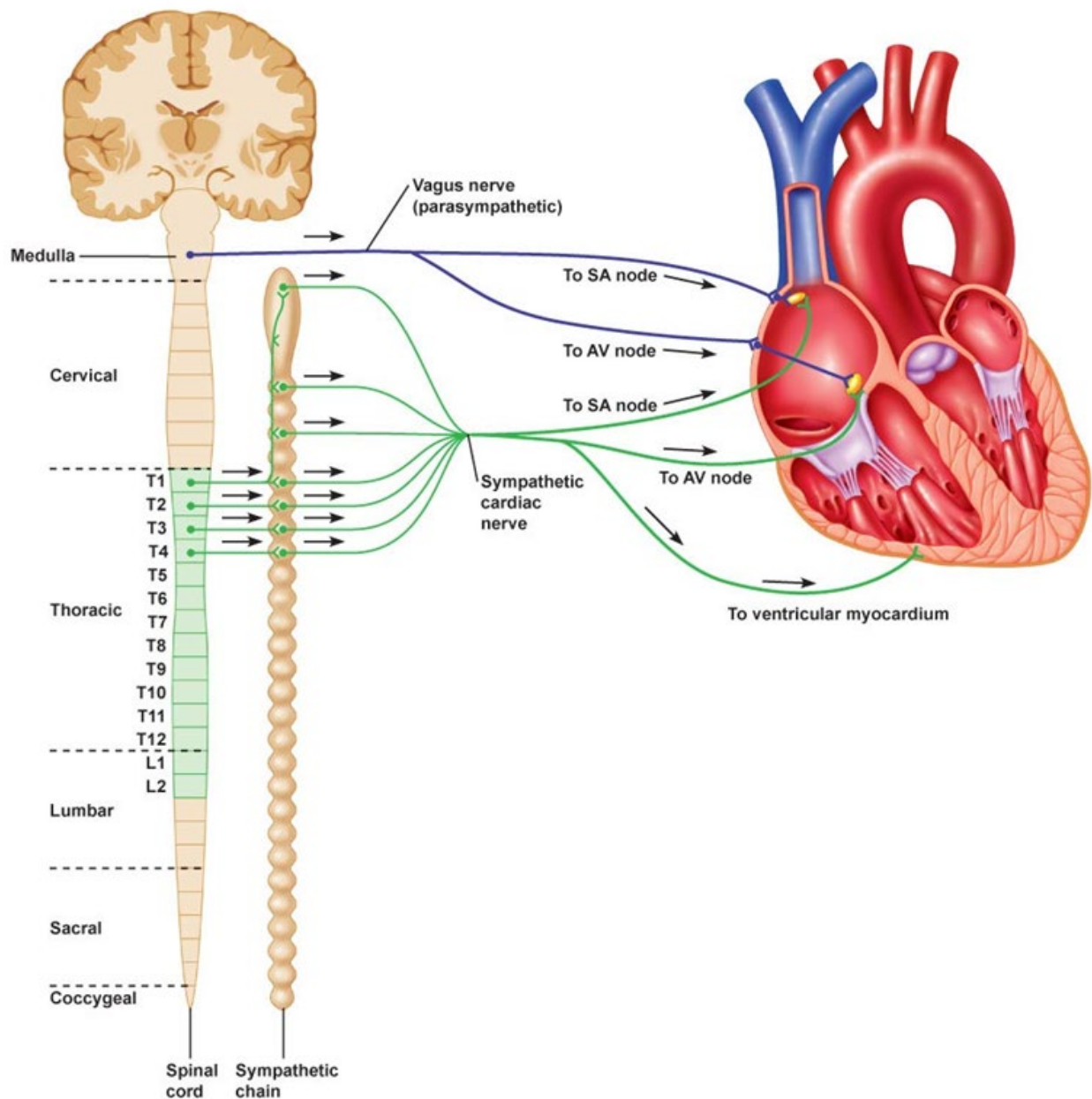
1.6.1. Sympathetic nervous system

The sympathetic nervous system is responsible for the fight-or-flight response. The sympathetic preganglionic nerves originate from the upper thoracic region of the spinal cord and synapse with postganglionic nerves in the sympathetic chain ganglia (Figure 7). The sympathetic postganglionic nerves innervate the heart through the liberation of norepinephrine which activates adrenergic receptors (9 different subtypes exist (Scofield, Deupree et al. 2000)) and trigger Gs protein signaling cascade (Gs-adenyl cyclase-cAMP-PKA) which can result in various biological actions. The human heart contains beta1, beta2, beta3, alpha1A and alpha1B subtypes. However, it is beta1 and beta2 which play the major roles under physiologic conditions. The activation of beta1 and beta2 induce the phosphorylation of several ionic channels and receptors (such as LTCC, RyR, HCN...) which result in positive chronotropic (increase heart rate), inotropic (increase force of contraction), bathmotropic (increase cardiac cells excitability) and dromotropic (increase conduction speed) effects (Triposkiadis, Karayannis et al. 2009).

1.6.2. Parasympathetic nervous system

The vagus nerve originates from the medulla oblongata and synapses with postganglionic neurons in the heart (Figure 7). The parasympathetic postganglionic nerves innervate the heart through the release of acetylcholine (ACh), a neurotransmitter which stimulates muscarinic receptors (mostly M2 in the heart) (Olshansky, Sabbah et al. 2008). When activated, M2 muscarinic receptors act as antagonists of the sympathetic nervous system through the activation of a Gi protein, which inhibits the adenylyl-cyclase, and reduces the levels of cAMP (cyclic adenosine monophosphate) and protein kinase A (PKA) in the cell. The parasympathetic

stimulation thus decreases the heart rate, reduces the contractile forces of the atrial cardiac muscle, and reduces the conduction velocity of the SA and AV nodes.



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Figure 7. Innervation of the heart by the autonomic nervous system.

1.7. The electrocardiogram (ECG)

It was in 1924 that Willem Einthoven was awarded the Nobel prize in Medicine for the invention of the electrocardiograph. This non-invasive device detects and amplifies the electrical activity of the heart during each cardiac cycle. The propagation of electrical activity (the sum of all cardiomyocytes action potentials) in the heart is recorded from electrodes directly placed on the skin and graphically represented as voltage increase and decrease between electrodes. The output from each pair of electrode is known as a lead and corresponds to the measure of the depolarization wave within a particular axis. While three electrodes placed on the limbs can suffice to measure an ECG, current ECG devices record 12-lead ECGs (Figure 8) by the use of 10 electrodes, of which 4 are placed on the limbs, and 6 on the chest. More recent techniques such as body surface mapping (BSM) even allow the recording of more than 80 leads in the form of a vest that is applied on the torso of the patient and provide colorimetric 3-D imaging (Taccardi, Punske et al. 1998). The analysis of ECG recording is used for both diagnosis and research purposes as it provides valuable information on possible heart abnormalities and arrhythmias in human and animals.

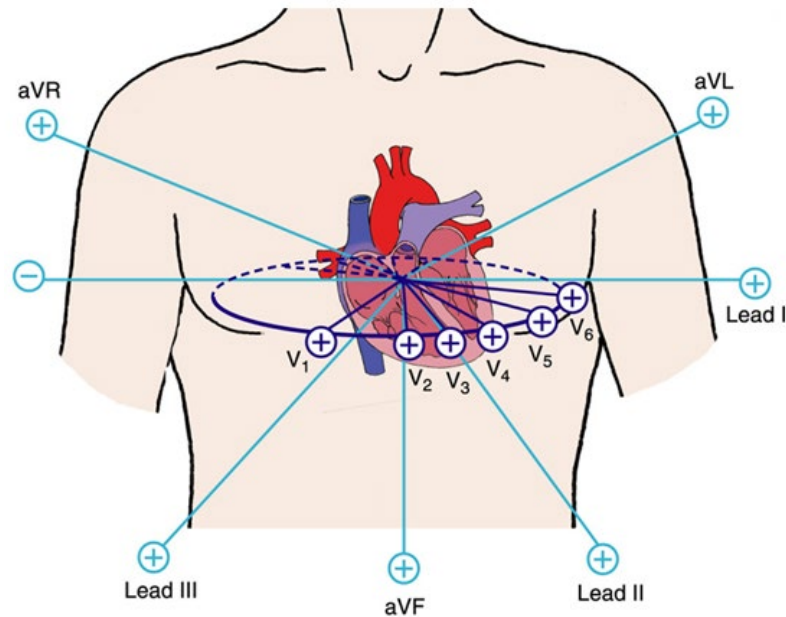


Figure 8. Leads, vectors, and position of a 12-lead electrocardiogram.

A typical ECG tracing of the cardiac cycle consists of a P wave, a QRS complex, a T wave, and a U wave (Figure 9).

The P wave is generally positive (depending on the recorded lead) and corresponds to the depolarization of the atria directed from the SA node towards the AV node. The duration of the PR interval represents the time required for the electrical impulse to travel from the atria to the ventricles.

The Q wave is a short and negative deflection which reflects the depolarization of the septum from left to right. The R wave is large and positive. It corresponds to the depolarization of the main mass of the right and left ventricles. The S wave is negative and reflects the end of the ventricular depolarization. Together, the QRS complex represents the depolarization of the ventricles.

The T wave is typically positive and represents the repolarization of the ventricles. The duration of the QT interval (return to isoelectric baseline) reflects the time required for the ventricles to be completely repolarized. Importantly, the prolongation of the cardiac repolarization duration (QT interval) is a surrogate biomarker for an increased risk of possibly lethal arrhythmias, such as torsades de pointes (TdP).

The U wave is the last and smallest wave of the electrocardiogram. It is generally positive but its physiological basis has not been clearly elucidated. Four major hypotheses may explain the underlying mechanism of the U-wave (Perez Riera, Ferreira et al. 2008; Hoppenfeld and Ashikaga 2010). These hypotheses describe the U-wave as:

- The repolarization of the Purkinje fibers system (Hoffman 1962).
- The delayed repolarization of the papillary muscles (Bufalari, Furbetta et al. 1956).
- After-potentials resulting from mechanoelectric feedback in the ventricular wall (Surawicz 1998).
- A delayed repolarization of the M-cells in the setting of transmural dispersion of repolarization (Antzelevitch and Sicouri 1994).

However, none of these proposed hypotheses has been universally accepted and further clinical and experimental research are still required to clarify the physiological genesis of the U-wave.

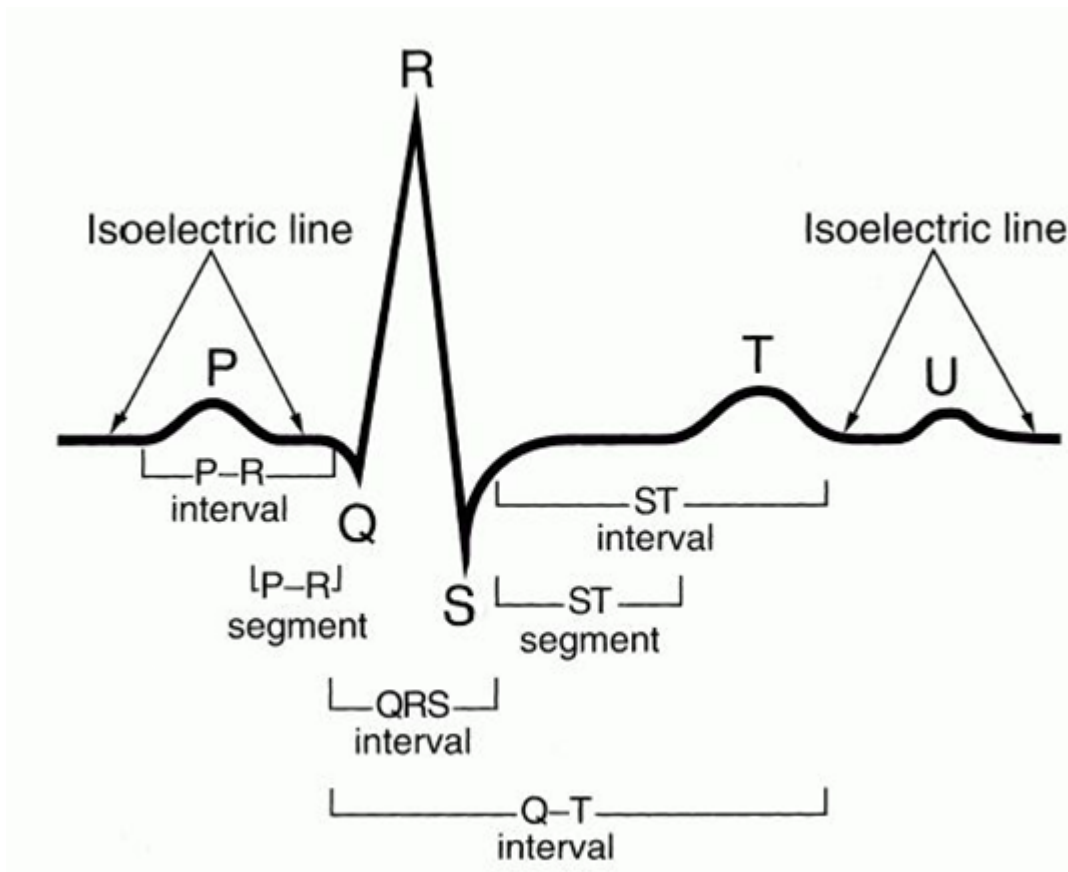


Figure 9. Waveforms and intervals of a typical ECG recording.

Nowadays, the ECG is one of the most used diagnostic tools as the analysis of waveform duration, voltage, and morphology provide insight whether anatomical regions have been impaired or if a drug candidate has the potential to modify cardiac conduction properties. The ECG is thus extensively used in both preclinical and clinical trials in order to unravel potential drug-induced changes in the ECG and risks of inducing adverse events, especially QT prolongation and torsades de pointes.

2. Long QT syndrome and Torsades de pointes

Long QT syndrome is a condition in which ventricular repolarization is delayed and creates an electrophysiological environment favorable to the development of arrhythmias, especially torsades de pointes (TdP), a polymorphic ventricular arrhythmia which is generally self-limited but can rarely degenerate into ventricular fibrillation and sudden death. The two major manifestations of LQTS are syncopal episodes (sometimes only dizziness due to the impaired cerebral circulation) and electrocardiographic abnormalities that usually occur in conditions of physical or emotional stress. The associated delayed ventricular repolarization can be either of congenital or acquired (drug-induced) origins (Roden, Lazzara et al. 1996; Viskin 1999) and translates on the surface electrocardiogram as a prolongation of the QT interval, measured from the start of the QRS complex (start of ventricular depolarization), to the end of the T-wave (end of the repolarization).

2.1. Congenital long QT syndrome

Congenital LQTS is hereditary with a prevalence estimated at 1 in 2000 births (Schwartz, Stramba-Badiale et al. 2009). The genetic basis of the disease was identified in 1957 by Jervell and Lange Nielsen (Jervell and Lange-Nielsen 1957). All the LQTS genes currently identified encode cardiac ion channel subunits or proteins involved in the modulation of ionic currents. Mutations in genes such as KCNQ1 (I_{Ks}), KCNH2 (I_{Kr} , hERG), SCN5A (I_{Na}), ANK2, KCNE1, KCNE2, KCNJ2, CACNA1c, CAV3, and SCN4B are characterized by a prolongation of the duration of the action potential and have been subdivided in LQTS subtypes from LQT1 to LQT10, in the aforementioned order. The relative prevalence of the subtypes is >50% for LQT1, 35-40% for LQT2, and 10-15% for LQT3. Interestingly, the characteristic syncopal episodes due to TdP are generally triggered under specific circumstances in a gene-specific manner. For example, most of the events for LQT1 patients occur during exercise or stress and are due to an impaired ability to shorten their QT interval during heart rate increases (due to mutations in I_{Ks}). In contrast, most of the events for LQT2 and LQT3 patients occur during rest or sleep. Lethal and non-lethal events follow the same gene-specific pattern (Schwartz, Priori et al. 2001).

The clinical diagnosis of LQTS is generally performed based on diagnostic criteria with relative points assigned to various electrocardiographic, clinical, and familial findings, such as the Schwartz score (Table 1) (Schwartz, Moss et al. 1993).

	Points
Electrocardiographic findings #	
A QTc*	
≥480 ms	3
460–479 ms	2
450–459 ms (in males)	1
B QTc* 4th minute of recovery from exercise stress test ≥480 ms	1
C Torsade de pointes*	2
D T wave alternans	1
E Notched T wave in 3 leads	1
F Low heart rate for age@	0.5
Clinical history	
A Syncope*	
With stress	2
Without stress	1
B Congenital deafness	0.5
Family history	
A Family members with definite LQTS\$	1
B Unexplained sudden cardiac death below age 30 among immediate family members\$	0.5

#In the absence of medications or disorders known to affect these electrocardiographic features.

*QTc calculated by Bazett's formula where $QTc = QT / \sqrt{RR}$.

*Mutually exclusive.

@Resting heart rate below the 2nd percentile for age.

\$The same family member cannot be counted in A and B.

SCORE: ≤1 point: low probability of LQTS.

1.5 to 3 points: intermediate probability of LQTS.

≥3.5 points high probability.

Table 1. LQTS diagnostic criteria: Schwartz score 1993-2011 (from Schwartz 2011).

The Schwartz score has been reported to have a high specificity (99%), but a rather low sensitivity (19%) for the identification of patients with LQTS (Hofman, Wilde et al. 2007). The low sensitivity is due to the inability of the Schwartz criteria to identify silent mutation carriers (Priori, Napolitano et al. 1999). Recently, a new criterion called the end-recovery QTc, in conjunction with an algorithm, has been proposed for the diagnosis of LQTS among asymptomatic patients or for the discrimination between LQT1 and LQT2 patients. The end-recovery QTc consists in comparing the QTc interval at rest and at 4 minutes after an exercise stress test (Sy, van der Werf et al. 2011) and has demonstrated relatively efficient recognition of patients with LQTS (Lodder and Wilde 2012; Obeyesekere, Sy et al. 2012). Subsequent genotyping is performed for

patients diagnosed or suspected of LQTS in order to identify or confirm the causative gene mutation. However, while most of the patients are diagnosed for LQTS after they (or a relative) developed symptoms, some patients have been reported with mutations which remain silent until they are challenged by QT-prolonging drug. These silent mutations are responsible for a reduced capability of the heart to adapt (see 2.3. reduced repolarization reserve) in the presence of other risk factors, thus increasing the risk of developing arrhythmias.

Currently, several advances are still needed to provide complete genotype-phenotype correlations, but in the near future, it should become possible to provide optimized treatments for LQTS patients on the basis of their individual mutation.

2.2. Acquired long QT syndrome

Acquired long QT syndrome is commonly induced by pharmacological agents, but can also result from cardiomyopathies (heart block, myocardial infarction, bradycardia), ionic deficit (hypokalemia, hypocalcemia, hypomagnesemia) or starvation. However, pharmacological agents remain the most common cause as more than 150 drugs have currently been referenced for having a potential to prolong the QT interval (AZCERT 2013). Although most of the recognized cases of drug-induced LQTS have been reported during therapy with class III antiarrhythmics, several other non-cardiac drug classes have been associated with a delay in cardiac repolarization and an increased risk of TdP. Accordingly, the issue of drug-induced LQTS has attracted a lot of attention from the drug regulatory authorities.

It was previously believed that the more affinity a drug had to block the hERG current (I_{Kr}), the more the QT interval was prolonged, and the more increased was the risk of arrhythmic events. However improvements in the understanding of the pathophysiology of drug-induced TdP have demonstrated that the QT interval prolongation and its extent are not the only determinants involved in the pathogenesis of TdP. While virtually all drugs inducing TdP and QT prolongation have been associated with a blockade of the outward K^+ currents (I_{Kr} and I_{Ks}), it is now well recognized that the induction of TdP does not solely result from the blockade of potassium current. Indeed, TdP may only occur when a combination of several risk factors creates a vulnerability window which favors the development of cardiac arrhythmic events susceptible of triggering torsades de pointes.

2.3. Drug-induced Torsades de pointes in hospital settings

The Arizona Center for Education & Research on Therapeutics (AZCERT) provides updated lists of drugs which are at risk, possible risk, or conditional risk of inducing TdP (www.qtdrugs.org). While the absolute incidence for each drug is difficult to establish as it generally requires millions of prescriptions due to the rarity of TdP events, drugs associated with TdP vary greatly in their risk for arrhythmias. The incidence of TdP with these drugs is generally very small and has been estimated to range from 0.001% to 8% (Drew, Ackerman et al. 2010). Accordingly, drugs associated with TdP may still be indicated in specific cases when the therapeutic benefits outweigh the possibility of drug-induced TdP.

Currently, there is no perfect quantitative risk index available for the prediction of TdP. The principal risk factor evaluated to predict TdP consists of measuring drug-associated QTc interval prolongation on periodically recorded 12-lead ECGs. It has been recognized that each 10 ms increase in QTc contributes to ~6% exponential increased risk of TdP in LQTS patients (Moss, Schwartz et al. 1991; Zareba, Moss et al. 1998). However, the lone evaluation of QTc prolongation in hospital setting remains inadequate due to the difficulty to accurately measure this interval. For example, suitable baseline values may not be available for chronically sick patients, continuous ECG monitoring may not be available depending on the hospital cardiac monitoring equipment, or the end of the T-wave may be obscured by pathologic or drug-induced abnormal waveforms.

Consequently, evaluating the risk of TdP in single hospitalized patients is a challenge that is generally best evaluated by the resource-intensive recognition of a vast array of risk factors associated with TdP.

2.4. Risk factors and repolarization reserve

Over the years, many risk factors have been identified for increasing the risk of TdP (Drew, Ackerman et al. 2010). These risk factors are QTc prolongation (Elming, Brendorp et al. 2002), female gender (Zeltser, Justo et al. 2003), advanced age (Yasuda, Nakazato et al. 2006), ionic deficits (K^+ , Mg^{2+} , Ca^{2+}) (Roden and Iansmith 1987; Akiyama, Batchelder et al. 1989), hyperglycemia (Pickham, Flowers et al. 2013), bradycardia (Roden 2004), heart disease (Pedersen, Elming et al. 2007), rapid intravenous infusion (Carlsson, Abrahamsson et al. 1993), treatment with diuretics (Fiset, Drolet et al. 1997), genetic predisposition (congenital LQTS) (Fitzgerald and Ackerman 2005), increased Transmural Dispersion of Repolarization (TDR; see 2.3.3.) and T wave morphology changes (Topilski, Rogowski et al. 2007).

Risk factors for torsades de pointes

- QTc > 500 ms
- LQT2-type ECG abnormalities: notch, long Tpeak-Tend
- QT-prolonging drugs
 - with rapid infusion by intravenous route
- Heart disease
- Advanced age
- Female sex
- Hypokalemia
- Hypomagnesemia
- Hypocalcemia
- Treatment with diuretics
- Impaired hepatic drug metabolism
- Bradycardia
- Premature complexes leading to short-long-short cycles
- Clinically silent congenital LQTS (reduced repolarization reserve)

Table 2. Risk factors for torsades de pointes (from Drew, 2010).

The concept of repolarization reserve was first introduced by Roden and translates a mechanism of compensation by other repolarization currents when one is impaired (Roden 1998). This means that the loss of one component (e.g. I_{Kr}) from the system (the repolarization), will not result in the failure of the repolarization (and produce QT prolongation). This concept started after several observations that a block of I_{Kr} was not always associated with an increased APD and risk of arrhythmias. Recently, Xiao et al. demonstrated that after the withdrawal of I_{Kr} inhibition (dofetilide), the action potentials were shorter in drug-exposed than control cells, due to a compensatory action of I_{Ks} which remained elevated (Xiao, Xiao et al. 2008). Accordingly, risk factors impairing ionic currents reduce the repolarization reserve (Figure 10), thus creating vulnerability in an individual repolarization system for which any added stress could be sufficient to trigger TdP.

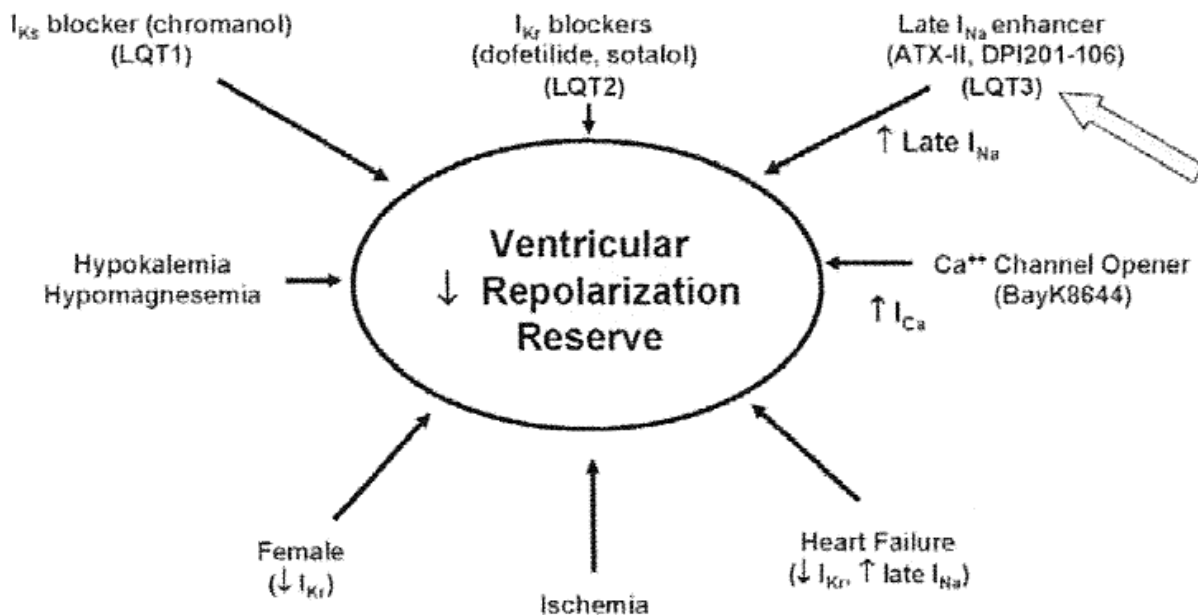


Figure 10. Conditions reducing the repolarization reserve in ventricular myocytes. The same conditions have been associated with an increased risk of TdP in patients administered with QT prolonging drugs (from Belardinelli 2005).

2.5. Torsades de pointes: Mechanisms

Torsades de pointes is a very rare but possibly lethal polymorphic ventricular tachycardia (180-250 bpm) characterized by a twisting of the QRS complex around the isoelectric baseline (Ben-David and Zipes 1993). Episodes of TdP generally occur after a short-long-short pattern of RR cycles which starts with a premature ventricular complex (PVC) triggered by an early

afterdepolarization (EAD). The PVC is followed by a compensatory pause terminated by a sinus beat with a prolonged QT interval and an exaggerated U wave. The TdP may occur when another PVC coincidentally trigger on the exaggerated U wave (Yap and Camm 2003) (Figure 11).

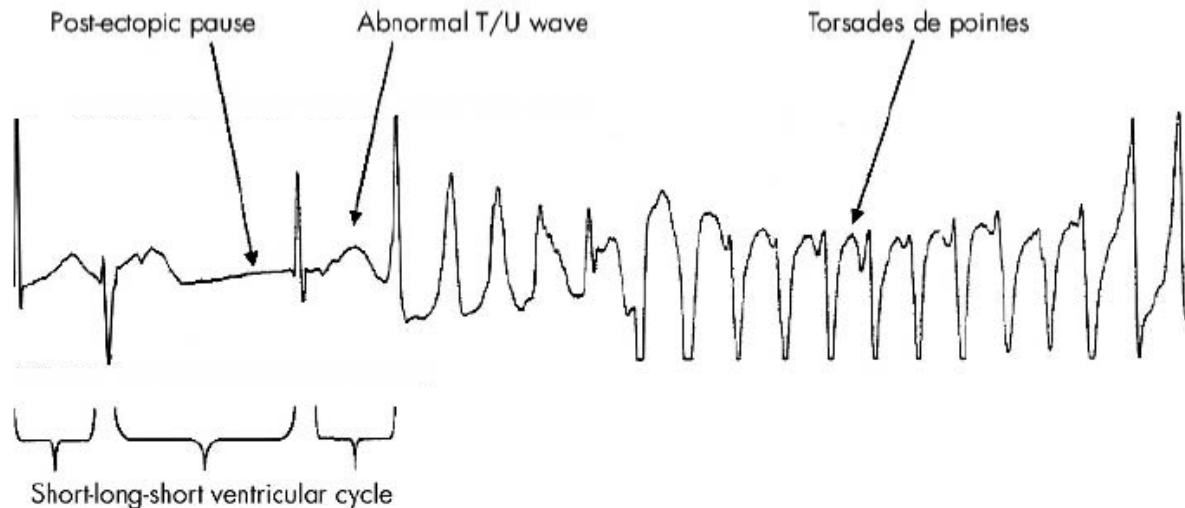
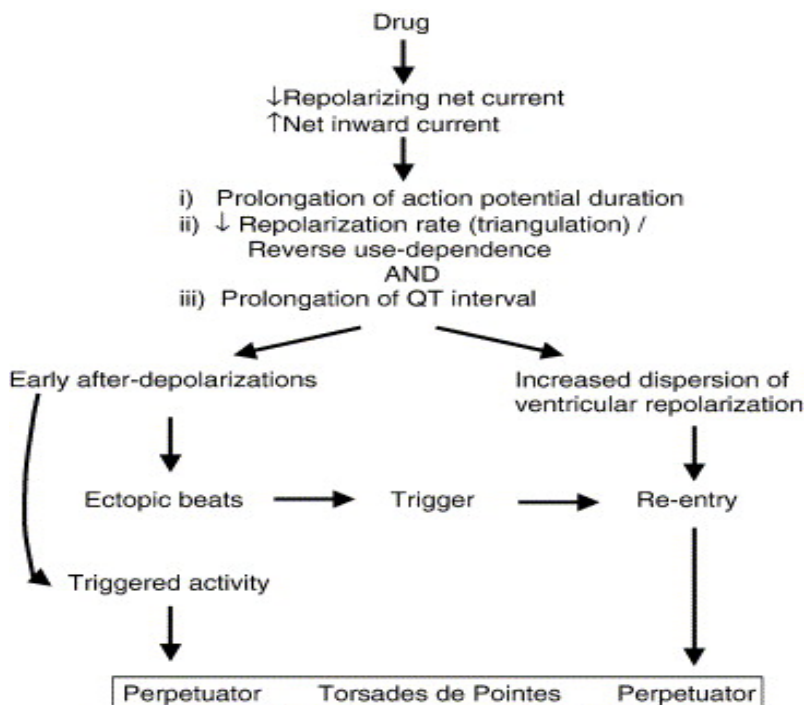


Figure 11. Drug-induced TdP with the typical short-long-short initiating ventricular cycle (from Yap 2003).

Once the TdP is triggered, the heart can no longer efficiently pump which results in a decrease in arterial blood pressure, and sometimes, syncope. In those cases when TdP does not spontaneously stop, it can further deteriorates into ventricular fibrillation, and sudden death. These arrhythmias generally occur when the heart demonstrates reduced repolarization reserve which favors the development of early/delayed after-depolarizations and re-entry phenomenon



susceptible to trigger torsades de pointes (Figure 12).

Figure 12. Mechanism of torsades de pointes (from Lawrence, 2005).

2.5.1. Early and delayed after-depolarization

After-depolarizations occur when a normal action potential triggers a second abnormal depolarization. Two types of after-depolarization have been described: delayed (DADs) and early after-depolarization (EADs). After-depolarizations generally occur in the setting of AP prolongation and are the result of ionic imbalance due to increased inward currents and/or decreased outward currents. The supplementary AP is either triggered during the phase 2 (plateau), or phase 3, for EADs, or during phase 4 for DADs. The ionic mechanism responsible for EAD is not yet entirely understood but involves the synergic actions of I_{CaL} and the Na-Ca exchange currents (I_{CNX}) which have the particular ability to progressively activate as the membrane voltage depolarizes (Weiss, Garfinkel et al. 2010). These channels are characterized by a window region which is a region of overlap between steady state activation (SSA) and inactivation (SSI). This means that inside a certain voltage window (-30 to 0 mV for I_{CaL}), when the repolarizing currents are slow enough; some of the channels that are being inactivated can reactivate sufficiently to reverse the repolarization and generate an EAD (Figure 13).

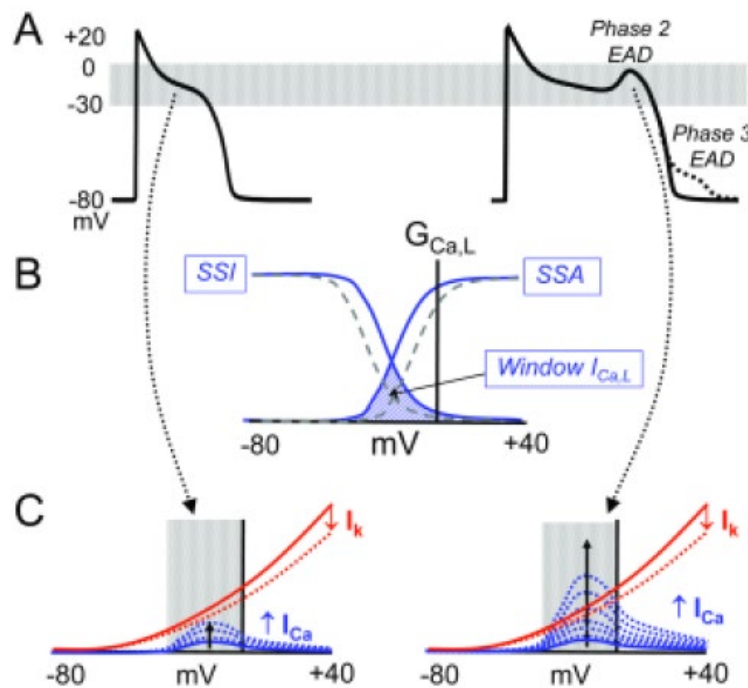


Figure 13. Mechanism of EAD. A. Normal action potential (left) and AP presenting an EAD in phase 2 or phase 3 (right). B. Plot of L-type Ca channel conductance (G_{CaL}) versus membrane voltage (mV). At the window I_{CaL} region, a fraction of Ca channels remain continuously open. C. Interaction between time-dependent I_{CaL} reactivation and time-dependent deactivation of

repolarization currents (I_K). When the repolarization rate is normal (left), I_{CaL} remain lower than I_K , thus resulting in an effective repolarization. When the repolarization rate is slow (right), I_{CaL} grow larger than I_K , thereby reversing the repolarization which triggers an EAD (from Weiss 2010).

The mechanism for DAD involves the sarcoplasmic reticulum calcium release, non-related to voltage-gated channels, which occurs during diastole. Under certain conditions, the Ca^{2+} load is increased and lead to a spontaneous release of calcium from the SR. The high concentration of calcium causes the activation of I_{CNX} current which results in an inward Na^+ current which depolarize the sarcolemma and triggers a DAD (Joung, Zhang et al. 2011; Johnson, Heijman et al. 2012).

When triggered, these ectopic beats increase the electrical heterogeneity and disorganize the uniform repolarization wave of the cardiac regions. This heterogeneity of repolarization produces the substrate that may induce reentry phenomenon, a known cause of arrhythmia (see 2.4.3).

Interestingly, single, isolated EAD-susceptible myocytes are not sufficient to trigger an EAD at the scale of the heart. A “source-sink mismatch” mechanism prevents an occasional cell predisposed to EAD to trigger a generalized premature ventricular complex by preventing the propagation of an isolated current to unsusceptible neighboring cells. A typical ventricular myocyte (the source) is generally coupled to 11 neighboring myocytes (the sink) (Hoyt, Cohen et al. 1989; Peters and Wit 1998). Simulations have demonstrated that a large number of adjacent myocytes are required to demonstrate a synchronized EAD in order to trigger an EAD at the heart scale. The number of required myocytes depends on the number of dimensions of the cardiac tissue, and range from ~70 for 1D cables (like the His-Purkinje system), to ~700K for 3D tissues. In addition, it has been shown that reduced repolarization reserve due to reduced gap junction conductance or fibrosis significantly decreases these numbers (Xie, Sato et al. 2010).

2.5.2. Transmural dispersion of repolarization

The ventricular myocardium is composed of different layers with distinct cell types. The epicardial, mid-myocardial (M), and endocardial cells differ by their electrophysiological characteristics and responses to pharmacological agents. Compared to epi- and endocardial cells, M cells are characterized by more prominent morphologic and action potential duration (APD) changes in response to drug-induced increases of I_{Na}/I_{CaL} or decreases of I_{Kr} currents (Figure 14).

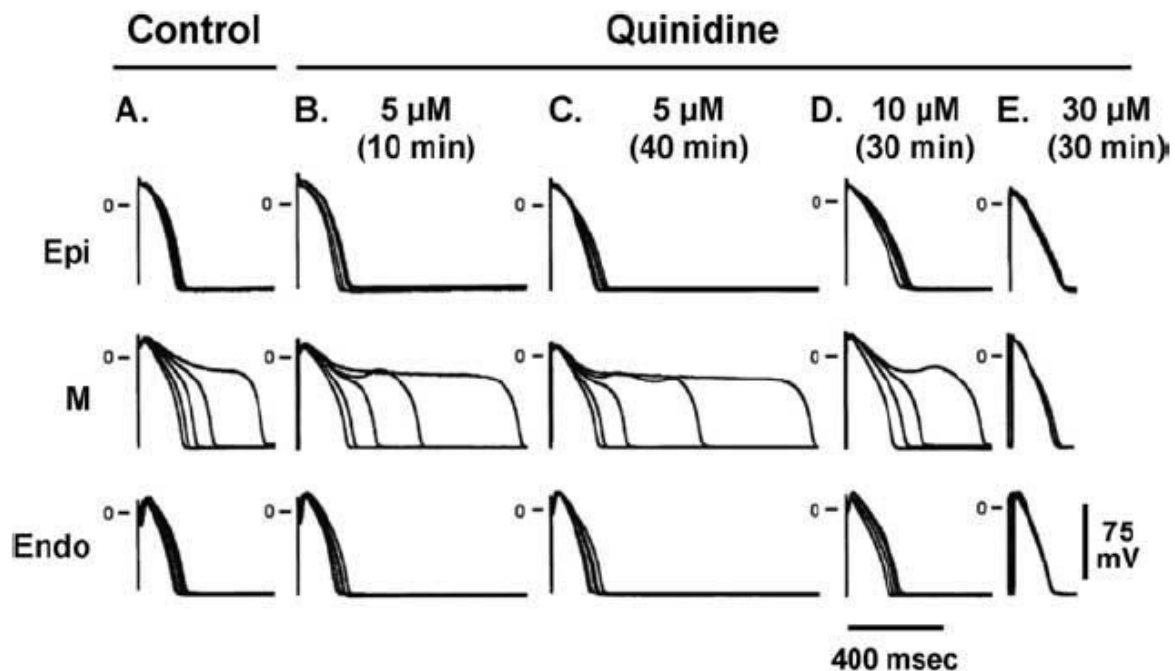


Figure 14. Action potential prolongation in epicardial (Epi), M, and endocardial (Endo) cells after quinidine administration. The quinidine-induced AP prolongation is quantitatively more increased in the M cells (from Antzelevitch, 1999).

This heterogeneity is related to an increase in tissue resistivity in the deep subepicardium (Yan, Shimizu et al. 1998) which is due to a transition in cell orientation (Antzelevitch 2007), an increased density of collagen (LeGrice, Smaill et al. 1995), and a reduced expression of connexin 43 (Poelzing, Akar et al. 2004; Yamada, Kanter et al. 2004). The increased tissue resistivity limits the degree of electrotonic interaction between myocardial layers, thus increasing the heterogeneity (Antzelevitch 2010). Moreover, while histologically similar to epi- and endocardial cells, the M cells resemble Purkinje cells regarding their pharmacological and electrophysiological properties. Like Purkinje fibers, M cells exhibit prominent APD prolongation

and develop EAD in response to I_{Kr} blockers, and DAD in response to drugs that induce a calcium overload in the cardiac cell. These features of M cells are due to the presence of lower I_{Ks} current and increased persistent sodium current and Na/Ca exchange current compared to the epi- and endocardial cells (Antzelevitch, Shimizu et al. 1999). Accordingly, the heterogeneous conduction velocities between the different layers is responsible for transmural dispersion of repolarization which creates vulnerable windows favorable to the development of abnormal cardiac events such as EAD, DAD, and reentry, that in turn triggers the initiation of TdP.

Amiodarone is a class III antiarrhythmic agent which has been associated with a low incidence of TdP and sodium pentobarbital is a widely used anesthetic known to prevent the development of TdP. Both amiodarone and sodium pentobarbital have been associated with a large QT prolongation but a reduced TDR (Sicouri, Moro et al. 1997; Shimizu, McMahon et al. 1999), which prevented the induction of TdP. In contrast, sotalolol, dofetilide, and erythromycin are pure I_{Kr} blockers known to induce TdP. These drugs were associated with dose-dependent QT and TDR prolongation (Figure 15) (Antzelevitch 2005). Collectively, these examples demonstrate that only drugs associated with increased TDR have the potential to increase the risk of TdP. These findings suggest that TDR might be a better predictor for TdP liabilities than the QT interval prolongation (Hondeghem 2008).

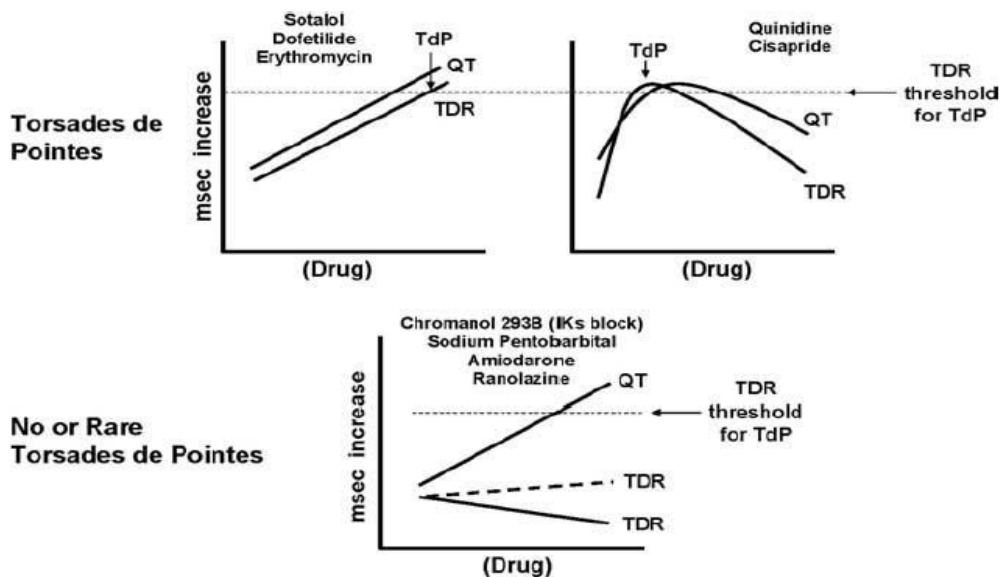


Figure 15. Transmural dispersion of repolarization and QT interval prolongation as surrogate biomarkers of TdP liabilities. While both proarrhythmic and non-proarrhythmic drug groups demonstrated increased QT prolongation, only proarrhythmic drugs demonstrated an increased TDR (from Antzelevitch 2005).

The TDR has been measured on the ECG as the interval from the peak of the T-wave to the end of the T-wave ($T_{p-e} = T_{peak} - T_{end}$; Figure 16) or the T_{p-e}/QT ratio (Yan, Rials et al. 2001; Joshi, Dimino et al. 2004; Liu, Brown et al. 2006). However, it has been noted that the T_{p-e} interval is unlikely to provide an absolute measure of the TDR *in-vivo*, as not only the transmural gradients but also the apico-basal and interventricular gradients contributes to the genesis of the T wave (Xia, Liang et al. 2005). This hypothesis was recently confirmed by studies in rabbit (Arteyeva, Goshka et al. 2013) and dog hearts (Izumi, Chinushi et al. 2012) which concluded that the T_{peak} to T_{end} interval translated a combination of apico-basal and transmural dispersion.

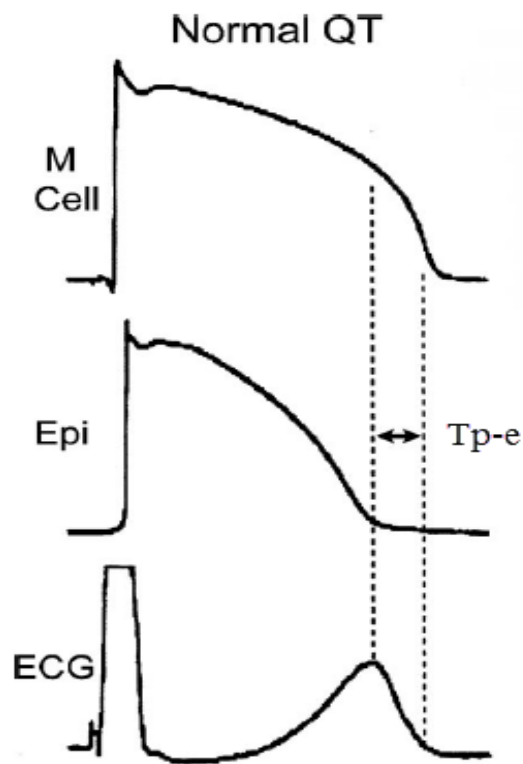


Figure 16. The $T_{peak}-T_{end}$ interval as an electrocardiographic measurement of TDR

2.5.3. Re-entry

Reentrant arrhythmias are defined as the continuous propagation of an excitatory impulse which travels in a circular path, reactivating its site of origin and creating a self-sustained circuit. This type of arrhythmia can only occur over small or large regions of the heart if several conditions are fulfilled: first, adjacent tissue or pathways must be joined to form a circuit and have different conduction and refractoriness properties. Each pathway of the circuit must be able to conduct an electrical impulse in both anterograde and retrograde directions. A unidirectional block must also be present so that anterograde conduction is prohibited in a region while retrograde conduction remains possible (Figure 17A; Pathway 1). The block can be due to either physiologic (refractory period) or pathologic (repolarization abnormalities) conditions. The conduction velocity of the unblocked pathway (2) must be slow enough to enable reactivation of neighboring cells in pathway 3, then 1. In other words, when a region is blocked due to normal tissue in refractory period or trauma-induced damage, the impulse resulting from a premature beat propagates through the pathway with the slower conduction speed (Pathway 2) and activates the neighboring cells from pathway 3 and 1 which are no longer refractory (due to the block) and can be reactivated. The retrograde impulse travels back to the point where it connects with pathway 2, which, due to its shorter recovery period, will be able to be reactivated, thus translating into a sustained re-entry circuit rhythm responsible for maintenance of cardiac arrhythmias (Yan, Wu et al. 2001).

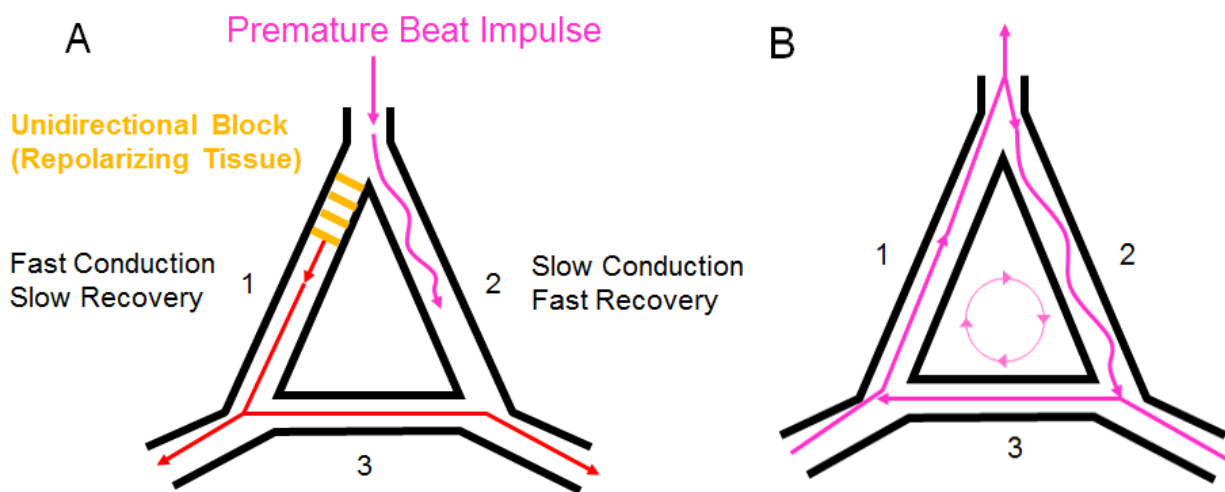


Figure 17. Mechanism of reentry. Subsequent to a premature beat impulse, the unidirectional block (A) results in a sustained circuit responsible for the maintenance of cardiac arrhythmias (B).

3. Safety Pharmacology

The main goals of safety pharmacology are to determine whether a drug is likely to be associated with adverse events as well as to provide a continuous risk/benefit assessment throughout the development of a drug (Pugsley, Authier et al. 2008). In order to reduce the unnecessary use of animal models (the 3 R's principles (Russel and Burch 1959)) and ensure the protection of human subjects enrolled in clinical studies, industry and regulators from the United States, Europe, and Japan have worked together in the context of the International Conference on Harmonization (ICH) in an effort to provide scientific and technical recommendations for the conduct of preclinical and clinical safety pharmacology studies.

3.1. Preclinical studies

According to ICH guidelines S7A (FDA 2005) and S7B (FDA 2005), each drug in development has to go through a core battery (Figure 18) of CNS, respiratory, and cardiovascular tests in order to demonstrate relative safety to induce adverse effects before first-in-human assays, especially those which can result in serious vital organ injury. The characterization of a drug's relative safety relies on the determination of exposure-adverse effect relationships in several *in-vitro*, *ex-vivo*, and *in-vivo* preclinical models in order to provide an integrated risk assessment. While some of the preclinical models and biomarkers may not always have predictive value in man, these tests collectively enable the regulators to take an enlightened, yet subjective, decision as to whether a drug can be tested in human without exposing the subjects to an unreasonable risk.

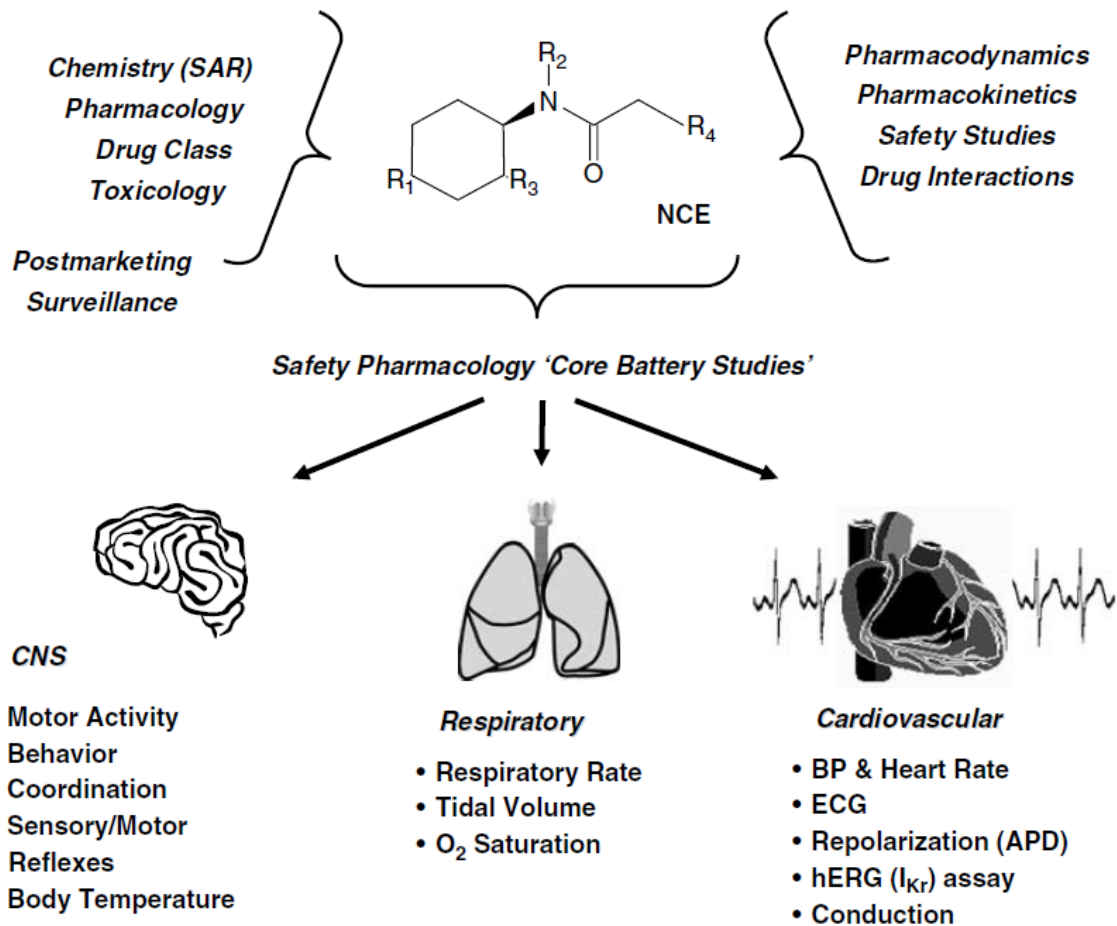


Figure 18. Multidisciplinary evaluations required for a new chemical entity (NCE) in safety pharmacology (from Pugsley, 2008).

Preclinical models demonstrating good translational power are very important as they greatly facilitate decision making (Figure 19) whether the development of a drug should be discontinued or not (e.g. if a compound is likely to demonstrate a positive signal in a TQT study). An ideal preclinical model would be one that has high sensitivity (correctly detects positive effect), high specificity (correctly detects negative effect), is well validated and is easily applicable (Lawrence, Pollard et al. 2005). However, the complexity of the TdP induction mechanisms, the rarity of the events, and the imperfection of its current accepted biomarker (i.e. QT interval prolongation) have made further development of a highly predictive model very difficult. Despite continuous efforts to fulfill this unmet need, none of the currently available preclinical model provides ideal predictive power for the torsadogenic potential of a drug (Shah 2008; Leishman, Beck et al. 2011). Based on ICH S7B recommendations, current practice generally consists of the

assessment of TdP liability through the surrogate measurement of a drug's potential to inhibit I_{Kr} current and prolong the QT interval.

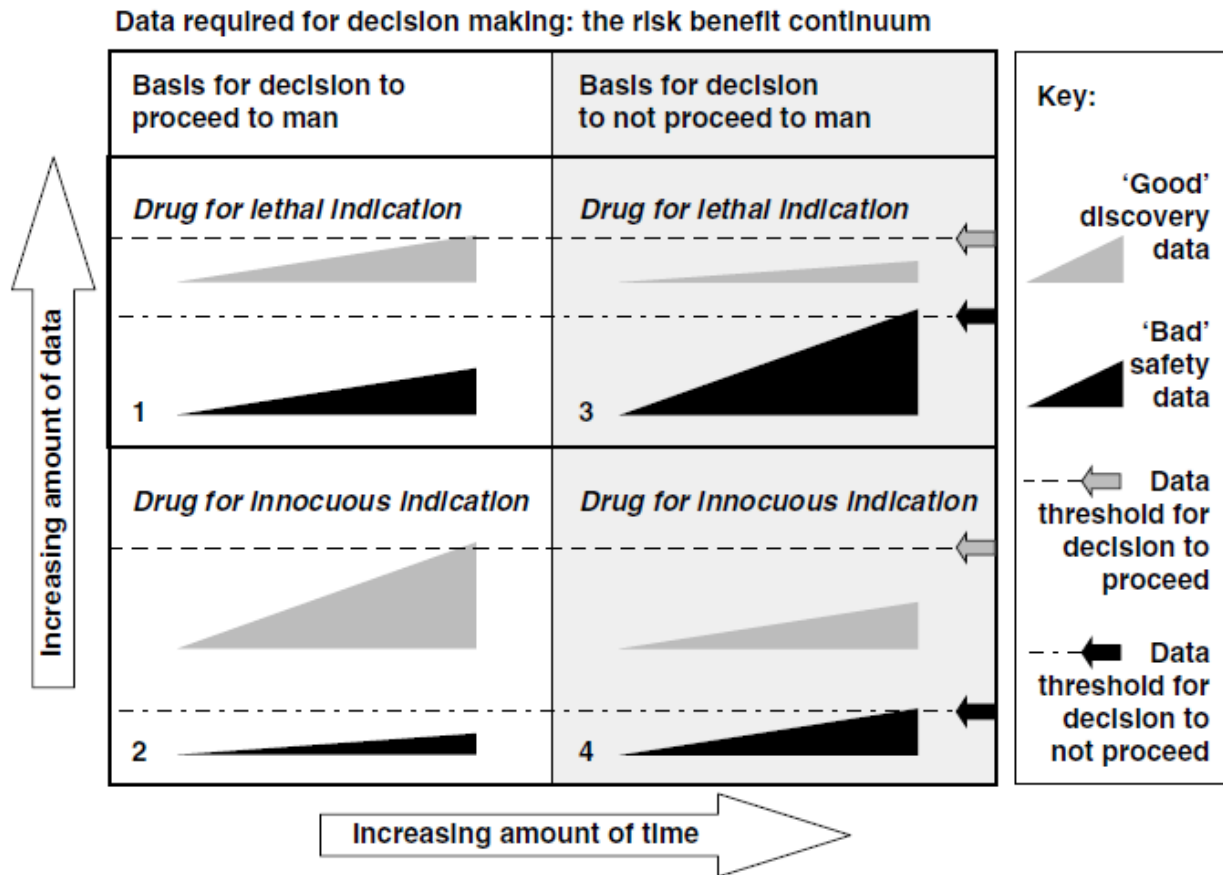


Figure 19. Risk/benefit continuum. 1. For a drug with lethal indication, “bad” safety data are more acceptable and decision to proceed in man will be made even when the drug exhibits as much “good” as “bad” safety data. **2.** However, for an innocuous indication, the “good” safety data must be largely superior to the “bad” in order to proceed in man with the drug. **3.** Similarly, for a drug for a lethal indication to be discontinued, the “bad” safety data must largely outweigh the “good”. **4.** For innocuous indications, the drug is killed whenever a moderate “bad” safety data threshold is crossed (from Pugsley 2008).

For the prediction of QT prolongation in man, the most recent reports have demonstrated improved, but still insufficient translation of the routinely used preclinical repolarization assays (i.e. hERG (Webster, Leishman et al. 2002; Redfern, Carlsson et al. 2003; Wallis 2010), perfused rabbit heart (Lawrence, Bridgland-Taylor et al. 2006) and telemetered dogs (Toyoshima, Kanno et al. 2005; Hanson, Bass et al. 2006)) to man (HESI 2012). Moreover, the use of surrogate biomarkers is inherently associated with occasional false positive results.

Accordingly, several proarrhythmia models and new biomarkers have been or are currently being developed by different groups to supplement the current practice and provide a more integrated assessment of torsadogenic risk. The development of more predictive models or biomarkers would reduce the potential risk of discontinuing the development of safe drugs that could save lives (Lawrence, Pollard et al. 2005; Thomsen, Matz et al. 2006). These new alternative parameters, including TRlaD (Hondegheem 2005), transmural dispersion of repolarization (Antzelevitch 2005), beat-to-beat variability of repolarization (Thomsen, Verduyn et al. 2004), and electro-mechanical window (van der Linde, Van Deuren et al. 2010; Vargas 2010; Guns, Johnson et al. 2012), although appealing, are not yet being routinely used due to a current lack a validation, experience, and clear understanding of the mechanisms underlying TdP. In addition, as was encouraged in the ICH S7B, several proarrhythmia models such as the dog model of chronic AV block (Oros, Beekman et al. 2008) or the methoxamine-sensitized rabbit (Carlsson 2008; Jacobson, Carlsson et al. 2011), have been developed to directly evaluate the torsadogenic risk. However, these models are not routinely used for screening purpose due to lower throughput compared to other available approaches.

Altogether, despite the large number of promising preclinical techniques proposed to assess TdP liability, none of them have been currently validated and accepted as a standard methodology (Pugsley, Hancox et al. 2008).

3.1.1. *In-vitro* models: hERG

The ventricular repolarization process, measured as the QT interval, is mainly (but not exclusively) dependent on the I_{Kr} current. In 1995, Curran et al. demonstrated that the human ether-à-go-go related gene, hERG, encodes the alpha-subunit of I_{Kr} current, which loss of function was responsible for the LQT2 syndrome. Accordingly, determination of the IC_{50} -values (drug concentration resulting in the inhibition of 50% of the I_{Kr} current) during *in-vitro* experiments on human embryonic kidney cells (HEK293) or Chinese hamster ovary (CHO) cells transfected with hERG became a valuable tool to evaluate the affinity of new drugs for the hERG channel and potential torsadogenic risk. Although drug-induced blockade of hERG is very sensitive, this approach lacks specificity and correlates poorly with the TdP liability of a drug (Redfern, Carlsson et al. 2003; Milberg, Ramtin et al. 2004). Some compounds such as verapamil, ranolazine, clozapine, sodium pentobarbital, and fluoxetine have been demonstrated to block hERG, but these agents were not associated with TdP (Fossa, DePasquale et al. 2002;

Belardinelli, Antzelevitch et al. 2003; Redfern, Carlsson et al. 2003; Antzelevitch 2004; Antzelevitch, Belardinelli et al. 2004; Shimizu and Horie 2011). Indeed, some drugs which interact with multiple ion channels can either increase the TdP liability, or have the multiple ion channels effects being compensatory with no increased risk of TdP. Moreover, some compounds have been associated with TdP in patients without binding to the hERG channel directly. Such molecules were associated with a reduced trafficking of I_{Kr} -related proteins (Cordes, Sun et al. 2005; Kuryshv, Ficker et al. 2005; Katchman, Koerner et al. 2006; Dennis, Wang et al. 2007). Furthermore, alterations of the QTc interval have been reported to be, in some cases, indirectly mediated through the activation of the autonomic nervous system which is unlikely to increase the proarrhythmic risk (Berger, Patel et al. 2005). However, while I_{Kr} block has been shown to be poorly predictive of TdP in man, it is generally a good predictor of QT interval prolongation in man (Webster, Leishman et al. 2002; Wallis 2010).

Accordingly, the main advantage of *in-vitro* models is to enable a quick screening process on a large number of molecules for the detection of potential I_{Kr} blocking effects that might result in a positive TQT study in man. However, the results does not always translate to the human and the development and validation of new methods to evaluate the hERG channel, such as *in silico* methods (computerized models, e.g. quantitative structure-activity relationship QSAR) (Sanguinetti and Mitcheson 2005; Taboureau and Jorgensen 2011; Hoefen, Reumann et al. 2012), the use of human induced pluripotent (iPSC-CMs (Guo, Abrams et al. 2011; Ma, Guo et al. 2011)), embryonic (hESC-CM (Braam, Tertoolen et al. 2010)) stem cell-derived-cardiomyocytes, or biomimetic microchips containing living human cells which replicate complex organ-level functions (organs-on-chips (Huh, Hamilton et al. 2011; Huh, Torisawa et al. 2012; van der Meer and van den Berg 2012)) could provide an early and integrated characterization of the compound profile. These advanced techniques, in the near future, might help the discrimination between torsadogenic and non-torsadogenic drugs, possibly reducing the current attrition rate (Belardinelli, Shryock et al. 2005; Lawrence, Pollard et al. 2005; Cavero 2012; Liang, Lan et al. 2013).

3.1.2. Ex-vivo models

3.1.2.1. Hondeghem isolated heart model

The isolated heart model, popularized through the SCREENIT system (Hondeghem, Carlsson et al. 2001; Hondeghem and Hoffmann 2003) is a Langendorff-perfused female rabbit heart (it can also be applied to other species (Cheng and Incardona 2009)) which has been developed to investigate the correlation between APD prolongation and arrhythmia to discriminate between pro- and non-arrhythmic compounds. In this model, the bundle of His is sectioned, the heart is stimulated, and contact electrodes enable the recording of a monophasic action potential (MAP). Based on the premise that APD prolongation is not the sole predisposing factor of TdP, the measure of APD prolongation is accompanied by other surrogate measures of proarrhythmia such as triangulation (defined as the prolongation of APD_{90-30} (Valentin, Hoffmann et al. 2004); Figure 20), reverse-use dependence (increased effects of the compound at lower stimulation rates), instability (beat-to-beat variability (Fossa, Wisialowski et al. 2004) and dispersion of the APD (TRIaD).

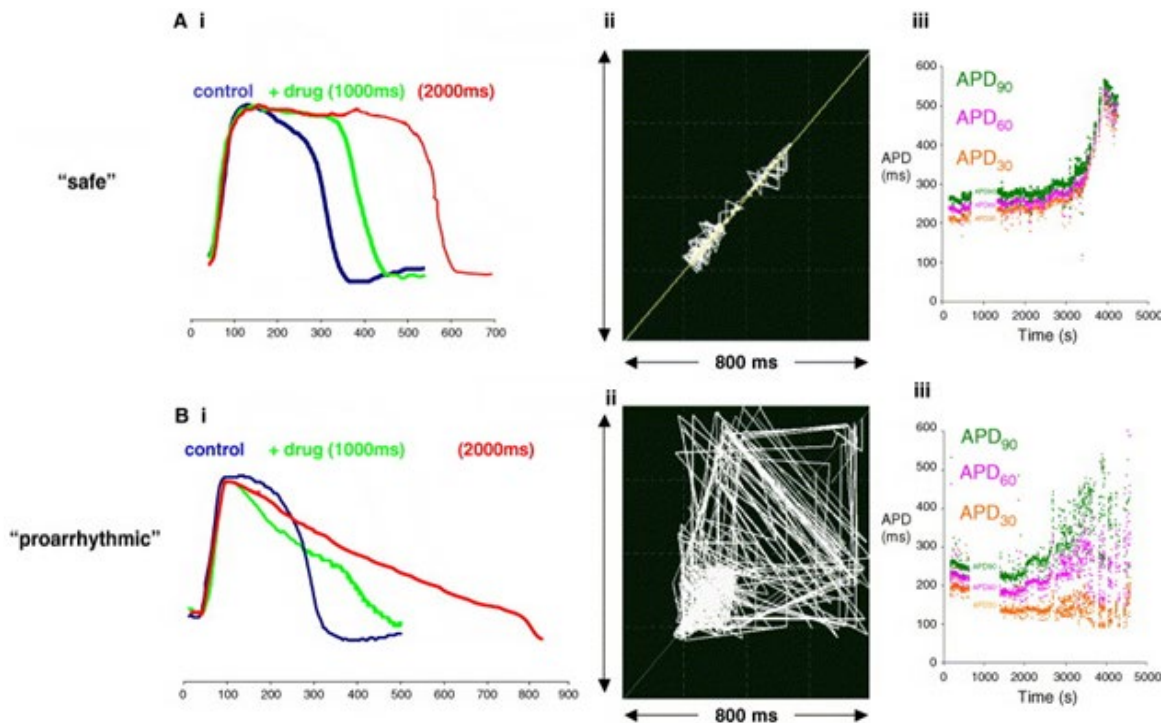


Figure 20. Triangulation of the APD: a surrogate biomarker of TdP liability. When a drug prolongs the APD, risk of arrhythmia is only increased when the morphology of the AP is modified. When the prolongation occurs during phase 2, the repolarization is only delayed but not altered. This phenomenon does not increase the triangulation and has not been associated with TdP. However, when the repolarization phase of the AP is altered during phase 3, the triangulation is increased and result in an increased risk of proarrhythmia (From Lawrence, 2005).

Through the use of an automated algorithm, SCREENIT automatically analyzes TRLad proarrhythmia indices and provides an assessment of the torsadogenic potential of the drug. The advantages of this model are its high predictivity (Valentin, Hoffmann et al. 2004; Lawrence, Bridgland-Taylor et al. 2006), its relative technical simplicity, and the ability to analyze the overall shape of the action potential as well as the stability of the APD prolongation compared to the typical strict APD prolongation measurements (Roche, Renauleaud et al. 2010).

3.1.2.2. Ventricular wedge preparation

After the discovery of the unique electrophysiological properties of M cells (Antzelevitch, Sicouri et al. 1991; Sicouri and Antzelevitch 1991) and in order to better understand their physiological role, Yan and Antzelevitch developed the arterially perfused ventricular wedge preparation by using canine left ventricle (Yan and Antzelevitch 1996). This model consists of the implantation of extracellular electrodes in the endocardial, mid-myocardial, and epicardial tissues in order to study the electrical heterogeneity of the myocardium which is known to play a role in the induction of TdP (Figure 21). The technique generates a pseudo-ECG where the QT interval, T-wave morphology, and arrhythmias can be monitored. Over the years, the wedge preparation has also been developed in other species, such as the rabbit (Yan, Rials et al. 2001; Chen, Cordes et al. 2006; Liu, Brown et al. 2006; Guo, Zhou et al. 2008), which is being increasingly adopted in safety pharmacology as it is now thought to be more predictive than the dog for studying drug-induced TdP. Indeed, the rabbit ventricular endocardium (especially the female rabbit) is characterized by a very small I_{Ks} current (Xu, Rials et al. 2001; Xu, Salata et al. 2002), which facilitates the detection of weak QT-prolonging effects and increases the occurrence of EADs due to a reduced repolarization reserve (Liu, Choi et al. 2005). In addition, this technique also enables tight control over the experimental conditions, as factors such as bradycardia, hypokalemia, hypomagnesemia and autonomic stimulation can be modulated to further reduce the repolarization reserve (Vos, van Opstal et al. 2001). In this model, the QT interval, the Tp-e interval, and the incidence of EAD and EAD-dependent phenomena (R-on-T extrasystoles, and TdP) are used as the main parameters for estimating TdP liability (Joshi, Dimino et al. 2004; Belardinelli, Shryock et al. 2005). The main advantage of this model is to provide an electrophysiological basis through the interactions of different myocardial cells or tissues in order to reflect the spatial dispersion of repolarization throughout the heart. This method generates a "TdP score" which takes into account the critical factors associated with the development of TdP (QT interval, APD, EADs, Tp-e, use-dependence...), and has been associated with highly

predictive results for human torsadogens (100% specificity and 90% sensitivity (Antzelevitch 2004)). The major drawback associated with the perfused ventricular wedge preparation resides in its technical complexity which currently limits its routine use in most laboratories.

Arterially Perfused Left Ventricular Wedge

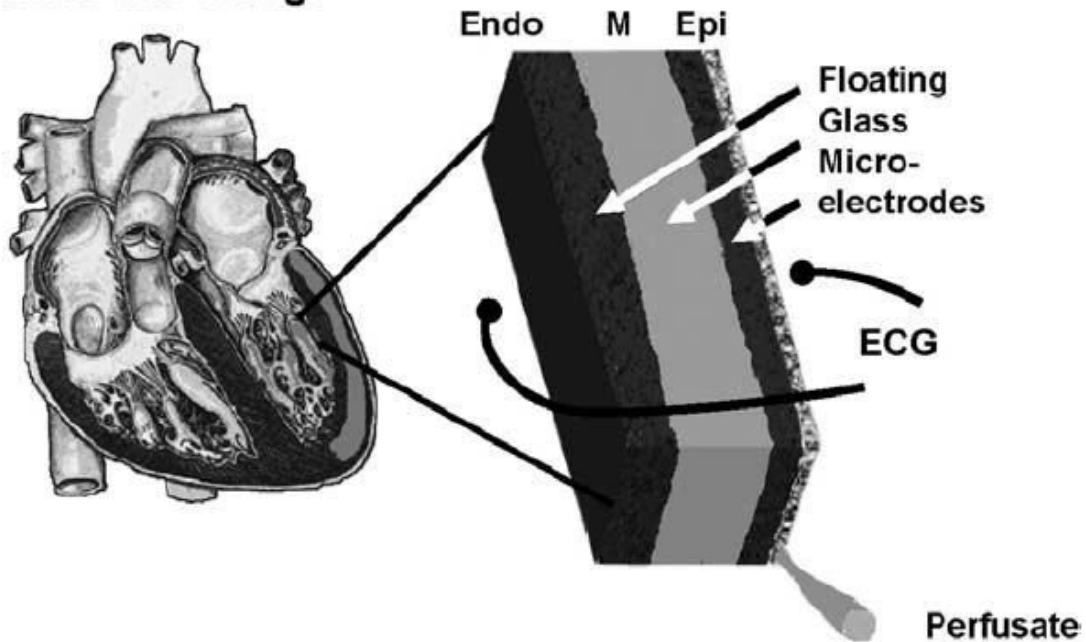


Figure 21. Schematic of arterially perfused canine left ventricular wedge preparation. This technique enables the recording of ECG and AP of the three cellular layers (from Antzelevitch 2005).

3.1.2.3. Purkinje fibers

The Purkinje fiber model is a stable multicellular preparation consisting of a single cellular population of Purkinje fibers extracted from rabbit or canine heart. Compared to the hERG model which is restricted to the I_{Kr} current, the advantage of this preparation is that it allows the analysis of all major ionic currents. The main parameter recorded is generally the APD, however, due to the increasing evidence demonstrating that ionic channel inhibition does not always translate into APD prolongation nor torsades de pointes, other parameters such as the triangulation or the reverse-use dependence are now routinely measured. Accordingly, this model allows a good detection of compounds demonstrating multiple ion channel effects. Of

note, the emergence of efforts combining the better understanding of TdP induction mechanism, computer algorithms, and large preclinical and clinical databases (SCREENIT, TdPscreen (Champeroux, Viaud et al. 2005)) are likely one of the most predictive method currently available.

3.1.3. *In-vivo* models

Although *in-vitro* and *ex-vivo* models generally provide predictive results, one must bear in mind their specific limitations. The lack of autonomic, metabolic, and hormonal influences precludes these experimental techniques from reliably mimicking the *in-vivo* conditions. Accordingly, the goal of *in-vivo* animal studies is to obtain information whether a safety concern (or its absence) previously detected in *in-vitro* models (e.g. hERG channel block) translates into a functional liability in an integrated animal organism, and further into a human risk. For this purpose, several *in-vivo* models have been developed.

3.1.3.1. TdP models

Several groups have tried to develop *in-vivo* models to directly examine TdP proarrhythmia events in conscious or anesthetized animals (rabbit, dog, and mouse) demonstrating TdP-like polymorphic ventricular tachycardia after torsadogenic drug administration (Carlsson, Abrahamsson et al. 1993; Weissenburger, Davy et al. 1993; Eckardt, Haverkamp et al. 1998; Salama and London 2007). The variety of specialized models that have been developed are to date not well accepted by regulatory agencies nor recommended for routine evaluation due to the lack of a clear understanding of the key proarrhythmic mechanisms, the high variability of TdP occurrence, and the difficulty to model the clinical settings of a TdP induction (Lawrence, Pollard et al. 2005; Hoffmann and Warner 2006). Accordingly, the lack of experience and the inherent complexity currently precludes the routine and reliable use of *in-vivo* proarrhythmia models. However, with wider experience and better understanding of the TdP mechanism, these models might become very important tools in the near future.

3.1.3.2. Animal studies

Preclinical assays designed to predict drug-induced proarrhythmic effects in man have been performed in several species, such as dogs, non-human primates (NHPs), guinea pigs, swine, rabbit, ferret, and minipigs. However, experience has shown that telemetered dogs and NHPs are the most sensitive models (Toyoshima, Kanno et al. 2005; Hanson, Bass et al. 2006; Wallis 2010) and allow the recording of clean and noise-free ECG signals (Cavero 2012). While recent technological advances in Jacketed External Telemetry (JET) have greatly improved the reliability of skin surface electrodes, their use was generally not recommended as surface electrodes sometimes provided ECG signals of poor quality. Accordingly, telemetered (implanted) conscious animals are generally preferred. From a cardiovascular (CV) perspective, the dog is the most commonly used non-rodent species and is considered by some to be the most appropriate species for this purpose (Greaves 1998; Gralinski 2003; Friedrichs, Patmore et al. 2005; Lindgren, Bass et al. 2008; Markert, Shen et al. 2011; Leishman, Beck et al. 2012). Dogs are less expensive and easier to handle than nonhuman primates, are amenable to multiple routes of drug administration, and have pharmacokinetic similarities to humans to support human dose and exposure projections (Martignoni, Groothuis et al. 2006). Moreover, dogs have a high positive concordance among preclinical species for predicting human toxicity (Olson, Betton et al. 2000). Canine cardiac ion channel activity and distribution are similar to humans (Wymore, Gintant et al. 1997; Gralinski 2003), and responses in the dog are consistent with clinical outcomes for positive and negative agents (Miyazaki, Watanabe et al. 2005; Hanson, Bass et al. 2006). Another positive feature of dogs is their size, which supports surgical instrumentation for cardiovascular testing (Eckardt, Haverkamp et al. 1998; Guth 2007), and intra-thoracic placement of ECG leads that generate high amplitude ECG tracings largely devoid of movement artifacts (Holzgreffe, Cavero et al. 2007).

Animal studies must be properly designed in order to assess changes in blood pressure, heart rate, and QTc intervals with sufficient study power and required assay sensitivity (through the use of a positive control). The formulae applied for QT-rate correction are critical for the measurement of drug-induced QT prolongation. The ICH S7B describes the Bazett and Fridericia formulae as “the most common” approaches but warn that they might provide misleading data, especially in presence of a heart rate effect. This is the consequence of three points: first, the slope of the QT/RR relationship (or β value) of 0.5 for Bazett and 0.333 for Fridericia have been established from human populations and do not translate well in all animal species. Second, QT/RR relationships are both species and individuals specific, and the use of

any population-based corrections is thus imbedded with potentially large measurement errors. And third, Bazett and Fridericia formulae normalize the QTc to an RR of 1000 ms (or 60 bpm), the average resting RR in man. While this reference rate of 1000 ms is appropriate in man as it is commonly observed in normal physiological conditions, some species exhibit averaged resting RR intervals of 260 or 400 ms. Accordingly, the use of QTcF or QTcB in these species normalizes the QTc to an RR which is never observed under normal physiological conditions, and may thus result into large measurement errors.

The availability of ECG recordings over long periods of time has enabled the development of new methodologies of QT/QTc analysis. It is now generally accepted that individual QT-rate correction is the most appropriate correction method (Malik 2001). Holzgrefe (Holzgrefe, Cavero et al. 2007) has demonstrated that distribution-based analysis can provide reliable individual QT-rate correction (QTca) in various species and for the entire duration of the ECG recording. Fossa (Fossa 2004) developed a method which uses similar beat-to-beat ECG measurements but does not require any QT correction. His method assesses the QT-RR relationship over 24 h through the comparison of the centroids of beat-to-beat data “clouds” (QTbtb).

The years subsequent to the adoption of the ICH S7B and E14 guidance have witnessed an ever increasing emphasis on the accurate and early detection of repolarization liabilities. To this end, substantial effort has been devoted to the characterization of *in-vivo* preclinical repolarization models and their translation to man. Despite this rapidly expanding body of preclinical work, current regulatory cardiovascular safety assessment still relies principally on clinical results due to the lack of reliable and predictive translation of preclinical results to man (HESI 2012).

3.2. Clinical studies

3.2.1. Thorough QT study

Since the implementation of ICH E14 guidance in May 2005, virtually all new drugs in clinical development are required to demonstrate relative safety for the risk of inducing torsades de pointes (TdP), assessed as QT/QTc prolongation (the regulatory accepted surrogate for the risk of TdP) measured during a thorough QT (TQT) study. This single reference trial, which closely resembles the “gold standard” telemetry dog study in terms of design and conditions, is intended to quantify the drug-induced delay in cardiac repolarization (measured as the QT interval

prolongation) in order to identify drugs which may have the potential to lead to lethal arrhythmias. In other words, the purpose of the TQT study is not the quantification of the risk of drug-induced TdP, but restrict to the qualitative detection of a small threshold pharmacological QT prolonging effect of ~5 ms that has been empirically defined by experts during the preparation of the E14 guidance. The purpose of the ICH recommendations was to ensure public safety by providing a consistent methodology for the clinical evaluation of the effects of new therapeutics on cardiac repolarization, during a well-standardized TQT study. However, the recommendations remained intentionally “opened” to allow the use of newly developed methodologies of QT measurement and analysis when these would eventually become available. The TQT studies are generally performed in healthy subjects, include both a positive (typically moxifloxacin) and a negative control (a placebo), continuous 12-lead ECG recording (Holter devices), protocol designed time points (for plasma drug levels and ECG extractions), and use appropriate sample size (~40-80 subjects for a cross-over study (Yan, Zhang et al. 2010)) and statistical models (typically mixed effect models) in order to reliably exclude a threshold pharmacologic effect on cardiac repolarization at each time point. A negative thorough QT study is one that excludes a QT/QTc prolongation effect of ~5 ms evidenced by an upper bound of the 95% confidence interval (CI) excluding 10 ms at all protocol scheduled time points (Figure 22).

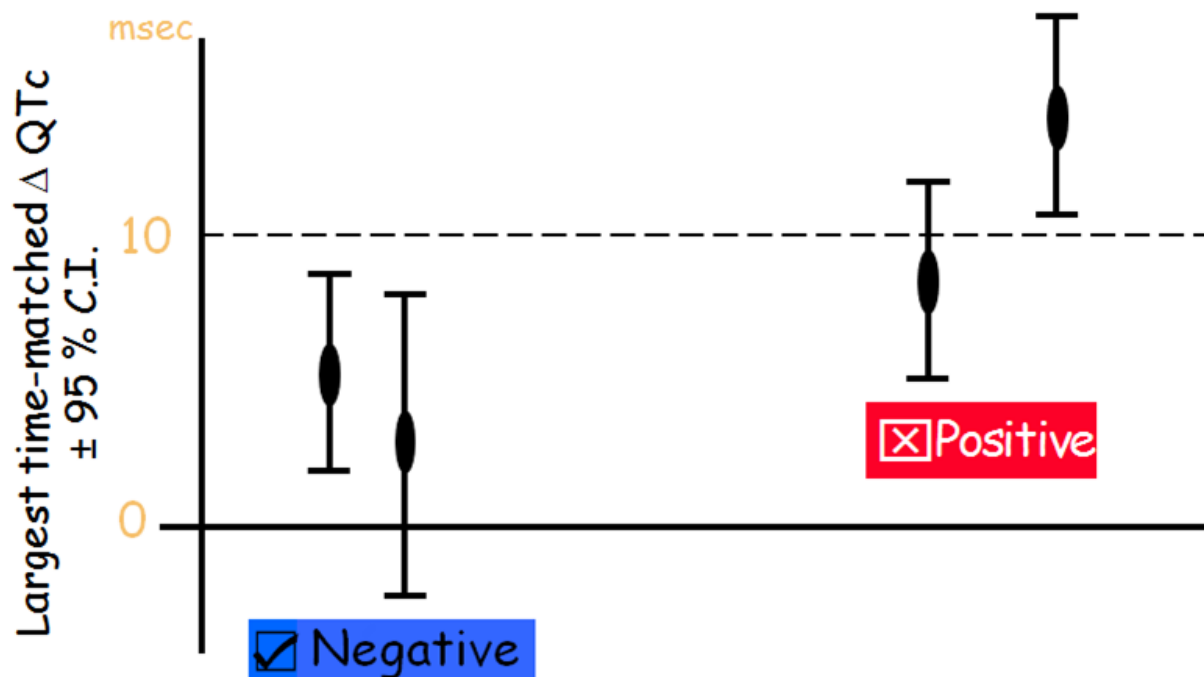


Figure 22. Evaluation of threshold pharmacologic effects in thorough QT studies.

In the case where at least one time point exhibits a 95% CI > 10 ms, the TQT study is concluded to be “positive” and almost always requires an extended ECG safety evaluation during phase II and III of drug development. Extensive ECG monitoring is generally not required for drug candidates with negative TQT studies, where standard ECG monitoring is sufficient. Furthermore, to establish assay sensitivity, the positive control (moxifloxacin) must demonstrate that the lower 95% one-sided confidence interval of the moxifloxacin effect is >5 ms at one or more time-points around 1 to 4 hours after dosing (Zhang 2008; FDA 2012).

The TQT study has rapidly become well-standardized and most sponsors have generally opted to outsource data analysis to ECG core laboratories specialized in semi-automated (SA) ECG annotations performed by well-trained cardiologists. While central readings by ECG core laboratories have been long thought to be the best available choice in order to reduce the variability associated with QT measurement and analysis, it remains to be determined whether this practice is still the current optimal methodology, particularly with regard to the rapid evolution of ECG measurements techniques.

3.2.2. Early QT assessment

Thorough QT studies are generally conducted in parallel to Phase 2 studies, once the clinical pharmacokinetic profile and therapeutic dose range of the drug have been determined, as it enables making the “go or no-go” decision before starting the expensive Phase 3 studies. Taking into account the large costs (~US \$ 1M per study) and the low cost-effectiveness (Bouvy, Koopmanschap et al. 2011) associated with thorough QT/QTc studies, it has been recently proposed to perform “Early QT assessments” by recording high quality ECG data and modeling exposure-response (ER) relationships from First-in-man single ascending dose (SAD) and multiple ascending dose (MAD) studies in healthy volunteers (Darpo and Garnett 2012). However, due to the small number of subjects included in these studies (generally 6 treated, and 2 placebos), assuring the same level of confidence with an alternative “early QT assessment” may be a challenging task. Accordingly, it is likely that the current Thorough QT study will remain a standard requirement for foreseeable future.

4. The QT interval

4.1. Biomarker

The use of the QT interval prolongation as a biomarker for the risk of TdP has been widely criticized (Bednar, Harrigan et al. 2001; Sager 2008). TdP has been linked to delayed cardiac repolarization measured as prolongation of the QT interval on the ECG. Due to the fact that the occurrence of TdP is always associated with QT interval prolongation, and that almost all drugs that produced TdP have demonstrated the ability to block I_{Kr} current (hERG), drug-induced QT prolongation has been accepted as the primary biomarker to predict possible drug-induced torsadogenic liability (Bednar, Harrigan et al. 2001; Joshi, Dimino et al. 2004). The main problem with this conceptualization resides in the fact that even if all compounds that induce TdP prolong the QT interval, the reverse (i.e. that all compounds that prolong the QT interval induce TdP) isn't true (Antzelevitch and Shimizu 2002; Belardinelli, Antzelevitch et al. 2003; Yap and Camm 2003). In addition, there is no clear correlation between the extent of the QT prolongation and the risk of TdP. Indeed, many examples have been established, such as ziprasidone and haloperidol. While both drugs are associated with a QTc increase, ziprasidone demonstrated a larger QTc increase compared to haloperidol, but has not been associated with TdP while haloperidol has been (Sager 2008). Other examples such as moxifloxacin, amiodarone, or verapamil (Roden 2004; Morganroth, Dimarco et al. 2005), are compounds associated with QT prolongation that do not induce TdP. On the other hand, terfenadine only causes mild QT interval prolongation, but is associated with a significant risk of TdP (Fossa, DePasquale et al. 2002; Ando, Hombo et al. 2005).

These examples demonstrate that QT interval prolongation is a poor indicator of torsadogenic potential. However, one important concept is that despite the fact that the QT interval prolongation is a poor biomarker of the torsadogenic risk; the absence of QT interval prolongation remains a good biomarker of compounds which very likely lack any proarrhythmic potential. So, in the absence of a gold-standard assay to detect proarrhythmic risk, the regulators have made the choice to detect all drugs which lack QT prolonging effects (and thus, are likely to be safe) and to classify all the compounds that prolong the QT interval as "potentially unsafe" until proven otherwise. In other words, it is better to discard false positives (reject safe drugs) than taking the risk of accepting false negatives (authorize dangerous drugs). While this concept has been shown to effectively prevent potentially harmful drugs from reaching

the market, it has also likely resulted in the discontinuation of many safe and potentially lives-saving drugs.

In recent years, the implementation of regulatory requirements regarding the potential for drug-induced cardiac arrhythmia have resulted in withdrawals, relabeling, black-box warnings, or restricted access of approved drugs and has been the most common cause of non-clinical drug development termination (Shah 2002).

Accordingly, determining the optimal approach and the best indicators of TdP liability remains an unmet necessity that might only be met once the mechanisms of TdP are completely understood. It is likely that in the near future, complementary analysis of alternative ECG biomarkers, such as TDR, short-term variability (STV) (Hinterseer, Thomsen et al. 2008; Hinterseer, Beckmann et al. 2010), T-wave loop morphology (Zabel and Malik 2002; Couderc 2009), and ECG restitution (Fossa, Wisialowski et al. 2007) will play a role as an integrative TdP liability assessment designed to differentiate proarrhythmic from non-proarrhythmic compounds. At the present time, the assessment of the QT interval prolongation alone cannot differentiate proarrhythmic from non-proarrhythmic drugs, but, until a better surrogate for TdP liability becomes widely accepted and validated (which will likely take many years), QT interval prolongation assessed during the Thorough QT study will likely remain the only regulatory standard (Fenichel, Malik et al. 2004; Pugsley, Authier et al. 2008).

4.2. QT interval measurement from surface ECG

Accurate and precise QT interval measurements are fundamental for the evaluation of the proarrhythmic risk in drug development. However, multiple physiological, technical, and clinical factors have the potential to influence QT interval measurement variability. Under physiological conditions, the QT interval is modulated by the heart rate, both in terms of duration (QT rate-dependence) and adaptation (QT hysteresis), and by the autonomic nervous system (heart rate-independent). Other influences such as the gender, age, circadian pattern and genetic variations have been associated with QT interval differences and contribute to the variable nature of the cardiac repolarization. Furthermore, the determination of the T-wave offset, either manually or automatically, is always associated with some uncertainty due to the incomplete understanding of the recovery process and its projection on the body surface (Kautzner 2002). Technical issues such as the ECG recording device resolution, electrical noise, low amplitudes, merged T-U waves, baseline drift, and reader's experience and/or automated algorithm performance can also

confound accurate and precise QT measurements. The administration of pharmacological compounds which affect the waveform morphologies, or modify other physiological cardiac properties can result in abnormal waveforms which can be very difficult to measure, for both human readers and automated algorithms. Collectively, the interaction of these multiple sources of variability makes reliable QT measurements a real challenge.

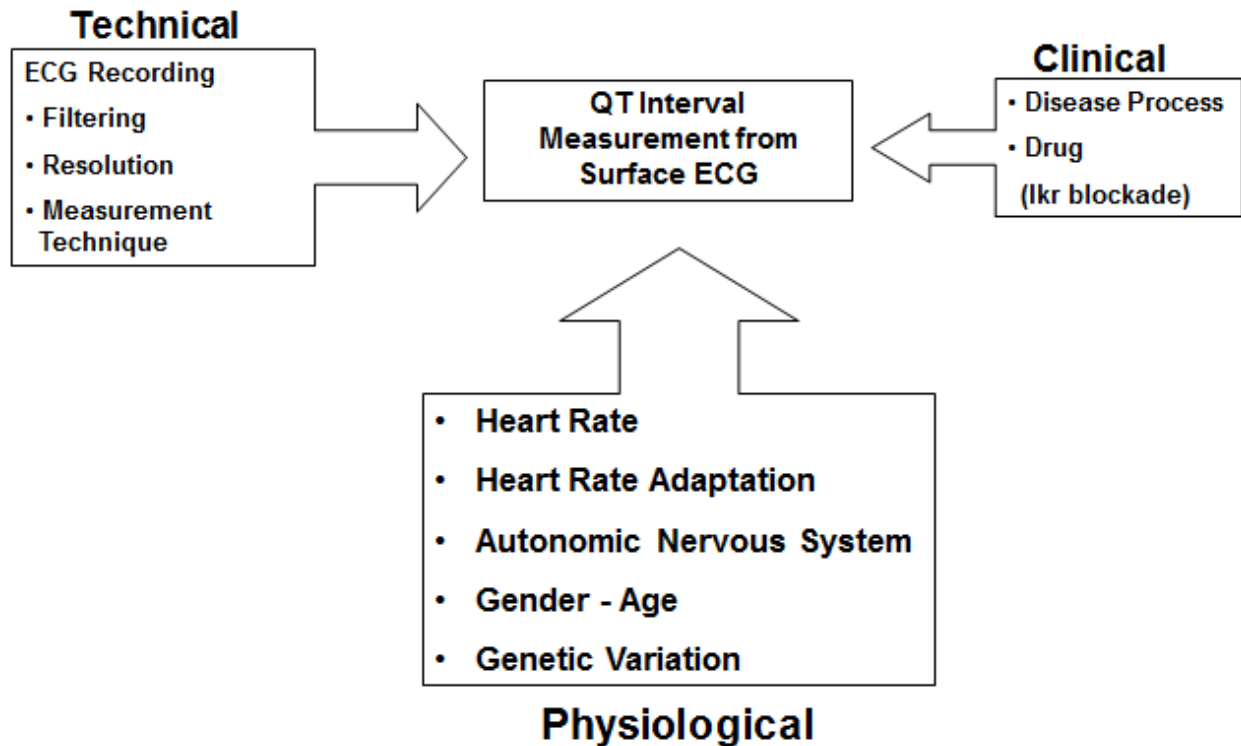


Figure 23. Sources of variability influencing the QT interval measurement.

4.3. QT-RR relationship

Ventricular repolarization is dependent on and varies proportionally with cardiac cycle length. This means that when the RR interval increases, the QT interval increases as well. The rate-dependency of the QT interval has long been recognized (Bazett 1920) and is the result of cardiomyocyte ionic channel kinetics which vary at different cycle lengths and/or through the modulation of the autonomic nervous system (Ahnve and Vallin 1982; Browne, Prystowsky et al. 1983). In order to compare QT intervals within and between subjects, a rate-correction is required. In 1920, Bazett (Bazett 1920) first established a “universal” (or fixed) rate-correction formula. This “universal” formula has been used for many years and is still sometimes used despite its well-recognized drawbacks. Indeed, it is currently widely accepted that the QT-RR

relationship is individual-specific (Batchvarov and Malik 2002), which in other words, means that each individual has his own optimized rate-correction formula (inter-subject variability), and that this formula does not vary much (intra-subject stability) unless in presence of pathologies or drug-induced effects. It is likely that the inter-individual differences are the manifestation of individual-specific distributions of ionic channels in cardiac tissues which vary based on each individual genotype (Malik, Hnatkova et al. 2008). The QT-RR relationship also follows circadian patterns where the slope of the relationship is generally steeper during day compared to nocturnal periods. These effects are due to the circadian modulation of sympatho-vagal balance, where the sympathetic tone is more pronounced during the day, and the vagal tone more pronounced at night (Smetana, Batchvarov et al. 2003). Accordingly, using fixed correction formula is inherently associated with potentially large measurement errors and the modeling an individual QT-RR relationship for each subject is now accepted as the most appropriate correction method. Recently, further investigations in individual rate-correction have demonstrated that QT-RR curvatures were subject-specific, meaning that using an individual-specific regression model (linear, log linear...) is important to reduce the measurement error in the presence of drugs exhibiting large effects on heart rate (Malik, Hnatkova et al. 2012).

However, other approaches have also been proposed to avoid the need for rate-correction. These methods, such as the bin method, ECG restitution, transmural dispersion of repolarization, and the short-term variability purport to prevent the need for rate-correction techniques that are usually rather complex. These methods are described thereafter.

4.4. QT hysteresis

Another fundamental physiologic property of the heart is the delay in the adaptation of the QT interval to an abrupt change in RR. This lag in the adaptation of the QT interval is called QT hysteresis and has been previously approximated in man (~2.5 min) in studies employing constant pacing rates (Lau, Freedman et al. 1988). Other studies employing long-term ECG recordings without fixed pacing rates have reported that differences in the QT/RR hysteresis profile could stratify the risk of arrhythmia in survivors of myocardial infarction (Pueyo, Smetana et al. 2004; Smetana, Pueyo et al. 2004). More recently, the QT-RR hysteresis profiles were investigated in healthy subjects and demonstrated that QT-RR hysteresis profiles were highly individual and that the combination of individual hysteresis and rate-correction might lead to improvements in cardiac repolarization studies (Malik, Hnatkova et al. 2008). In addition, it has

recently been reported that QT-RR hysteresis is also mediated by differential autonomic nervous system effects (Pelchovitz, Ng et al. 2012). Accordingly, the QT-RR hysteresis effects on QT dynamicity are quite complex and several methods have been implemented to either model (through mathematical formulae) or avoid (by selecting periods of HR stability) the effects of hysteresis.

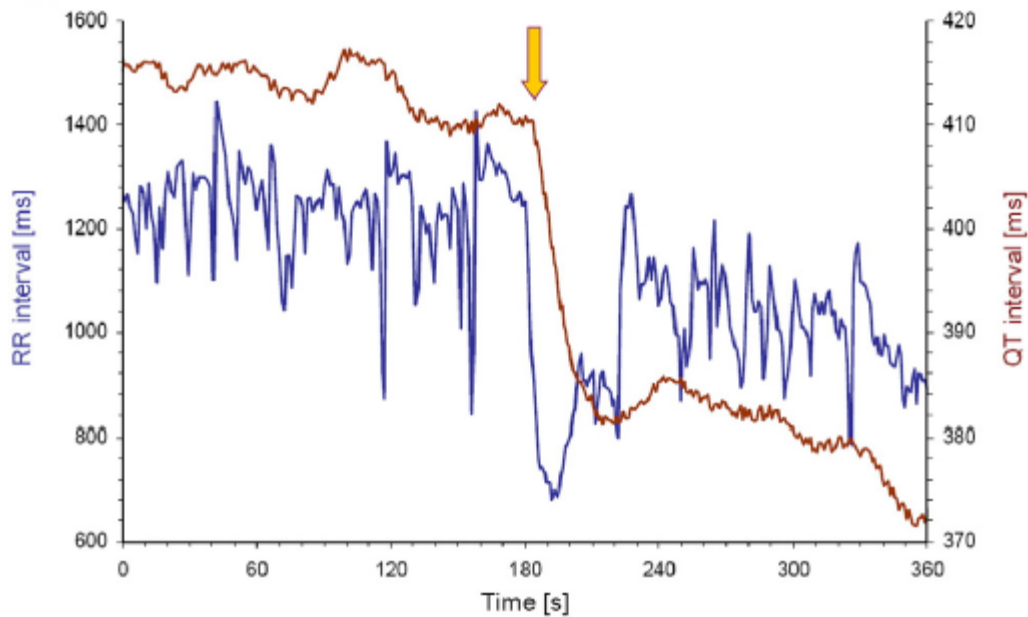


Figure 24. QT hysteresis. At 180 seconds (arrow), the subject changed abruptly from supine to unsupported sitting position. The RR (blue) almost immediately shortened but the QT interval (red) only gradually shortened to the new level of heart rate (from Garnett 2012).

4.5. Heart-rate independent influences

Although the heart rate is the main driver of the cardiac repolarization duration, other rate-independent covariates have been identified for influencing the QT interval (Magnano, Holleran et al. 2002). The autonomic nervous system, and more precisely, the balance between sympathetic and vagal tones, has been associated with changes in the QT interval duration. Generally, increases in sympathetic tone shorten the QT interval and increases in vagal tone increase the QT interval. These effects are evident especially during exercise (Davidowski and Wolf 1984) or periods of sleep (Browne, Prystowsky et al. 1983) and meals (Nagy, DeMeersman et al. 1997).

Accordingly, each fixed RR interval duration is associated with a large population of possible QT interval durations, thus resulting in typical QT-RR clouds, as presented in Figure 25.

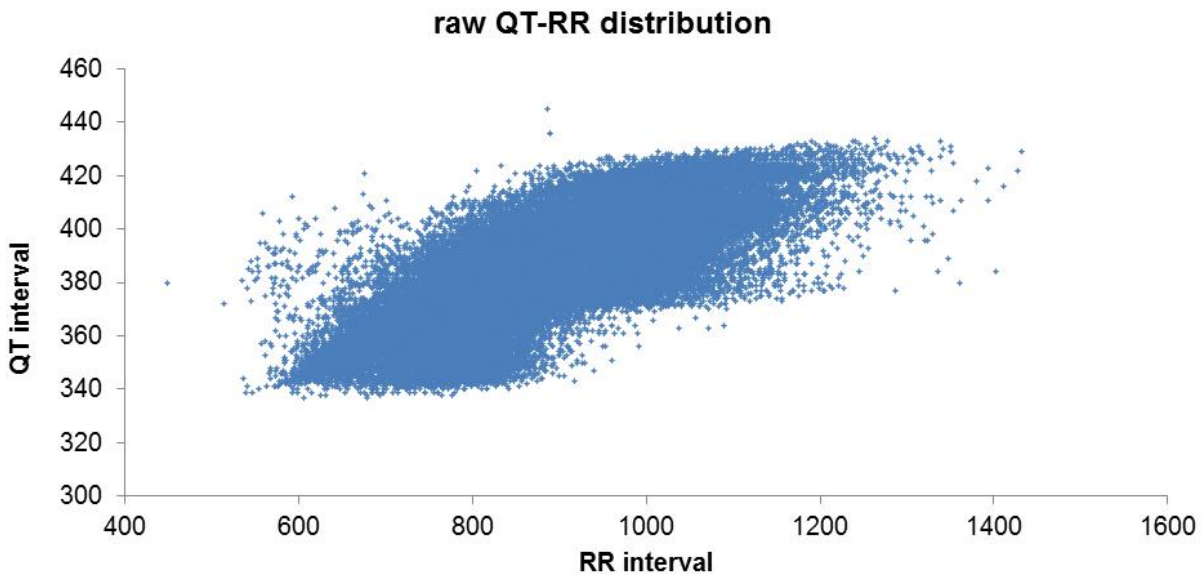


Figure 25. The typical QT-RR clouds resulting from multiple physiological influences over a 24 hour period.

4.6. Correction methods

4.6.1. Rate-correction

In the absence of specific regulatory guidance, sponsors have been free to invoke multiple *in-vivo* repolarization models and have typically employed QT rate-correction procedures which were based on historical precedent rather than relevant and objective scientific observations.

4.6.1.1. Fixed rate-correction

Many mathematical models have been developed to describe the QT-RR relationship. The simplest and most commonly used rate-correction formulae were log-linear regressions established from ~35 and ~50 subjects in 1920 by Bazett (QTcB) (Bazett 1920) and Fridericia (QTcF) (55), respectively. The formulae were $QTcB = QT/RR^{1/2}$ and $QTcF = QT/RR^{1/3}$. These fixed rate-correction formulae were established to be universally applied for all individuals. Other mathematical models, such as the linear regressions (e.g. Framingham formula: $QTcFr =$

$QT+0.154(1-RR)$ or Hodges $QTcHodges = QT+1.75(HR-60)$) have been investigated as potential improved formulae. In total, more than 70 fixed formulas have been established and compared (Kawataki, Kashima et al. 1984; Ahnve 1985; Sagie, Larson et al. 1992; Funck-Brentano and Jaillon 1993), but none of them have been able to demonstrate consistent superiority over the others. In time, it became apparent that none of these models could ever universally describe the relationship between QT and RR intervals, as it was subsequently demonstrated that each individual is best characterized by a specific rate-correction formula.

4.6.1.2. Study-specific rate-correction

The study-specific rate-correction (QTcS) (Malik, Hnatkova et al. 2004) consists of applying a mathematical model (linear, log-linear, hyperbolic, logarithmic, or any other) to the QT and RR data extracted from a study population in order to extract an α or β parameter which will serve for the derivation of a QTcS value which will then be applied to all patients within the study. This method was used as a surrogate for individual rate-correction methods when the numbers of available individual QT-RR pairs were insufficient to reliably model individual rate-corrections formulae. This is typically the case during analyses which are restricted to a few sparse ECG extractions at scheduled time points (the standard ECG core laboratory procedure). While this practice is generally associated with less residual error compared to fixed rate-correction formulae, it can still be further improved with individual rate-correction formulae.

4.6.1.3. Individual rate-correction

That cardiac properties are highly individual have been extensively described (Batchvarov and Malik 2002) and the use of individual rate-correction (QTcI) is now accepted as the most reliable practice (Funck-Brentano and Jaillon 1993; Hnatkova and Malik 1999; Malik, Hnatkova et al. 2004). In addition, the hysteresis profiles as well as the selection of an optimized mathematical model to be applied to assess the QT-RR regression have also been demonstrated to be best characterized on an individual basis (Malik, Hnatkova et al. 2008; Malik, Hnatkova et al. 2012). Another approach, using a Bayesian hierarchical measurement error model, has been developed and suggests that it might be superior to commonly used frequentist methods (Chen and Zhao 2010). However, the question of the analysis period used to measure the baseline QT-RR regression currently remains a matter of debate.

4.6.1.3.1. Analysis periods and circadian patterns

The optimal period on which the QT-RR regression must be applied has not yet been characterized. Some studies have demonstrated that the slope of the QT-RR regression is steeper during the day than during the night (Extramiana, Maison-Blanche et al. 1999). Accordingly, whether individual QT-RR regression should be applied on 24 h (Holzgreffe, Ferber et al. 2012), separated between day and night, or restricted to day data (Malik, Hnatkova et al. 2008) or time windows (Strachan, Hughes et al. 2009) is still a matter of debate. The selection of ECGs preceded by heart rate stability has been extensively used to reduce the influence of hysteresis (Extramiana, Badilini et al. 2007; Malik, Hnatkova et al. 2008; Garnett, Zhu et al. 2012) and reduce the variability of the QTc measurement. In addition, whether off-drug QT-RR relationship or on-drug QT-RR relationship should be used for the derivation of the on-drug QTc has also been a matter of discussion (Extramiana, Badilini et al. 2007). The high heterogeneity of practice for the derivation of individual rate-correction results from the current lack of a gold-standard methodology. The derivation of an individual rate-correction is performed in several steps:

1. The selection of the mathematical model to apply (linear, exponential, logarithmic, subject-specific curvature...) for the measurement of the individual QT-RR regression.
2. The selection of the data periods on which the model is applied (24 h, day, night, preceded by HR stability, restricted to supine periods, on- or off-drug, beat-to-beat or averaged values) to derive an individual correction factor.
3. The application of a QTc formula implementing the derived correction factor to the raw dataset (beat-to-beat or averaged values).

Accordingly, the number of subject-specific rate-correction methodologies possibly applicable is almost infinite, and, while it is possible to determine an optimized mathematical model to apply to a specific dataset (Malik, Hnatkova et al. 2012), it is likely that this “optimized mathematical model” (e.g. linear) determined for a specific dataset (e.g. baseline diurnal) from an individual will differ if another data selection is made (e.g. baseline periods of stability).

4.6.2. Hysteresis-correction

Recently, Malik proposed a method to correct for hysteresis (Malik, Hnatkova et al. 2008; Malik, Hnatkova et al. 2009). The subjects were required to perform a pre-study effort tests (cycling) to evaluate their individual hysteresis profile with an optimized algorithm. Once the individual QT-RR hysteresis parameters were characterized, the formula

$$RR^H = \sum_{i=1 \dots n} (e^{-\lambda(i-1)/n} - e^{-\lambda i/n}) / (1 - e^{-\lambda}) RR_i$$

was applied, where RR_i is the i th RR interval preceding the QT interval measurement and n is the duration of the known history of the measurement.

When effort tests are not amenable, hysteresis effect may be accounted for by implementing an averaged value of the preceding RR in the QTc interval formula such as

$$QTc = QT / \overline{RR}^\beta$$

where \overline{RR} is the mean RR over a predefined period.

4.7. ECG recording and QT measurement methods

The reliable measurement of ECG intervals and waveforms are of great importance as it is required to assess both safety and efficacy of new drugs in development. Whether these measurements should be performed manually (by cardiologist), automatically (with computer programs), or semi-automatically (computer-assisted) have been a matter of debate. Compared to manual measurements, computerized ECG measurement platforms were designed to provide several advantages such as improved reproducibility (no intra-observer variability), improved time-efficiency, and reduced manpower-requirements and associated costs. The counterpart to these advantages is that automatic computer algorithms are not devoid of measurements errors, especially due to the difficulty to reliably mark the end of the T-wave in presence of noise, low amplitude, merged T-U waves, or circadian and drug-induced T-wave morphological changes (Hnatkova, Gang et al. 2006; Zhou and Wei 2011). Accordingly, semi-automated methods have been implemented as they combine human visualization and adjustments with computer algorithm reproducibility and time-efficiency.

The QT interval is defined as the duration from the start of the Q wave to the end of the T wave. While the measurement of the Q-onset is generally simple and similar between readers, the

assessment of the end of the T-wave is a subtle task for which even cardiologists sometimes fail to agree (Viskin, Rosovski et al. 2005). Accordingly, due to inherent subjectivity associated with the QT interval measurement, it is imperative that all ECG measurements for a specific subject are performed by a single reader. This “subjectivity” is also somewhat found in computerized measurements as each algorithm was developed by different groups of people and designed based on different principles of waveform measurement. Indeed, computer algorithms based on tangent, threshold, median beats, superimposed beats, pattern recognition, or Hidden Markov Models methods are likely to detect and mark the ECG waveforms differently, which would result in slight measurement differences between algorithms (Panicker, Karnad et al. 2009). Accordingly, proper baseline- and/or placebo adjustments are very important when comparing ECG measurements methods as it provides a means to evaluate possible over- or underestimation of a QTc effect between methods.

Although fully automated QT analysis applications have been available for several years, it has been reported that first generation algorithms were associated with numerous measurement errors due to low amplitude waveforms, electrical noise, baseline drift, and T-wave morphological changes (Glancy, Weston et al. 1996; McLaughlin, Campbell et al. 1996; Morganroth 2001; Kautzner 2002; Hnatkova, Gang et al. 2006; Zhou and Wei 2011). Accordingly, they were not routinely accepted by regulatory agencies for primary TQT analysis and sparse SA measurements performed by ECG core laboratories have become a widely accepted standard. Over time, computerized methods have been refined, and, recently, several studies have reported reliable QT measurements with several commercially available or internally developed software applications (Hnatkova, Gang et al. 2006; Extramiana, Badilini et al. 2007; Sarapa, Gussak et al. 2009; Strachan, Hughes et al. 2009; Tyl, Kabbaj et al. 2009; Couderc, Garnett et al. 2011; Meyer, Ferber et al. 2012). These automated methods have been designed to facilitate accurate cardiac repolarization assessment by combining a computerized ECG interval measurement with a refined QT/QTc analysis methodology. When compared to reference SA or manual evaluations, these applications and analytical methods have demonstrated equivalent QT/QTc assessments during TQT studies with generally reduced variability (Hnatkova, Gang et al. 2006; Sarapa, Gussak et al. 2009; Strachan, Hughes et al. 2009; Tyl, Kabbaj et al. 2009; Couderc, Garnett et al. 2011; Green, Kligfield et al. 2012; Meyer, Ferber et al. 2012).

4.8. Analytical methods

4.8.1. QT interval duration

4.8.1.1. ECG core laboratories

Since the implementation of the ICH-E14 which stated (in 2005) that the “thorough QT/QTc study” would warrant particularly careful attention to interval measurement. At present, this would usually involve the measurement by a few skilled readers (whether or not assisted by computer) operating from a centralized ECG laboratory”, pharmaceutical companies have generally outsourced TQT study data analysis to ECG core laboratories which specialize in semi-automated (SA) ECG annotations performed by well-trained cardiologists. The analyses performed by such laboratories are well standardized and typically consists of semi-automatically adjudicated annotations of 3-9 beats obtained during periods of heart rate stability (± 2 bpm) extracted at predefined time points prospectively defined in the study protocol (Malik, Hnatkova et al. 2008). Although this methodology provides reliable QT interval measurements during the specified intervals, it has many well recognized and, to date, unresolved limitations including the exclusion of the majority of the recorded data, insufficient temporal resolution, substantial time and resource (manpower, cost) requirements, inter-observer variability, and possible measurement error when only few QT intervals are selected for a specific RR interval (Fossa 2004; Extramiana, Badilini et al. 2007; Strachan, Hughes et al. 2009; Holzgrefe, Ferber et al. 2012). Importantly, ECG core labs have to rely on suboptimal rate-corrections such as fixed or study specific rate-corrections as the sparse QT-RR data derived from fully manual or SA ECG analysis precludes the derivation and use of individual QT-rate corrections. Accordingly, while ECG core labs generally provide reliable data analyses, there is still room for substantial improvement.

4.8.1.2. Distribution-based analysis

The main advantages of distribution-based analysis are the inclusion of almost all recorded ECG complexes (>70000 beats per 24 h) and the integration of the probabilistic nature of the QT interval into the analysis. The property of *rate-independent* QT variability stipulates that multiple, discrete QT intervals exist for each RR interval where the QT interval exists as a probabilistic value which may be reliably estimated only as the mean of a sufficiently large number of ECG complexes associated to each RR value. However, when probabilistic QT values are substituted

for discrete point measurements in any of the common QT-RR regression models, the resulting rate-correction coefficients dissociate the effects of heart rate from the raw QT interval to a near mathematical certainty. While this finding has been independently confirmed by several investigators (Champeroux-Pascal 2009; Champeroux, Ouille et al. 2010; Honda, Komatsu et al. 2010; Komatsu, Honda et al. 2010), prior methodologies have not incorporated the property of rate-independent QT variability into the associated QT-RR regression models, resulting in increased intra- and inter-study QT variability.

For each 24 h dataset, derivation of an individual rate correction is performed by first assigning each raw QT value to a RR bin of 10 ms, and then apply a log-linear regression model ($\log(QT) = \beta \cdot \log(RR) + \alpha$) to derive the QT-RR relationship parameters from the average QT and RR of each bin. The slope (β) value derived from the off-drug dataset is then applied in the formula $QT_{ca} = QT / RR^{\beta}$ for each recorded QT value (Holzgreffe, Cavero et al. 2007). The large amount of data generated is then condensed into a suitable time scale (10 or 5 min segments) to depict the time-dependent effects of the analyzed drugs. This method, first evaluated in dog and cynomolgus monkey, demonstrated reliable results and a good applicability independently of the species analyzed.

4.8.1.3. Rate binning method

The rate binning method was designed to avoid the need of QT-rate correction by comparing uncorrected on and off-drug QT interval at similar heart rates. For a selected period of time (usually a period around C_{max}) each individual ECG complexes are pooled into a specific RR bin based on their associated RR value (Badilini, Maison-Blanche et al. 1999). All complexes preceded by heart rate stability which are present in a specific RR bin are then averaged into a “median beat”. A median beat is a single waveform representative of all associated complexes. It is generally well-defined (due to the averaging) and allows the use of highly automated measurements followed by operator review. This rate binning method thus allows the comparison of uncorrected QT intervals at a normalized RR (generally 1000 ms) which is particularly suited to assess QT interval prolongation in presence of a concomitant drug-induced effect on heart rate (Extramiana, Maison-Blanche et al. 2006). The main problem with the rate-binning method consists of the fact that a period of interest has to be preselected, resulting in the loss of time-dependent effects. However, a variant, named time-binning has been developed which enables the computation of median beats from pooled complexes over periods of time instead of RR-bins (Extramiana, Badilini et al. 2007).

4.8.1.4. Dynamic QT beat-to-beat analysis

The dynamic QT beat-to-beat analysis (or QTbtb) has been developed to differentiate normal heart rate and autonomic effects from drug-induced repolarization effects without the use of QT rate-correction formulae in both preclinical (Fossa, DePasquale et al. 2002) and clinical studies (Fossa, Wisialowski et al. 2007; Fossa, Langdon et al. 2011). To this end, QTbtb method relies on the intra-individual comparison between on-drug QT-RR data and “normal” off-drug (baseline) QT-RR data (referred as “clouds”). During the baseline recording, all sources of physiologic variability are included (hysteresis, autonomic tone, protocol scheduled events, day, night...) and serve for the evaluation of the 97.5% upper reference bound, which represents the “normal” physiologic autonomic boundaries of a specific subject. Then, the on-drug QT-RR data clouds can be superimposed to the off-drug data to detect the proportion of outlier beats that cross the reference upper bound and might have the potential to impair the repolarization. Nominal time points of interest (generally 5-min) can also be selected and compared to the off-drug 24 h data cloud. In addition to the proportion of outliers, a QTbtb value can also be derived as the centroid of the 5-min data cloud (calculated as the median QT at the median RR interval), and compared to the centroid of all baseline beats within the same RR range (± 12 ms) (Fossa 2004). More recently, the same beat-to-beat technique has also been used to assess the QT-TQ interval relationship, or ECG restitution, which translates the heart recovery from one beat to another and has been associated with potentially increased arrhythmia vulnerability (Fossa, Wisialowski et al. 2006; Fossa, Wisialowski et al. 2007). The main advantages of this method are that it includes all 24 h QT-RR data, provides supplementary information on potential increased proarrhythmia risk, and does not require rate-correction methods.

4.8.2. T-wave morphology

The analysis of the T-wave morphology is currently considered a complementary measure to the extensively used QT interval duration. Several approaches for the analysis of T-wave morphology have been developed and include the determination of the duration, amplitude, area, slopes, Q to T_{peak}, T_{p-e}, T-loop morphology and vectorcardiograms (Fayn and Rubel 1988; Kanters, Fanoë et al. 2004; Couderc, Zhou et al. 2008; Graff, Andersen et al. 2009; Haarmark, Graff et al. 2010). Recently, it has been reported that the T-wave morphology descriptors were independent of heart rate, age, and gender (Haarmark, Graff et al. 2010). With more experience and the development of computer algorithms which quantify the repolarization morphological abnormalities, the analysis of the T-wave morphology might hold promises as a complementary

way to differentiate drugs that effectively increase the risk of proarrhythmia, or to discriminate between different LQTS genotypes (Neyroud, Maison-Blanche et al. 1998; Vaglio, Couderc et al. 2008).

4.8.3. Transmural dispersion of repolarization

Since the discovery that epicardial, mid-myocardial, and endocardial cells (i.e. the layers of the ventricular myocardium) were characterized by differential responses to pharmacological agents, many studies have been performed to evaluate the use of transmural dispersion of repolarization, measured as the Tp-e interval on the ECG, as a potential biomarker of proarrhythmia. In isolated heart models (Milberg, Hilker et al. 2007) and anesthetized dogs (Gallacher, Van de Water et al. 2007), an increased TDR has been associated with drug-induced TdP. Moreover, the transmural dispersion of repolarization is thought by many authors to be a superior biomarker of TdP as it has demonstrated the ability to segregate proarrhythmic drugs from non-proarrhythmic drugs more efficiently than the QT interval prolongation (Antzelevitch 2005; Shah, Kluger et al. 2007). In a recent study evaluating the predictivity of TDR versus QT prolongation, the effects of two proarrhythmic drugs (bepridil and E4031) and two non-proarrhythmic drugs (risperidone and verapamil) were compared in canine ventricular wedge preparations. While all drugs increased the QT interval (high sensitivity, poor specificity), only proarrhythmic drugs increased the TDR (high sensitivity, high specificity). This study suggests that TDR may represent a more reliable preclinical biomarker of proarrhythmic risk compared to the QT interval prolongation (Said, Wilson et al. 2012).

4.8.4. Arrhythmia detection

Several automated arrhythmia detection algorithms have been developed in patients and animals demonstrating ventricular premature beats (Meurs, Spier et al. 2001; Chan, Wang et al. 2010), atrial fibrillation (Babaeizadeh, Gregg et al. 2009), premature supra-ventricular complexes (Ince, Kiranyaz et al. 2009), and multiple arrhythmias (Ozbay 2009). However, their application in preclinical studies remains limited because freely moving animals exhibit substantial noise and ECG artifacts that have to be reviewed in order to determine whether the detected arrhythmic events are accurate. While further development of such tools may facilitate the evaluation of the proarrhythmic potential of drug candidates, their use currently remains limited.

4.8.5. Spectral analysis / Heart rate variability

The heart rate variability (HRV) has been shown to be an important non-invasive tool which enables the evaluation of autonomic nervous system responses. In isolated heart models or after pharmacological block of the ANS, the heart attains its *intrinsic heart rate* and remains relatively stable. In the healthy heart, high heart rate variability is a sign of good cardiovascular health as it reflects the heart's ability to rapidly adapt to the internal and external stimuli. Accordingly, the inability to maintain a capacity to adapt can be measured as a decreased HRV and is generally associated with a poor medical outcome (Rowan, Campen et al. 2007). HRV is measured as the degree of difference between successive heart beats and can be analyzed in both the time and frequency domains. The application of a fast Fourier transform (FFT) enables the measurement of the power spectrum of heart rate variability. Spectral analyses of HRV (see figure 26) are segmented into low frequencies (LF) and high frequencies (HF). Due to their different nerve diameters and neurotransmitters rates of release and removal, the two branches of the autonomic nervous system (sympathetic and parasympathetic) affect the heart with different latencies. This enables the distinction between sympathetic and vagal tone effects (van de Borne, Montano et al. 1997). HF bands are predominantly of parasympathetic origin, and LF bands are a combination of both sympathetic and vagal influences. Accordingly, the HF/LF ratio is generally used as a biomarker of the sympatho-vagal balance to evaluate drug effects on the ANS (Champeroux, Martel et al. 2013). However, the major drawback associated with HRV as a biomarker consists of its complex interpretation which may lead to misuse or inappropriate conclusions. Indeed, the HRV alone is generally not sufficient to provide meaningful results without other clinical tests (Stein and Kleiger 1999).

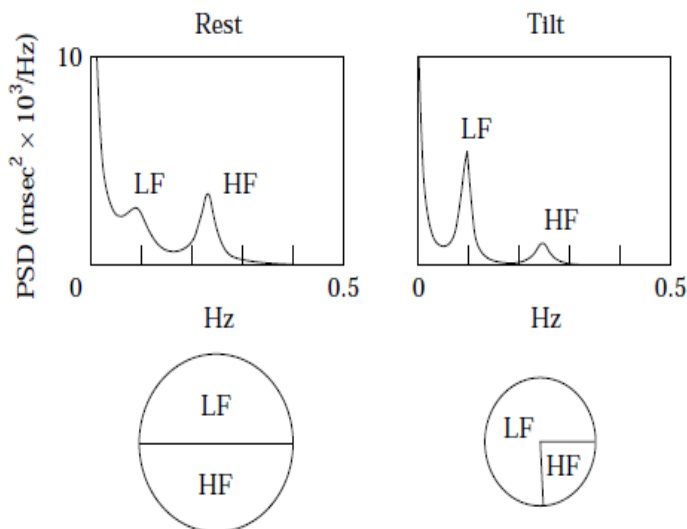


Figure 26. Spectral analysis of RR interval variability in healthy subject at rest and during 90° head-up tilt. At rest, two components of similar power are detectable at low and high frequencies. During tilt, LF component becomes predominant with reduced HF components, demonstrating an increase of sympathetic and decrease in parasympathetic influences (from Task Force of the European Society of

4.9. Statistical methods

The determination of an optimal statistical procedure to evaluate the QT prolongation liability of a compound has been a matter of debate since the implementation of ICH E14 guidance. The main topic of discussion has been the possible false positive or negative that might be associated with the use of a particular statistical test over another. In 2005, the E14 Statistics Group recommended that an intersection-union test (IUT) would be appropriate to exclude a clinically significant effect on the QT interval. The IUT was suggested for its practicality and conservatism regarding QT prolongation assessments. After baseline and placebo adjustments, the QTc data are analyzed using an analysis of variance (ANOVA) to estimate the drug-induced effects and their associated 95% confidence intervals. A statistically significant QT prolonging effect is excluded when the CIs of the baseline- and placebo- adjusted QTc values are inferior to 10 ms at each measured time point. The IUT is defined as follows:

$$H_0 : \cup \{ m_{D(i)} - m_{P(i)} \} > 10, i = 1, 2, \dots, k$$

$$H_1 : \cap \{ m_{D(i)} - m_{P(i)} \} \leq 10, i = 1, 2, \dots, k$$

Where K is the number of time points, and $\mu_{D(i)}$ and $\mu_{P(i)}$ are the mean changes from baseline of QTc for the study drug and the placebo at time point i, respectively.

Accordingly, to accept H_0 and declare the study “negative”, all 1-sided 95% confidence intervals of the mean differences must be inferior to 10 ms.

In 2008, the ICH E14 implementation working group (IWT) issued a Questions & Answers document which was further updated in April 2012 (FDA 2012). Among other, the IWT provided recommendations regarding the evaluation of assay sensitivity for which the test is defined as follows:

$$H_0 : \cap \{ m_{M(i)} - m_{P(i)} \} \leq 5, i = 1, 2, 3, 4$$

$$H_1 : \cup \{ m_{M(i)} - m_{P(i)} \} > 5, i = 1, 2, 3, 4$$

Where $i=1,2,3,4$ are the time points are 1, 2, 3, and 4 h after moxifloxacin oral administration, as these time points generally correspond to the Tmax of moxifloxacin (the optimal time points are

different for intravenous administration). $\mu_{M(i)}$ and $\mu_{P(i)}$ are the mean changes from baseline of QTc for moxifloxacin and placebo at time point i , respectively.

Similarly, to reject H_0 and declare the assay sensitivity “established”, all 1-sided 95% confidence intervals of the mean moxifloxacin differences around T_{max} must be superior to 5 ms. However, depending on the study design (especially with parallel design) and the sample size, substantial false positive rates were observed (Hutmacher, Chapel et al. 2008). Importantly, clinical concern is defined as a mean QT prolongation of ~5 ms. In this regard, the IUT does not directly provide an estimate of the pharmacologic effect of the drug but relies on the maximum difference to exclude an effect. The maximum difference of the means by time point is a potentially poor estimate of a general QT prolongation difference (Hutmacher, Chapel et al. 2008). Many approaches have been proposed in order to improve the reliability of the statistical tests, however, none of them have demonstrated clear improvement compared to the IUT (Tian, Qiao et al. 2010).

Objectives

Today, due to the various influences which may impact the accurate and precise measurement of the QT interval, one of principal unanswered questions is whether the primary TQT study analysis as currently assessed with gold standard, semi-automated methodologies, is optimal, and if not, can it be further optimized?

The objectives of the current work is to evaluate the applicability of advanced computerized ECG measurement technologies in conjunction with continuous QT/QTc analysis methodologies to provide improved fully automated cardiac repolarization assessments during clinical trials which yield results which are comparable to, or exceed, those currently obtained with more laborious and resource-intensive SA methods.

In Parts I and II, continuous ECG analyses employing multiple ECG measurement platforms are compared to ECG core laboratory analysis obtained with SA methodology. Parallel comparisons are performed after treatment with placebo, moxifloxacin (the standard clinical reference agent), and saquinavir-boosted-ritonavir (a drug characterized by T-wave morphological changes).

Confirmation of accurate and precise continuous ECG analysis employing multiple advanced methodologies would provide powerful new methods characterized by:

- Improved power and temporal resolution due to the inclusion of all recorded ECG complexes
- More detailed and reliable time-dependent measurements of possible drug effects
- Decreased variability due to the use of more suited QT rate correction methods
- Decreased time and resource (manpower, cost) requirements

Due to the current lack of standardization for continuous ECG assessments, Part III evaluates various physiological, technical, and pharmacological influences and their impact on the reproducibility and accuracy of cardiac repolarization assessments. The goal of these evaluations is to provide recommendations for an optimal use of continuous ECG evaluation methods.

Materials and Methods

1. QT studies

The following clinical studies were conducted in the Institut de Pharmacologie Clinique Roche (IPCR), also named Clinical Pharmacology Unit (CPU) in Strasbourg, France.

1.1.NP21249 – Saquinavir – TQT

1.1.1. Background, Rationale, and Objectives

Human Immunodeficiency Virus (HIV), the etiological agent of the Acquired Immunodeficiency Syndrome (AIDS), causes a chronic infection leading to progressive dysfunction of the immune system. The HIV protease is a constitutive enzyme of HIV that processes viral proteins essential for the maturation of infectious virions. Thus, HIV protease plays a vital role in the viral life cycle and represents a key target for intervention in the development of novel therapeutic agents for AIDS.

Saquinavir (RO31-8959, SQV) is a potent inhibitor of human immunodeficiency virus (HIV-1) viral protease. Two formulations of SQV are currently marketed for oral administration, Invirase® 200 mg (hard capsule) and Invirase 500 mg film-coated tablets. The oral bioavailability of SQV is limited by extensive first pass metabolism, mediated primarily by CYP3A4. Ritonavir (Norvir®, RTV), a protease inhibitor (PI) with antiviral activity against HIV-1 and HIV-2, is also a potent inhibitor of CYP3A4 and P-glycoprotein (P-gp), responsible for the resistance to treatments with PI. The concomitant administration of SQV and RTV has demonstrated large increases of SQV exposure due to the inhibition of the CYP3A4. SQV is indicated for the treatment of HIV-1 infected adult patients, and should be administered only in combination with low dose RTV and other antiretroviral medicinal products. The approved therapeutic dose in the United States and European Union is SQV 1000 mg twice daily (BID) in combination with RTV 100 mg BID.

The potential for QT interval prolongation with the saquinavir was thought to be low based on the results obtained during Roche preclinical investigations and spontaneous reports in Roche clinical safety databases. Furthermore, the results obtained from a TQT study evaluating the effects of ritonavir (RTV) demonstrated that a single dose of 100 mg of RTV was not associated with QTc prolongation in healthy subjects (Sarapa, Nickens et al. 2008). Nevertheless, several HIV protease inhibitors, including saquinavir and ritonavir, were suspected to cause drug-

induced QT prolongation. Further investigation demonstrated that both SQV and RTV were associated with dose-dependent block of hERG channels (I_{Kr} current) in HEK293 cells *in-vitro* which suggested that HIV protease inhibitors could increase the risk of TdP (Anson, Weaver et al. 2005). Subsequent to these findings, the FDA requested that all manufacturers of protease inhibitors conduct a TQT study assessing the potential to prolong the QT/QTc interval of their drugs.

Accordingly, the primary objective was to evaluate whether saquinavir boosted with ritonavir (SQR) had a threshold pharmacological effect on cardiac repolarization, as detected by changes in the QT/QTc interval on 12-lead electrocardiograms.

1.1.2. Study design

Sixty healthy subjects were enrolled and 24 h continuous 12 lead digital ECGs (1000 Hz, Mortara H12+ Holter devices, Mortara Instruments, Milwaukee, WI) were acquired on Day -1 (pretreatment baseline) and Day 3 (on-drug) for each period. The choice of monitoring ECGs on day 3 was based on an internal Roche report (Multiple ascending dose study) which demonstrated maximal mean plasma concentrations of SQV metabolites and RTV on day 3. The study was performed at a single center (IPCR), employing a double-blind, randomized, 4-period, 4-way crossover design. All subjects received each of 4 treatments by random assignment from a 4-period Williams' square design with a washout period of at least 7 days between consecutive treatments. Each of the 4 periods consisted of one pretreatment baseline day, followed by 3 days of multiple dosing. The treatments were saquinavir (1000 or 1500 mg) in association with ritonavir (100 mg), placebo, and moxifloxacin. Moxifloxacin was given as a single dose (400 mg) on Day 3 to confirm the assay sensitivity.

A: Saquinavir boosted ritonavir 1000/100 mg (SQR1000)

B: Saquinavir boosted ritonavir 1500/100 mg (SQR1500)

C: Moxifloxacin 400 mg (Moxi400)

D: Placebo

Eligible subjects reported to the IPCR on the morning of Day -2 following an overnight fast of approximately 10 h for clinical laboratory testing and triplicate paper (safety) 12-lead ECG recordings, and were admitted to the unit on the evening of Day-2 for the preparation of baseline

assessments on Day -1. Subjects who remained eligible were fasted overnight. On Day -1, subjects were given a standard breakfast 30 minutes prior to dosing and completed breakfast at least 10 minutes prior to dosing. The time-matched baseline 12-lead continuous digital ECG began 30 minutes later, from -24 h to -0.5 h prior to dosing on Day 1. Triplicate ECGs from the continuous ECG recording data were extracted at -22, -21, -20, -19, -18, -16, -12, -8, -4, -1, -0.75 and -0.5 h prior to dosing on Day 1. Subjects were at rest and supine for 10 minutes prior to and 5 minutes after ECG extraction time point with all distractions minimized. On Days 1 to 3 of each treatment period, subjects remained at the ICPR and study drug was administered. On Day 3, subjects were given a standard breakfast 30 minutes prior to dosing and required to complete breakfast at least 10 minutes prior to dosing. The 12-lead continuous digital ECG recording was repeated from just prior to the morning dose of study medication up through 24 h post-dose, with continuous ECG data extracted at 2, 3, 4, 5, 6, 8, 12, 16 and 20 h post-dose. Blood samples were collected for measurement of SQV, SQV metabolites (M4, M6, M10), RTV, and Moxi400 on Day 3 immediately prior to the morning dose of study medication, and at 2, 3, 4, 5, 6, 8, and 12 h post-dose. Safety laboratory tests were performed on Day 4 after the 24 h digital ECG recording. Blood pressure and pulse rate were measured on Days -1 and 3 at specified time points. Adverse events were monitored throughout the study. All subjects gave written informed consent to the protocol which was reviewed and approved by the independent Investigational Review Board (Comité de Protection des Personnes 1, Strasbourg Cedex, France) which operates in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines.

1.2. BP22464

1.2.1. Background, Rationale and Objectives

Type 2 diabetes is a metabolic disorder characterized by elevated blood glucose levels. In addition to hyperglycemia, type 2 diabetes is typically accompanied by other metabolic and cardiovascular risk factors, including hypertension, dyslipidemia, and accelerated atherosclerosis. Because of its chronic complications, type 2 diabetes causes significant morbidity and mortality in affected individuals. The prevalence of type 2 diabetes is increasing worldwide in both industrialized and developing countries. Approximately 366 million people worldwide are predicted to be diagnosed with type 2 diabetes by 2030 (Wild, Roglic et al. 2004). The current pharmacological therapies for type 2 diabetes are often inefficient at achieving glycemic control partly because they address only one of several underlying defects. In addition,

some of the anti-diabetic medications are associated with undesirable side-effects (i.e., weight gain, water retention, worsening lipid profiles or hypoglycemia). Moreover some medications are contraindicated in some patients or require specific monitoring. Thus, there is the need for new antidiabetic therapies that are both more effective and better tolerated in mono- and combination therapy than currently available antidiabetic medications. The availability of such therapies with different mechanisms of action could lead to novel combination therapies that change the current treatment paradigm. RO4998452 is a selective inhibitor of the sodium glucose co-transporter 2 (SGLT2). It is being co-developed by Chugai Pharmaceutical Co and F. Hoffmann-La Roche Ltd for the treatment of type 2 diabetes. SGLT2 is expressed in the renal tubules and is responsible for reabsorption of bulk glucose from the renal filtrate. Inhibition of SGLT2 reduces renal reabsorption of glucose and promotes urinary glucose excretion thereby ameliorating blood glucose levels in patients with type 2 diabetes.

The effects of RO4998452 (10, 30, 100 mg/kg) on systolic and diastolic blood pressures, heart rate, electrocardiogram (ECG; intervals PR, QRS, RR, QT/QTc), and body temperature were assessed in conscious telemetered cynomolgus monkeys where no abnormal findings were reported. Further investigations were performed on the K⁺-current using *in-vitro* whole-cell patch-clamp method on human embryonic kidney (HEK) cells expressing the hERG channel. No abnormal findings were noted up to 100 µmol/L. Previous clinical experience in 4 studies demonstrated that RO4998452 was well tolerated in a total of 93 healthy subjects and 32 type 2 diabetic patients where no clinically significant changes in ECGs were reported. Accordingly, based on the clinical and the preclinical information available at the time, RO4998452 showed no potential for QT prolongation within the anticipated clinical dose range. However, regulatory confirmation from a TQT study that clinical doses of RO4998452 do not cause clinically relevant increases in QT interval duration was still required.

The primary objective was to evaluate whether RO4998452 had a threshold pharmacological effect on cardiac repolarization as detected by changes in the study corrected QT interval (QTcS) following a single dose administration.

1.2.2. Study design

Fifty two healthy subjects were enrolled (27 men, 24 women) and 24 h continuous 12 lead digital ECGs (1000 Hz, Mortara H12+ Holter devices, Mortara Instruments, Milwaukee, WI) were acquired on Day 1 (on-drug) for each period. There were no continuous ECG recordings on day -1. The study was performed at a single center, employing a double-blind, double-dummy,

randomized, 4-way crossover design. All subjects received each of 4 treatments by random assignment from a 4-period Williams' square design with a washout period of at least 7 days between consecutive treatments. Each of the 4 periods consisted of oral single doses. The treatments were RO4998452 (40 or 400 mg), placebo, and moxifloxacin (400 mg).

A: RO4998452 40 mg (SGLT40)

B: RO4998452 400 mg (SGLT400)

C: Moxifloxacin 400 mg (Moxi400)

D: Placebo

Eligible subjects were admitted to the ICPR on Day -1. Subjects were randomized to one of the four treatment sequences. After starting continuous monitoring of pharmacodynamic ECGs and after recording vital signs and safety 12-lead ECG, the study drug was administered. Blood samples were taken for pharmacokinetic analysis and blood glucose measurements, and triplicate pharmacodynamic 12-lead ECGs were extracted. Vital signs were also recorded at regular intervals up to 24 hours. Concomitant medications and adverse events were monitored continuously until 24 hours after dosing, when the subjects were allowed to leave the ICPR. Subjects attended a follow-up visit 7-14 days later. A washout period of at least seven days was observed between treatment periods. Adverse events were monitored throughout the study. All subjects gave written informed consent to the protocol which was reviewed and approved by the independent Investigational Review Board (Comité de Protection des Personnes 1, Strasbourg Cedex, France) which operates in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines.

1.3. BP22693

1.3.1. Background, Rationale, and Objectives

Major Depressive Disorder represents one of the most common and proliferating health problems worldwide (Wong and Licinio 2001; Wong and Licinio 2004) and with increasing longevity, its prevalence is estimated to be 15-20% (Kessler, Berglund et al. 2003). Approximately 340 million people worldwide are expected to suffer from Major Depressive Disorder (Kessler, McGonagle et al. 1994) and nearly 35-40% of suicides are considered to be related to major depression as an underlying cause (Vijayakumar 2005). Moreover it is estimated that only 60% to 70% of patients respond to approved antidepressants and only 30-50% achieve full remission.

In the last ten years, the treatment for depression has focused mainly on serotonin, as can be seen from the introduction of selective serotonin reuptake inhibitors (SSRIs), such as fluoxetine, sertraline, citalopram, and paroxetine, and selective serotonin and noradrenaline reuptake inhibitors (SNRIs) with a dual action on serotonin and noradrenaline (e.g. venlafaxine and duloxetine) (Meyer, Wilson et al. 2004). However, their main limitations include the failure to achieve complete symptom remission in 40%-60% of all patients for whom they are prescribed, delayed onset of action and long-term side effects, such as sexual dysfunction or weight gain.

RO5200628 is a chemically unique agent under development by F. Hoffmann-La Roche Ltd as a “broad spectrum” antidepressant. As an inhibitor of serotonin, norepinephrine, and dopamine reuptake, it exhibits antidepressant-like actions in animal models. A triple reuptake inhibitor adds the element of dopamine transporter blockade to a dual (serotonin and noradrenaline) reuptake inhibitor. The potential advantages of treatment with triple reuptake inhibitor (TRI) compared to traditional antidepressants may include faster onset of action, greater efficacy compared to SSRI/SNRI and better tolerability with less effect on sexual dysfunction and body weight gain as seen with SSRIs.

In animal models of depression, RO5200628 was active in the mouse tail-suspension test at 1 mg/kg, p.o. (minimal effective dose: MED), in the rat forced swim test at 10 mg/kg, p.o. (MED) and in the cynomolgus monkey DRL (Differential Reinforcement for Low Rate) procedure at 1 mg/kg, p.o., as well as 0.03 mg/kg, i.m (MED). RO5200628 did not induce or reduce anxiety-like activity as the compound was inactive on the rat elevated plus maze test for doses up to 10 mg/kg, p.o.

Prior to initiating studies in patients with depression, it is important to assess the tolerability and safety of RO5200628 and characterize its pharmacokinetic properties in healthy subjects. This was the first study with RO5200628 in man. The study was conducted in two parts. In part I, the study aim was to assess the safety, tolerability, pharmacokinetics and pharmacodynamics of a range of single oral doses, and if possible to determine the maximum tolerated dose (MTD). Part II assessed the effect of a single oral dose of RO5200628 or matching placebo on blood pressure, heart rate and ECG parameters. The dose to be tested in part II was selected based on the safety, tolerability and pharmacokinetics obtained from part I and on any other supportive information (e.g. PD data). The dose had to be lower or equal to the highest well tolerated dose in part I. The effect of the compound on pharmacodynamic parameters (i.e. Contingent Negative Variation) was investigated in part II in an exploratory manner. The results from part I study were used to determine the appropriate dose(s) to be used for a subsequent multiple dose study with RO5200628.

The primary objectives were, in part I, to assess the tolerability and safety of single oral doses of RO5200628 in healthy male subjects and to determine, if achievable, the maximum tolerated dose (MTD). In part II, the objectives were to assess the effect of a single dose (selected from part I) of RO5200628 on systolic and diastolic blood pressure and ECG parameters using a larger cohort.

1.3.2. Study design

1.3.2.1. Part I: Single Ascending Dose (SAD)

Seventy-two male healthy subjects were enrolled (8 in each dose group and assuming a maximum of 9 dose groups) and 24 h continuous 12 lead digital ECGs (1000 Hz, Mortara H12+ Holter devices, Mortara Instruments, Milwaukee, WI) were acquired on Day -1 and Day 1. The study was performed at a single center, employing a double-blind, randomized, placebo-controlled, parallel group, single ascending dose design. The study had an adaptive design with ongoing assessment of available safety, tolerability, pharmacokinetics and pharmacodynamics. Triplicate ECG measurements as well as triplicate measurements of blood pressure were obtained. Each dose group was constituted of 6 active and 2 placebo subjects. A single oral dose of RO5200628 or matching placebo was administered on the morning of study Day 1 after an overnight fasting of at least 8 hours. A standard light lunch was provided approximately four

hours after dosing. Dose escalation decisions were based on tolerability and safety of the previous dose(s), and on available pharmacokinetic parameters.

Dose groups were:

Dose group 1: RO5200628 0.3 mg (TRI-0.3) or matching placebo

Dose group 2: RO5200628 1 mg (TRI-1) or matching placebo

Dose group 3: RO5200628 3 mg (TRI-3) or matching placebo

Dose group 4: RO5200628 10 mg (TRI-10) or matching placebo

Dose group 5: RO5200628 30 mg (TRI-30) or matching placebo

Dose group 6: RO5200628 60 mg (TRI-60) or matching placebo

Dose group 7: RO5200628 90 mg (TRI-90) or matching placebo

Two additional dose groups were added to better characterize the MTD:

Dose group 8: RO5200628 120 mg (TRI-120) or matching placebo

Dose group 9: RO5200628 45 mg (TRI-45) or matching placebo

In each dose group, subjects were dosed using a staggered approach by dividing the group into 2 cohorts to allow for safety evaluation. The two cohorts were dosed with an interval of approximately 24 hours to enable the review of the safety and tolerability data of cohort 1. Dose escalation decisions were made by the Roche Clinical Pharmacologist and the Principal Investigator (or designee). The dose was not escalated further if the tolerability or safety at the preceding dose level was not acceptable as judged by the Investigator and the Clinical Pharmacologist. Subjects were admitted to the IPCR on Day -2 in the afternoon. Study drug was given the morning of Day 1 and subjects stayed in the IPCR for approximately 48 hours after the study drug had been administered. Subjects were discharged after completion of the 48-hour post-dose evaluations following a medical check-up by a physician and attended a follow-up visit 7-14 days later.

1.3.2.2. Part II: Effect of RO5200628 on blood pressure and ECG intervals

Twenty-two male healthy subjects were enrolled and 24 h continuous 12 lead digital ECGs were acquired on Day -1 and Day 1. The study was performed at a single center, employing a double-blind, randomized, placebo-controlled, two-period, two-way crossover design. Each subject received 2 treatments with at least 2 weeks of washout between the administrations. After reviewing the available data from the first 7 groups in part I, the 60 mg dose was selected as the dose to be administered in part II. This dose was selected as it provided an adequate safety confidence in relation to the predicted efficacious exposure.

Treatment A: RO5200628 60 mg (TRI-60)

Treatment B: Placebo

Subjects were admitted to the IPCR on Day -2 in the afternoon. Study drug was given the morning of Day 1 and subjects stayed in the IPCR for approximately 48 hours after the study drug had been administered. Subjects were discharged after completion of the 48-hour post-dose evaluations following a medical check-up by a physician and attended a follow-up visit 7-14 days later. Adverse events were monitored throughout the study. All subjects gave written informed consent to the protocol which was reviewed and approved by the independent Investigational Review Board (Comité de Protection des Personnes 1, Strasbourg Cedex, France) which operates in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines.

2. QT measurement methods

2.1. Semi-automated

The analysis performed by ECG core laboratories are well standardized and typically consists of semi-automatically adjudicated annotations of 3-9 beats obtained during periods of heart rate stability (± 2 bpm) extracted at predefined time points prospectively defined in the study protocol (Figure 27). For each nominal protocol-specified time point where the subjects are required to lay supine for 15 minutes to minimize variability, a 5 min period preceded by 2 min of stable heart rate (± 2 bpm) is selected. Three discrete 10s intervals demonstrating minimal noise and at least 20s apart from each other are selected from each 5 min period. Triplicates ECGs are quantitatively evaluated from each of the three selected 10s intervals. Calipers are automatically placed for each beat using a tangent- or threshold-based algorithm, and subsequently adjusted by an experienced cardiologist as necessary. A study-specific QT rate correction (QTcS) (Malik 2001) is then applied to the mean of each triplicate value such that the QTcS for each designated time point is represented by the mean of 3 triplicate values (9 aggregate complexes).

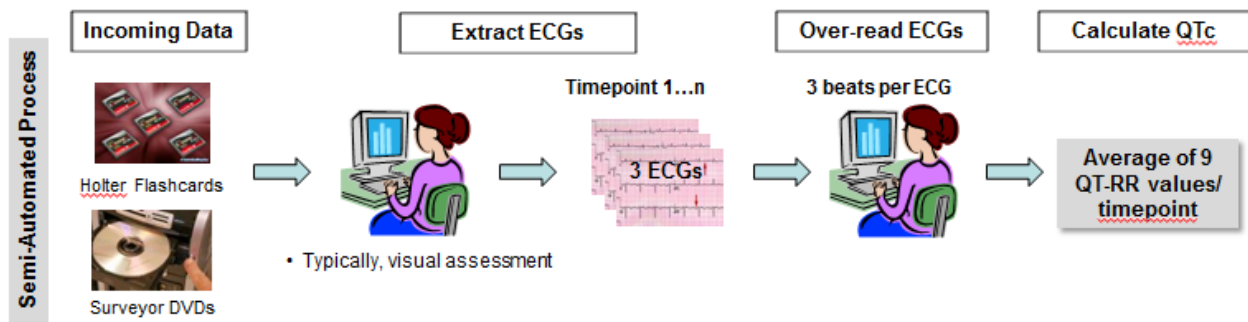


Figure 27. Typical process of semi-automated methods by ECG core laboratories. Three ECG extractions are performed from continuous recordings at each protocol-scheduled time point. Then, each extracted ECG is over-read and adjusted if necessary by an experienced operator or cardiologist. QT and RR values are averaged for the calculation of the QTc interval at each specific time point.

2.2. Continuous ECG measurement platforms and analysis software

Once the studies were completed, all high resolution (1000 Hz) ECG recordings were available on Holter flashcards. These recordings are encoded into a Mortara proprietary format, .H12, which is not directly readable by non-Mortara software applications. Each software application required that the .H12 Holter files be converted into a readable software-specific format prior to the analysis.

2.2.1. Ponemah

2.2.1.1. Step 1. Binary conversion

The Ponemah software uses a binary file input format. The necessary .bin files were obtained through the conversion of .H12 files with SuperECG software (Figure 28), a non-commercial program which was developed by Mortara specifically for scientists interested in analyzing Mortara Holter recordings with systems that could not read the proprietary Mortara format. SuperECG sequentially converts 1 h Mortara (.H12) files into 1 h generic binary (.bin) files.

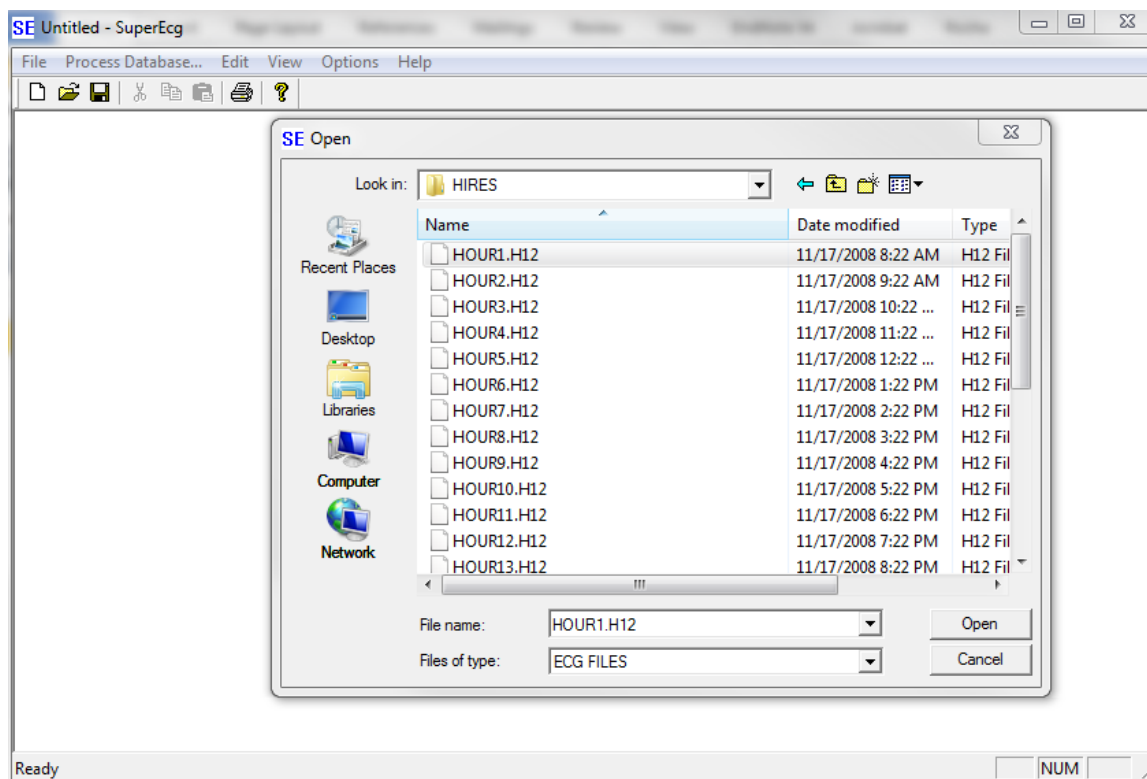
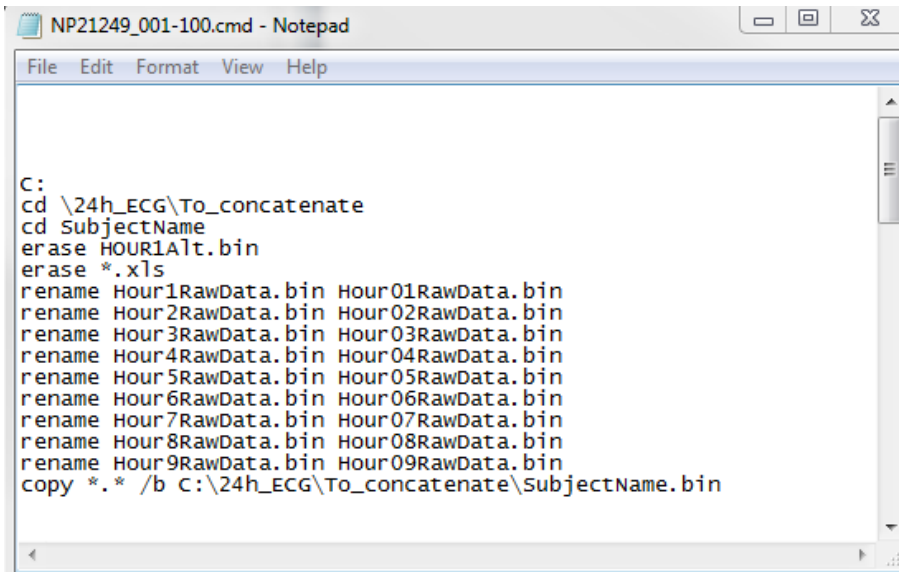


Figure 28. SuperECG software interface. This software enables the conversion of each .H12 files to binary file by selecting the first .H12 file,

Once the conversion has been performed, the 1 h binary files are concatenated into one unique binary file which includes the entire 24 h recording. This process is done using the DOS command “copy *.* /b” (Figure 29).

. indicates all files present in the selected folder

/b indicates a binary file.



```
NP21249_001-100.cmd - Notepad
File Edit Format View Help
C:
cd \24h_ECG\To_concatenate
cd SubjectName
erase HOUR1Alt.bin
erase *.xls
rename Hour1RawData.bin Hour01RawData.bin
rename Hour2RawData.bin Hour02RawData.bin
rename Hour3RawData.bin Hour03RawData.bin
rename Hour4RawData.bin Hour04RawData.bin
rename Hour5RawData.bin Hour05RawData.bin
rename Hour6RawData.bin Hour06RawData.bin
rename Hour7RawData.bin Hour07RawData.bin
rename Hour8RawData.bin Hour08RawData.bin
rename Hour9RawData.bin Hour09RawData.bin
copy *.* /b C:\24h_ECG\To_concatenate\SubjectName.bin
```

Figure 29. Concatenation script. The concatenation process enables the creation of a single 24 h file combining each of the previously converted 1h files. The “erase” and “rename” commands are used to delete unwanted files and to order the holter files in the correct order, respectively.

2.2.1.2. Step 2. ECG Replay in Ponemah

The resulting “SubjectName.bin” is then selected for Replay in the Ponemah software (Figure 30A). The replay process enables the Ponemah system to read the ECG binary file and creates Ponemah .RAW and .DRa files. The .RAW files (~1.4 Gb for 24 h) are the .bin ECG data converted into the Ponemah file format. These raw files are then submitted for further analysis with the Ponemah pattern recognition module.

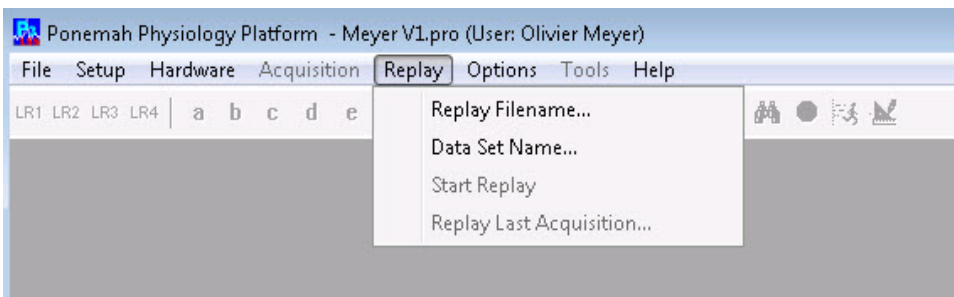


Figure 30A. Ponemah interface, ECG replay process.

During the replaying process, a .DRa file is created and consists of 24 h of continuous ECG waveform measurements based on the fully-automated Ponemah algorithm analysis (ALG). The Ponemah ECG analysis algorithm (ALG) first establishes the isoelectric baseline between the preceding and subsequent QRS complexes through the use of a straight line fit. The signal deflection relative to this baseline establishes the location of the T-wave. T-end location is then determined by fitting a 4th order polynomial to the T-wave. The relative slopes between T-wave curve fit and the baseline over the T region are used to establish a threshold for the identification of T-end. Working forward in time from the peak of the T-wave, the analysis calculates the slope at which the T-wave approaches the previously established baseline. T-end is then marked once the rate of change relative to the baseline drops below a user-defined threshold. Aberrant waveforms, typically due to either physiologic or environmental noise or lead faults, are automatically identified and rejected employing a post hoc combination of Ponemah algorithm noise rejection filters and algorithm performance descriptors (Figure 30B).

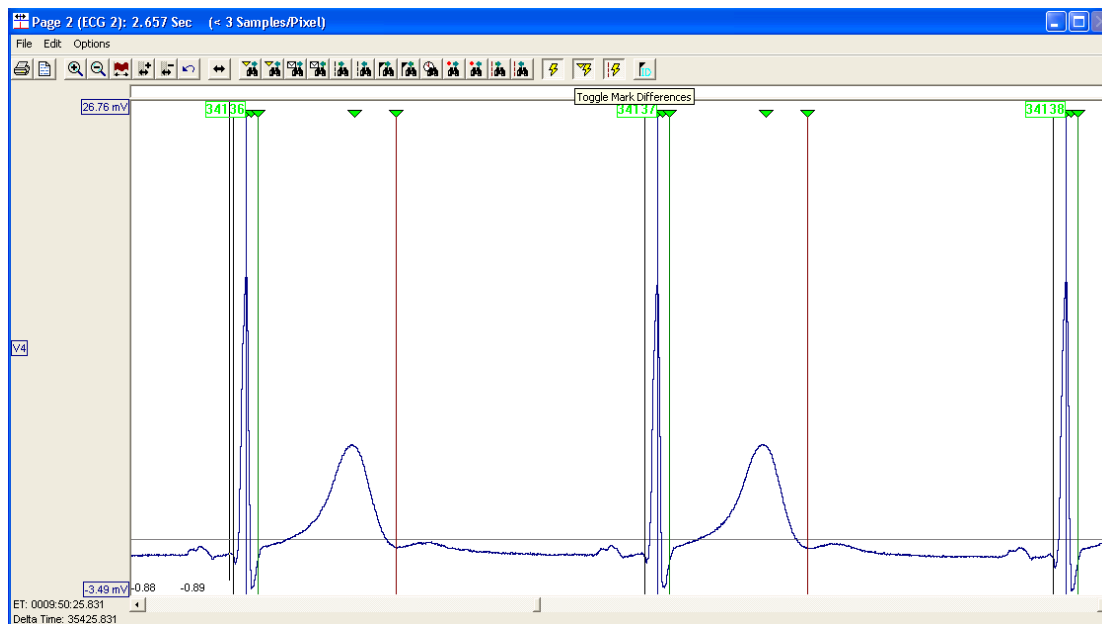


Figure 30B. Ponemah interface, QT interval measurement. The ECG waveform fiduciary marks can be visualized for the whole 24 h recording.

2.2.1.3. Step 3. Application of pattern recognition

To further analyze the ECG traces with the pattern recognition analysis (PRO) module (Figure 31), the .RAW files must be replayed in order to create a review (.RVW) file. Opening the review file with PRO enables the user to compare and automatically adjust ALG-derived fiduciary marks to manually adjudicated candidate waveforms (templates). Template waveforms are added as needed to individual libraries for each subject for each treatment day such that at least 90% of the total waveforms are recognized and included in the analysis.

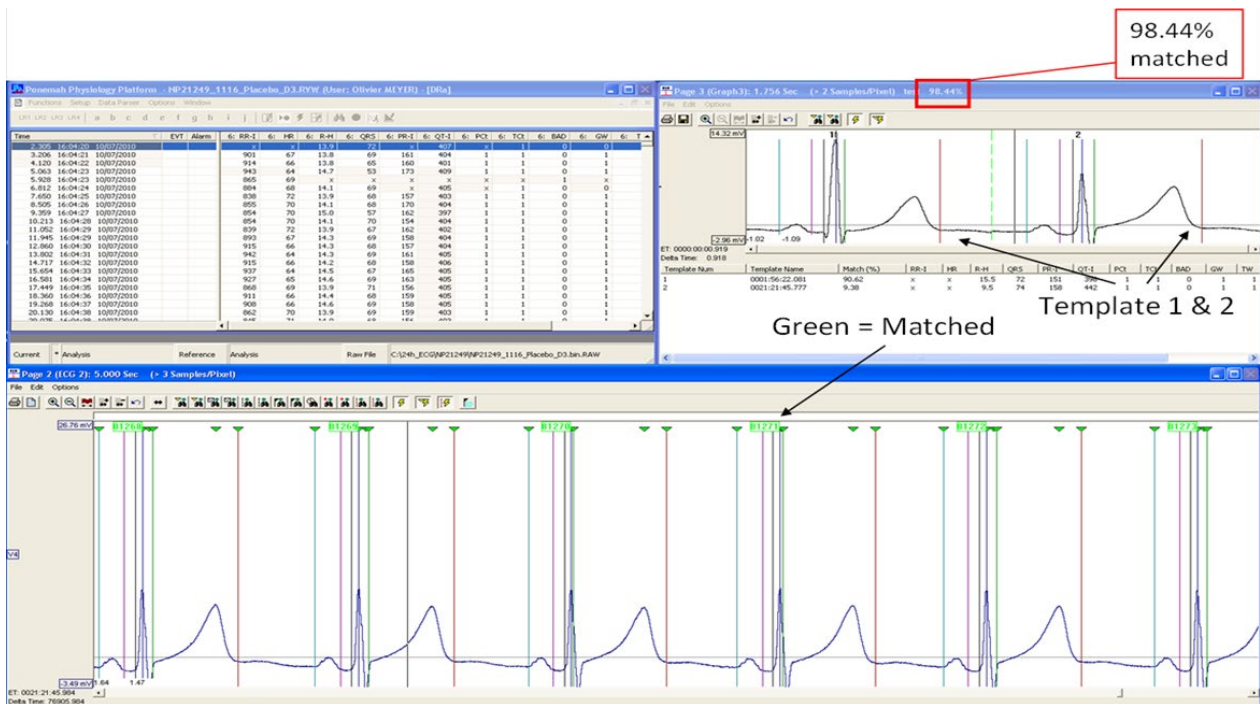


Figure 31. Ponemah's pattern recognition module. The subject-specific library (top right) includes templates which serve as the basis to measure the 24 h continuous ECG recording (bottom). In this example, 2 templates were sufficient for the recognition of 98.44% of all waveforms. When the pattern recognition process is completed, the ECG intervals are saved in the new DRa file (top left).

In practice, PRO compares specified regions of each ECG complex to the corresponding individual library and assigns the corresponding template region with the highest conformational match. Each waveform region (P, Q, S, and T) in an ECG complex is characterized by a search window (+/- 40 ms for the T-wave) centered on its assigned template mark position (Figure 32). Fiduciary marks are subsequently adjudicated by PRO when the assigned template attributes are recognized within the raw waveform search windows.

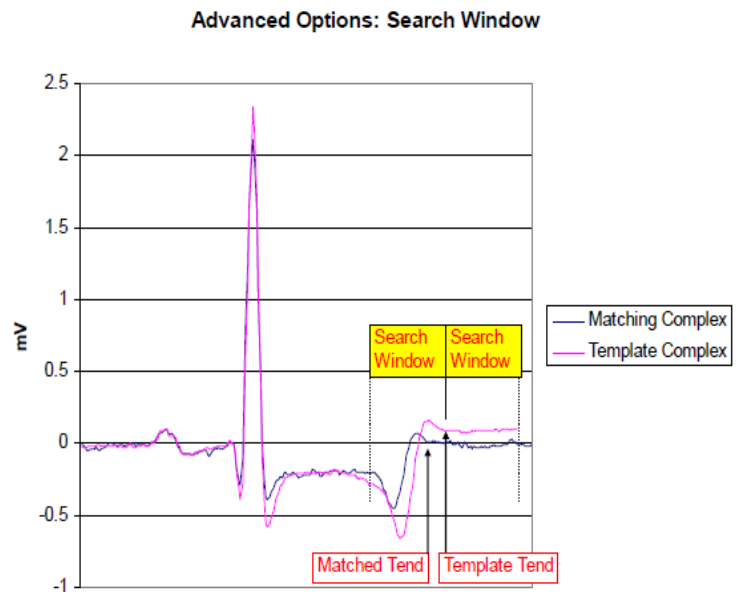
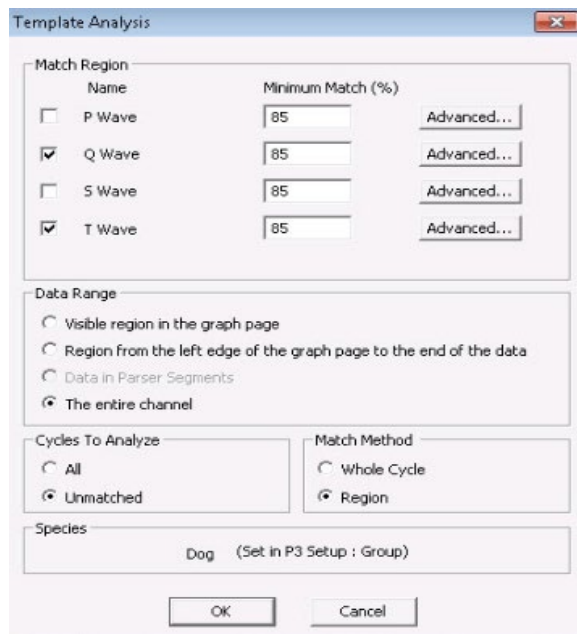


Figure 32. Ponemah PRO's waveform analysis regions (left) and search windows (right). The ECG waveform is characterized by regions for each wave which can be activated to apply the pattern recognition module. In the current analysis, only Q and T waves were evaluated with PRO.

In the current studies, PRO analysis was applied to the T-wave and Q-wave regions, such that P, R, and S waves were derived by ALG for each ECG complex. Individual template libraries were constructed from the baseline and, when needed, on-drug data. Templates were selected from well-inscribed, noise-free ECG complexes and T-end marks were manually adjusted. Individual libraries for each subject were subsequently used to identify and measure similar T-waves throughout each individual ECG dataset. T-wave regions which demonstrated $\geq 85\%$ conformational match to a template of the corresponding individual library were automatically measured and included in the final data analysis. Complexes with $< 85\%$ conformational match were excluded from analysis (DSI 2010).

For the SQR study (NP21249), this analysis resulted in the creation of 4.3 ± 1.1 (Range: 2 – 6) templates per subject. The selection of these templates enabled to match $91 \pm 9\%$ (Range: 46 – 100; Median: 94%) of the recorded data, thus resulting in the exclusion of $\sim 9\%$ of bad quality data. Note that recordings characterized by low template matching values ($< 90\%$) were due to portions of ECG where the P, Q, S and T waves were not measurable, but artifactual R waves were still detected by the automated algorithm.

2.2.1.4. Step 4. ECG data analysis

2.2.1.4.1. Importation in Excel

All the beat-to-beat values for each 24 h Holter dataset obtained with ALG and PRO analysis were stored in .DRa files. These files are then imported and processed with Microsoft Excel 2010. In order to process the large amount of data obtained from 24 h beat-to-beat recordings using a standardized and reproducible methodology, an algorithm was created through Excel macros using Visual Basic for Applications (VBA).

2.2.1.4.2. Calculation of the slope (β) of individual QT/RR relationship

The first step of the data processing is to sort the QT/RR pairs according to RR values in successive 10 ms RR increments (e.g. 1000-1010, 1010-1020, etc...). This binning step facilitates the management of large datasets as a regression based on more than 70000 QT-RR pairs may not be feasible with some software. The 10 ms RR bins are readily obtained employing the Excel Roundup function.

$$=\text{ROUNDUP}((\text{RR}/1000),2)*1000 \quad [1]$$

For data expressed in ms, equation [1] will assign all RR values to the ascending 10 ms bin associated with native RR value (e.g. a RR of 801 ms will be associated to the bin "810"). The equation converts the RR ms value to decimal seconds, rounds this value up to 2 significant digits, and then converts the rounded value to the associated RR bin in ms. In practice, equation [1] is copied through the entire data array in a separate column.

The second step is the calculation of the mean QT and RR values associated with each 10 ms RR increment. This can be readily done with the Excel PivotTable function used to query the dataset by 10 ms RR bin. For each 10 ms bin, the PivotTable generates the associated mean raw QT, mean raw RR, and count of QT/RR pairs. The bins for which the mean QT and RR values are obtained from less than 250 QT/RR pairs are excluded from the analysis. This minimum number of QT/RR pairs have been demonstrated as sufficient to avoid the possible bias due to the QT hysteresis, short term QT interval variability triggered by autonomic nervous system changes, and intrinsic rate-independent variability (Holzgreffe, Cavero et al. 2007).

Once these mean QT and RR values per 10 ms RR increment have been obtained, a log-log transformation is employed to linearize possible curvilinear raw QT-RR relationships (Figure 33). The individual distribution-based QT rate-correction factors (β) and associated coefficients of determination (R^2) are then derived from the following log-linear regression model

$$\log(\text{QT}) = \beta \cdot \log(\text{RR}) + \alpha \quad [2]$$

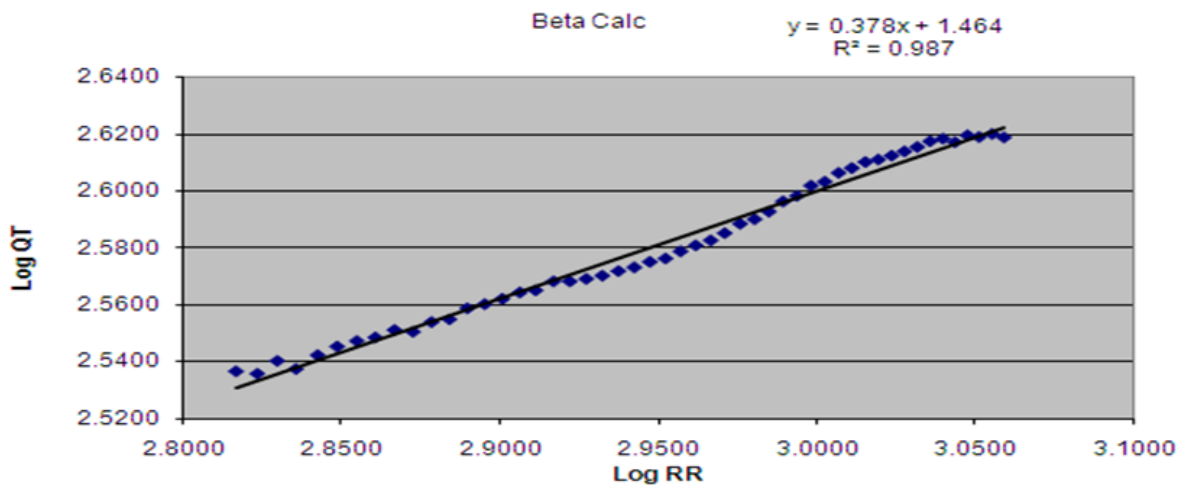


Figure 33. Log-linear QT-RR relationship with β and R^2 values. The β (0.378) and R^2 (0.987) values are extracted from the trendline parameters using a linear regression of the log of QT and RR.

No differential weighting is applied since the regression analysis is performed from data calculated at regular RR intervals (10 ms). Extreme QT-RR values will therefore have the same weight in the slope calculation as the mid-range values even if the latter are derived from a larger number of beats. The probabilistic nature of the QT interval stipulates that a minimum number of beats must be present to accurately locate the mean of the associated raw QT distribution. Once this minimum number has been satisfied, differential weighting has no further impact (Holzgreffe, Cavero et al. 2007).

To ensure the reliability of the QTca determinations, datasets demonstrating β values with an associated $R^2 < 0.9$ were deemed unacceptable and excluded from analysis.

2.2.1.4.3. Calculation of QTca values

When the baseline (off-drug; Day -1) β value is obtained, the QT rate-correction is achieved by application of formula [3] to each beat-to-beat raw QT and RR pair from each on-drug periods

$$QTca = QT / RR^\beta \quad [3]$$

The individual β value determined under treatment-free conditions is applied to both off- and on-drug 24 h recordings from each period. However, when a baseline day (Day -1) is not available (BP22464), the β value obtained during active placebo treatment may be used for derivation of the baseline QT/RR relationship.

Note: For non-human species, formula [4] is applied

$$QTca = QT / (RR/RRref)^\beta \quad [4]$$

Formula [3] is a simplified version of formula [4] which is due to the fact that the reference RR is 1000 ms (1 second) in man. However the RRref value is a fixed value depending on the species and should correspond to an appropriate physiological mid-range RR value (Table 3)

Species	RR ref (ms)	Heart Rate (bpm)
human	1000	60
dog	750	80
minipig	750	80
cynomolgus	500	120
marmoset	400	150
guinea pig	260	230

Table 3. Species-specific RR reference cycle lengths within the normal physiological range.

The resulting QTca values are fully dissociated from short term changes in heart rate. However, it should be mentioned that the distribution-based method does not mask the influence of the autonomic nervous system on the QTca interval in the presence of large RR interval changes. Indeed, the sympathetic nervous system is known to shorten the QT interval independently from heart rate through a direct action on the action potential duration in the ventricles. This explains why QTca shortening can be still observed during short episodes of sympathetic activation. These phenomena can be observed during periods of physical activity, blood sampling, meals, etc.

2.2.1.4.4. Time-matching and time segments

The large amount of data generated by continuous ECG analysis requires that beat-to-beat data be condensed into a suitable time scale for the presentation of summary data. The time matching is performed based on the “elapsed time”, which starts from 0, and is record into the DRa file. For each 24 h recording, the Holter start time (when the Holter recording device was turned on) has to be manually entered into the spreadsheet in order to calculate the real time. The real time then serves as the basis for the construction of an experimental time line indexed such that the time of dosing occurs at 0 h (time-matching) as well as the creation of contiguous 5-min time segments throughout the 24 h for the presentation of the summary data.

Time segments are calculated as

$$=\text{ROUNDUP}((\text{ElapsedTime} - \text{DosingTime})/(60*60*24)/(5/(24*60)),0)$$

$(60*60*24)/(5/(24*60))$ -> This formula segments the elapsed time (sec) into 5-min segments starting from the dosing time (sec) to 5 min segments. In this manner, each real time value from 0 to 5 min will be converted to a number between 0 and 1. The roundup function rounds all of these values to “1”. Accordingly, all real time values between 0 and 5 min will be labeled “1”, real time values between 5 and 10 minutes will be labeled “2”, and so on. Predose values will be labeled as “-1”, “-2”, etc...

Note: A time segment of 10 minutes would be measured as:

$$=\text{ROUNDUP}((\text{ElapsedTime} - \text{DosingTime})/(60*60*24)/(10/(24*60)),0)$$

The subsequent use of the Excel PivotTable function enables the extraction of the mean value of each evaluated ECG parameter (QT, RR, QRS, etc..) based on contiguous, time-matched 5-min segments. The “count of QT” for each 5-min time segment enables identification of under-represented segments with a threshold for inclusion in the final analysis is generally set to >50 beats per 5-min time segment. For each 24 h recording for each subject, the summary PivotTable including all mean parameters over time is then copied into a summary file to facilitate further analysis.

2.2.2. WinAtrec

2.2.2.1. Step 1: ISHNE conversion

Prior to analysis with WinAtrec, the Mortara Holter .H12 files must be converted into ISHNE (International Society for Holter and Noninvasive Electrocardiology) format (Badilini 2001) through the use of software provided by AMPS (HS2ISHNE; Figure 34). This software requires that the Mortara H-scribe application be installed. H-scribe is Mortara proprietary software which enables the review of the Holter recordings and the generation of reports. In the current context, the H-scribe software was only used for its export function which allows HS2ISHNE to communicate with H-scribe in order to convert the Mortara Holter files (.H12) into ISHNE files (.ecg). This application works via an interface or command line. However, the interface does not provide the possibility to sequentially convert multiple files as it is restricted to one-by-one conversion.

Example of command line:

```
HS2ISHNE.exe -f "F:\NP21249_Raw\NP21249_1002_Moxi400_D-1\PAT" -tRHI -o"F:\NP21249_ecg" -p"1002_Moxi400_D-1" -a0 -b -h"C:\Program Files\Mortara Instrument Inc\H-Scribe"
```

Where `-f` is the input file, `-tRHI` refer to raw files recorded at 1000 Hz, `-o` is the output folder, `-p` is the output file name, `-a0` saves a TXT format, `-b` saves annotations in the ISHNE binary format, and `-h` is the folder where H-scribe is installed.

Accordingly, batch files constituted of n command lines were constructed to sequentially convert the Mortara Holter files into WinAtrec readable ISHNE files (where n is the number of 24 h Holter recordings).

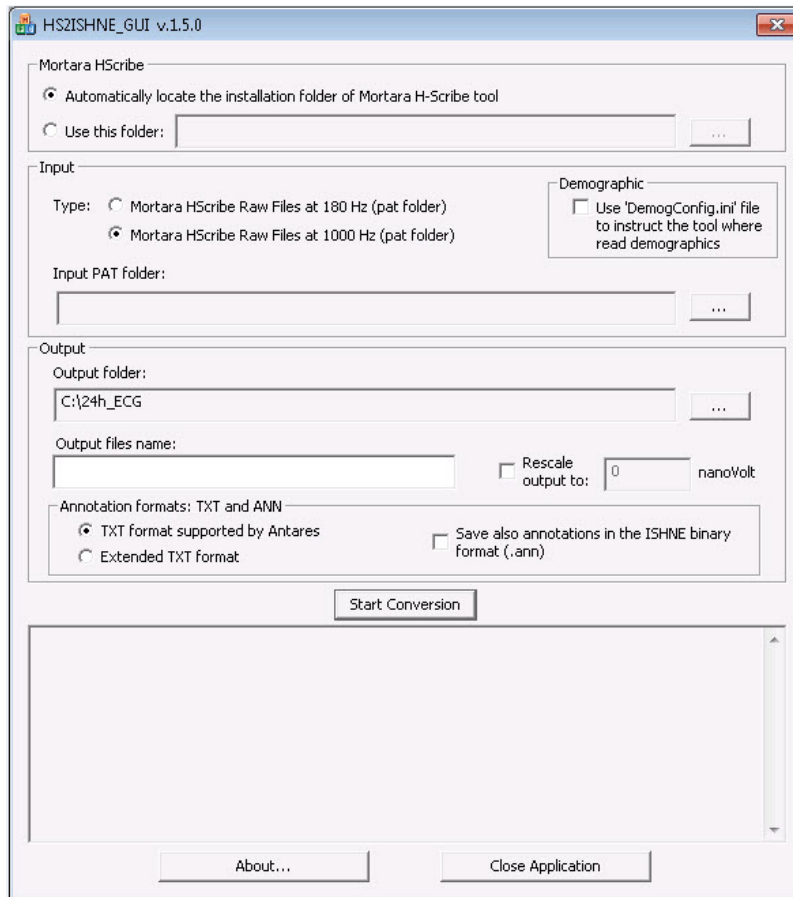


Figure 34. HS2ISHNE interface. Enable the conversion of Mortara into ISHNE files for the evaluation with WinAtrec.

2.2.2.2. Step 2: Analysis with WinAtrec2

Once the Mortara Holter files have been converted to the ISHNE format, the analysis of the ISHNE files is performed by the software WinAtrec2. This application also works via command lines with various arguments. In order to analyze the 24 h recordings, WinAtrec uses an intermediary .csv (comma separated values) file on which are compiled the information regarding the analyses that will be performed (Figure 35).

	A	B	C	D	E
1	HolterName	Period1 Start	Period1 End	Period1 Enabled	Period2 S
2	NP21249_1002_Moxi400_D-1	2008-11-17 07.28.00	2008-11-18 05.40.00	YES	2008-11-1
3	NP21249_1002_Moxi400_D3	2008-11-20 07.43.00	2008-11-21 05.40.00	YES	2008-11-1
4	NP21249_1002_Placebo_D-1	2008-11-07 07.53.00	2008-11-08 05.40.00	YES	2008-11-0
5	NP21249_1002_Placebo_D3	2008-11-10 07.23.00	2008-11-11 05.40.00	YES	2008-11-1
6	NP21249_1002_SQV1000_D-1	2008-12-07 07.28.00	2008-12-08 05.40.00	YES	2008-12-0
7	NP21249_1002_SQV1000_D3	2008-12-10 07.23.00	2008-12-11 05.40.00	YES	2008-12-1
8	NP21249_1002_SQV1500_D-1	2008-11-27 07.28.00	2008-11-28 05.40.00	YES	2008-11-1
9	NP21249_1002_SQV1500_D3	2008-11-30 07.27.00	2008-12-01 05.40.00	YES	2008-11-1
10	NP21249_1003_Moxi400_D-1	2008-12-07 07.28.00	2008-12-08 05.50.00	YES	2008-12-0

Figure 35. Organization of the analysis information file. Each analysis period start and end times must be carefully entered in the information file prior to analysis.

WinAtrec allows the analysis of 3 periods for the rate-binning analysis, and 1 period of time-binning analysis. Accordingly, each of the n lines in the .csv file must be carefully completed with the dates of Holter recordings, as well as the starting and ending time of each period to be analyzed.

When the intermediary .csv file has been prepared and reviewed, a batch file containing the n command lines is created.

Example of command line:

```
WinAtrec2 -h "C:\winatrec\BP22464_10004_Moxi400.ecg" -1 V4 -2 V3 -3 V2 -t 60 -r 10 -m 10 -s 1000 -n 3 -p "C:\apps\AMPS\Winatrec2\H_P_sh.csv"
```

Where $-h$ is the input file, -1 -2 and -3 are the three channels/leads to be analyzed, $-t$ is the time bin resolution (in seconds), $-r$ is the rate bin resolution (in ms), $-m$ is the minimum number of beats per bin, $-s$ is the resampling rate (in Hz), $-n$ is the max noise level, and $-p$ is the intermediary .CSV file location.

The n command lines are then launched and create n analysis folders which contain WinAtrec ECG waveform measurements of each bin and median beat.

In the current studies, .CSV file was constructed so that all 24 hours were analyzed for each ECG recording. Subsequent WinAtrec2 analysis was performed employing the parameters displayed in the example above. Lead V4 (with lead V3 and V2 as back-ups) was chosen as it demonstrated the most appropriate signal-to-noise ratio and to facilitate the direct comparison

with Ponemah PRO analysis. For the other parameters, default values were used as recommended by the software provider.

2.2.2.3. Step 3: Analysis review with WinAtrec

The ECG waveform measurements performed during step 2 have to be loaded into the WinAtrec interface (Figure 36) in order to be reviewed and possibly adjusted.

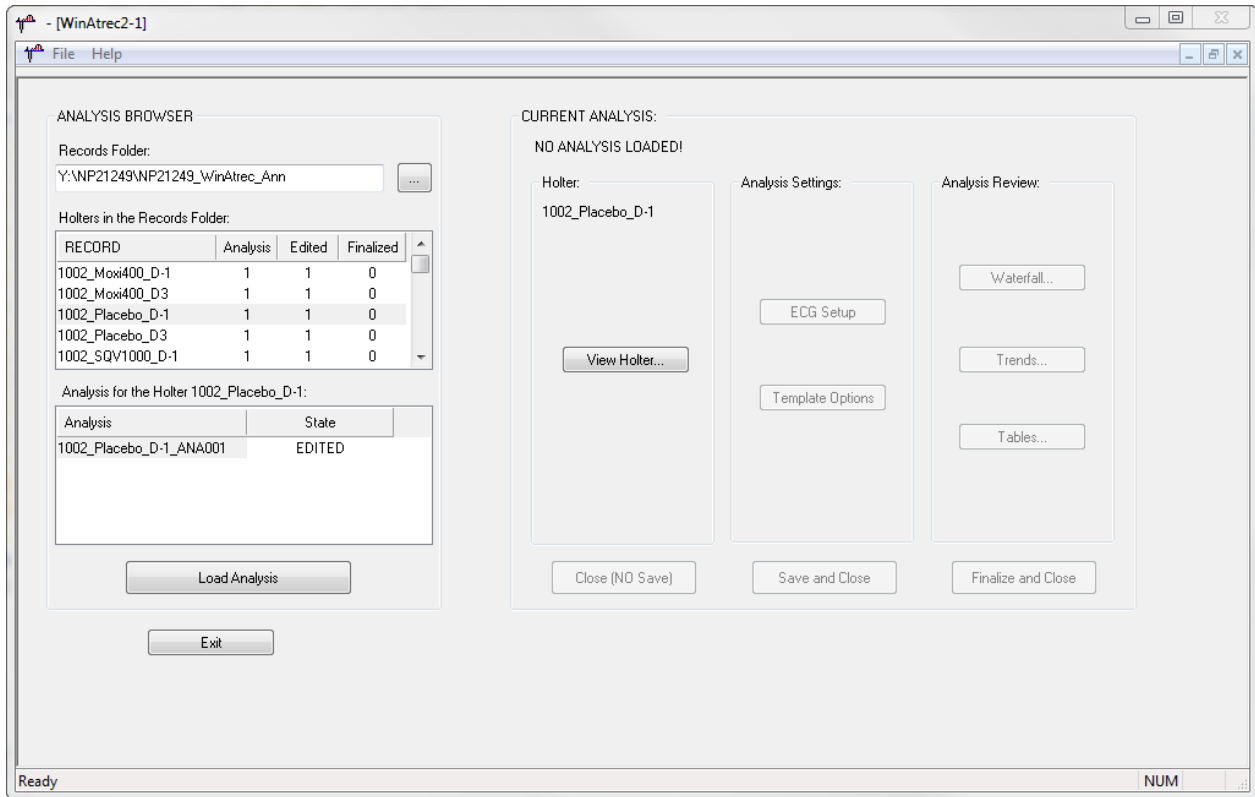


Figure 36. WinAtrec interface. After the analysis step, WinAtrec interface enables to load the analysis and visualize and review the ECG.

Once it has been loaded, ECG waveform measurements for both rate-binning and time-binning analyses can be reviewed and adjusted (Figure 37A and B).

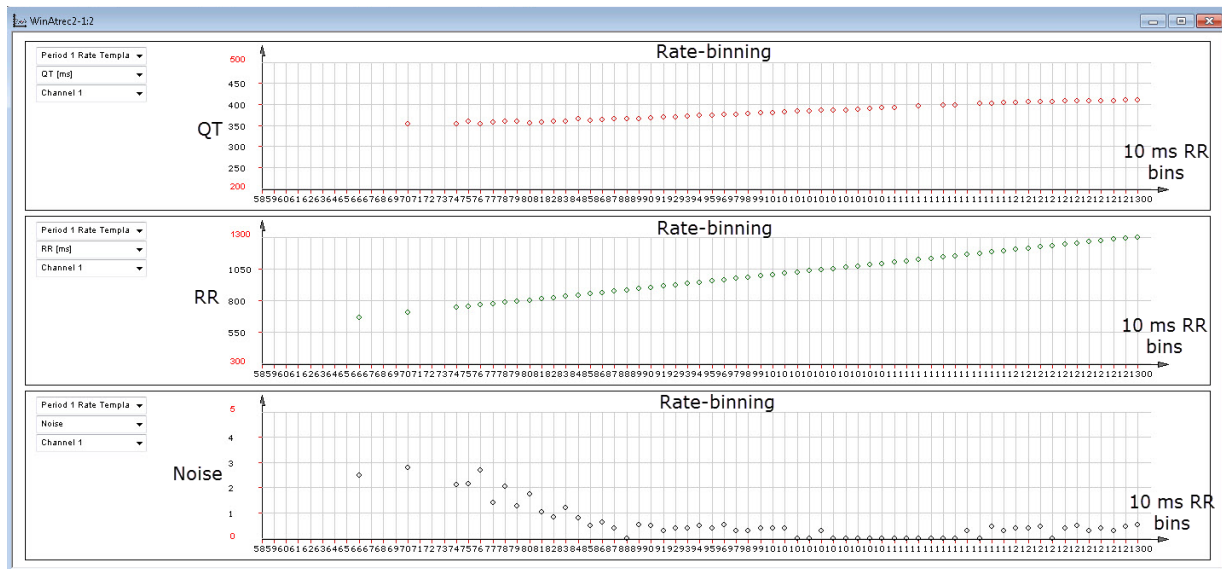


Figure 37A. Rate-binning reviewing and adjustment interface. Each median beat is reviewed and adjusted (see Figure 37B)

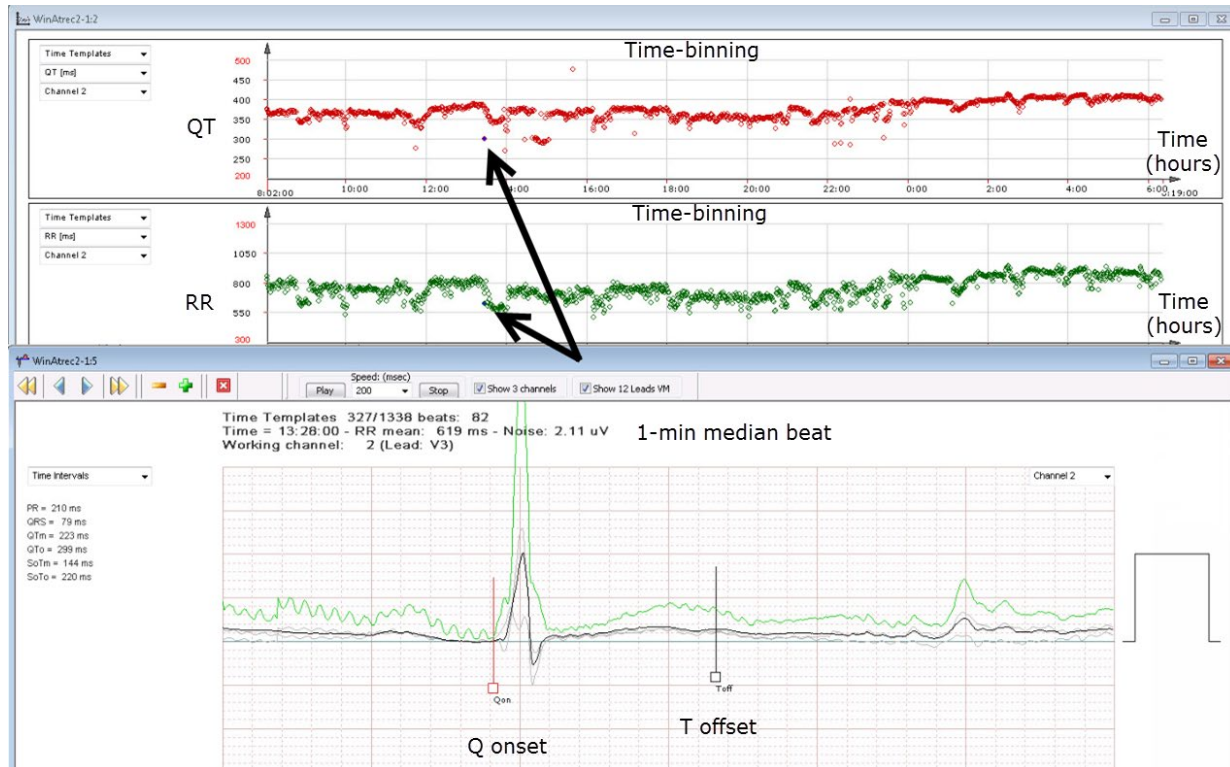


Figure 37B. Time-binning reviewing and adjustment interface. Fiduciary marks for each median beat can be reviewed. In the current example, the selected median beat (arrow) demonstrates a low QT value with an associated normal RR, suggesting an algorithm-based measurement error. When the specific median beat is reviewed, an algorithm-based measurement error was present, likely due to noise, notched T-wave, and low amplitude. Such errors are then corrected by changing the T-offset caliper, or discarded when the operator cannot confidently adjust the T-offset.

When each measurement has been reviewed and validated, the analyses are saved as text files (.txt) which can be imported in Excel for further analysis. In the current studies, direct quantification of erroneous measurements which required manual adjudication could not be effectively assessed. The number of measurement errors was highly dependent on ECG quality and treatment effects and could range from 0 to 15-20% for any given recording. For example, in presence of SQR-induced T-wave morphological changes (notched T-wave), some subjects exhibited periods where all their QT measurements were anomalous due to an abnormal T-wave. Importantly, WinAtrec2 reduced the number of QT measurement that must be reviewed as each QT value is created from a 60 sec time period. Accordingly, the number of QT measurement that required manual correction could be estimated to be ~15 QT values per ECG recording.

2.2.2.4. Step 4. ECG data analysis

Similar to Ponemah DRa files (see section 2.2.1.4), WinAtrec text files are imported into Excel where an algorithm (also created in Excel VBA) calculates the slope (β) of the individual QT/RR relationship, applies the baseline β value for the QTcl median derivation, and provides mean time-matched parameters over contiguous 5-min time segments which are subsequently exported to a summary file for further analysis.

2.2.3. BioQT

2.2.3.1. Step 1: XML conversion

BioQT requires that Mortara Holter files (.H12) be converted into the XML format. This process is performed using n command lines which directly access the H-scribe system.

Example of command line:

```
"C:\Program Files\Mortara Instrument Inc\H-Scribe\Hsp.exe" /X 0 /W /HI /FORCE /S  
E:\NP21249_Raw\NP21249_1002_Moxi400_D-1\PAT /D E:\NP21249_XML
```

Where `/X 0` indicates that the entire recording must be exported, `/W` specify that the waveforms must be included in the XML files, `/HI` specify that it is high resolution (1000 Hz) data, `/FORCE` forces the export of high resolution data in case of truncation at the end of the data, `/S` specifies the output directory.

Accordingly, batch files constituted of n command lines were constructed to sequentially convert the Mortara Holter files into BioQT readable XML files.

2.2.3.2. Step 2: BioQT analysis

BioQT requires that Matlab (Figure 38) be installed to function. Matlab is a programming language which enables complex data manipulation, graphical representations, and statistical analysis on which the BioQT algorithms are based.

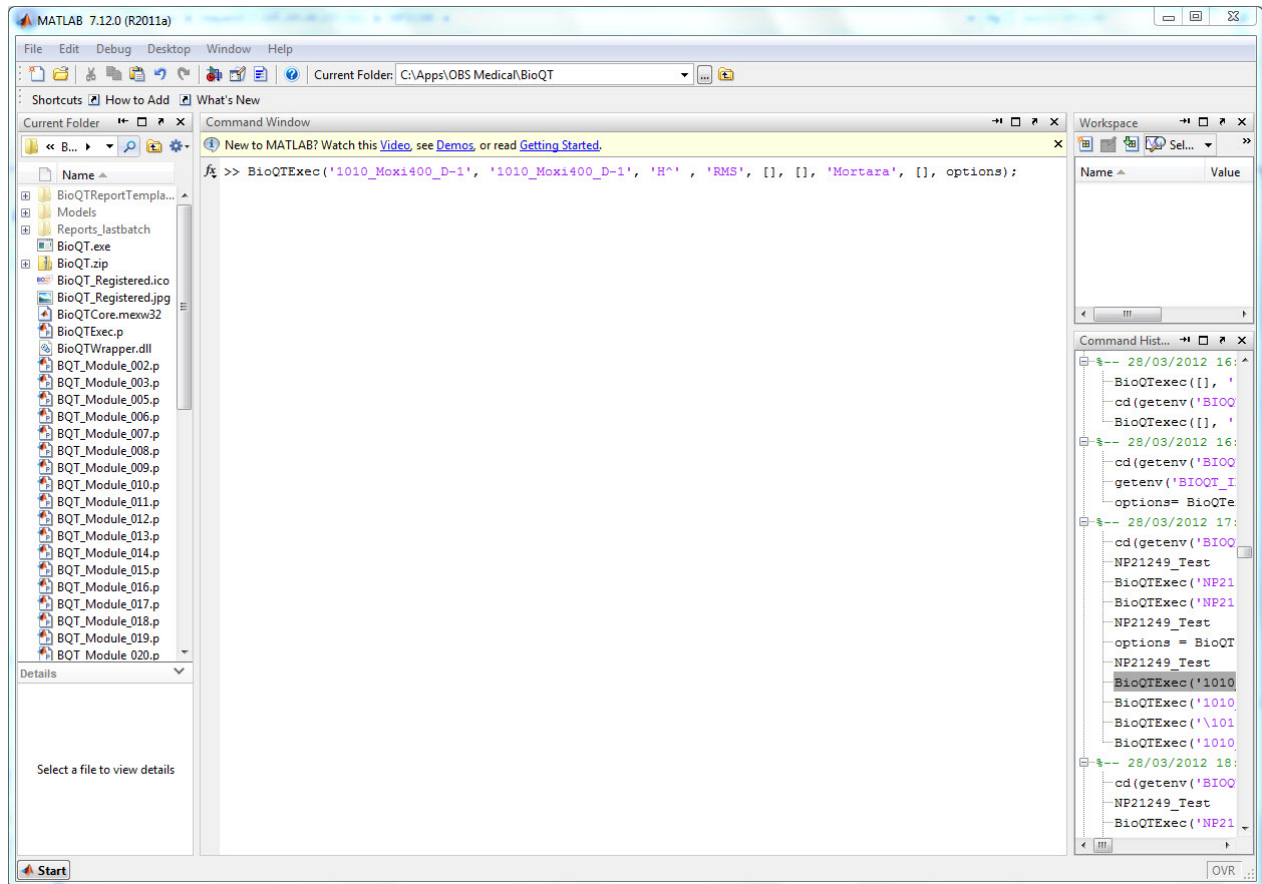


Figure 38. Matlab interface enables BioQT command lines and performs the analyses.

The analysis is initiated through the use of a script composed of n command lines

Example of command line:

```
BioQTExec([], '1002_SQV1000_D-1', '1002_SQV1000_D-1', 'RMS', [], [], 'Mortara', [])
```

With BioQTExec(Input_Directory, Patient_Name, Files_Name, Lead, DoseTime, StartTime, Format, Template_File)

Where Files_Name is an expression present in the 24 XML files, Lead is either lead II or RMS (Multiple lead analysis as a mean square root of the leads I, II, V1-V6), Format is either ISHNE, Mortara, or FDA, and Template_File represents the name of the MS Word template to use for the report generation.

In the current studies, the multiple lead analysis (RMS) was employed as it was the most suitable alternative to single lead V4 which is not supported by the software. Default values for

analysis parameters were employed as suggested by the software provider. The completion of BioQT fully-automated analysis generates both an Excel and a Word report. The Excel report provides the beat-to-beat depiction of the elapsed time, heart rate, QT, QTcF, QTclC, QRS, PR, Pconf, QRS conf, and T conf, where “conf” are proprietary ‘coefficient of confidence’ values for the measurement of the associated parameters. The Word report provides detailed information on the recorded parameters and automatically computes figures and tables. However, the default format of the Word report provides 15 min segments which are not time-matched such that the 0 h does not correspond to the time of dosing. Accordingly, the Word reports were not used in the current analysis. The BioQT Excel reports were analyzed with an algorithm (created through Excel VBA) to provide mean time-matched parameters over contiguous 5-min time segments which were subsequently exported to a summary file for further analysis. Individually corrected values (QTclC) were directly measured by the BioQT algorithm and no manual adjustments were performed. The exclusion of possibly unreliable measurements was performed solely on the basis of the proprietary confidence measures. However, in order to compare the different ECG measurement platforms using the same methodologies, an Excel algorithm (similar to section 2.2.1.4) was used to calculate the slope (β) of the individual QT/RR relationships.

Differences	Methods			
	BioQT	Ponemah PRO	WinAtrec	ECG core laboratory
Analysis methodology	Entirely machine-read	Machine-read based on operator-selected and adjusted templates	Machine-read, then adjusted by operator	Machine-read, then adjusted by cardiologist
Holter leads analyzed	Multiple Lead Analysis	Lead V4	Lead V4 (back-up: V3, V2, 12L)	Lead II (back-up: V2, V5)
Raw ECG data measured	All beats	All beats	1-min median beats	9 beats extracted per time point
Raw QT measurement algorithm	Hidden Markov Model	Pattern recognition	Threshold-based	Tangent-based
Raw QT measurement reliability	Self-check with <i>confidence measures</i>	Visual verification of appropriate template application	Visual verification of all median beats and adjustment when needed	Visual verification of all extracted beats and adjustment when needed
Raw QT measurement excluded	<i>Confidence measure</i> <0.7	<85% conformational match	Median beats from <10 beats Noise level >3	None
Number of valid raw QT measurements	~25,000,000	~30,000,000	~590,000	~44,000
Number of time point analyzed	~100,000	~106,000	~113,000	~4,900
Heart rate correction	Fixed (QTcF) and individual from time variation of QT/RR relationships (QTclC)	Fixed (QTcF) and individual from distribution-based analysis (QTca)	Fixed (QTcF) and individual from <i>rate-binning</i> analysis (QTclmedian)	Fixed (QTcF) and study-specific (QTcS)
QT/RR regression	Linear regression	Log-linear regression	Log-linear regression	Linear regression
Period used to measure QT/RR correction factors	4h on-treatment moving periods	24h baseline recordings	24h baseline recordings	Pooled baseline measurements
Corrected QT measurement excluded	QTcF values differing from mean QTcF by >3 times the SD of QTcF	None	None	None

Table 4. Summary of differences between methods for study NP21249.

3. Summary data analysis

3.1. VBA excel macros

VBA programming enables recurrent and consistent spreadsheet manipulations which would not be amenable manually. The range of use of Excel VBA is relatively wide as it is not only restricted to Excel, but also applies to other Microsoft Office applications. This is of particular importance as the data handling inherent to the analysis of continuous Holter ECG recordings is very time-consuming and must be automated to reduce the time associated with technical data manipulation. Excel VBA routines can be designed to move, copy, rename, and create a large number of files, folders, or subfolders.

One typical example among many (Figure 39):

SuperECG converts the Holter ECG .H12 files into bin files. The Mortara .H12 files are generally contained into folders designated as c:/Holter/Pat0001/HIRES (where Pat0001 is the blinded name of a 24 h recording). When SuperECG converts the files, it creates a generic subfolder named "PAT" such as the output folder of the converted files will be c:/Holter/Pat0001/HIRES/PAT. The end result is hundreds of bin files contained in folders where the path is c:/Holter/PAT0XXX/HIRES/PAT where "XXX" is the blinded name. The obvious problem with this structure is that the subfolders containing the files of interest are not identifiable (they are all named "PAT").

VBA programming can create a macro that will select all folders in c:/Holter/, and rename all 3rd order subfolders named "PAT" to the name of the 1st order subfolder (the blinded name "PAT0XXX"). Such a macro enables the automated renaming of hundreds of files that would otherwise require hours of manual entry, in only few seconds.

```

Sub Renaming_Folder_To_Foldername ()

On Error Resume Next
Dim o: Set o = CreateObject("Scripting.FileSystemObject")
Dim tonRep, Fi, Fo, Fj, Fk, Nn: tonRep = "C:\24h_ECG" ' source folder
Dim Gf: Set Gf = o.GetFolder(tonRep)
  For Each Fo In Gf.SubFolders 'Folder
    For Each Fi In Fo.SubFolders 'First subfolder
      For Each Fj In Fi.SubFolders 'Second subfolder
        For Each Fk In Fj.SubFolders 'Third subfolders
          If Fk.Name = "pat" Then

            End If
            Fn = o.GetBaseName(Fo.Name)
            Nn = Replace(Fi.Name, o.GetBaseName(Fi), Fn)
            Fk.Name = Nn

          Next
        Next
      Next
    Next

  End Sub

```

Figure 39. Example of a VBA excel macro which enables the renaming of subfolders.

While the VBA programming is helpful to optimize the basic work of data handling, it also allows the creation of analysis algorithms that are applied to the raw ECG waveform measurement files (Ponemah: DRa files, WinAtrec: txt files, BioQT: Excel reports). The raw ECG waveform files are presented differently depending on the software. However, VBA macros enable the user to rearrange, copy, and filter several parameters, apply various formulae, segment the data into bins of RR, calculate the QT/RR relationship, report the β value into another formula, calculate the QTca, QTcF, QTcB, QTca on-drug, separate day and night, set the dosing times and create time segments of various lengths, create a PivotTable presenting all mean parameters per time segment, copy the table and all other parameters of interest into a separate summary file, save and close the 24 h beat-to-beat excel file, and import the next raw ECG file to be analyzed. The fact that all these actions are performed automatically and consistently with minimal manual input dramatically reduces the risk of user error and increases time-efficiency.

At the end of the process, the beat-to-beat files (~100 Mb per 24 h recording) are stored, and the summary file containing all subjects and periods is used for analysis.

One advantage of these VBA routines is that a new algorithm can be programmed at any moment to reanalyze all the beat-to-beat files using a loop function. This function can enable the

opening of every previously analyzed file in a specific folder, reanalyze the files (using a different QTc formula for example), create a new summary file, then close and save the result. Reanalysis can be run overnight (entirely automated) as all the necessary manual inputs (Holter real start time, time matching, and predose β) have already been performed.

Note: During the course of my PhD, I have extensively used VBA programming and created multiple macros (~10K lines of code) for a wide variety of purposes.

3.2. Statistical analysis

The utilization of continuous ECG analysis consists of repeated measurements over time which are correlated and sometimes result in missing values at certain time points. Standard ANOVA models are not suited for correlated datasets characterized by randomly missing measurements as they assume that all measurements have uncorrelated errors. However, when multiple measurements are performed, the independence can no longer be assumed, which then requires more complex models, such as mixed effects models. The advantage of the mixed effects models is that they are more flexible and can handle uneven spacing of repeated measurements. These models consist of the use of fixed and random effects in the same analysis. Fixed effects are effects that would be used again if the experiment was repeated (e.g. treatments) and random effects are effects that are a sample from a population (e.g. subjects). Accordingly, mixed effects models are the most appropriate as they enable the integration of the variability associated with subjects or time points to provide correct estimates of treatment or method effects in presence of correlated errors related to the continuous measurements.

In addition to mixed effects models, standard descriptive statistics and Bland-Altman comparison were extensively used for the evaluation of treatment and method effects.

3.2.1. Analyse-it

Analyse-it is a third party statistical module for Excel (Figure 40). It provides a range of statistical parametric and non-parametric statistical tests, such as descriptive statistics, ANOVA, Bland & Altman, chi-square, Wilcoxon and linear regression.

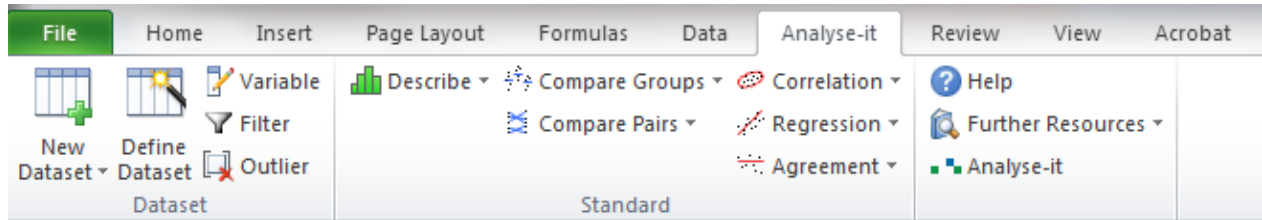


Figure 40. Analyse-it interface.

3.2.2. R

R is a programming language used for statistical analyses and graphical representations (www.r-project.org). This language is widely used by statisticians as it offers an extensive array of features. The advantages of this statistical software are that it is freely distributed with a large user community that actively contributes to implementation of new statistical techniques and packages. The major drawback associated with R is that it is a rather complicated and demanding tool. Learning the R language, codes, and commands is required in order to perform the statistical tests.

Note: During the course of my PhD, I mainly used R to apply linear mixed effects models including fixed and random effects.

The function `lmer` enables the user to fit a linear mixed effects model. First, it is important to know what statistical test is to be applied to the data. The next step consists of understanding how to write the desired test, which arguments are to be used, and how they interact with one another.

For example:

If the purpose is to apply a linear mixed effects model to see the effect of $\Delta\Delta\text{QTcF}$ for each method and treatment, with time as a fixed effect and subjects as a random effect.

```
Lmer( DDQTcF ~ -1 + Method * Treatment + Time + ( 1|sujet ) )
```


In this example, the DDQTcF is the baseline- and placebo-adjusted QTcF (it could also be the RR, QTca, QRS, etc.) for the saquinavir study which consisted of three drug treatments: two doses of SQR and one dose of Moxifloxacin. There were 3 methods, BioQT, Ponemah, and WinAtrec. With the *lmer* function, when the argument “-1” is not present, the model calculates the values as differences. Because the 3 methods were used independently, the effect of each method is required. Accordingly, “-1” was added to the argument.

To see the effect of each method for each treatment, the model requires the use of “Method * Treatment” in order to obtain the interaction of both methods and treatments. The omission of such interaction would result in modeling the effects of the methods irrespectively of the specific treatments, which could mask certain treatment-related differences between methods. This is of particular importance when the treatments consist of different drugs with variable effects on waveforms morphology which may not be consistently evaluated between the methods. To account for the correlation of contiguous time segments, the time is modeled as a fixed effect (“+ Time”), and, because the subjects are a random selection from a larger population, the subjects are modeled as a random effect (“+ 1|sujet”).

We obtain the following results:

```

R Console
> lmer(DDQTcF ~ -1+ Method*Treatment + Time + (1|sujet),data=mydata,na.action=na.omit)
Linear mixed model fit by REML
Formula: DDQTcF ~ -1 + Method * Treatment + Time + (1 | sujet)
Data: mydata
   AIC   BIC logLik deviance REMLdev
711507 711621 -355742  711458  711483
Random effects:
Groups   Name              Variance Std.Dev.
sujet    (Intercept)      54.899   7.4094
Residual                    122.227  11.0556
Number of obs: 93031, groups: sujet, 55

Fixed effects:
              Estimate Std. Error t value
MethodBioQT      5.795126   1.006713   5.76
MethodPonemah    5.527490   1.005901   5.50
MethodWinAtrec   6.969161   1.005115   6.93
TreatmentSQV1000 2.121093   0.167995  12.63
TreatmentSQV1500 9.339176   0.167997  55.59
Time              0.173528   0.005844  29.69
MethodPonemah:TreatmentSQV1000 0.955353   0.228936   4.17
MethodWinAtrec:TreatmentSQV1000 2.880538   0.220419  13.07
MethodPonemah:TreatmentSQV1500 2.579616   0.226056  11.41
MethodWinAtrec:TreatmentSQV1500 5.681450   0.218949  25.95

Correlation of Fixed Effects:
              MthBQT MthdPn MthdWA TSQV10 TSQV15 Time   MP:TSQV10 MWA:TSQV10
MethodPonmh  0.988
MethodWnAtrc 0.988  0.989
TrtmSQV1000 -0.077  0.000  0.000
TrtmSQV1500 -0.078 -0.002 -0.002  0.464
Time         -0.059 -0.057 -0.058 -0.002 -0.009
MP:TSQV1000  0.056 -0.051  0.000 -0.731 -0.340  0.006
MWA:TSQV100  0.058  0.000 -0.045 -0.759 -0.351  0.003  0.557
MP:TSQV1500  0.057 -0.051  0.000 -0.343 -0.735  0.005  0.477  0.261
MWA:TSQV150  0.059  0.000 -0.045 -0.353 -0.761  0.010  0.260  0.477
MP:TSQV15
MethodPonmh
MethodWnAtrc
TrtmSQV1000
TrtmSQV1500
Time
MP:TSQV1000
MWA:TSQV100
MP:TSQV1500
MWA:TSQV150  0.563
> |

```

Figure 41. R interface presenting mixed effects model results.

Were MethodBioQT, MethodPonemah, and MethodWinAtrec are the mean moxifloxacin effects over the 24 h (the moxifloxacin results are given first because it is the first treatment in the list of the treatments). TreatmentSQV1000 and TreatmentSQV1500 are the differences relative to the moxifloxacin treatment. Time is the fixed effect of time, MethodPonemah:TreatmentSQV1000 is the difference to BioQT (which is the first method in the list of the methods) for the treatment SQV1000, and so on.

Accordingly, the mean effects can be calculated as:

Mean effect of BioQT Moxi = MethodBioQT + Time

Mean effect of BioQT SQR1000 = MethodBioQT + Time + TreatmentSQV1000

Mean effect of BioQT SQR1500 = MethodBioQT + Time + TreatmentSQV1500

Mean effect of Ponemah Moxi = MethodPonemah + Time

Mean effect of Ponemah SQR1000 = MethodPonemah + Time + TreatmentSQV1000 +
MethodPonemah:TreatmentSQV1000

Mean effect of Ponemah SQR1500 = MethodPonemah + Time + TreatmentSQV1500 +
MethodPonemah:TreatmentSQV1500

Mean effect of WinAtrec Moxi = MethodWinAtrec + Time

Mean effect of WinAtrec SQR1000 = MethodWinAtrec + Time + TreatmentSQV1000 +
MethodWinAtrec:TreatmentSQV1000

Mean effect of WinAtrec SQR1500 = MethodWinAtrec + Time + TreatmentSQV1500 +
MethodWinAtrec:TreatmentSQV1500

The results obtained by the linear mixed effects model then serve for the computation of credible intervals which are used to determine the significance of the difference using Bayesian statistics.

The following function are used where

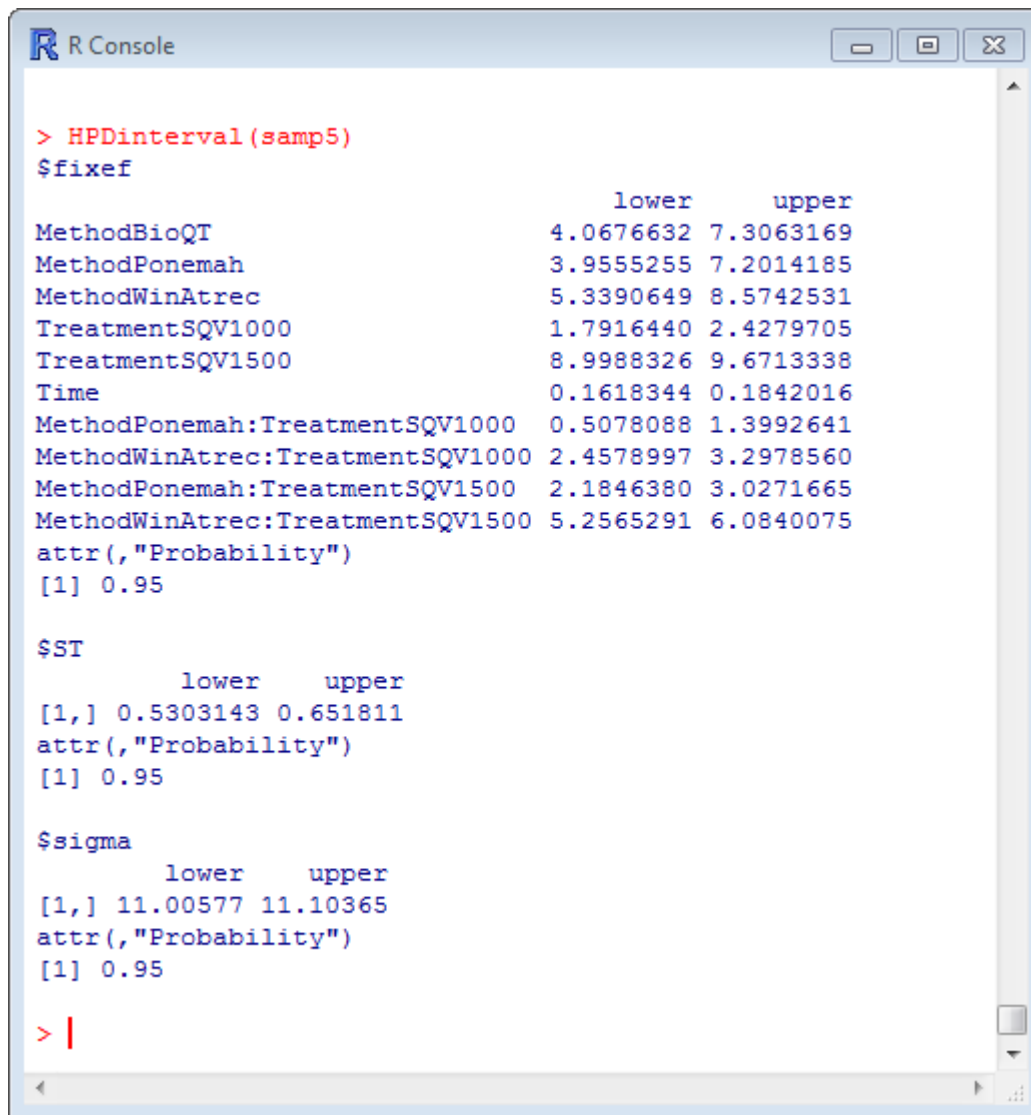
```
model<- Lmer( DDQTcF ~ -1 + Method * Treatment + Time + ( 1|sujet), data)
```

```
samp5<-mcmcscamp(model, n=1000,deviance=true)
```

```
HPDinterval(samp5)
```

mcmcscamp generates a sample from the posterior distribution of the parameters of the mixed effects model using a Markov Chain Monte Carlo (MCMC) method. In other words, *mcmcscamp* simulates multiple sequences of the Markov Chain Monte Carlo method.

HPDinterval creates Highest Posterior Density (HPD) intervals for the parameters in an MCMC sample.



```
> HPDinterval(samp5)
$fixef
              lower      upper
MethodBioQT      4.0676632  7.3063169
MethodPonemah     3.9555255  7.2014185
MethodWinAtrec    5.3390649  8.5742531
TreatmentSQV1000  1.7916440  2.4279705
TreatmentSQV1500  8.9988326  9.6713338
Time              0.1618344  0.1842016
MethodPonemah:TreatmentSQV1000 0.5078088  1.3992641
MethodWinAtrec:TreatmentSQV1000 2.4578997  3.2978560
MethodPonemah:TreatmentSQV1500 2.1846380  3.0271665
MethodWinAtrec:TreatmentSQV1500 5.2565291  6.0840075
attr(,"Probability")
[1] 0.95

$ST
      lower      upper
[1,] 0.5303143 0.651811
attr(,"Probability")
[1] 0.95

$sigma
      lower      upper
[1,] 11.00577 11.10365
attr(,"Probability")
[1] 0.95

> |
```

Figure 42. R interface presenting Bayesian 95% credible intervals.

These 95% credible intervals (the analogous of the confidence intervals in frequentist statistics) are measured because the linear mixed effect model function *lmer* cannot provide p values. Significance is established when the credible intervals between treatments and/or methods do not overlap.

3.3. Matlab

Matlab is a programming language which enables complex data manipulation, graphical representations and statistical analysis. For the current project Matlab was used solely as a background operating environment for BioQT.

In short, for the current project, VBA programming language and Excel was extensively used for data handling, analysis, and visualization, R for statistical analysis, and Matlab for BioQT analysis.

Results

1. Part I: Validation of pattern recognition methodology for continuous ECG measurements

1.1. Rationale

The continuous analysis of ECG waveforms requires that ECG measurements be performed automatically. However, measuring the ECG intervals automatically remains a challenge as technical, physiological, and clinical influences may affect the accurate and reproducible measurements performed by automated software applications. The first and primordial step required in order to perform a precise evaluation of drug-induced effects on cardiac repolarization is to ensure that all raw ECG intervals are measured appropriately. Fully-automated ECG measurement platforms devoid of review and adjustment features have generally been associated with substantial measurement errors in presence of atypical waveform morphologies which has limited the wide use and acceptance of continuous ECG analysis methods for the evaluation of TdP liability (McLaughlin, Campbell et al. 1996; Malik and Camm 2001). Recently, a pattern recognition module (PRO) has been developed which enables the measurement of thousands of beats based on few manually selected and adjusted templates. Thus, it has been hypothesized that pattern recognition analysis may provide reliable raw QT interval measurement, even in presence of drug-induced waveform morphological changes. Accordingly, the current primary endpoint was to evaluate the performance and potential improvements associated with pattern recognition analysis (PRO) in comparison to a fully-automated method (ALG) and the conventional ECG core laboratory semi-automated method (SA) in three different studies which included drugs with and without effects on the waveform morphology. As a secondary endpoint, all analyses were performed using distribution-based analysis (Holzgreffe, Ferber et al. 2012) in order to further validate this methodology as an appropriate practice for continuous ECG assessments of TdP liability in clinical studies.

1.2. Publication Summary

Currently, the accurate clinical assessment of potential drug-induced QT interval prolongation remains a major concern for regulatory agencies as all new drug candidates are required to demonstrate relative safety for the risk of inducing Torsades de pointes (TdP), indirectly assessed as an estimation of their potential to prolong the QT interval. Pharmaceutical companies generally outsource TQT data analysis to ECG core laboratories which specialize in semi-automated (SA) ECG annotations performed by well-trained cardiologists. While the analysis performed by such laboratories is currently accepted as the gold standard, it has many well recognized limitations which may be resolved through the use of computerized ECG measurement methods. However, computerized QT measurements employing T-wave detection algorithms (ALG) are not well accepted by regulatory agencies for the primary analysis of thorough QT (TQT) studies. It has been hypothesized that the newly developed pattern recognition software (PRO) which matches ECG waveforms to user-defined templates may improve this situation.

The performance of current computerized methods (ALG and PRO) were evaluated by direct comparison of the reference moxifloxacin-induced QT prolonging effect with parallel time-matched values obtained by an ECG core laboratory during the primary TQT analysis.

Compared to ALG, PRO reduced the frequency of T-end measurement errors (5.6% vs. 0.1%), reduced the intra-individual QT variability (12.6 ± 5.9 vs. 4.9 ± 1.1 ms) and allowed the recovery of 3/58 subjects that exhibited an unacceptable (<0.9) R^2 .

PRO has the potential to accelerate the drug development process by simultaneously reducing manpower requirements, study costs associated with semi-automated measurements, and the number of human volunteers at risk, while simultaneously improving repolarization risk assessments. The current results support the use of pattern recognition methods in conjunction with distribution-based QT analysis as an alternative to resource-intensive semi-automated analyses currently performed by ECG core laboratories.

1.3. Publication ALG vs. PRO – NP21249



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Pattern recognition analysis of digital ECGs: Decreased QT measurement error and improved precision compared to semi-automated methods[☆]

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Abstract

Background and Purpose: Machine-read QT measurements employing T-wave detection algorithms (ALG) are not accepted by regulatory agencies for the primary analysis of thorough QT (TQT) studies. Newly developed pattern recognition software (PRO) which matches ECG waveforms to user-defined templates may improve this situation.

Methods: We compared RR, QT, QTc, QT variability, T-end measurement errors, and individual QT rate correction factors and their associated coefficients of determination (R^2) following ALG and PRO analysis. Machine-read QTc values were compared with core laboratory semi-automated (SA) values for verification.

Results: Compared to ALG, PRO reduced the frequency of T-end measurement errors (5.6% vs. 0.1%), reduced the intra-individual QT variability (12.6 ± 5.9 vs. 4.9 ± 1.1 ms) and allowed the recovery of 3/58 subjects that exhibited an unacceptable (<0.9) R^2 .

Conclusions: PRO adjusted for ALG-based T-end measurement errors and provided an accurate and precise automated method for continuous QT analysis, thus offering an alternative to resource-intensive semi-automated analyses currently performed by ECG core laboratories.

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Keywords:

Automated QT analysis; Pattern recognition; QT measurement error; Distribution-based analysis; QT interval; Ponemah

Introduction

Currently, the accurate clinical assessment of potential drug-induced QT interval prolongation remains a major concern for regulatory agencies. Rarely, prolongation of ventricular repolarization may lead to torsade de pointes (TdP), a life-threatening polymorphic ventricular tachycardia. Despite several attempts to develop new and more reliable biomarkers predictive of TdP,^{1–3} the assessment of cardiac repolarization during a thorough QT (TQT) study remains the gold standard where virtually all new drug candidates are required to demonstrate relative safety for the risk of inducing TdP, indirectly assessed as an estimation of their potential to prolong the QT interval.

Pharmaceutical companies generally outsource TQT data analysis to ECG core laboratories which specialize in semi-

automated (SA) ECG annotations performed by well-trained cardiologists. The analysis performed by such laboratories typically consists of semi-automatically adjudicated annotations of 3–9 beats obtained during periods of heart rate stability (± 2 bpm) extracted at predefined time points prospectively defined in the study protocol.⁴ Although this methodology provides reliable QT interval measurements during the specified intervals, it has many well recognized and, to date, unresolved limitations including the exclusion of the majority of the recorded data, insufficient temporal resolution, substantial time and resource (manpower, cost) requirements, and inter-observer variability.^{5–8} Importantly, the sparse QT–RR data derived from fully manual or SA ECG analysis precludes the derivation and use of individual QT rate corrections, now generally accepted as the most accurate and reliable method to control for the effects of heart rate on the QT interval.⁹

Most of the fully automated T-end detection algorithms employ traditional analytical approaches (e.g. first-derivative adaptive threshold, tangent-based return to baseline) which usually perform well in noise-free, stable ECG recordings.^{10,11} However, electrical noise, baseline drift,

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and T-wave morphological changes will unavoidably lower the accuracy of algorithm-based ECG interval measurements, particularly affecting the reproducible determination of the T-end offset.^{12,13} Recently developed pattern recognition software enables reliable machine-read analysis of continuous ECGs by matching individual ECG waveforms to user-selected, noise-free, well-inscribed, and manually adjudicated ECG complexes which serve as reference waveform analysis templates.

In this study, we considered a well-characterized automated ECG analysis algorithm,¹⁴ and assessed potential improvements in raw QT interval measurement accuracy and precision with the addition of an ECG pattern recognition module (Ponemah Physiology Platform, ver. 5.0, Data Sciences International, St. Paul, MN). The algorithm (ALG) and pattern recognition (PRO) modules were compared by performing parallel analyses of continuous ECG data collected from all periods of a TQT study which included placebo, two doses of saquinavir, a QT-prolonging drug that induces changes in T-wave morphology,¹⁵ and the standard clinical reference agent, moxifloxacin. The current machine-read methods were validated by direct comparison of the reference moxifloxacin-induced QT-prolonging effect with parallel time-matched values obtained by an ECG core laboratory during the primary TQT analysis. Confirmation of accurate and precise fully automated QT analytical method, particularly in the setting of variable T-wave morphology,¹⁶ would provide a powerful new method for continuous beat-to-beat ECG analysis, possibly supplanting the current labor intensive manual adjudication of sparse ECG samples. Accordingly, the goals of the current study were twofold: (1) to directly compare the accuracy and precision of ALG and PRO T-end determinations in the presence of drug-induced QT prolongation and/or T-wave morphological changes and (2) to characterize and compare the ability of both machine-read analyses to detect benchmark moxifloxacin-induced QTc changes previously adjudicated by an ECG core laboratory employing SA analysis of beats extracted from a common data set.

Materials and methods

Study design

Data used in this study were extracted from a TQT study previously evaluated by an ECG core laboratory. Sixty healthy subjects were enrolled and 24-h continuous 12-lead digital ECGs (1000 Hz, Mortara H12+ Holter devices, Mortara Instruments, Milwaukee, WI) were acquired on day -1 (pretreatment baseline) and day 3 (on-drug) for each period. The study was performed at a single center, employing a double-blind, randomized, four-period, four-way crossover design. All subjects received each of four treatments by random assignment from a four-period Williams' square design with a washout period of at least 7 days between consecutive treatments. Each of the four periods consisted of one pretreatment baseline day, followed by 3 days of multiple dosing. The treatments were saquinavir (1000 or 1500 mg) in association with ritonavir (100 mg), placebo, and moxifloxacin. Moxifloxacin was given as a single dose (400 mg) on day 3 to confirm the assay sensitivity.

Of the 60 subjects enrolled, 58 (37 men and 21 women; mean age 34±11 years) had sufficient continuous ECG coverage (≥ 18 h) for inclusion in the current analysis. For the primary SA analysis, the subjects were required to lay supine for 15 min prior to scheduled ECG extractions performed at -1, -0.75, -0.5, 2, 3, 4, 5, 6, 8, and 12 h relative to dose administration. Standardized meals were provided at -0.5, 4, and 12 h relative to dose administration. All subjects gave written informed consent to the protocol which was reviewed and approved by the independent Investigational Review Board (Comité de Protection des Personnes 1, Strasbourg Cedex, France) which operates in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines.¹⁷

Automated ECG analysis

Whereas lead II ECG waveforms were used for the core ECG laboratory SA measurements, lead V₄ was selected for all machine-read ECG analyses as this lead was characterized by decreased motion artifacts, higher T-wave amplitudes, and consistently well-inscribed T-offsets compared to lead II. Prior to replay and analysis, Mortara H12+ ECG data from lead V₄ were converted to a generic binary format employing SuperECG software (ver. 3.1, Mortara Instruments, Milwaukee, WI). Both machine-read analyses were performed employing the Data Sciences International (DSI) Ponemah analysis system as subsequently described.

The Ponemah ECG analysis algorithm (ALG) first establishes the isoelectric baseline between the preceding and subsequent QRS complexes through the use of a straight line fit. The signal deflection relative to this baseline establishes the location of the T-wave. T-end location is then determined by fitting a fourth-order polynomial to the T-wave. The relative slopes between T-wave curve fit and the baseline over the T region are used to establish a threshold for the identification of T-end. Working forward in time from the peak of the T-wave, the analysis calculates the slope at which the T-wave approaches the previously established baseline. T-end is then marked once the rate of change relative to the baseline drops below a user-defined threshold. Aberrant waveforms, typically due to either physiologic or environmental noise or lead faults, were automatically identified and rejected employing a post hoc combination of Ponemah algorithm noise rejection filters and algorithm performance descriptors.

Pattern recognition analysis (PRO) was performed employing a supplemental tool recently developed for the machine-read Ponemah ECG algorithm. PRO compares and automatically adjusts ALG-derived fiducial marks to manually adjudicated candidate waveforms (templates). Template waveforms were added as needed to individual libraries for each subject and each treatment day such that at least 90% of the total waveforms were recognized and included in the analysis. In practice, PRO compares specified regions of each ECG complex to the corresponding individual library and assigns the corresponding template region with the highest conformational match. Each

waveform region (P, Q, S, and T) in an ECG complex is characterized by a search window (± 40 ms for the T-wave) centered on its assigned template mark position. Fiduciary marks are subsequently adjudicated by PRO when the assigned template attributes are recognized within the raw waveform search windows.

In the current study, we restricted PRO analysis to the T-wave region, such that P, Q, R, and S waves were derived by ALG for each ECG complex. Individual template libraries were constructed from the baseline and, when needed, on-drug data. Templates were selected from well-inscribed, noise-free ECG complexes and T-end marks were manually adjusted by one of the authors (O.M.). Individual libraries for each subject were subsequently used to identify and measure similar T-waves throughout each individual ECG data set. T-wave regions which demonstrated $\geq 85\%$ conformational match to a template of the corresponding individual library were automatically measured and included in the final data analysis. Complexes with $< 85\%$ conformational match were excluded from analysis.¹⁸

Continuous ECG analysis

As previously described, individual 24-h Holter data sets were machine-read by Ponemah software and the resulting beat-to-beat values from both methods (ALG and PRO) were imported and processed with a custom spreadsheet (Microsoft Excel 2007, Redmond, WA). For both methods, all measured raw QT and RR values from each baseline Holter recording were utilized for the derivation of individual QT rate correction factors (β) and associated coefficient of determination (R^2) using the following log-linear regression model.

$$\log(\text{QT}) = \beta \cdot \log(\text{RR}) + \alpha$$

Distribution-based rate-corrected QT (QTca) values were subsequently derived as $\text{QTca} = \text{QT}/\text{RR}^\beta$ for each raw QT value from each data set as previously described.⁷ To ensure the reliability of the QTca determinations, data sets demonstrating β values with an associated $R^2 < 0.9$ were deemed unacceptable and excluded from analysis. The large amount of data generated by continuous ECG analysis requires that beat-to-beat data be condensed into a suitable time scale for the presentation of summary data. In this study we divided each 24-h period into a series of contiguous 10-min segments and added an experimental time line indexed such that the time of dosing (placebo, moxifloxacin, or saquinavir) occurred at 0 h. All continuous data (RR, raw QT, and QTca) for each time segment were represented by the average value of all measured beats within a given segment. To ensure consistent quality for the values thus obtained, we required that each segment contained at least 250 valid beats. If this minimum number was not attained, the corresponding segment was excluded from the summary analysis.⁷ Summary data, expressed as consecutive 10-min mean values, are presented as conventional x - y plots to reveal possible time-dependent changes.

Semi-automated ECG analysis

The primary ECG analysis was performed by an ECG core laboratory using SA analysis. For each nominal protocol-specified time point (Section 2.1), a 5-min period preceded by 2 min of stable heart rate (± 2 bpm) was selected. Three discrete 10-s intervals demonstrating minimal noise and at least 20 s apart from each other were selected from each 5-min period. Triplicate ECGs were quantitatively evaluated from each of the three selected 10-s intervals. Calipers were automatically placed for each beat using a tangent-based algorithm, and subsequently adjusted by an experienced cardiologist as necessary. A study-specific QT rate correction (QTcS)⁹ was applied to the mean of each triplicate value such that the QTcS for each designated time point was represented by the mean of three triplicate values (nine aggregate complexes).

Statistical analysis

In the current study, the stability and reproducibility of T-end determinations were compared for both ALG and PRO using two approaches. First, T-end adjudication was visually characterized with both methods for 12 K beats obtained as groups of 100 successive complexes extracted from periods of uncontrolled physical activity between 3 and 4 h post-dose for 30 randomly selected subjects on day 3 of each study period. Beats were classified as either *valid* or *measurement error* by one of the authors (O.M.). *Measurement errors* were only declared in the case of indisputable T-end inaccuracies > 30 ms. Second, we compared the intra-individual QT standard deviations (SD) for successive 2-min segments obtained from the baseline (off-drug) 24-h Holter ECG recordings for each subject. Significant difference between methods was assessed using a pairwise Wilcoxon test and the Rüger correction for multiplicity.¹⁹ To further characterize the differences between ALG and PRO, baseline (day -1) QT-RR β -values and coefficients of determination (R^2) were compared using the pairwise Wilcoxon test where a p value ≤ 0.05 was considered statistically significant.²⁰ On-drug effects assessed by ALG and PRO were compared as the baseline-adjusted, placebo-corrected QTca and RR (double delta; $\Delta\Delta\text{QTca}$, $\Delta\Delta\text{RR}$) and their associated confidence intervals (CI). The baseline-adjusted QTca and RR (single delta; ΔQTca , ΔRR) were calculated by subtracting all post-dose values from their respective 1-h individual baselines obtained as the average value from -90 to -30 min. The $\Delta\Delta\text{QTca}$ and $\Delta\Delta\text{RR}$ were calculated as the time-matched difference between the on-treatment and the placebo ΔQTca and ΔRR . Significant $\Delta\Delta\text{QTca}$ difference between ALG and PRO was assessed by fitting linear mixed-effects models for each time segment, with *method* (PRO or ALG) as a fixed effect, and *subject* as random effect, in conjunction with the Rüger correction for multiplicity. The reliability and precision of the machine-read methods (ALG and PRO) were assessed by comparison of the respective $\Delta\Delta\text{QTca}$ and SD to the corresponding $\Delta\Delta\text{QTcS}$ and SD reported with ECG core laboratory SA analysis for the moxifloxacin periods. Systematic differences in QT measurements between ALG, PRO, and SA were

assessed using Bland–Altman analysis on baseline QT mean values at the protocol-specified time points and are expressed as bias \pm 95% limits of agreements. Adequate assay sensitivity was determined as described in ICH E14. Statistical analyses were performed using R (R Development Core Team, 2009) and the R package *nlme* (Bates and Maechler, 2009) or Analyse-it Software (version 2.26, Leeds, United Kingdom). Where applicable, data are expressed as the mean \pm SD.

Results

Raw QT and RR interval analysis

For all subjects and periods, PRO analysis matched 91% \pm 10% of all beats employing 4.2 \pm 1.3 templates (range 2–7), yielding \sim 72-K valid beats for each 24-h period. Generally, unmatched complexes were due to either electrical noise or environmental disturbances.

Visual characterization of T-end adjudication with both ALG and PRO yielded 671 and 10 measurement errors, corresponding to incidence rates of 5.6% and 0.1%, respectively ($p < 0.001$, chi-squared test). ALG measurement errors usually consisted of T-end declarations in the middle

of a non-fused U-wave or notched T-wave, resulting in inaccurate QT measurement that varied by $\geq \pm 100$ ms as depicted in Fig. 1. In contrast, PRO measurement errors were infrequent and typically resulted from matching an ECG complex to a suboptimal template.

The intra-individual SDs of the baseline QT calculated for consecutive 2-min segments were decreased with PRO compared to ALG (12.6 \pm 5.9 vs. 4.9 \pm 1.1 ms, ALG vs. PRO, respectively, $p < 0.001$). As expected, when this analysis was restricted to supine ECG extraction periods, both ALG and PRO demonstrated improved stability compared to periods of less well-controlled physical activity. However, PRO still demonstrated an incremental and significant reduction in raw QT variability compared to ALG (7.2 \pm 1.7 vs. 4.1 \pm 0.3 ms, ALG vs. PRO, respectively, all $p < 0.001$) during supine periods, as depicted in Fig. 2.

Parallel analysis of the baseline (day -1) 24-h ECGs confirmed equivalent RR detection with both methods (mean RR=920 \pm 97 vs. 937 \pm 101 ms, ALG and PRO, $p = \text{NS}$), which was further confirmed with Bland–Altman analysis at the scheduled time points where the mean bias was 0.6 \pm 22.1 ms. RR interval measured by SA demonstrated slightly increased values compared to machine-read methods where Bland–Altman analysis demonstrated

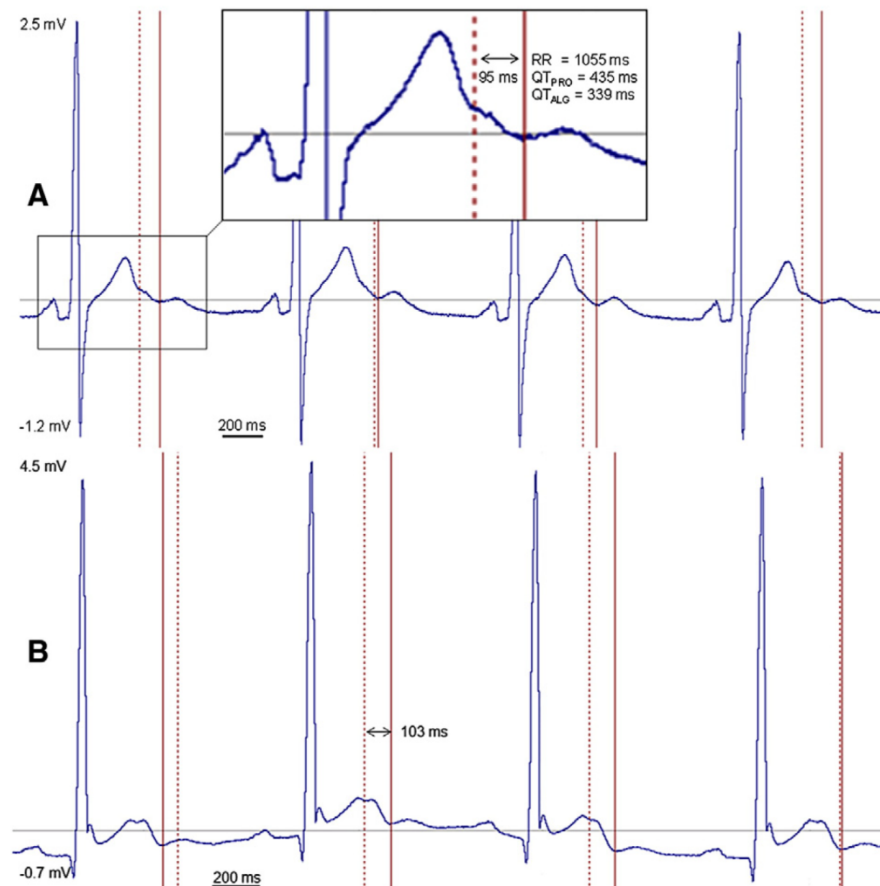


Fig. 1. Impact of T-wave morphology on T-end determination with ALG and PRO. Representative individual examples of morphologically-related T-end determination errors and inconsistencies with ALG observed from subjects demonstrating excessive variability during off-drug periods (A) or following administration of SQR 1500 mg (B). ALG (dotted line) inconsistently marked T-end due to variable T-wave morphologies, which resulted in differences as large as 100 ms between two successive beats. In contrast, PRO (solid line) consistently marked T-end in the context of notched T-wave with concomitant baseline shift.

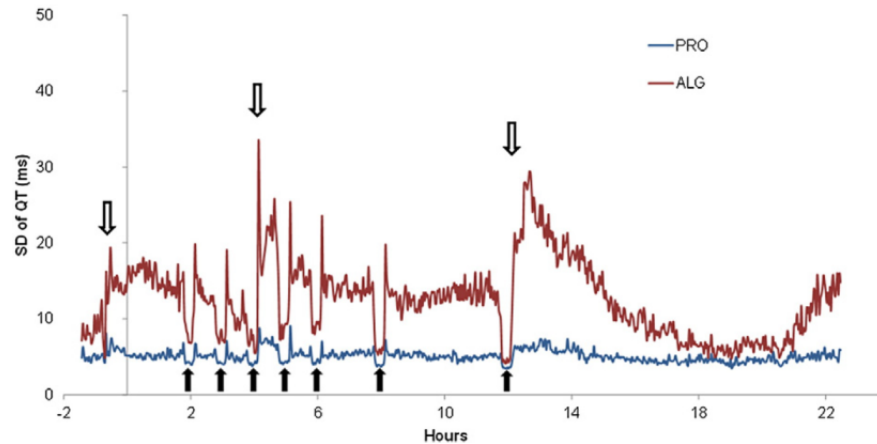


Fig. 2. Intra-individual raw QT variability: comparison of ALG and PRO. ALG-associated increases in SD were due to T-end measurement errors which were not observed with PRO (see Fig. 1). With both methods, variability was reduced during predefined supine ECG extraction periods (filled arrows) and increased during meals (–0.5, 4, and 12 h; open arrows).

24.6±65.6 and 24.1±65.6 ms vs. ALG and PRO, respectively. Compared to ALG, PRO demonstrated consistently increased QT values (376±20 vs. 403±22 ms, ALG vs. PRO, respectively, $p<0.001$). At the scheduled time points, corresponding raw QT values derived with ALG and SA were very close (Bland–Altman SA vs. ALG=–0.7±22 ms). In contrast, PRO demonstrated increased values compared to ALG and SA (Bland–Altman SA vs. PRO=23.7±25.2 ms; ALG vs. PRO=24.2±22.5 ms).

QT–RR relationships

QT–RR relationships derived with both ALG and PRO analyses yielded similar β values (0.303±0.06 vs. 0.294±0.05, ALG and PRO, respectively, $p=NS$). As expected, individual β values demonstrated large inter-individual variations and low intra-individual variability with both methods. The mean coefficients of determination (R^2) for the baseline QT–RR relationships was improved with PRO compared to ALG (0.981±0.01 and 0.963±0.05, respectively, $p<0.001$), and PRO yielded no individual $R^2<0.95$.

In 3 of 58 subjects, ALG demonstrated inconsistent T-end adjudications with differences as large as 100 ms between two successive beats (Fig. 1). As the associated ALG-derived QT–RR R^2 for these subjects was 0.78±0.09, they were, by definition, considered unacceptable for study inclusion due to excessive QT variability. Parallel PRO evaluations of these subjects reduced raw QT variability and markedly improved the R^2 to 0.98±0.01.

Treatment effects—RR and QTc interval

Both doses of saquinavir/ritonavir (SQR) demonstrated similar significant decreases of ~50 ms in $\Delta\Delta RR$ compared to both placebo and moxifloxacin. No differences were apparent between placebo and moxifloxacin, nor between the two doses of SQR.

As depicted in Fig. 3, moxifloxacin-associated $\Delta\Delta QTc$ prolongation commenced ~1 h after dosing, with the maximum increase occurring during a 1-h period centered around 4 h post-dose. Similar $\Delta\Delta QTc$ increases were observed at 4 h post-dose with both ALG and PRO (12.5 vs.

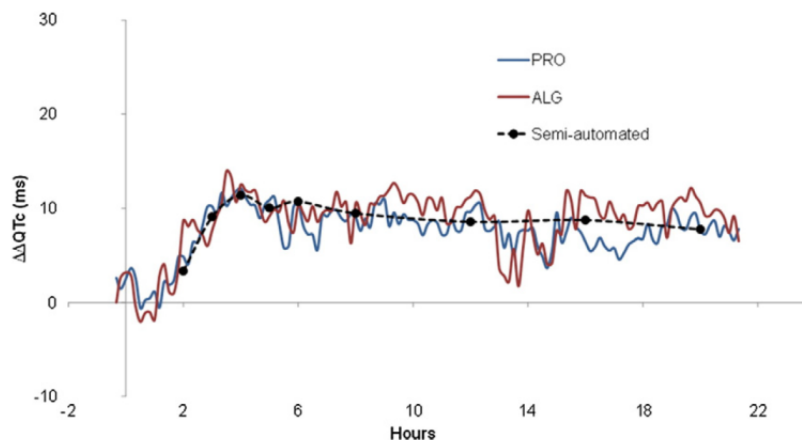


Fig. 3. Effects of moxifloxacin: parallel comparison of ALG, PRO, and semi-automated QT analysis. Machine-read and semi-automated methods demonstrated similar $\Delta\Delta QTc$ moxifloxacin-induced increases over time, confirming the sensitivity of the machine-read ECG evaluations to detect small drug-induced QTc effects.

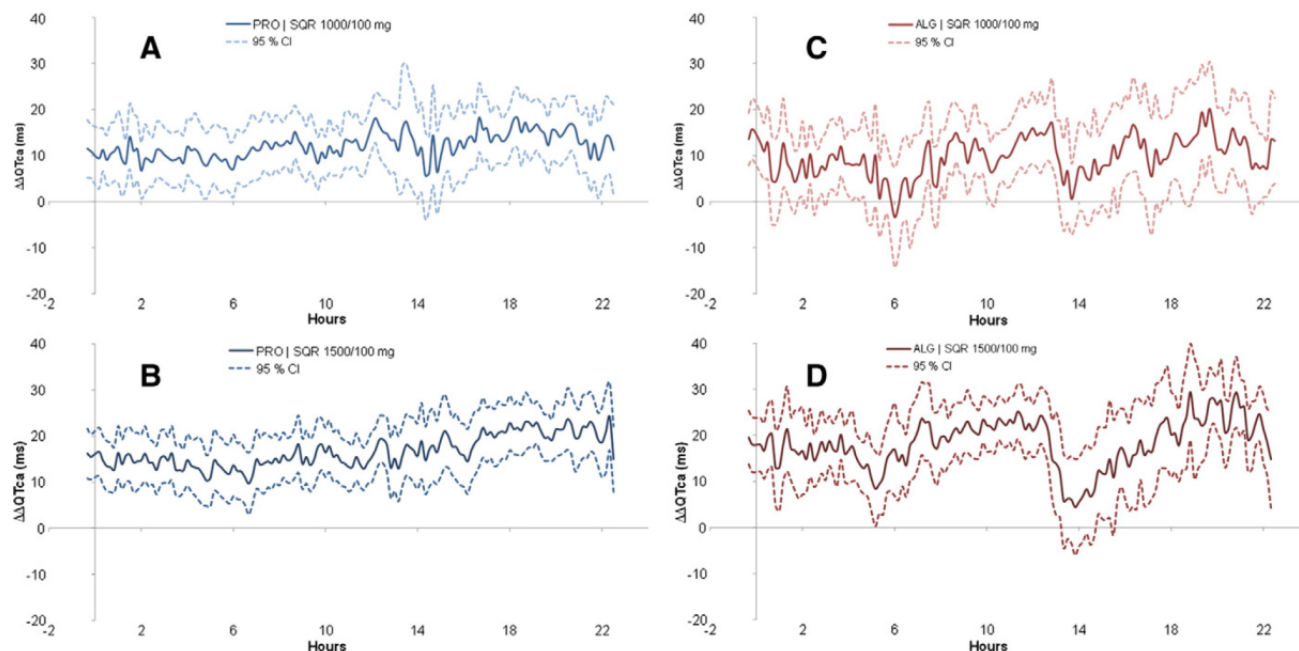


Fig. 4. Effects of saquinavir on $\Delta\Delta QTc$: comparison of ALG and PRO. While both methods detected dose-dependent increases in QTc with SQR, both doses of SQR with PRO (A and B) exhibited markedly improved QTc stability and improved confidence intervals over the entire 24-h period compared to ALG (C and D) which was characterized by pronounced variability, especially after food intake at 4 and 12 h.

12.1 ms, ALG and PRO, respectively). SA yielded a similar mean $\Delta\Delta QTc$ increase of 12.2 ms at 4 h post-dose. For the moxifloxacin periods, PRO analysis significantly reduced QT variability where the mean inter-individual SDs during the supine ECG extraction periods were 15 ± 4.4 , 9.1 ± 0.5 , and 10.2 ± 1.1 ms for ALG, PRO, and SA methods, respectively ($p < 0.011$, PRO vs. SA, pairwise Wilcoxon test). The predefined test for E14 assay sensitivity was satisfied by both machine-read methods.

As depicted in Fig. 4, SQR demonstrated similar dose-dependent increases in $\Delta\Delta QTc$ with both methods where the mean $\Delta\Delta QTc$ increases were 9.2 ± 4.9 and 10.4 ± 2.9 ms with the 1000/100 mg SQR and 17.2 ± 5.6 vs. 15.4 ± 3.7 ms with 1500/100 mg SQR, assessed by ALG and PRO, respectively. With ALG, decreases in $\Delta\Delta QTc$ were apparent with both SQR doses commencing at ~ 4 h and 12 h post-dose and were attributed to incorrect T-end measurements due to the combination of drug and/or meal-induced T-wave morphological changes (Fig. 1B). In contrast, similar decreases were not apparent with PRO, which demonstrated a concomitant decrease in $\Delta\Delta QTc$ variability where the mean inter-individual SDs for 1500 mg SQR were 24.6 ± 4.9 and 17.9 ± 1.9 ms, ALG and PRO, respectively. A descriptive summary for ALG and PRO analysis is presented in Table 1.

Discussion

While the feasibility of reliable machine-read QT interval measurements has been previously demonstrated,^{5–8,10,13} this methodology is not yet routinely accepted by the regulatory agencies as a standalone clinical ECG evaluation

method. Natural and drug-induced changes in T-wave morphology remain a major concern when employing machine-read analysis of continuously digitized ECGs. Currently, there is no clinical evidence to support the notion that a machine-read analysis, which performs well during stable and well-controlled periods, will continue to reliably adjudicate T-end in the presence of environmental or pharmacologic effects which may extensively alter the cardiac repolarization properties with possible associated changes in T-wave morphology. Accordingly, the current TQT study was selected for two reasons. First, the current ECG recordings were characterized by substantial T-wave morphological variability, attributed to the periods where subjects were allowed to move freely, and to SQR-induced T-wave morphological changes.¹⁵ As such, this data set provided for the extensive characterization of both ALG and PRO performance on ECG waveforms characterized by variable T-wave morphologies in conjunction with variable baseline noise levels. Second, the study data had been previously analyzed by an ECG core laboratory which

Table 1
Comparison of ALG and PRO.

	ALG	PRO
Measurement error	5.6%	0.1%
Coefficient of determination, R^2	0.963 ± 0.05	0.981 ± 0.01
Baseline QT(ms)	376 ± 20	403 ± 22
Baseline intra-individual SD of QT—24 h (ms)	12.6 ± 5.9	4.9 ± 1.1
Baseline intra-individual SD of QT—time points (ms)	7.2 ± 1.7	4.1 ± 0.3
Moxifloxacin inter-individual SD of $\Delta\Delta QTc$ —time points (ms)	15 ± 4.4	9.1 ± 0.5

permitted the direct comparison of the current results to an accepted regulatory standard.

In the current study, the utilization of pattern recognition analysis provided for the consistent measurement of the raw QT and RR intervals for >90% of all ECG complexes recorded during the study, irrespective of environmental or drug-induced changes in T-wave morphology. Compared to ALG and SA values, the mean raw QT interval was significantly longer with PRO, a direct consequence of methodological differences in T-end determination. With ALG, T-end was declared when the slope of the T-wave at which the rate of change relative to the baseline dropped below ALG default threshold, and resulted in a systematically shorter QT interval compared to PRO where templates were user-adjusted such that T-end was declared, by convention, at the nadir of T-wave and any non-fused U-wave. However, ALG threshold position is user-adjustable and the use of another threshold position could likely reduce the systematic difference observed between ALG and PRO measurements. SA analysis used an automated tangent-based algorithm to place calipers which were subsequently manually over-read and adjusted by a cardiologist as needed. Tangent-based algorithm adjudicates T-end where a tangential line from the descending portion of the T-wave crosses the isoelectric baseline, which contributed to the difference obtained with user-adjusted PRO measurements. Importantly, as TQT study analysis relies on baseline- and placebo-adjusted QTc values, the absolute accuracy of the raw QT measurements is of less importance than precision for the reliable detection of a drug-induced change in QTc.

Visual confirmation of T-end determination with machine-read methods demonstrated increased rates for obvious inaccurate T-end adjudications (see Section 2) with ALG compared to PRO (5.6% vs. 0.1% respectively). As it was logistically impossible to visually characterize the T-end placement of each ECG complex recorded in this study (>35MM beats), a surrogate assessment of beat-to-beat QT interval variability was derived as the intra-individual standard deviation associated with both ALG and PRO analysis for successive 2-min intervals spanning the entire 24-h collections. As expected, raw QT interval variability was relatively low with both evaluation methods during the well-controlled supine ECG extraction periods.²¹ Importantly, during the less well-controlled periods (~95% of the recorded data), such as when subjects were allowed to move freely, raw QT variability was markedly increased with ALG due to variable T-wave morphologies which subsequently manifested as inconsistent beat-to-beat T-end adjudications and/or punctual measurement errors. In contrast, PRO consistently adjudicated complex T-wave morphologies, which enabled reliable QT measurements, even during periods of uncontrolled physical activity.

In conjunction with machine-based evaluations, the application of distribution-based analysis allowed for the precise characterization of QT–RR relationships and the derivation of individual QT rate-correction factors (β) using a previously described log–log–linear model^{7,22} for all subjects. Mean β values for both ALG and PRO were virtually identical and allowed for the derivation of

completely heart rate-dissociated QT (QTca) values, affirming the ability of distribution-based analysis to derive reliable individual QT–RR β values, even from continuous data characterized by high residual variability. Of note, PRO analysis resulted in a further, incremental, improvement in the QT–RR coefficients of determination (0.963 ± 0.05 vs. 0.981 ± 0.01 , ALG and PRO, respectively). The reliability of β values determination is derived from the process of distribution-based analysis which employs all available baseline QT–RR pairs (>70 K) to describe the individual QT–RR relationships. With this implementation, several thousands of incorrectly marked beats could potentially decrease the QT–RR coefficient of determination (R^2), but would not result in any substantial change in the associated regression slope (β). ALG analysis yielded three subjects who exhibited excessive raw QT variability during baseline ECG analysis, which would, by definition, result in the exclusion of these subjects from the final study population. The associated ECGs were characterized by inconsistent ALG-based T-end adjudications and were effectively recovered and rendered suitable for study inclusion with PRO analysis.

In typical ECG core laboratory practice, possible drug effects are based on values derived from semi-automated measurements of small numbers of replicate ECGs extracted at scheduled time points. As shown in Fig. 3, at common time points, the current baseline and placebo-adjusted $\Delta\Delta$ QTca values for moxifloxacin were coincident with the values reported with SA, confirming equivalent sensitivity for all methods to detect a small moxifloxacin-induced change in QTc. Importantly, compared to both ALG and SA, PRO analysis was characterized by a significant reduction in the inter-individual SD of $\Delta\Delta$ QTc during well-controlled supine periods (mean SD = 15 ± 4.4 , 10.2 ± 1.1 , 9.1 ± 0.5 ms for ALG, SA, and PRO, respectively). Following treatment with SQR, the ALG-derived $\Delta\Delta$ QTca demonstrated artifactual decreases with both doses at ~4 and 12 h post-dose (Fig. 4), which were likely associated with meal-induced autonomic, glucose, and/or C-peptide effects,²³ coupled with a direct SQR effect. The resulting atypical T-wave morphologies were inconsistently evaluated by ALG whereas PRO consistently and correctly adjudicated T-end (Fig. 1B). These results demonstrate that operator-based visual adjustments are crucial to assure both accuracy and precision in the application of fully automated pattern recognition measurement algorithms. The latter is of particular importance when the protocol includes substantial periods of unrestrained physical activities or employs drugs associated with T-wave morphological changes.

Conclusions

Machine-read ECG methodologies are rapidly evolving and, as demonstrated for the current TQT study, advanced measurement algorithms can precisely replicate the results of semi-automated visual interpretations currently performed by core laboratory cardiologists, but with reduced QT variability and improved temporal resolution. The current

results support the use of pattern recognition methods when evaluating continuously recorded digital ECGs, especially when the study involves the analysis of a drug which could affect T-wave morphology and/or includes sustained periods of uncontrolled physical activity. As such, PRO, in conjunction with distribution-based QT analysis, has the potential to accelerate the drug development process by simultaneously reducing manpower requirements, study costs associated with semi-automated measurements, and the number of human volunteers at risk, while simultaneously improving repolarization risk assessments.

Limitations

The current study assessed moxifloxacin-induced QTca changes based on machine-read values derived from continuous QT measurements from lead V₄ which were then compared to moxifloxacin-induced QTcS changes based on semi-automated lead II QT measurements performed by an ECG core laboratory. Comparison of repolarization results obtained in different leads and employing different methodologies should be interpreted with caution. In the current study, lead V₄ offered optimal waveform amplitudes for the machine-read analysis. While the use of lead II for the current machine-based analysis yields similar results for other studies (data not shown), the use of any suboptimal lead may degrade the performance of the current automated algorithms, which are improved with higher signal amplitudes. Importantly, in the current study $\Delta\Delta QT_{ca}$ (lead V₄) and $\Delta\Delta QT_{cS}$ (lead II) were virtually identical, demonstrating vector-independent moxifloxacin sensitivity when the respective lead-dependent QT values were normalized and expressed as the individual change from baseline in the normal healthy heart. Moreover, despite the absolute reduction in measurement error obtained with PRO, the current retrospective analysis cannot quantify the extent to which the current variability reduction was affected by minimization of the T-end position variability with PRO.

References

- Antzelevitch C. Role of transmural dispersion of repolarization in the genesis of drug-induced torsades de pointes. *Heart Rhythm* 2005;2:S9.
- Shah RR. Drug-induced QT, dispersion: does it predict the risk of torsade de pointes? *J Electrocardiol* 2005;38:10.
- Smetana P, Pueyo E, Hnatkova K, et al. Individual patterns of dynamic QT/RR relationship in survivors of acute myocardial infarction and their relationship to antiarrhythmic efficacy of amiodarone. *J Cardiovasc Electrophysiol* 2004;15:1147.
- Malik M, Hnatkova K, Ford J, Madge D, Near-Thorough QT. Study as part of a first-in-man study. *J Clin Pharmacol* 2008;48:1146.
- Fossa AA. Dynamic beat-to-beat modeling of the QT–RR interval relationship: analysis of QT prolongation during alterations of autonomic state versus human ether a-go-go-related gene inhibition. *J Pharmacol Exp Ther* 2004;312:1.
- Extramiana F, Badilini F, Sarapa N, Lecnhardt A, Maison-Blanche P. Contrasting time- and rate-based approaches for the assessment of drug-induced QT changes. *J Clin Pharmacol* 2007;47:1129.
- Holzgrefe HH, Ferber G, Morrison R, et al. Characterization of the human QT interval: novel distribution-based assessment of the repolarization effects of moxifloxacin. *J Clin Pharmacol* 2012;52:1222.
- Strachan IG, Hughes NP, Poonawala MH, Mason JW, Tarassenko L. Automated QT analysis that learns from cardiologist annotations. *Ann Noninvasive Electrocardiol* 2009;14(Suppl 1):S9.
- Malik M. Problems of heart rate correction in assessment of drug-induced QT interval prolongation. *J Cardiovasc Electrophysiol* 2001;12:411.
- Sarapa N, Gussak I, Vajdic B, et al. Comparison of QTinno, a fully automated electrocardiographic analysis program, to semiautomated electrocardiographic analysis methods in a drug safety study in healthy subjects. *J Electrocardiol* 2009;42:358.
- Tyl B, Azzam S, Blanco N, Wheeler W. Improvement and limitation of the reliability of automated QT measurement by recent algorithms. *J Electrocardiol* 2011;44:320.
- Zhou X, Wei D. A multidifferentiator-based approach to the reliable determination of T-wave offset in electrocardiograms. *J Electrocardiol* 2011;44:330.
- Hnatkova K, Gang Y, Batchvarov VN, Malik M. Precision of QT interval measurement by advanced electrocardiographic equipment. *Pacing Clin Electrophysiol* 2006;29:1277.
- Chui RW, Vargas HM. A comparison of three software platforms for automated ECG analysis. *J Pharmacol Toxicol Methods* 2009;60:28.
- Zhang X, Jordan P, Cristea L, et al. Thorough QT/QTc study of ritonavir-boosted saquinavir following multiple-dose administration of therapeutic and supratherapeutic doses in healthy participants. *J Clin Pharmacol* 2012;52:520.
- Glancy JM, Weston PJ, Bhullar HK, et al. Reproducibility and automatic measurement of QT dispersion. *Eur Heart J* 1996;17:1035.
- Puri KS, Suresh KR, Gogtay NJ, Thatte UM. Declaration of Helsinki, 2008: implications for stakeholders in research. *J Postgrad Med* 2009;55:131.
- DSI. Template Analysis Manual (MU00279_Template Analysis Manual), Revision nn, Copyright Data Sciences International, 20nn. 2010.
- Abt K. Planning controlled clinical trials on the basis of descriptive data analysis. *Stat Med* 1991;10:777.
- Lehmann EL. Nonparametrics: statistical methods based on ranks. San Francisco: Holden-Day, Inc.; 1975.
- Salvi V, Karnad DR, Panicker GK, et al. Comparison of 5 methods of QT interval measurements on electrocardiograms from a thorough QT/QTc study: effect on assay sensitivity and categorical outliers☆. *J Electrocardiol* 2011;44:96.
- Funck-Brentano C, Jaillon P. Rate-corrected QT interval: techniques and limitations. *Am J Cardiol* 1993;72:17B.
- Taubel J, Lorch U, Ferber G, et al. Insulin at normal physiological levels does not prolong QTc interval in thorough QT studies performed in healthy volunteers. *Br J Clin Pharmacol* 2012 [Epub ahead of Print].

1.4. Supplementary analysis on other QT studies

1.4.1. BP22464

To further evaluate the differences between ALG and PRO, similar comparisons were performed on a TQT study with a SGLT2 inhibitor, a drug which was not associated with T-wave morphological change or QT/QTC prolongation.

With both ALG and PRO, the circadian patterns of Δ QT and Δ RR were virtually superimposable (Figure 43-44). The protocol-induced changes in QT and RR were evident and resulted in expected increases and decreases during supine ECG extraction periods, meals, and sleep.

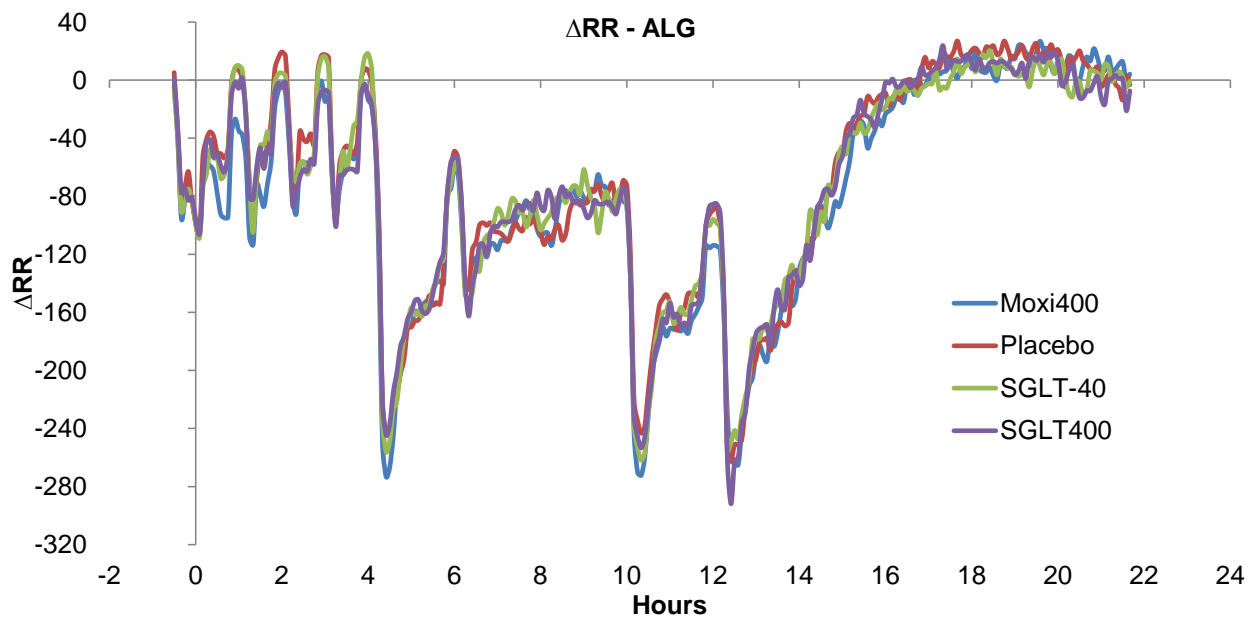


Figure 43A. ALG-derived Δ RR interval with all treatments. The excursions noted during the first 12 hours represent postural changes before and after protocol-directed ECG extractions where the patients were required to lie supine for 10 min prior to the recording, or meals (4 h and 12 h), resulting in repeated changes in adrenergic tone. There were no treatment-related RR effects.

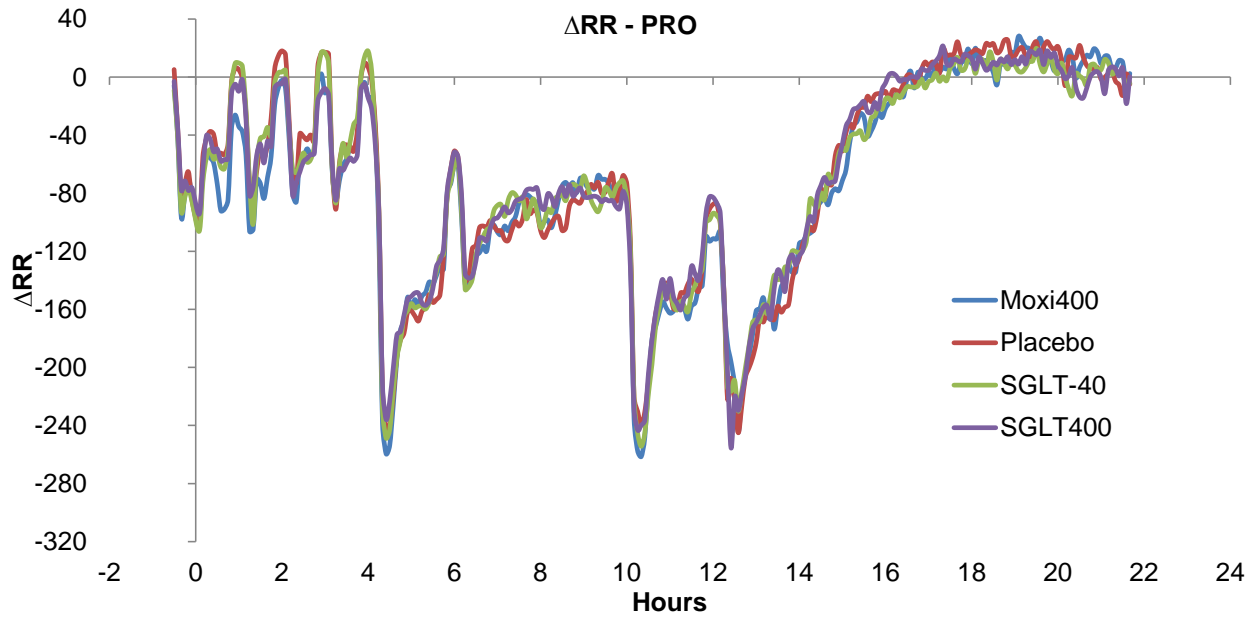


Figure 43B. PRO-derived ΔRR interval with all treatments.

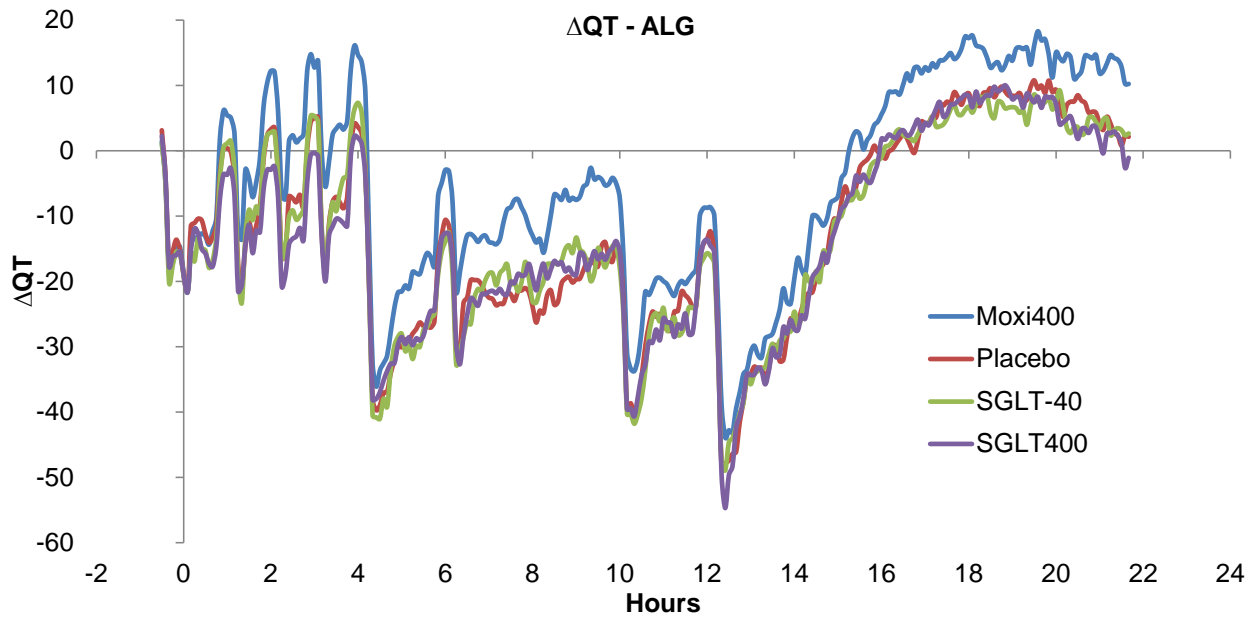


Figure 44A. ALG-derived ΔQT interval with all treatments. The raw QT interval changed in parallel with the RR interval (Figure 43) where protocol-associated changes in adrenergic tone were clearly evident. Moxifloxacin elicited an expected small increase in the raw QT interval.

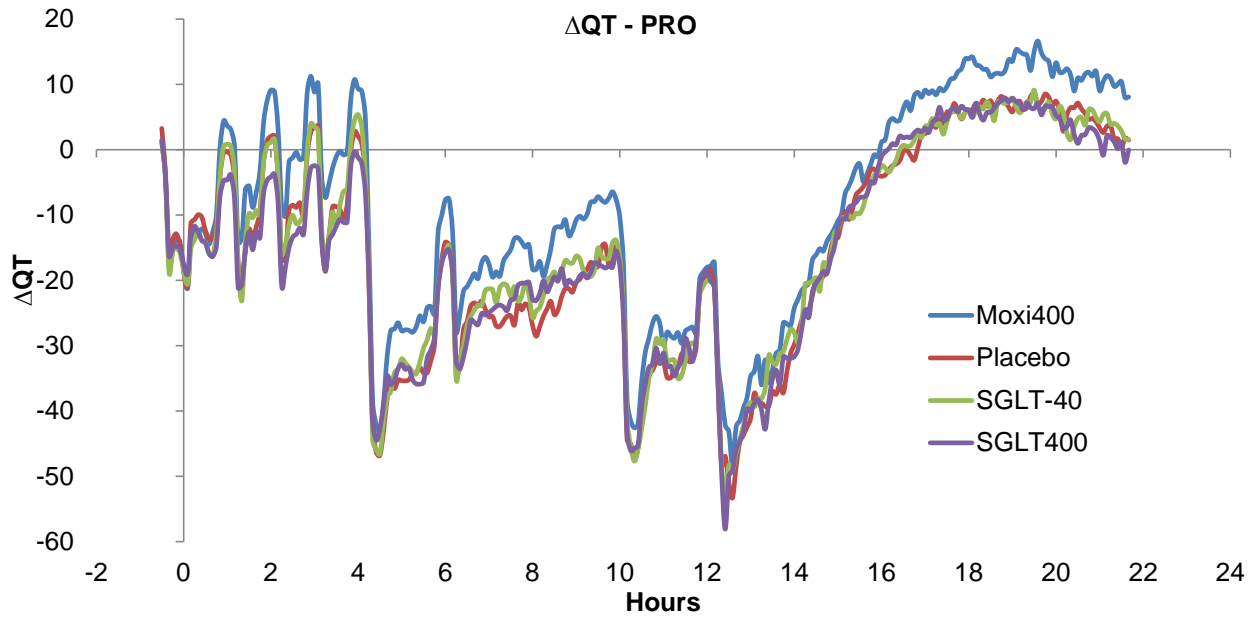


Figure 44B. PRO-derived Δ QT interval with all treatments.

The comparison of intra-individual intra-time point (2-min) standard deviation (intra-SD) measured during placebo treatment demonstrated patterns similar to the saquinavir study (Figure 45). The intra-SD was decreased with PRO compared to ALG (9.0 ± 3.9 vs. 5.0 ± 0.9 ms, ALG vs. PRO, respectively, $p < 0.001$). As expected, for both ALG and PRO, the variability was increased during periods of unrestrained activities and remained stable during supine periods. However, the intra-SD increase with ALG was quantitatively larger than PRO during periods of unrestrained activity, suggesting that PRO is more appropriate for continuous ECG analysis.

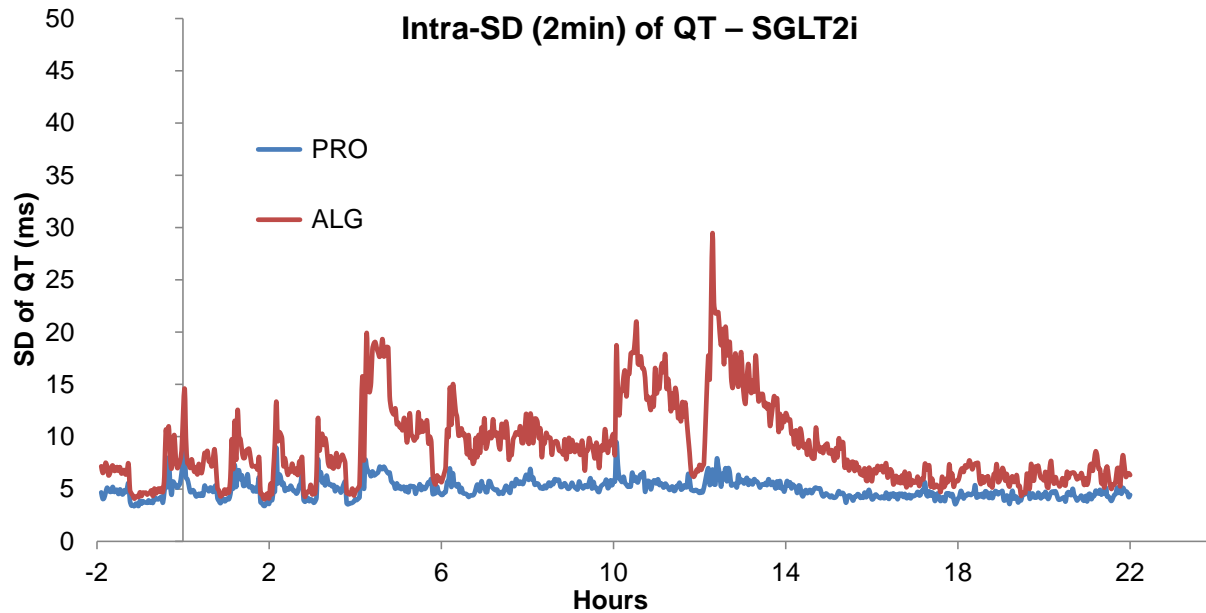


Figure 45. Intra-Individual Raw QT Variability: Comparison of ALG and PRO. ALG-associated increases in SD were due to T-end measurement errors which were not observed with PRO. With both methods, variability was reduced during predefined supine ECG extraction periods and increased during meals.

Study	Mean SD of QT over 2min \pm SD	
	ALG	PRO
NP21249	12.91 \pm 4.49	5.15 \pm 0.63
BP22464	9.01 \pm 3.86	4.98 \pm 0.85

Table 5. Mean intra-SD of QT over 2-min time segments. The variability associated with QT measurements was dramatically reduced with PRO compared to ALG.

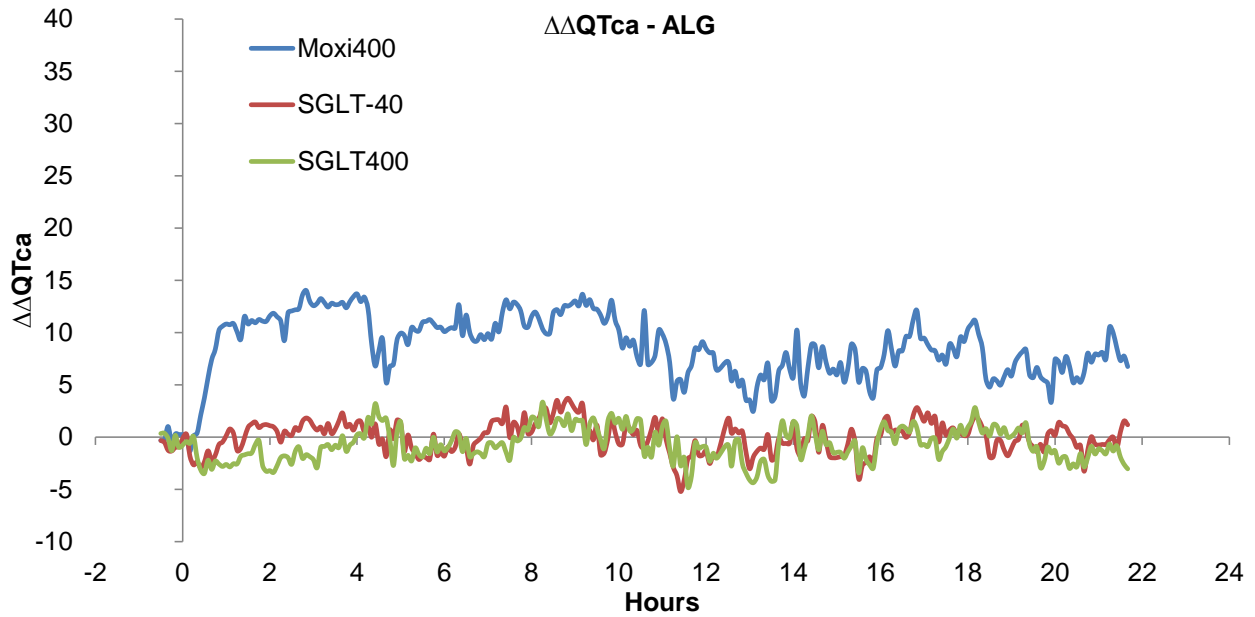


Figure 46A. ALG-derived $\Delta\Delta QTc$ interval with all treatments. Application of QTca revealed a persistent moxifloxacin-related QTca increase of 10-14 ms which persisted for ~ 22 h post-dose, consistent with the associated moxifloxacin plasma levels in this study. These results confirmed TQT assay sensitivity as required by ICH E14.

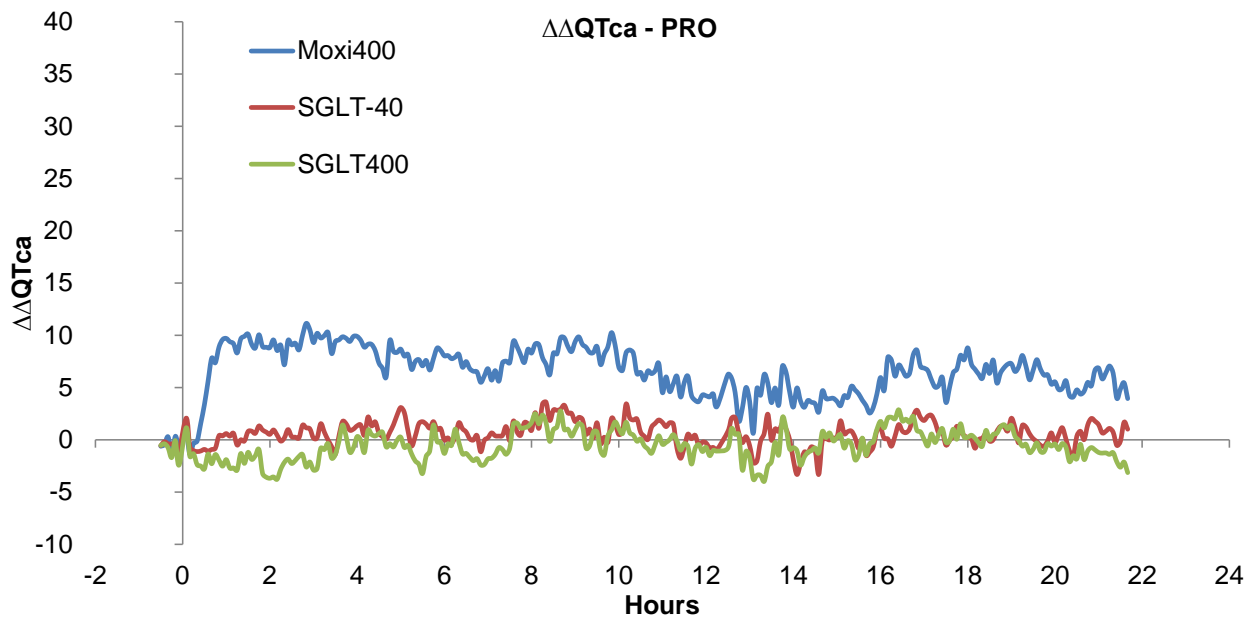


Figure 46B. PRO-derived $\Delta\Delta QTc$ interval with all treatments. Similar drug-effects were observed over the 24 hour time-course.

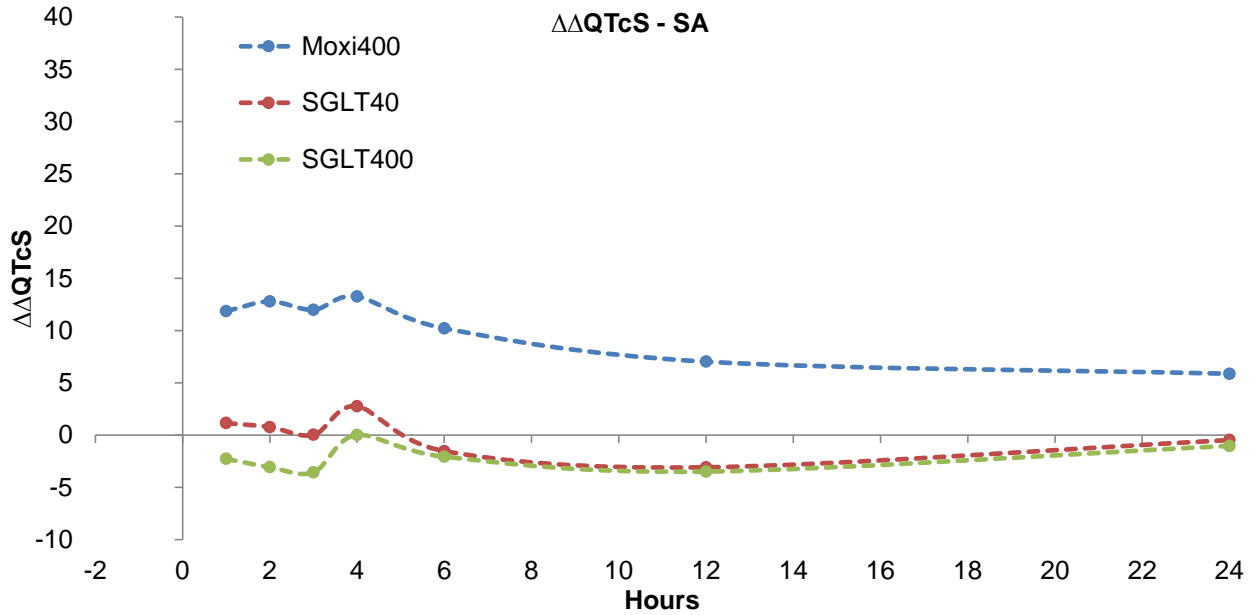


Figure 46C. SA-derived $\Delta\Delta\text{QTcS}$ interval with all treatments. Similar drug-effects were observed over the 24 hour time-course.

The $\Delta\Delta\text{QTc}$ for all treatments with the three methods (Fig 46A, B, and C) each demonstrated the expected QTc increase with moxifloxacin over time. The administration of SGLT2 inhibitor did not result in QT prolongation with either dose.

ALG				
Treatment	n	Mean effect	SD	CI
Moxi400	44.1 ± 3.2	8.6 ± 3.2	10.0 ± 2.7	3.1 ± 0.9
SGLT40	43.7 ± 3.8	0.0 ± 1.5	9.2 ± 2.6	2.8 ± 0.9
SGLT400	43.6 ± 2.8	-0.7 ± 1.6	9.7 ± 2.7	3.0 ± 0.9

PRO				
Treatment	n	Mean effect	SD	CI
Moxi400	44.3 ± 4.5	6.5 ± 2.5	9.3 ± 1.9	2.9 ± 0.7
SGLT40	44.4 ± 4.8	0.6 ± 1.2	8.3 ± 1.8	2.5 ± 0.6
SGLT400	44.6 ± 4.1	-0.6 ± 1.4	8.9 ± 1.7	2.7 ± 0.6

SA				
Treatment	n	Mean effect	SD	CI
Moxi400	50.7 ± 0.5	10.3 ± 2.9	7.6 ± 0.8	2.1 ± 0.2
SGLT40	51.9 ± 0.4	-0.1 ± 1.9	7.0 ± 0.8	2.0 ± 0.2
SGLT400	51.9 ± 0.4	-2.2 ± 1.3	7.0 ± 1.1	1.9 ± 0.3

Table 6. Descriptive statistics of $\Delta\Delta\text{QTca}$ for each treatment with all methods. Compared to ALG, PRO demonstrated reduced variability and slightly reduced mean moxifloxacin-induced effects (~2 ms).

Descriptive statistics associated with PRO demonstrated slightly decreased standard deviation and confidence intervals (Table 6). The impact of the substantial intra-SD decrease with PRO compared to ALG (9.0±3.9 vs. 5.0±0.9 ms) resulted in reduced analysis results variability (Confidence intervals: 3.0 vs. 2.7 ms, ALG and PRO, respectively). The reduced variability with PRO can be observed in figures 46A and B where the $\Delta\Delta\text{QTca}$ continuous effect was smoother compared to ALG. However, the ALG analysis was still associated with acceptable variability, suggesting that even in presence of several ECG measurement errors, continuous ECG measurement methodologies employing millions of beats provides acceptable results with limited variability as all methods yielded the same conclusions.

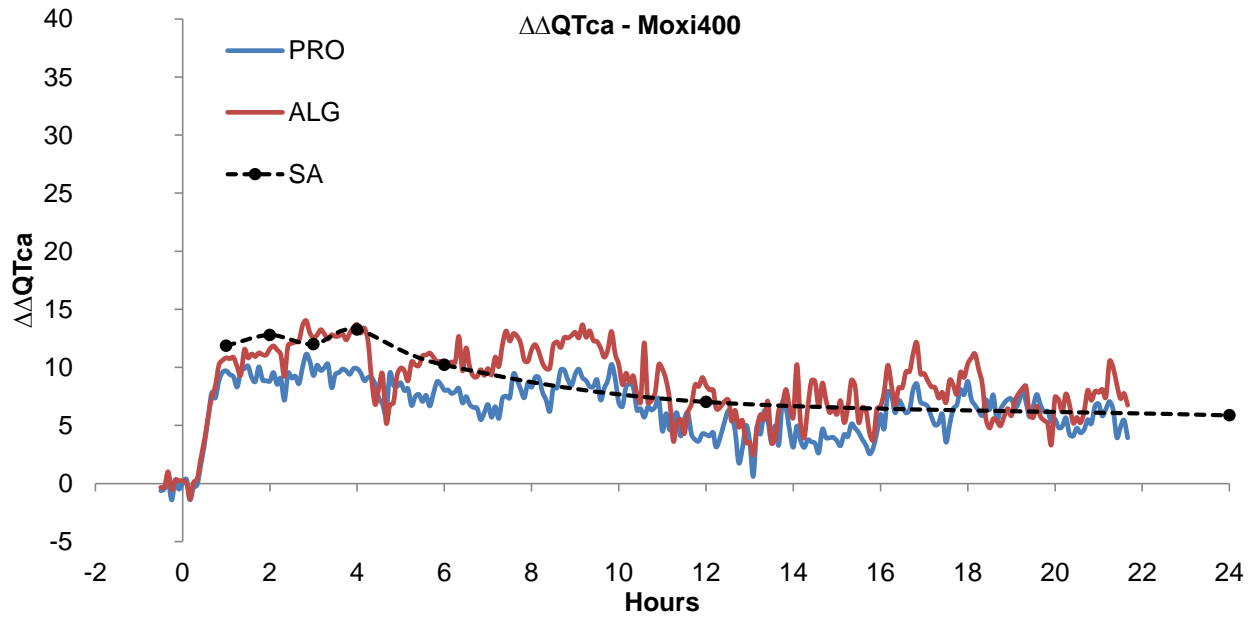


Figure 47. Moxifloxacin-induced $\Delta\Delta\text{QTca}$ effect with all methods. The moxifloxacin-induced effects were quantitatively smaller with PRO compared to ALG and SA.

Importantly, while both SGLT2 inhibitor doses demonstrated similar negative effects, PRO demonstrated a consistently smaller quantitative $\Delta\Delta\text{QTca}$ increase with moxifloxacin of ~2 ms compared to ALG and SA (Table 6, Figure 47). This difference is likely the consequence of a method-specific difference in the evaluation of the T-end when the QT is prolonged.

1.4.2. BP22693

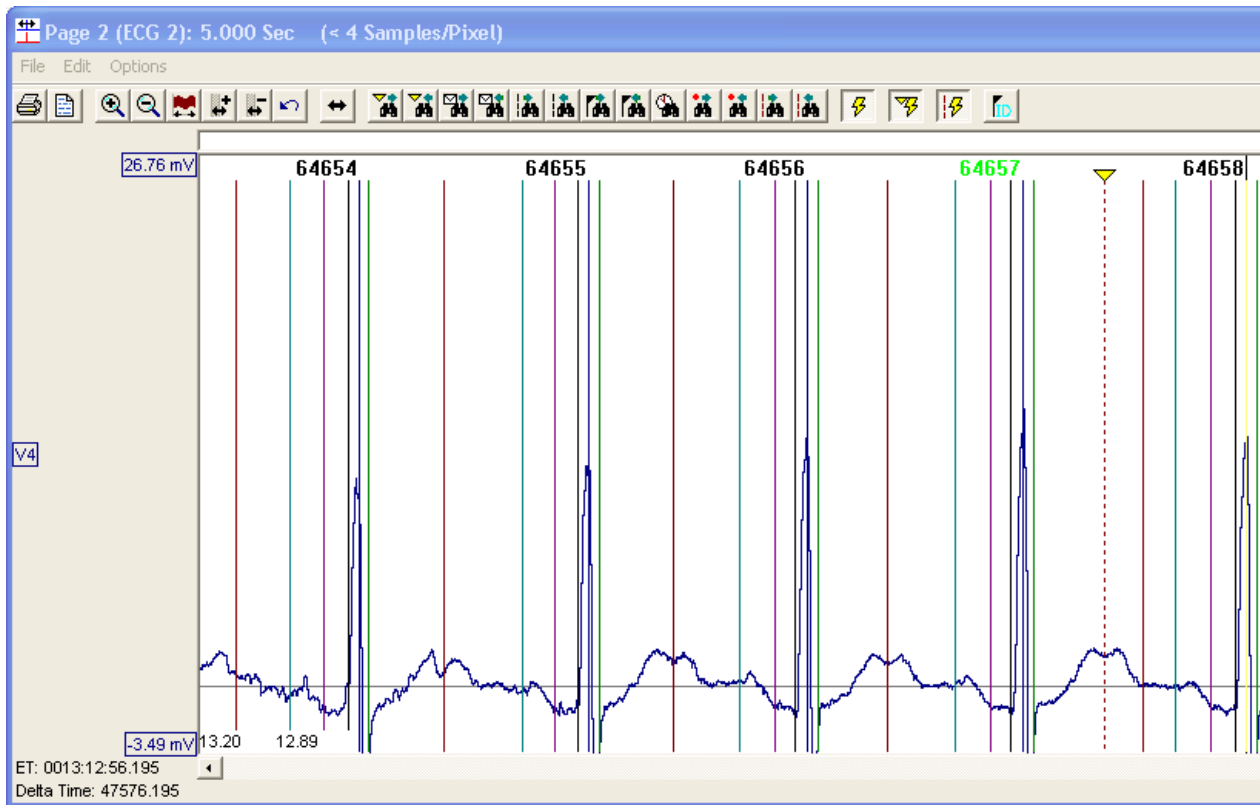


Figure 48. Example of algorithm-based ECG measurements errors with TRI (120 mg). The automated algorithm inaccurately marked the end of the T-wave due to a notched morphology. When PRO was applied (green complex), the erroneous mark (dotted line) was effectively adjusted.

At the highest dose regimen (120 mg), the Triple Reuptake Inhibitor induced T-wave morphological changes (biphasic T-waves) which resulted in numerous substantial measurement errors. The application of PRO effectively corrected these measurement errors.

1.5. Conclusion

In Part I, it has been demonstrated that the use of the pattern recognition method enabled effective adjustments which corrected the ECG measurement errors that are generally associated with fully-automated algorithm-based measurements. The pattern recognition method provided reproducible ECG measurements, even during periods of meals, blood sampling, and unrestrained physical activities which were characterized by extensive variability with ALG. In addition, the application of distribution-based analysis for the continuous evaluation of drug-induced QT prolongation effects enabled the precise derivation of individual correction factors which fully dissociated the QT from the effects of heart rate over the continuous 24 hour recordings. When compared to the gold standard SA method, PRO provided generally equivalent results and similar conclusions. Accordingly, PRO, in conjunction with distribution-based analysis provides a reliable alternative to the conventional SA method for TdP liability assessment as it reduces the study cost and manpower requirements, while increasing temporal resolution.

2. Part II: Comparison of methods

2.1. Rationale

The analyses described in Part I demonstrated that algorithm-based measurement errors could be corrected through the use of pattern recognition methodology. This comparison was performed with a single ECG measurement software application, Ponemah (ALG and PRO). While the correction of ECG measurement errors with PRO effectively reduced the variability associated with the detection of drug-induced effects on the QT interval with ALG, several other advanced QT measurement methods employing various combination of algorithms and features have been developed (Hnatkova, Gang et al. 2006; Sarapa, Gussak et al. 2009; Strachan, Hughes et al. 2009; Tyl, Kabbaj et al. 2009; Couderc, Garnett et al. 2011; Green, Kligfield et al. 2012; Meyer, Ferber et al. 2012). Most of these new methods have been compared to the standard manual and/or semi-automated measurements performed by cardiologists and yielded equivalent QT/QTc assessments. However, these ECG measurement methods have never been directly compared using continuous analysis on the same datasets characterized by consistent QTc prolongation with and without T-wave morphological changes. It was thus hypothesized that other advanced continuous ECG measurement platforms using different methodological approaches to measure and analyze the ECG waveforms may also provide reliable TdP liability assessment.

In Part II, three computerized ECG measurement methods (BioQT, Ponemah PRO, WinAtrec) were selected for extensive comparison in the parallel evaluation of a TQT study which included a drug which induces T-wave morphological changes. Although conceptually similar, with all methods providing continuous computerized QT interval measurements and heart-rate corrections, each of these applications incorporates unique vendor-specific analytical features. The comprehensive understanding of these features and their possible impact on cardiac repolarization assessments is an important step towards the standardization of continuous ECG analysis methodologies.

Briefly, BioQT is fully automated (no operator adjustments are required) and employs wavelet transforms and a Hidden Markov Model (HMM) to measure and self-check the QT intervals. The QT measurements for each beat are then individually heart rate-corrected based on an on-treatment sliding QT/RR regression (QTc_{ic}) (Strachan, Hughes et al. 2009). Ponemah PRO uses a pattern recognition algorithm where the QT interval for each beat is measured based on individual operator-adjusted templates. Distribution-based analysis is then applied to obtain

individual heart rate-corrected QT values based on the off-drug QT/RR relationship (QTca) (Holzgreffe, Ferber et al. 2012; Meyer, Ferber et al. 2012). WinAtrec *time-binning* analysis automatically computes and measures 60 s and single lead median beats from the continuous digital recordings which are then reviewed and adjusted by an operator. Individual heart rate-corrections (QTcl_{median}) are then applied to each median beat based on an associated off-drug *rate-binning* analysis (Extramiana, Badilini et al. 2007). Accordingly, Part II consisted in assessing the performance of three state-of-the-art continuous ECG measurement methods in the evaluation of a dataset characterized by abnormal waveform morphology and the evaluation of possible method-specific differences and their possible impact on cardiac repolarization assessments.

2.2. Publication Summary

Technological advances now enable the recording of high resolution, continuous digital ECGs which can be automatically annotated by various software applications and algorithms to perform detailed and precise cardiac repolarization assessments. Although fully automated QT analysis applications have been available for several years, methodologies employing the fully automated analysis of large datasets (millions of beats) are not yet routinely accepted by regulatory agencies for primary TQT analysis where sparse SA measurements performed by ECG core laboratories remain the gold standard. In the current study, three state-of-art computerized methods for continuous QT/QTc analysis were evaluated:

- BioQT (ver. 1.2, OBS Medical, Oxford)
- Ponemah with Pattern Recognition Option (ver. 5.0, Data Sciences International, ST. Paul, Minn.)
- WinAtrec (ver. 1.2.0, AMPS LLC, New York)

The objective of the current study was to assess the applicability of continuous QT/QTc analyses performed with three state-of-the-art QT measurement software applications to replicate the results and conclusions reported by an ECG core laboratory for a reference TQT study.

Despite slight vendor-specific differences in measurement variability, confidence intervals, number of valid measurements, and $\Delta\Delta\text{QTc}$ effect, the three ECG analytical software applications demonstrated requisite assay sensitivity and replicated the SA core-laboratory conclusions, with greatly improved temporal resolution and reduced analytical costs.

In conclusion, these continuous analysis methodologies provide previously unavailable temporal resolution for repolarization risk assessment, and enable the use of powerful additional analytical tools. With broader experience, these data suggest that current SA methodologies could be effectively replaced by fully automated ECG analysis.

2.3. Publication PRO vs. BioQT vs. WinAtrec



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Comparative TQT analysis with three fully-automated platforms: Comparison to core laboratory semi-automated results[☆]

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Background and purpose: Technological advances in machine-read QT measurement now enable detailed and precise cardiac repolarization assessments. This study assessed the applicability of three state-of-art ECG measurement applications to provide reliable continuous analyses from data obtained in a positive thorough QT study previously characterized with sparse semi-automated measurements performed by an ECG core laboratory.

Methods: Continuous RR, QT, QTc measurements, and individual QT/RR relationships and their associated intra- and inter-subject variability were derived in parallel with BioQT, Ponemah PRO, and WinAtrec analysis software.

Results: Despite slight vendor-specific differences in measurement variability and QTc, all machine-read methods demonstrated requisite assay sensitivity and yielded similar conclusions in accordance with SA analysis.

Conclusions: Three commercially available ECG analytical software applications reliably detected the drug-induced QT prolonging effects and replicated the SA core-laboratory conclusions, with greatly improved temporal resolution and reduced analytical costs. With broader experience, these data suggest that current SA methodologies could be effectively replaced by fully automated ECG analysis.

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Keywords:

Fully-automated QT analysis; QT measurement; Thorough QT study; ECG core laboratory

Introduction

Since the implementation of ICH E14 guidance, virtually all new drugs in clinical development have been required to demonstrate relative safety for the risk of inducing torsades de pointes (TdP), assessed as QT/QTc prolongation measured during thorough QT (TQT) studies. The assessment of cardiac repolarization during TQT studies is well-standardized and most sponsors generally outsource data analysis to ECG core laboratories specialized in semi-automated (SA) ECG annotations performed by well-trained cardiologists.

Technological advances now enable the recording of high resolution, continuous digital ECGs which can be automatically annotated by various software applications and algorithms.^{1–7} While most companies are currently working towards improving the precision of the sparse measurements

performed at scheduled time points,^{8–10} the inclusion of all the beats recorded during a TQT study would dramatically improve the temporal resolution of a study, providing more detailed and reliable time-dependent measurements of possible drug effects which are not possible with sparse sampling. However, consistent beat-to-beat automated QT measurement remains a complicated task as electrical noise, baseline drift, and T-wave morphological changes can greatly impact the accuracy and precision of computer-based ECG measurements.^{1,11} Currently, there are several commercially available or internally developed software applications which have been designed to facilitate accurate cardiac repolarization assessment by combining a machine-read ECG interval measurement with a refined QT/QTc analysis methodology. When compared to reference SA or manual evaluations, these applications have demonstrated equivalent QT/QTc assessments during TQT studies with generally reduced variability.^{1–6,12} Although fully automated QT analysis applications have been available for several years, methodologies employing the fully automated analysis of large datasets (millions of beats) are not yet routinely

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accepted by regulatory agencies for primary TQT analysis where sparse SA measurements performed by ECG core laboratories remain the gold standard. In the current study, three different machine-read methods for continuous QT/QTc analysis were characterized for their ability to accurately replicate the core laboratory results of a reference TQT study. The automated software applications evaluated were:

- BioQT (ver. 1.2, OBS Medical, Oxford)
- Ponemah with Pattern Recognition Option (ver. 5.0, Data Sciences International, ST. Paul, Minn.)
- WinAtrec (ver. 1.2.0, AMPS LLC, New York)

Briefly, BioQT is fully automated (no operator adjustments are required) and employs wavelet transforms and a Hidden Markov Model (HMM) to measure and self-check the QT intervals. The QT measurements for each beat are then individually heart rate-corrected based on an on-treatment sliding QT/RR regression (QTcIc).⁶ Ponemah PRO uses a pattern recognition algorithm where the QT interval for each beat is measured based on individual operator-adjusted templates. Distribution-based analysis is then applied to obtain individual heart rate-corrected QT values based on the off-drug QT/RR relationship (QTca).^{4,13} WinAtrec *time-binning* analysis automatically computes and measures 60 s and single lead median beats from the continuous digital recordings which are then reviewed and adjusted by an operator. Individual heart rate-corrections (QTcI_{median}) are then applied to each median beat based on an associated off-drug *rate-binning* analysis.⁷ The objective of the current study was to assess the applicability of continuous QT/QTc analyses performed with three state-of-the-art QT measurement software applications to replicate the results and conclusions reported by an ECG core laboratory for a reference TQT study. The current TQT study included placebo, two doses of saquinavir (SQR), a QT-prolonging drug that induces changes in T-wave morphology,¹⁴ and the standard clinical reference agent, moxifloxacin.

Materials and methods

Study design

Briefly, data used in this study were extracted from a TQT study previously evaluated by an ECG core laboratory. Both the current study design and semi-automated methodologies have been described in detail elsewhere.⁴

Machine-Read QT measurements

It is well-recognized that selection of the lead to be analyzed with machine-read methods is critical as electrical noise, baseline drift, and T-wave morphological changes will impact the accurate and reproducible determination of T-end offset.^{2,15} Accordingly, lead V4 was selected for all current machine-read ECG analyses as this lead was uniformly characterized by decreased motion artifacts, higher T-wave amplitudes, consistently well-inscribed T-offsets compared

to the other available leads, and has been shown to be appropriate when lead II is less suitable.¹⁶

BioQT is a fully-automated application which first computes a wavelet representation of the raw ECG signal by application of an undecimated wavelet transform (UWT). The UWT provides a time–frequency description of the ECG signal which is subsequently analyzed employing a Hidden Markov Model trained to segment and measures the ECG signal based on a database of reference ECG waveforms. The probabilistic nature of the Hidden Markov Model allows the computation of a proprietary *confidence measure* which quantifies the closeness of each analyzed waveform to the reference waveform database in order to identify and reject unreliable measurements characterized by either excessive noise or unusual waveform morphologies. BioQT provides an interface to review the different ECG waveform patterns and the associated caliper placements, allowing the user to visually confirm and adjust measurements when necessary. This review feature was not implemented in the present study such that all current BioQT data were comprised of fully automated measurements. All ECG complexes demonstrating *confidence measures* < 0.7 were excluded from the analysis.⁶

Ponemah PRO automatically adjudicates beat-to-beat fiducial marks based on conformational match to manually selected candidate waveforms (templates). The current waveform measurements were obtained as previously described⁴ with the further addition of all Q-wave regions which demonstrated $\geq 85\%$ conformational match to a template of the corresponding individual library.

WinAtrec software provides both *rate-binning* (the so-called “bin” method⁷) and *time-binning* analysis. *Rate-binning* analysis stratifies each QT measurement preceded by heart rate stability into 10 ms RR bins based on its associated RR value. Heart rate stability was defined as $RR - RR_{30s} \pm 20$ ms where RR_{30s} was the mean RR measured over a 30 s period preceding the measured QT. WinAtrec constructs median beats by aligning all beats within a specific RR bin to create a new, noise-reduced, approximated beat representative of the underlying ECG complexes. The second averaging method, *time-binning*, builds median beats from all beats measured within a given time period, irrespective of heart rate stability.⁷ Q-onset and T-offset calipers are automatically placed employing a threshold approach, and subsequently verified and/or adjusted by an operator (OM). In the current study, *time-binning* was performed using 60 s median beats. Importantly, WinAtrec allows the simultaneous analysis of a twelve lead (12L) composite lead and three operator-selected single leads without increasing the processing time. For the 12L composite lead, all leads are superimposed and an artificial lead designated as *vector magnitude* is computed by the algorithm as the square-root of the sum of the square of each lead. To avoid the excessive intra-reader variability associated with intensive manual adjustments, either lead V3, V2, or the 12L composite lead was used as a backup when lead V4 demonstrated excessive variability and/or algorithm-based measurement errors (17% of the subjects). Median beats computed from less than 10 beats or demonstrating a noise level > 3 μ V were excluded from the analysis.

Heart rate corrections

Each of the machine-read methods implemented vendor-specific variations for the application of individual (QTcI) and Fridericia (QTcF) rate-correction formulae, as well as various unique filtering parameters.

BioQT

Fridericia's formula was applied as $QTcF = QT/(RR_{50})^{1/3}$ for each beat where RR_{50} was the mean RR of 50 complexes centered on the QT to be measured. Additionally, an individual heart rate-correction based on the time variation of the on-treatment QT/RR relationship was derived as $QTcIc = QT + \Delta(QT/RR) \times (1000 - RR_{50})$ where $\Delta(QT/RR)$ was the gradient of the observed QT/RR linear regression over a 4 h sliding window centered on the beat to be corrected. A second level of beat exclusion was applied to QTcF where each derived QTcF which differed from the mean QTcF by more than three standard deviations was excluded from the analysis. This additional exclusion was not applied to the QTcIc.

To further investigate the individual QT/RR relationships obtained across methods, distribution-based log-linear individual β values and the associated R^2 (below) were derived from all QT and RR values obtained on day -1 with BioQT.

Ponemah PRO

Fridericia's formula was applied as $QTcF = QT/RR^{1/3}$ for each complex. All measured raw QT and RR values from each baseline Holter recording were utilized for the derivation of distribution-based individual QT rate-correction factors (β) and associated coefficients of determination (R^2) using a log-linear regression model [1].

$$\log(QT) = \beta \cdot \log(RR) + \alpha \quad [1]$$

Distribution-based rate-corrected QT (QTca) values were subsequently derived as $QTca = QT/RR^\beta$ for each raw QT value from each dataset as previously described.¹³

WinAtrec

The Fridericia formula was applied as $QTcF = QT_{\text{median}}/(RR_{\text{median}})^{1/3}$ for each median beat. For *rate-binning* analysis, each individual complex preceded by heart rate stability was stratified according to the preceding RR interval. A median beat representative of all QT intervals assigned to a specific RR bin was then computed, providing a QT-RR relationship derived from median beats. In the current study, a log-linear regression model [1] was applied to the *rate-binning*-derived QT/RR values. The resulting individual β values obtained from baseline 24 h recordings were then used for the derivation of an individually rate-corrected QT (QTcI_{median}) obtained as $QTcI_{\text{median}} = QT_{\text{median}}/(RR_{\text{median}})^\beta$ for each 60 s *time-binning* measurement.

Continuous ECG analysis

The large amount of data generated by continuous ECG analysis requires that beat-to-beat data be condensed into a suitable time scale for the presentation of summary data. In this study we divided each 24 h period into a series of consecutive 5 min segments and added an experimental time line indexed

such that the time of dosing (placebo, moxifloxacin, or saquinavir) occurred at 0 h. All continuous data (RR, raw QT, and QTc) for each time segment were represented by the average value of all measured beats or median beats within a given segment. To ensure consistent quality for the values thus obtained with the machine-read methods, we required that each 5 min segment contain at least 50 valid beats. This threshold value was selected as it provided a reasonable compromise between reliable time point representation and unnecessary data exclusion. If this minimum number was not attained, the corresponding segment was excluded from the summary analysis. Summary data, expressed as consecutive 5 min mean values, are presented as conventional x-y plots to reveal possible time-dependent changes.

Statistical analysis

For each method, raw measurement values were imported and processed with a custom spreadsheet (Microsoft Excel 2010, Redmond, Washington). Significant treatment-related differences between methods were analyzed using a linear mixed effects model with *method* (BioQT, Ponemah PRO, and WinAtrec), *treatment*, and *time* as fixed effects, and *subject* as random effect, in conjunction with Markov chain Monte Carlo credible intervals.¹⁷ On-drug effects assessed by machine-read methods were compared as the baseline-adjusted, placebo-corrected QTc and RR (double delta; $\Delta\Delta QTc$, $\Delta\Delta RR$) and their associated descriptive statistics including the number of measurements, standard deviations (SD), and confidence intervals (CI). For each time point, the baseline-adjusted QTc and RR (single delta; ΔQTc , ΔRR) and the $\Delta\Delta QTc$ and $\Delta\Delta RR$ were calculated as follows:

$$\begin{aligned} \Delta QTc_{\text{drug}} &= QTc_{\text{drug}} - QTc_{\text{predose baseline}} \\ \Delta QTc_{\text{placebo}} &= QTc_{\text{placebo}} - QTc_{\text{predose baseline}} \\ \Delta\Delta QTc_{\text{drug}} &= \Delta QTc_{\text{drug}} - \Delta QTc_{\text{placebo}} \end{aligned}$$

where *predose baseline* corresponded to the 1 h average value from -90 to -30 min obtained during the baseline recording (day -1). The reliability and performance of the machine-read methods were assessed by parallel Bland-Altman comparisons of moxifloxacin-induced changes in $\Delta\Delta QTc$ (QTcF and method-specific QTcI) and the associated statistical parameters with the ECG core laboratory SA analysis results at each scheduled time point. The variability and precision associated with each method were described as the mean intra-individual intra-time point SD, number of measurements, CI, and inter-subject SD for all 5 min segments. To further characterize potential systematic differences between methods, baseline (day -1) QT, RR, log-linear QT-RR β -values, and coefficients of determination (R^2) were compared using Bland-Altman analyses and expressed as bias + 95% limits of agreement. Adequate assay sensitivity was assessed as described in ICH E14.¹⁸ Statistical analyses were performed using R (R Development Core Team, 2009) and the R package *lme4* (Bates, Macchler, & Bolker, 2011) or Analyse-it Software (version 2.26, Leeds, United Kingdom). Where applicable, data are expressed as the mean \pm SD.

Results

ECG detection and processing

The current machine-read ECG analysis platforms implemented time-efficient measurement and review processes that were amenable to processing 24 h digital ECG datasets. Parallel beat-to-beat analysis with both BioQT and Ponemah PRO included ~25 and 30 MM valid beat measurements, respectively. With WinAtrec, the use of 60 s median beats condensed ~36 MM raw beats into 530 K valid median beat measurements. In comparison, the SA analysis performed by the ECG core laboratory resulted in the measurement of ~44 K beats (~0.1% of the total beats recorded) for the entire study.

Raw QT and RR interval analysis

Parallel analysis of baseline (day -1) intervals and parameters is depicted in Table 1 and confirms equivalent RR detection with all methods where Bland–Altman analysis demonstrated mean RR bias of -2.3 ± 55 ms (BioQT vs. Ponemah), -9.7 ± 62.9 ms (BioQT vs. WinAtrec) and -7.4 ± 56 ms (Ponemah vs. WinAtrec). RR values at scheduled time points assessed by SA were consistently increased compared to continuous methods, likely a consequence of the selection of replicates of 3 beats extracted during periods of heart rate stability. QT interval measurements were equivalent between BioQT and WinAtrec where the mean Bland–Altman bias was 2.8 ± 15 ms. However, while BioQT, WinAtrec, and SA were generally equivalent (Bland–Altman; data not shown), Ponemah PRO consistently demonstrated increased QT intervals compared to the other methods (Bland–Altman: BioQT vs. Ponemah: 22.9 ± 20.7 ms; WinAtrec vs. Ponemah 20.3 ± 22 ms; SA vs. Ponemah 23.7 ± 25.2). The consistently longer raw QT intervals obtained with Ponemah PRO were due to the use of operator-adjusted waveform templates where T-end was declared at the nadir of the T- and U-wave, resulting in longer QT measurements than the tangent-, threshold- or HMM-based algorithms employed with SA, WinAtrec and BioQT, respectively.

The intra-individual, intra-time point standard deviation (Intra-SD) was employed to assess the stability and reproducibility of QT/QTc measurements during short periods where QT variability was expected to remain constrained due to the stabilizing influence of QT hysteresis.¹⁹ The intra-SD was

limited with all methods, confirming that each analytical method measured the QT interval with high precision. As expected, intra-SD with BioQT and Ponemah PRO (5.0 ± 3.4 and 4.8 ± 3.6 ms, respectively) was slightly higher than with WinAtrec and SA (3.4 ± 3.2 and 3.7 ± 2.7 ms), due to the increased RR variability associated with beat-to-beat analysis compared to median beats (WinAtrec), or the average of triplicate complexes selected during periods of heart rate stability (SA). The intra-SD for both the QTcF and QTcA obtained with Ponemah PRO was increased compared to BioQT, WinAtrec, and SA (Table 1). The intra-SD differences obtained across methods were likely the consequence of methodological variations of RR averaging implemented in the QTc correction formulae. With Ponemah PRO, each QTc value was derived from each raw QT and RR pair. In contrast, the use of RR₅₀ with BioQT, RR_{median} with WinAtrec, and the average of RR measurements extracted from periods of RR stability with SA artificially reduces the intrinsic beat-to-beat RR variability which minimized the impact of the hysteresis on the QTc measurement.

QT–RR relationships

Even though BioQT employs an internal linear regression model to derive its associated QTc values, individual distribution-based log-linear QT/RR relationships were derived for each machine-read method to enable parallel assessment of potential measurement differences.¹³ With the three machine-read methods, individual β values measured from baseline (day -1) were generally equivalent, further confirmed by Bland–Altman analysis which demonstrated bias of -0.011 ± 0.075 (BioQT vs. Ponemah), 0.021 ± 0.085 (BioQT vs. WinAtrec), and -0.032 ± 0.1 (WinAtrec vs. Ponemah). The associated coefficients of correlation (R^2) were 0.982 ± 0.014 , 0.982 ± 0.013 , and 0.974 ± 0.017 for BioQT, Ponemah, and WinAtrec, respectively, demonstrating that reliable individual rate-correction factors were derived with each method, even during on-drug periods, where all R^2 values demonstrated reliable goodness-of-fit (Table 2).

Treatment effects

As depicted in Fig. 1, moxifloxacin-associated $\Delta\Delta$ QTc (QTcI and QTcF) prolongation and $\Delta\Delta$ RRR profiles were similar with all methods, and each of the investigated methods demonstrated the requisite assay sensitivity as described in the E14 guidance.¹⁸ In contrast, parallel

Table 1
Mean off-drug (Day -1) parameters for continuous methods and ECG core laboratory.

Baseline day -1		BioQT	Ponemah PRO	WinAtrec	ECG core laboratory
Raw intervals	n	50593	53382	56303	2114
	QT	387.1 ± 26.8	409.1 ± 29.3	388.6 ± 27.5	386.1 ± 26.6
	RR	938.8 ± 71.1	930.6 ± 70.3	924.8 ± 76.2	993.2 ± 146.9
Intra-individual intra-time point standard deviation	n	50593	53382	55204	2114
	QT	5.0 ± 3.4	4.8 ± 3.6	3.4 ± 3.2	3.7 ± 2.7
	QTcF	4.6 ± 2.0	9.1 ± 3.8	4.0 ± 3.2	4.6 ± 2.7
	QTcI	4.5 ± 2.8	7.5 ± 3.4	3.7 ± 3.0	N/A
	RR	64.8 ± 30.1	67.2 ± 31.7	34.3 ± 28.8	35.2 ± 24.7

QTcI corresponds to: QTcI for BioQT, QTcA for Ponemah PRO, and QTcMedian for WinAtrec.

Table 2
Mean individual QT/RR relationship correction factor (β) and associated coefficient of correlation (R^2).

QT/RR relationship	BioQT	Ponemah PRO	WinAtrec	
β Baseline (day – 1)	0.286 \pm 0.052	0.275 \pm 0.052	0.306 \pm 0.052	
	Placebo	0.303 \pm 0.053	0.293 \pm 0.046	0.315 \pm 0.051
	Moxi400	0.312 \pm 0.054	0.304 \pm 0.055	0.335 \pm 0.051
	SQR1000	0.336 \pm 0.068	0.345 \pm 0.074	0.384 \pm 0.072
	SQR1500	0.340 \pm 0.071	0.348 \pm 0.076	0.394 \pm 0.068
R^2 Baseline (day – 1)	0.982 \pm 0.014	0.982 \pm 0.013	0.974 \pm 0.017	
	Placebo	0.983 \pm 0.016	0.975 \pm 0.024	0.973 \pm 0.022
	Moxi400	0.980 \pm 0.022	0.979 \pm 0.019	0.976 \pm 0.020
	SQR1000	0.967 \pm 0.032	0.971 \pm 0.030	0.980 \pm 0.015
	SQR1500	0.971 \pm 0.023	0.976 \pm 0.018	0.977 \pm 0.019

analysis of SQR revealed quantitative methodological differences in $\Delta\Delta Q_{Tc}$. While both SA and WinAtrec detected mean Q_{Tc} dose-dependent increases of ~ 14 and ~ 23 ms for SQR1000 and SQR1500, respectively, both of the beat-to-beat measurement methods (BioQT and Ponemah PRO) demonstrated lesser mean $\Delta\Delta Q_{Tc}$ prolongations of ~ 10 and ~ 17 ms for SQR1000 and SQR1500, respectively (Table 3, Figs. 1–3). The increased SQR-induced $\Delta\Delta Q_{Tc}$ effects with SA and WinAtrec were consistent over the 24 h period and ranged from 4 to 10 ms compared to BioQT and Ponemah, depending upon the time point, as demonstrated in Table 4, which presents the analysis when restricted to the protocol-scheduled time points. In Figs. 1, 2 and 3, a dip was observed with WinAtrec at 13 h for all treatments. This decrease in $\Delta\Delta Q_{Tc}$ was the result of an aberrant and inconsistent Q_{Tc} increase during placebo treatment which translated to the $\Delta\Delta Q_{Tc}$ for all treatments. The aberrant Q_{Tc} increase with placebo occurred during low confidence periods of meals and/or unrestrained physical activities characterized by elevated noise and variability. As

such, the observed differences at this time point were considered outliers likely related to a method-specific difference in T-end declaration in the presence of abnormal T-wave morphology. While SA period analysis was based on 9 sparse scheduled time points which were fully populated with valid measurements (~ 4.9 K for 58 subjects), fully automated methods analyzed the entire 24 h datasets and consisted of 258 consecutive time points represented by ~ 110 K measurements. ECG analyses using fully automated methods must rely on a combination of algorithms, filters, and operator review to detect and reject unreliable measurements. The later steps result in missing values for some of the continuous time points. Similar to any large study, subjects had dyssynchronous starting times for digital ECG acquisition, resulting in under-represented time points at the beginning and end of the time-matched 24 h periods which were excluded from the overall analysis. As expected, SA provided valid measurements for virtually all the sparse time points where the drug-effect $\Delta\Delta Q_{Tc}$ analysis was based on mean values from 54.8 subjects per scheduled time point. In contrast, BioQT, Ponemah PRO, and WinAtrec $\Delta\Delta Q_{Tc}$ estimates were respectively based on mean values from 33.5, 39.7, and 47 subjects per 5 min time point for the entire 24 h (Table 3). These reductions in subject number reflect the application of conservative waveform filters with BioQT, the exclusion of unmatched complexes with Ponemah PRO, and the computation of median beats with WinAtrec.

Discussion

In the current study, continuous repolarization assessments were performed with three specialized QT measurement software applications which yielded similar conclusions, all

Table 3
Mean $\Delta\Delta Q_{Tc}$ treatment effect and associated variability from all time points.

Treatment	Correction	BioQT ^a				Ponemah PRO ^a			
		n	mean effect	SD	CI	n	mean effect	SD	CI
Moxi400	QTcF	36.6 \pm 5.3	7.9 \pm 2.4	7.9 \pm 1.0	2.7 \pm 0.4	40.8 \pm 4.6	7.8 \pm 2.3	10.6 \pm 1.3	3.4 \pm 0.6
	QTcl	36.6 \pm 5.3	9.1 \pm 2.8	11.1 \pm 1.7	3.8 \pm 0.6	40.8 \pm 4.6	7.9 \pm 2.3	11.3 \pm 1.3	3.6 \pm 0.6
SQR1000	QTcF	31.5 \pm 6.0	9.4 \pm 2.2	12.5 \pm 2.0	4.7 \pm 1.1	37.6 \pm 5.3	10.6 \pm 2.7	15.2 \pm 1.5	5.1 \pm 0.7
	QTcl	31.5 \pm 6.0	9.9 \pm 3.1	15.3 \pm 1.7	5.7 \pm 1.1	37.6 \pm 5.3	9.0 \pm 2.5	15.4 \pm 1.5	5.1 \pm 0.7
SQR1500	QTcF	32.4 \pm 5.9	16.2 \pm 2.1	12.1 \pm 1.4	4.4 \pm 0.9	40.7 \pm 4.9	19.5 \pm 2.6	17.7 \pm 1.4	5.6 \pm 0.6
	QTcl	32.4 \pm 5.9	16.6 \pm 2.9	15.3 \pm 2.0	5.6 \pm 1.1	40.7 \pm 4.9	17.5 \pm 2.7	19.0 \pm 1.5	6.1 \pm 0.7
		WinAtrec ^a				ECG core laboratory ^b			
		n	mean effect	SD	CI	n	mean effect	SD	CI
Moxi400	QTcF	47.3 \pm 1.9	9.3 \pm 2.9	8.6 \pm 1.0	2.5 \pm 0.3	53.1 \pm 2.1	9.5 \pm 2.4	10.2 \pm 1.1	2.8 \pm 0.3
	QTcl	47.3 \pm 1.9	9.5 \pm 2.9	8.9 \pm 1.0	2.6 \pm 0.3	53.8 \pm 2.0	9.1 \pm 2.4	10.3 \pm 1.0	3.2 \pm 0.0
SQR1000	QTcF	45.8 \pm 2.4	14.0 \pm 3.0	13.3 \pm 1.2	4.0 \pm 0.4	54.3 \pm 2.3	14.4 \pm 3.3	13.3 \pm 1.2	3.6 \pm 0.4
	QTcl	45.8 \pm 2.4	13.5 \pm 2.9	13.4 \pm 1.2	4.0 \pm 0.4	54.9 \pm 2.3	13.9 \pm 3.2	13.5 \pm 1.5	3.2 \pm 0.0
SQR1500	QTcF	47.9 \pm 2.8	23.8 \pm 3.1	15.2 \pm 1.2	4.4 \pm 0.3	56.2 \pm 2.3	22.8 \pm 4.1	14.4 \pm 1.5	3.9 \pm 0.4
	QTcl	47.9 \pm 2.8	23.3 \pm 3.1	15.2 \pm 1.2	4.4 \pm 0.4	56.7 \pm 2.2	22.7 \pm 4.3	14.3 \pm 1.5	3.1 \pm 0.0

n = Average subject number at each time point; Effect = Average drug-induced effect (ms) over 24 h; SD = Average of each time point associated between-subject standard deviation (ms); CI = Average of each time point associated confidence intervals (ms); QTcl corresponds to: QTcl for BioQT, QTcS for Ponemah PRO, QTclmedian for WinAtrec, QTcS for ECG core laboratory.

^a Average over 258 continuous time points.

^b Average over 9 protocol scheduled time points.

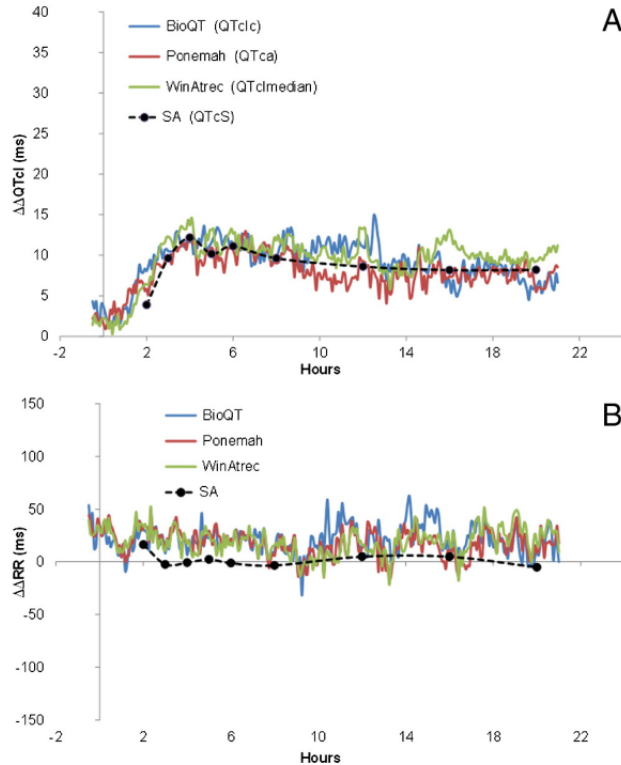


Fig. 1. Effects of moxifloxacin. The effects of moxifloxacin are presented for $\Delta\Delta QTcI$ (Panel A) and $\Delta\Delta RR$ (Panel B). Machine-read and semi-automated methods each demonstrated similar $\Delta\Delta QTcI$ and $\Delta\Delta RR$ moxifloxacin-induced increases over time, confirming that each method detected an equivalent reference pharmacological effect.

of which were in accordance with the reference SA results reported by an ECG core laboratory. Although each machine-read method would have been well suited for the analysis of the current study, continuous assessment of cardiac repolarization employing computer assisted techniques is an emerging trend and the comprehensive understanding of method-specific features and their possible impact is an important step toward the optimal utilization of such analyses.

BioQT

Continuous ECG analyses are generally a time-consuming process which consists of converting digital ECG data into a software-specific readable format, measuring ECG intervals with the software algorithm, and applying analysis/statistical algorithms to the resulting ECG measurements. As expected, a fully-automated method not requiring human intervention, such as BioQT, was more time-efficient than methods requiring visual verification and/or manual measurement adjustments. However, omitting the careful quality check performed by a skilled operator can occasionally result in the inclusion of systematic algorithm-based errors,⁴ even after the application of appropriate filters and automatic self-verification. Nonetheless, the BioQT algorithm, in conjunction with proprietary *confidence measures*, demonstrated robust fully-automated measurements where the intra-individual intra-time point SD for QT was tightly constrained, demonstrating that the need for extensive operator-

adjustments in the current study was not required. However, the application of the *confidence measure* excluded several million beats which were included with the other methods, thus resulting in a slight increase in the confidence intervals associated with drug effect evaluations due to reduced number of subject measurements for each 5 min time point. Moreover, unique to BioQT, was the utilization of RR_{50} for QTc derivation. This method minimizes intrinsic beat-to-beat RR variability and reduces the impact of hysteresis, resulting in a reduced intra-SD for QTc compared to Ponemah, which did not implement RR averaging into the QTc formula (Table 1). These results suggest that when effective QT-RR hysteresis correction cannot be implemented,²⁰ the use of RR averaging is likely to reduce the variability associated with QTc measurements.

Ponemah PRO

Ponemah PRO enables the user to finely adapt the software parameters in order to reliably and accurately measure morphologically abnormal, drug-induced, or species-specific waveforms. Operator-adjusted waveform templates resulted in a consistent raw QT increase of ~ 20 ms in the current analyses. However, as TQT study analysis relies on baseline- and placebo-adjusted ($\Delta\Delta$) QTc values, the absolute and relative differences in raw QT did not affect the detection of the drug effect, confirmed by the observation

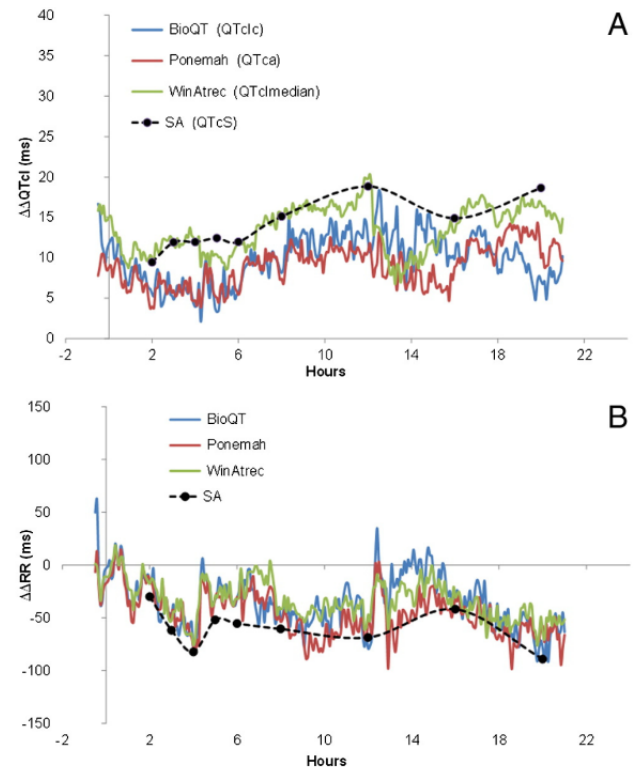


Fig. 2. Effects of SQR1000. The effects of SQR1000 are presented for $\Delta\Delta QTcI$ (Panel A) and $\Delta\Delta RR$ (Panel B). Both WinAtrec and SA demonstrated increased QT prolongation (~ 4 ms) compared to BioQT and Ponemah, likely a consequence of SQR-induced T-wave morphological changes which resulted in systematically different T-end adjudications with each method. All methods detected similar $\Delta\Delta RR$ changes.

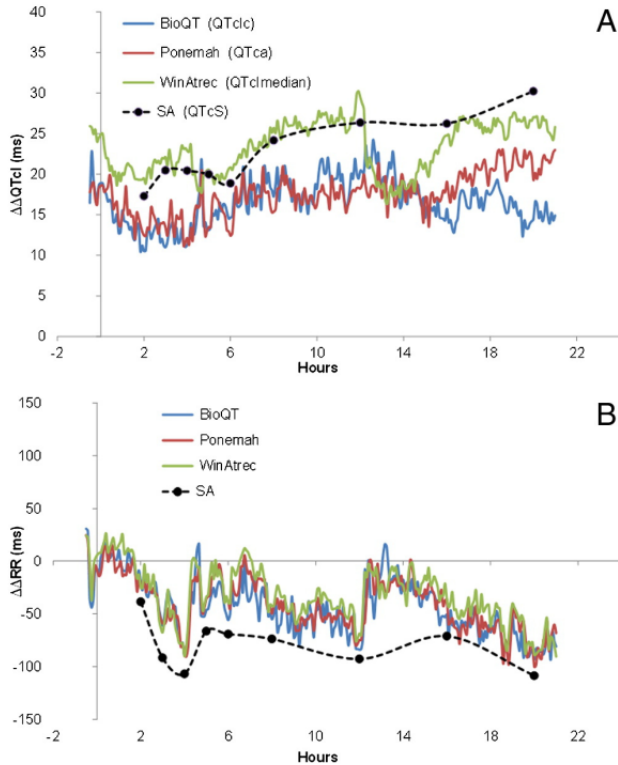


Fig. 3. Effects of SQR1500. The effects of SQR1500 are presented for $\Delta\Delta QTcI$ (Panel A) and $\Delta\Delta RRR$ (Panel B). Similar to Fig. 2, WinAtrec and SA demonstrated increased QT prolongation (~6 ms) compared to BioQT and Ponemah. All methods detected similar $\Delta\Delta RRR$ changes.

that all automated methods demonstrated similar moxifloxacin-associated $\Delta\Delta QTcI$ increases over time (Fig. 1). With Ponemah PRO, the templates are adjusted by the operator prior to the automated measurements and, thus, require that the operator be well-trained and experienced to limit any possible bias that may be introduced by intra-observer variability. Overall, Ponemah PRO was the most detailed and adaptable platform, but was also the most time and resource intensive of the evaluated automated methods.

WinAtrec

Unique to WinAtrec is the creation of median beats from all beats over a given time period. While this feature can

occasionally create abnormal median beats due to the averaging of normal and abnormal complexes, it generally facilitates the subsequent automated measurements and limits the number of required operator adjustments. In addition, the simultaneous analysis of supplementary leads allowed the recovery of a subject which demonstrated flat T-waves in lead V4, precluding the need to perform a second analysis. Compared to purely beat-to-beat methodologies, WinAtrec could arguably be slightly less well-suited when rapid or detailed ECG changes are of primary interest. However, in the current study, WinAtrec yielded increased numbers of subjects demonstrating a valid measurement per time point, reduced raw QT variability, and the most constrained confidence intervals. These findings demonstrate that the use of median beats can accelerate and facilitate the analysis of large numbers of ECG waveforms, and would thus be well-suited for TQT analyses where the main objective is to reliably detect a threshold pharmacologic effect on cardiac repolarization.

Individual heart rate correction

All machine-read methods demonstrated generally equivalent β values with uniformly high R^2 values (Table 2). With all methods, similar drug-induced effects were detected with both QTcI/QTcS and QTcF, again confirming that QTcF is generally a suitable rate-correction method in man when the drug-induced heart rate changes are not substantial.²¹ As expected, the QTcF rate-correction factor of 0.333 approximated the mean of QTcI and the QTcS β values (0.289 ± 0.053 and 0.325 , respectively). For both QTca and QTcI_{median}, the confidence intervals were similar to those obtained with QTcF for Ponemah PRO and WinAtrec, respectively (Table 3). In contrast, the application of QTcI_c for BioQT demonstrated increased CIs compared to QTcF. The use of the BioQT sliding on-drug QT/RR relationship measured over short observation periods may overcome possible QT/RR relationship variations in the presence of drug, between days, day and night, and even periods of the day.²² However, this approach also narrows the number of QT/RR pairs and RR ranges available for QT/RR modeling. Moreover, the application of QT/RR rate-correction factors derived from 4 h periods of on-drug data may further

Table 4
Mean $\Delta\Delta QTcI$ treatment effect at protocol-scheduled time points.

Treatment	Method	Mean $\Delta\Delta QTcI$									
		2 h	3 h	4 h	5 h	6 h	8 h	12 h	16 h	20 h	All Time points
Moxi400	BioQT	8.1	10.6	11.7	11.2	11.4	9.3	11.3	6.7	5.9	9.6
	Ponemah	6.0	9.6	11.5	10.7	10.8	9.0	9.4	7.2	6.6	9.0
	WinAtrec	6.3	12.0	13.8	12.7	12.9	11.8	13.3	12.6	9.4	11.6
	SA	3.9	9.6	12.2	10.2	11.1	9.6	8.6	8.2	8.2	9.1
SQR1000	BioQT	6.0	5.2	4.6	4.2	5.3	8.6	13.7	10.0	7.3	7.2
	Ponemah	4.2	6.1	4.7	5.4	5.9	7.8	10.6	8.0	12.4	7.2
	WinAtrec	10.0	12.3	12.3	10.3	11.5	15.5	19.9	13.7	16.7	13.6
	SA	9.4	11.9	11.9	12.4	11.9	15.1	18.9	14.9	18.7	13.9
SQR1500	BioQT	10.7	11.4	11.2	13.5	14.9	17.9	20.0	14.9	14.2	14.3
	Ponemah	13.1	13.8	11.6	13.0	12.8	15.6	18.3	18.3	21.4	15.3
	WinAtrec	19.4	22.0	23.1	20.4	20.6	25.5	29.9	24.6	26.7	23.6
	SA	17.3	20.5	20.4	20.0	18.9	24.2	26.4	26.2	30.2	22.7

confound the data interpretation as each 4 h period may be characterized by temporally variable drug concentrations which could result in unstable QT/RR relationships.

Treatment effects

While each automated method satisfied the requisite E14 assay sensitivity and detected similar moxifloxacin-induced $\Delta\Delta\text{QTc}$ increases, $\Delta\Delta\text{QTc}$ for SQR was consistently lower (~4 to 10 ms) with beat-to-beat methods (BioQT and Ponemah) compared to WinAtrac and SA (Figs. 2–3; Tables 3–4). These findings demonstrate that, even after careful review processes, the utilization of method-specific features (beat-to-beat, beat averages, and median beats) and/or T-end measurement algorithms (tangent, threshold, HMM, and pattern recognition) resulted in consistent and potentially substantial quantitative differences in $\Delta\Delta\text{QTc}$ when applied to abnormal waveforms, such as those associated with SQR-induced T-wave morphological changes. Importantly, while the SA method is the current gold standard, the various method-specific features employed in the 3 automated methods preclude any firm conclusions regarding the relative accuracy of the quantitative QTc assessments associated with each evaluated method. However, in light of the current results, it is possible that some of the evaluated methods would yield discordant conclusions for threshold pharmacologic effects on cardiac repolarization.

Conclusion

Despite slight vendor-specific differences in variability, confidence intervals, number of valid measurements, and $\Delta\Delta\text{QTc}$ effect, all current machine-read methods demonstrated the ability to reliably detect the drug-induced QT prolonging effects of both moxifloxacin and SQR, as previously characterized by an ECG core laboratory. Collectively, the current results demonstrate that three fully-automated ECG measurement applications replicated the conclusions obtained by conventional sparse core laboratory SA measurements. Moreover, these methodologies provide previously unavailable temporal resolution for repolarization risk assessment, and enable the use of powerful additional analytical tools, such as $\Delta\Delta\text{QTc}$ AUC over pre-specified intervals as a unitary endpoint based upon all observations. These capabilities offer the potential for greatly improved repolarization risk assessment while simultaneously reducing the associated manpower requirements and study costs. Fully automated ECG analysis now merits serious consideration as an adjunct or replacement for sparse SA methodologies.

Limitations

The current study demonstrates the drug-induced QT prolonging effects of moxifloxacin and SQR obtained with three fully-automated ECG measurement methodologies. While generally equivalent and expected drug effects were detected with all methods, generalization of the current results will require further validation with other drugs and

combinations that more significantly affect the heart rate or the QT/RR relationship.

References

1. Sarapa N, Gussak I, Vajdic B, et al. Comparison of QTinno, a fully automated electrocardiographic analysis program, to semiautomated electrocardiographic analysis methods in a drug safety study in healthy subjects. *J Electrocardiol* 2009;42:358.
2. Hnatkova K, Gang Y, Batchvarov VN, Malik M. Precision of QT interval measurement by advanced electrocardiographic equipment. *Pacing Clin Electrophysiol* 2006;29:1277.
3. Couderc JP, Garnett C, Li M, et al. Highly automated QT measurement techniques in 7 thorough QT studies implemented under ICH E14 guidelines. *Ann Noninvasive Electrocardiol* 2011;16:13.
4. Meyer O, Ferber G, Greig G, Holzgrefe HH. Pattern recognition analysis of digital ECGs: decreased QT measurement error and improved precision compared to semi-automated methods. *J Electrocardiol* 2013;46:118.
5. Tyl B, Kabbaj M, Fassi B, De Jode P, Wheeler W. Comparison of semiautomated and fully automated methods for QT measurement during a thorough QT/QTc study: variability and sample size considerations. *J Clin Pharmacol* 2009;49:905.
6. Strachan IG, Hughes NP, Poonawala MH, Mason JW, Tarassenko L. Automated QT analysis that learns from cardiologist annotations. *Ann Noninvasive Electrocardiol* 2009;14(Suppl 1):S9.
7. Extramiana F, Badilini F, Sarapa N, Leenhardt A, Maison-Blanche P. Contrasting time- and rate-based approaches for the assessment of drug-induced QT changes. *J Clin Pharmacol* 2007;47:1129.
8. Darpo B, Fossa AA, Couderc JP, et al. Improving the precision of QT measurements. *Cardiol J* 2011;18:401.
9. Hingorani P, Kamad DR, Ramasamy A, et al. Semiautomated QT interval measurement in electrocardiograms from a thorough QT study: comparison of the grouped and ungrouped superimposed median beat methods. *J Electrocardiol* 2012;45:225.
10. George S, Rodriguez I, Ipe D, et al. Computerized extraction of electrocardiograms from continuous 12-lead holter recordings reduces measurement variability in a thorough QT study. *J Clin Pharmacol* 2012;52:1891.
11. Tyl B, Azzam S, Blanco N, Wheeler W. Improvement and limitation of the reliability of automated QT measurement by recent algorithms. *J Electrocardiol* 2011;44:320.
12. Green CL, Kligfield P, George S, et al. Detection of QT prolongation using a novel electrocardiographic analysis algorithm applying intelligent automation: prospective blinded evaluation using the Cardiac Safety Research Consortium electrocardiographic database. *Am Heart J* 2012;163:365.
13. Holzgrefe HH, Ferber G, Morrison R, et al. Characterization of the human QT interval: novel distribution-based assessment of the repolarization effects of moxifloxacin. *J Clin Pharmacol* 2012;52:1222.
14. Zhang X, Jordan P, Cristea L, et al. Thorough QT/QTc study of ritonavir-boosted saquinavir following multiple-dose administration of therapeutic and supratherapeutic doses in healthy participants. *J Clin Pharmacol* 2012;52:520.
15. Zhou X, Wei D. A multidifferentiator-based approach to the reliable determination of T-wave offset in electrocardiograms. *J Electrocardiol* 2011;44:330.
16. Salvi V, Kamad DR, Kerkar V, et al. Choice of an alternative lead for QT interval measurement in serial ECGs when lead II is not suitable for analysis. *Indian Heart J* 2012;64:535.
17. Chen MH, Shao QM. Monte Carlo estimation of Bayesian credible and HPD intervals. *J Comput Graph Stat* 1999;8:69.
18. FDA. Guidance for industry: clinical evaluation of QT/QTc interval prolongation and proarrhythmic potential for non-antiarrhythmic drugs (E14). <http://www.fda.gov/downloads/RegulatoryInformation/Guidances/UCM129357.pdf> 2005.
19. Lau CP, Freedman AR, Fleming S, et al. Hysteresis of the ventricular paced QT interval in response to abrupt changes in pacing rate. *Cardiovasc Res* 1988;22:67.

20. Malik M, Hnatkova K, Novotny T, Schmidt G. Subject-specific profiles of QT/RR hysteresis. *Am J Physiol Heart Circ Physiol* 2008;295:H2356.
21. Gamett CE, Zhu H, Malik M, et al. Methodologies to characterize the QT/corrected QT interval in the presence of drug-induced heart rate changes or other autonomic effects. *Am Heart J* 2012; 163:912.
22. Bexton RS, Vallin HO, Camm AJ. Diurnal variation of the QT interval—influence of the autonomic nervous system. *Br Heart J* 1986;55:253.

2.4. Supplementary analysis on other QT studies

2.4.1. BP22464

A similar analysis was performed with the TQT study including the SGLT2 inhibitor. As previously described (Figure 46), the two doses of SGLT2 inhibitor were not associated with any QT/QTc interval prolongation. The analysis performed with WinAtrec was in accordance with the results previously obtained, as all methods demonstrated the expected moxifloxacin-induced QT prolongation and a negative SGLT2i-induced effect (Table 7). Interestingly, while ALG, WinAtrec, and SA results were virtually superimposable (Figure 49), the $\Delta\Delta\text{QTca}$ obtained with Ponemah PRO was consistently reduced by ~ 2 ms (Table 7). The $\Delta\Delta\text{QTca}$ effect was quantitatively reduced with Ponemah PRO compared to methods employing tangent (SA) and threshold ECG measurement algorithms (ALG, WinAtrec). Similar differences were also present with SQR (Table 4 from 2nd article) where both Ponemah PRO and BioQT demonstrated reduced quantitative $\Delta\Delta\text{QTca}$ effects after SQR administration. These results suggest that while each method yielded similar conclusions, quantitative QT prolongation differences are likely depending on the measurement method employed and the potential of the assessed drug to prolong the QT interval or alter the T-wave morphology.

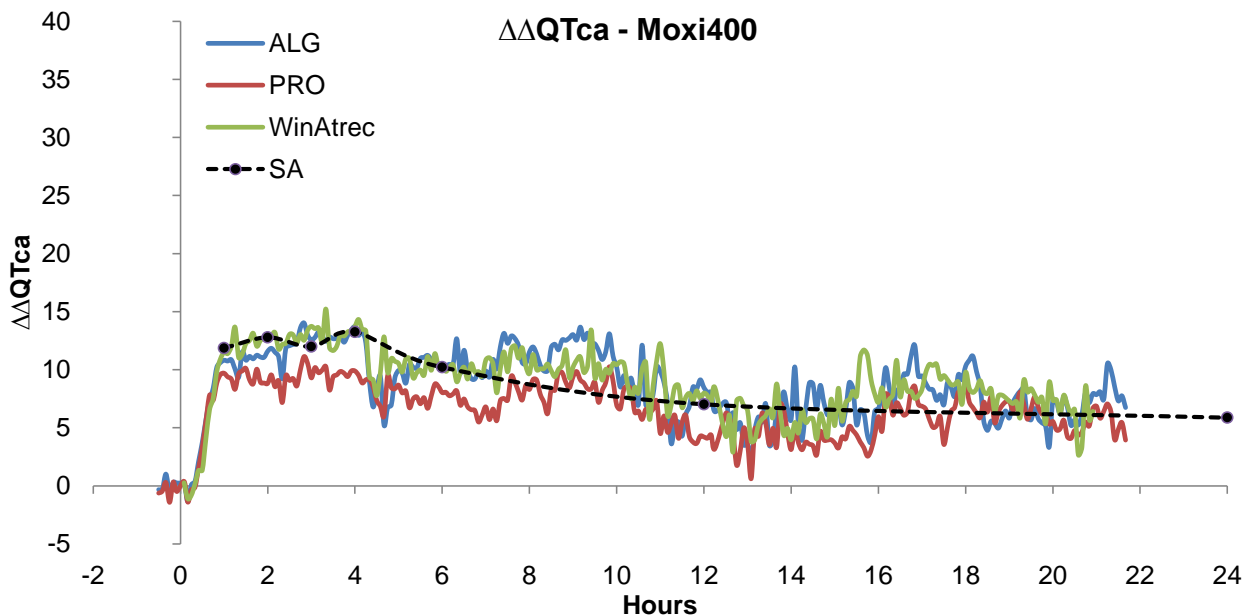


Figure 49. Moxifloxacin-induced $\Delta\Delta\text{QTca}$ effect with all methods (Figure 47 + WinAtrec). The application of PRO resulted in slightly reduced moxifloxacin-induced effects compared to ALG, WinAtrec, and SA.

Treatment	ALG				PRO			
	n	Mean effect	SD	CI	n	Mean effect	SD	CI
Moxi400	44.1 ± 3.2	8.6 ± 3.2	10.0 ± 2.7	3.1 ± 0.9	44.3 ± 4.5	6.5 ± 2.5	9.3 ± 1.9	2.9 ± 0.7
SGLT40	43.7 ± 3.8	0.0 ± 1.5	9.2 ± 2.6	2.8 ± 0.9	44.4 ± 4.8	0.6 ± 1.2	8.3 ± 1.8	2.5 ± 0.6
SGLT400	43.6 ± 2.8	-0.7 ± 1.6	9.7 ± 2.7	3.0 ± 0.9	44.6 ± 4.1	-0.6 ± 1.4	8.9 ± 1.7	2.7 ± 0.6

Treatment	WinAtrec				Semi-automated			
	n	Mean effect	SD	CI	n	Mean effect	SD	CI
Moxi400	44.0 ± 1.5	8.9 ± 2.9	9.1 ± 3.0	2.8 ± 1.0	50.7 ± 0.5	10.3 ± 2.9	7.6 ± 0.8	2.1 ± 0.2
SGLT40	47.6 ± 2.2	0.5 ± 1.5	7.9 ± 3.0	2.3 ± 0.9	51.9 ± 0.4	-0.1 ± 1.9	7.0 ± 0.8	2.0 ± 0.2
SGLT400	45.2 ± 1.7	-0.9 ± 1.4	7.8 ± 2.6	2.3 ± 0.8	51.9 ± 0.4	-2.2 ± 1.3	7.0 ± 1.1	1.9 ± 0.3

Table 7. Descriptive statistics of $\Delta\Delta\text{QTc}$ for each treatment with all methods (Table 6 + WinAtrec). The application of PRO resulted in slightly reduced moxifloxacin-induced effects compared to ALG, WinAtrec, and SA. WinAtrec provided the continuous analysis the most constrained variability.

2.5. Conclusion

The results obtained with 5 ECG measurement methods (BioQT, ALG, PRO, WinAtrec, SA) from 2 TQT studies employing a QT prolonging drug with associated T-wave morphology alterations (SQR) and a non-QT prolonging drug (SGLT2i) yielded similar comparative results. In both TQT studies, each method effectively detected the positive moxifloxacin-induced effect and demonstrated appropriate assay sensitivity. Both the positive SQR-induced and negative SGLT2i-induced effects were accurately detected with each method, demonstrating that continuous computerized methods can reliably supplant SA for TQT primary analyses. All evaluated methods were characterized by several unique vendor-specific features which can impact variability, confidence intervals, numbers of valid measurements, and the quantitative $\Delta\Delta\text{QTc}$ effect. Collectively, the current results demonstrate that continuous ECG analysis results obtained employing computerized software applications in conjunction with refined analytical methods are reliable and provide improved temporal resolution which is not possible with conventional sparse ECG sampling. The simultaneous reduction in the TQT associated costs and manpower requirements further supports the use of continuous ECG evaluation as an effective alternative to current resource intensive semi-automated methods.

3. Part III: Continuous ECG Analysis refinements and recommendations

3.1. Rationale

In the context of E14-compliant studies evaluating continuous drug effects on the QT interval, four principal improvement possibilities exist:

- The development or utilization of more precise ECG recording devices
- The utilization of better suited study protocols
- The improvement of QT interval measurement methods (manual or computerized)
- The refinement of analysis methodologies.

These 4 areas of improvement are all inter-connected. For example, the use of low frequency ECG recordings (<500 Hz) will reduce the performance of any algorithm to precisely measure the raw QT interval. Similarly, even perfectly measured raw QT intervals will provide highly variable results if the analysis methodology employs sub-optimal QTc rate-correction (e.g. QTcB) or inappropriate time resolution. It is therefore of primary importance to both the industry and the regulatory agencies that QT and QTc interval be precisely and accurately measured. Obtaining reliable and robust results with constrained confidence intervals is required in TQT studies as it reduces the risk of obtaining a positive signal due to an isolated time point characterized by high variability and wide confidence intervals which may exceed the 10 ms regulatory threshold. Many factors have the potential to increase or decrease the variability associated with a drug-effect assessment. The variability associated with QT measurements can be of two origins; some part of the QT variability is due to normal physiologic conditions, while the remaining part can be attributed to artificial (technical or methodological) causes, such as raw QT measurement imprecision, inappropriate hysteresis or rate-correction, or other bias introduced by the analyst. In Part III, major sources of variability (physiological, technical, and pharmacological) were reviewed in order to refine the current continuous ECG analysis methodology by reducing the inherent variability while maintaining high temporal resolution to provide robust cardiac repolarization assessments. Part III presented and summarized recommendations on how to reduce the methodological variability to improve the quality of the data without artificially smoothing the physiological variability which would result in a loss of scientific information.

3.2. Measurement adjustments and lead choice

The analysis of the SGLT2i study was performed on leads II, V2, V5, and 12L. Operator-adjustments were only performed for the 12L composite beat, while leads II, V2, and V5 consisted of the fully-automated measurements by the WinAtrec threshold algorithm. With leads II, V5, and 12L, the treatment effects with moxifloxacin and both doses of SGLT2i were very similar and only demonstrated QT prolongation effects with moxifloxacin (Figure 50A, B, and C). The comparison of leads II, V5, and 12L for moxifloxacin yielded virtually superimposable results, with increased variability during periods of lower confidence (unrestrained activity) for non-adjusted measurements (Figure 51). The results obtained after operator-adjustments demonstrated slightly reduced standard deviations and confidence intervals compared to fully-automated annotation of leads II and V5 (Table 8). Collectively, these results suggest that absent drug-induced T-wave morphological changes, WinAtrec still provides precise QT interval measurements with a limited number of measurements errors. While the operator-adjustments reduce the variability associated with the drug-effect measurement, WinAtrec resulted in consistent drug-induced effect evaluations irrespective of the selected lead or operator adjustments. These results further confirm that fully-automated methods are reliable when the presence of abnormal ECG waveforms is not expected (Fosser, Duczynski et al. 2009).

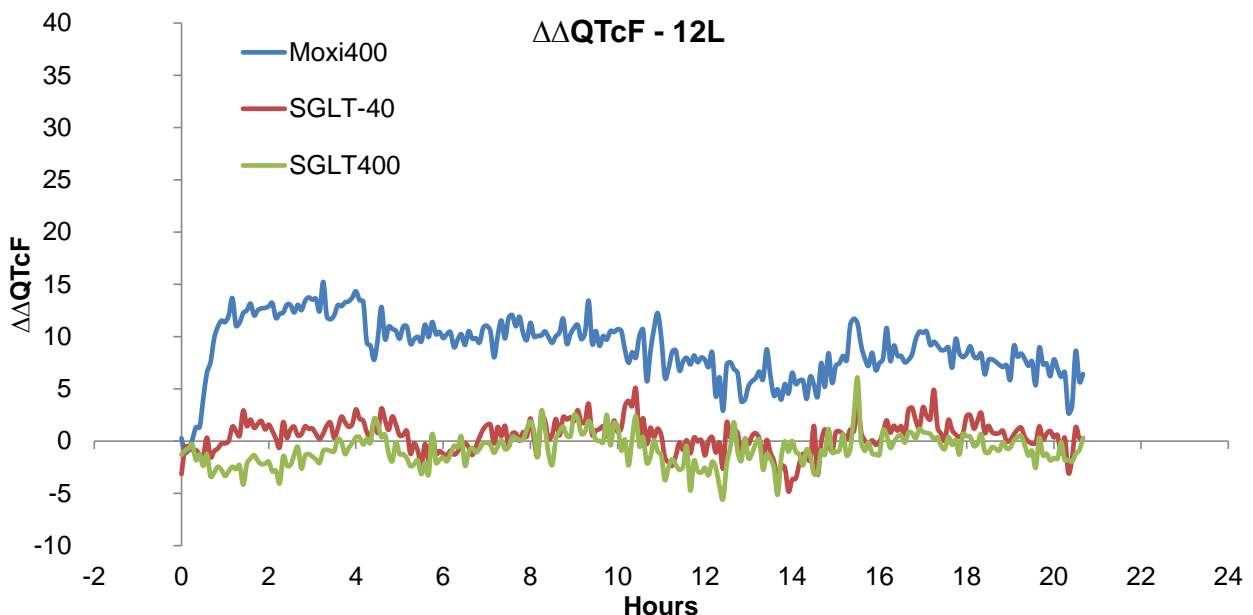


Figure 50A. WinAtrec-derived $\Delta\Delta\text{QTcF}$ for each treatment with 12L average. The 12L average were reviewed and adjusted. WinAtrec method demonstrated the expected moxifloxacin-related QTc increase of 10–14 ms and a negative QTc effect with SGLT2i.

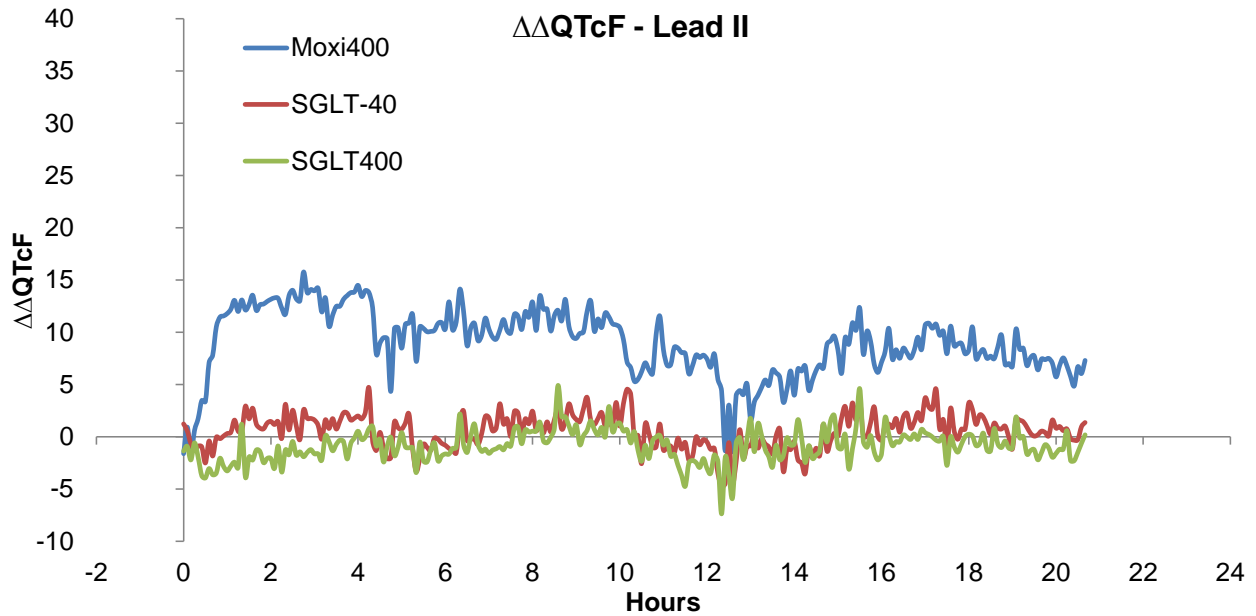


Figure 50B. WinAtrec-derived $\Delta\Delta\text{QTcF}$ for each treatment with lead II. Lead II was not reviewed or adjusted. Compared to figure 50A, the use of an unadjusted lead resulted in higher variability.

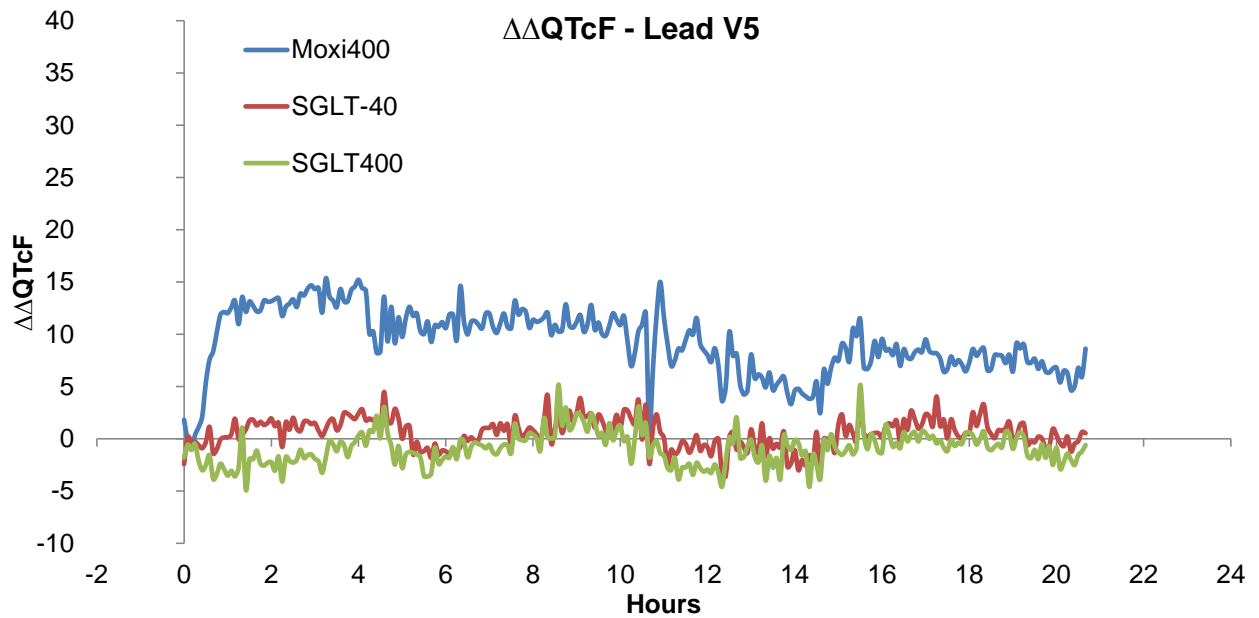


Figure 50C. WinAtrec-derived $\Delta\Delta\text{QTcF}$ for each treatment with lead V5. Lead V5 was not reviewed or adjusted. Compared to figure 50A, the use of an unadjusted lead resulted in higher variability.

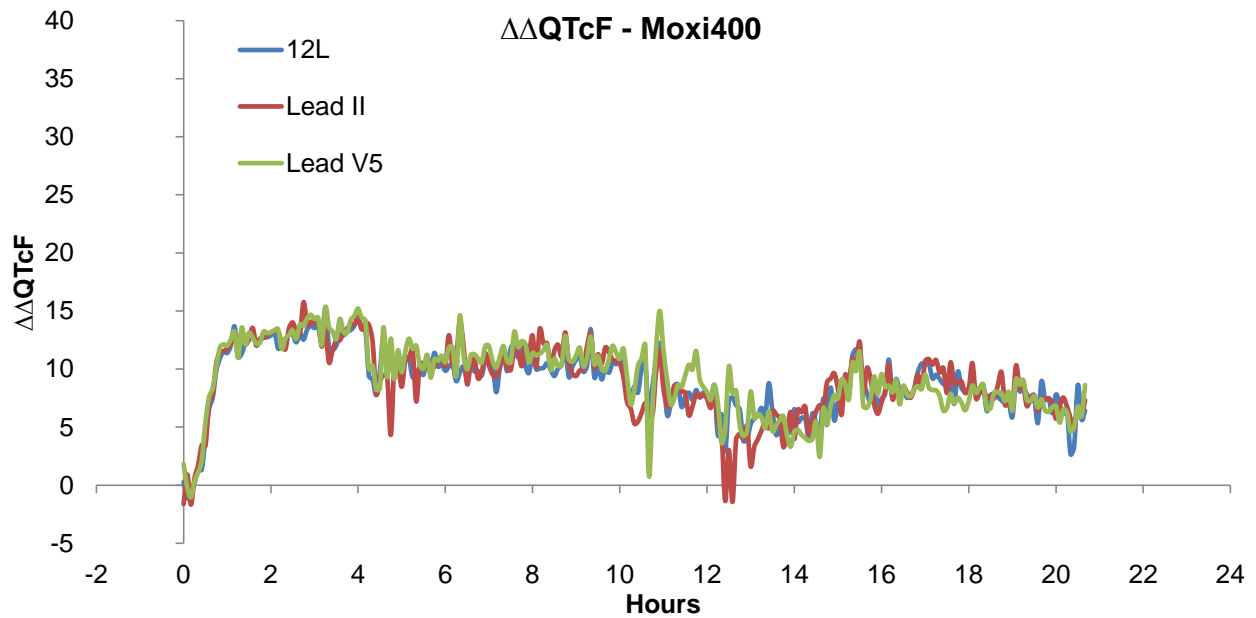


Figure 51. Moxifloxacin-induced $\Delta\Delta\text{QTcF}$ effects for each lead.

WinAtrec - Moxi400				
Lead	n	Mean effect	SD	CI
12L	44.0 ± 1.5	8.9 ± 2.9	9.1 ± 3.0	2.8 ± 1.0
V5	43.6 ± 2.1	9.3 ± 3.2	9.6 ± 3.5	2.9 ± 1.1
II	42.3 ± 3.8	9.0 ± 3.2	10.6 ± 4.7	3.3 ± 1.6

Table 8. Descriptive statistics of moxifloxacin-induced $\Delta\Delta\text{QTcF}$ effects for each lead. The reviewed and adjusted lead 12L demonstrated the most constrained confidence intervals and standard deviation.

3.3. Time segments

When analyzing continuous ECG recordings, the large amount of data generated requires that beat-to-beat data be condensed into a suitable time scale for the presentation of summary data. The length of the time segments translates into the time resolution of the results, reflected in the detail of the summary data, which may or may not depict rapid parameter changes. Generally, for longer time segments, the results are smoother, and variability is lower. However, while reducing the variability may be of interest, using longer time segments also obscure fast protocol driven effects. In contrast, reducing the length of the time segment may result in increased variability, less appropriate time matching, and increased data handling complexity.

Accordingly, a comparison was performed for both ΔQT_{ca} and $\Delta\Delta QT_{ca}$ using 1, 2, 3, 5, and 10 min time segments in order to assess the most appropriate balance between time resolution and variability (Figure 52-53, A, B, C, D, and E). Table 9 presents the mean standard deviation and confidence intervals associated with the evaluation of moxifloxacin and SQR drug effects for each time segment. As expected, the larger the time segment, the lower the standard deviation and confidence intervals. In addition, the number of subjects per time point was also increased with longer time segments. This was due to the fact that if, for example, a 3-minute period of high noise and variability cannot be reliably measured, it results in a gap in the continuous measurements. If this 3-min gap is included in a 5-min time segment, the 5-min time segment will likely be excluded due to insufficient number of beats to appropriately represent the time segment. However, if a 3-min gap is included in a 10-min segment, the 10-min segment will still be appropriately represented and included in the analysis.

When continuous ΔQT_{ca} and $\Delta\Delta QT_{ca}$ are segmented in 1, 2, 3, 5, and 10 minutes (Figure 52-53, A, B, C, D, and E), it becomes apparent that increasing the length of the time segment masks certain fast protocol induced effects. With 10 minutes, the squared shape of ΔQT_{ca} apparent for time segments inferior to 5-min are no longer visible. These increases correspond to semi-automated ECG extraction periods where subjects are required to lay supine during 15-min (at 2, 3, 4, 5, 6, 8, and 12 hours), resulting in a reduction of the sympathetic activity and inducing non-rate-dependent QT_c increases (Yeragani, Pohl et al. 2000). The time resolution offered by 10-min segments was then too large to detect the fast QT_c increases and decreases associated with these periods. In contrast, 5, 3, 2, and 1-min time segments all effectively represented the fast ΔQT_{ca} circadian and protocol-induced variations. However, the 3, 2, and 1-min segments did not yield any supplementary information compared to the 5-min time segments.

Collectively, the 5-min time segment resolution is the most appropriate as it provides the most acceptable balance between detailed continuous ECG evaluations over 24 hours and constrained variability.

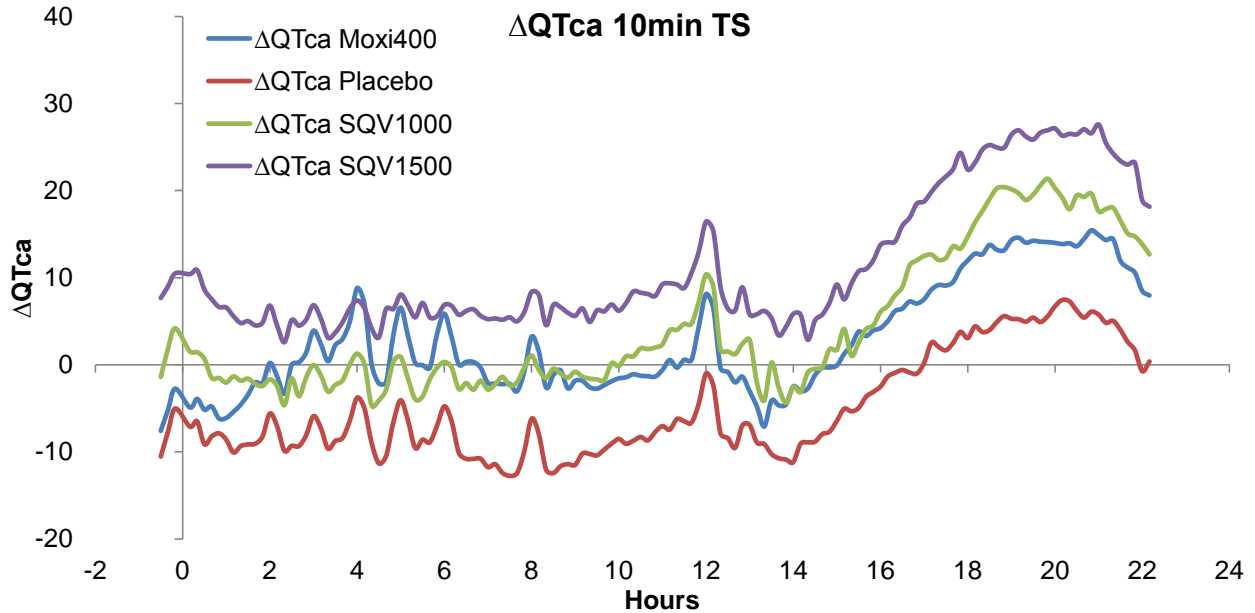


Figure 52A. PRO-derived ΔQTc for each treatment with 10 min time segments. The fast protocol-induced effects are smoothed by the 10-min time segments.

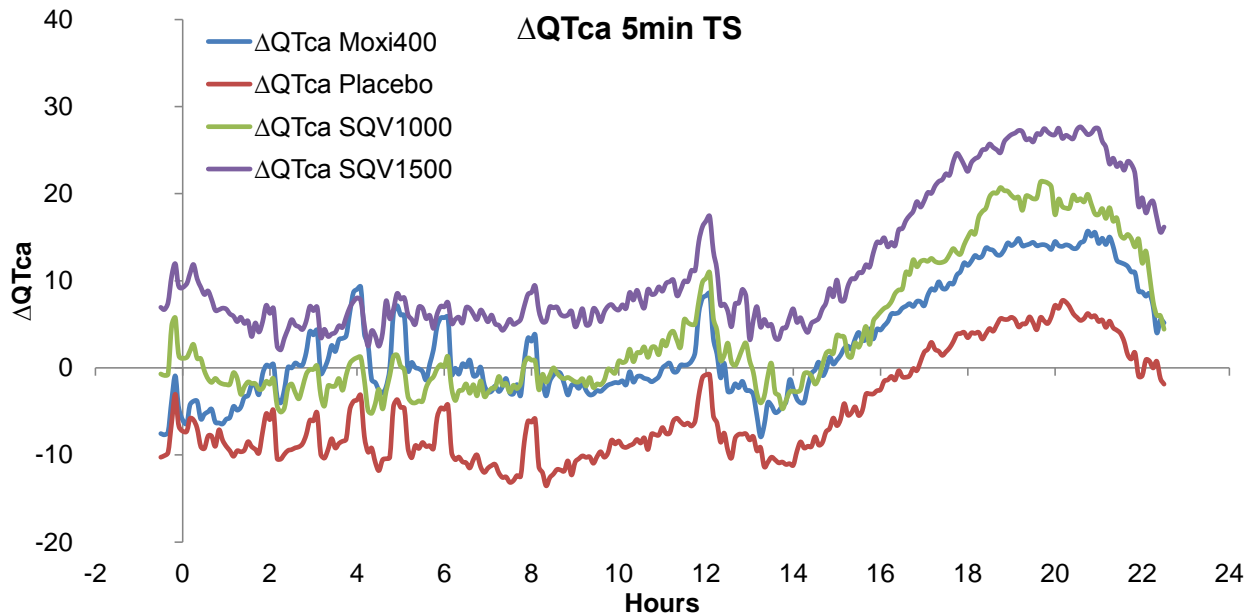


Figure 52B. PRO-derived ΔQTc for each treatment with 5 min time segments. The fast protocol-induced effects are well defined with 5-min time segments.

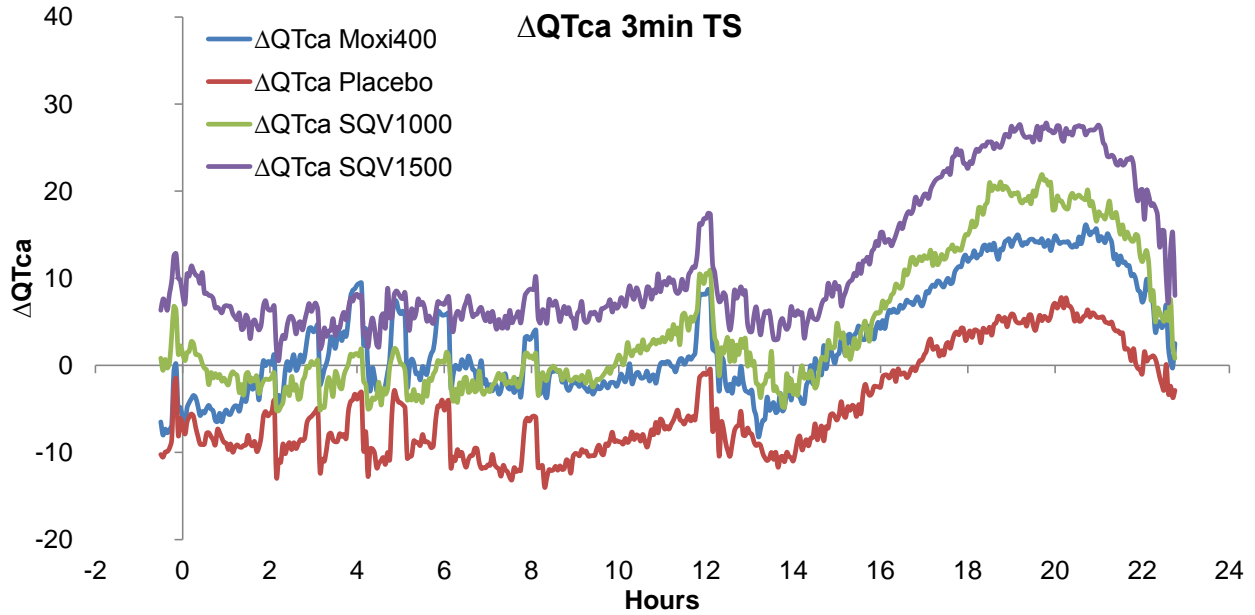


Figure 52C. PRO-derived Δ QTca for each treatment with 3 min time segments. The reduction in time segment length does not provide supplementary information, but results in increased variability.

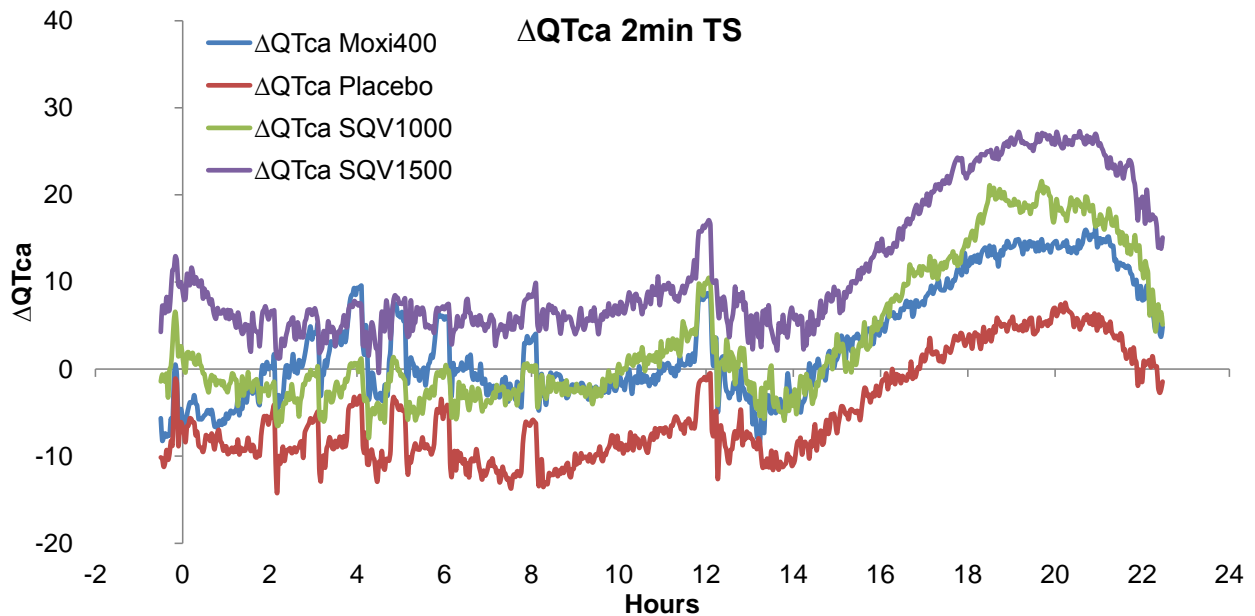


Figure 52D. PRO-derived Δ QTca for each treatment with 2 min time segments. The reduction in time segment length does not provide supplementary information, but results in increased variability.

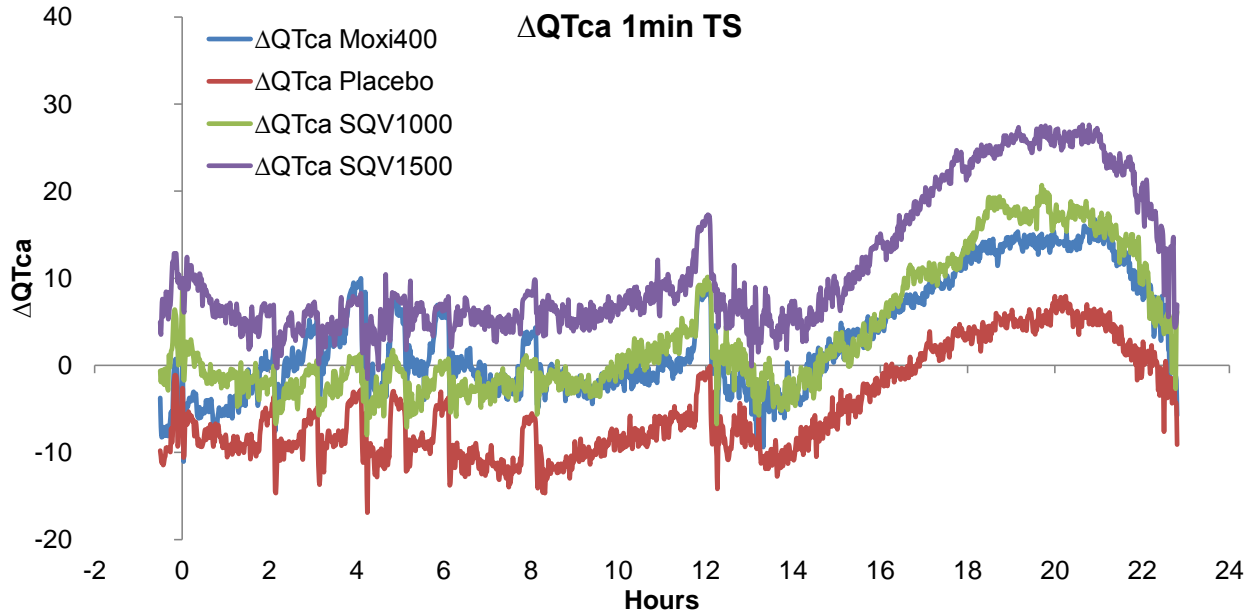


Figure 52E. PRO-derived $\Delta QTca$ for each treatment with 1 min time segments. The reduction in time segment length does not provide supplementary information, but results in increased variability.

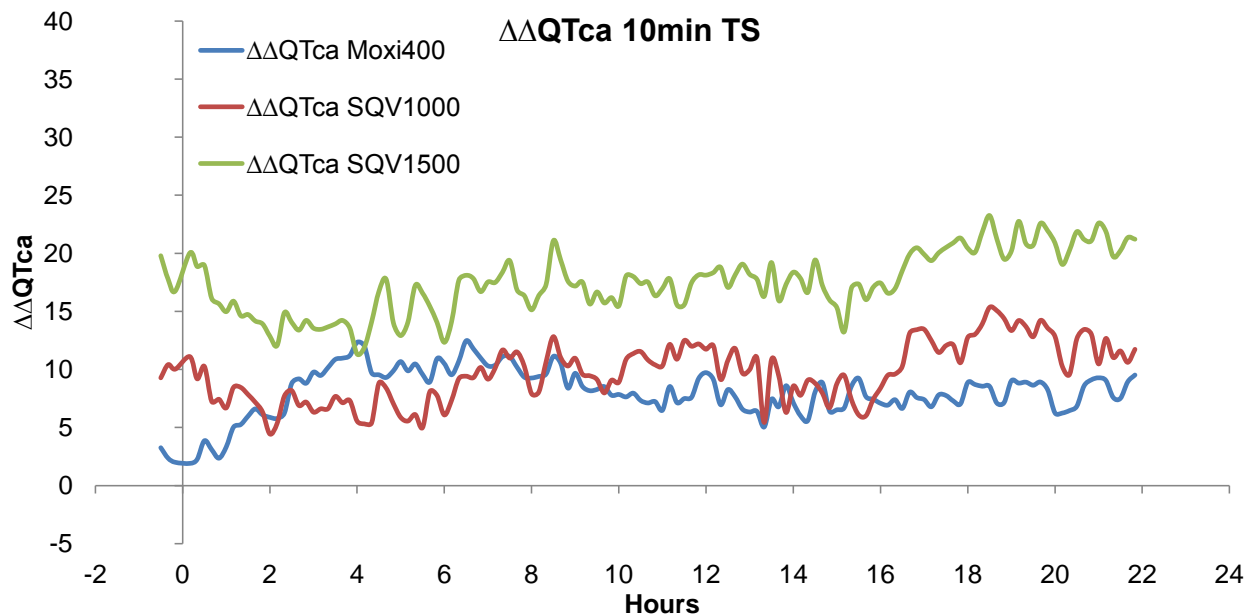


Figure 53A. PRO-derived $\Delta\Delta QTca$ for each treatment with 10 min time segments.

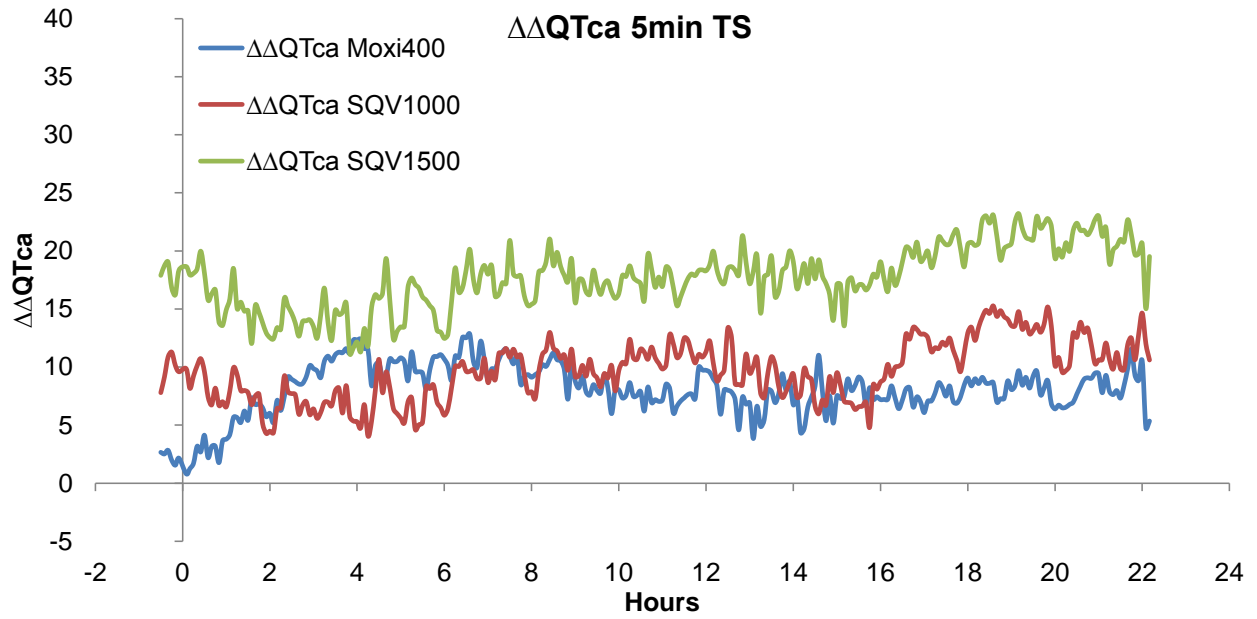


Figure 53B. PRO-derived $\Delta\Delta QTca$ for each treatment with 5 min time segments.

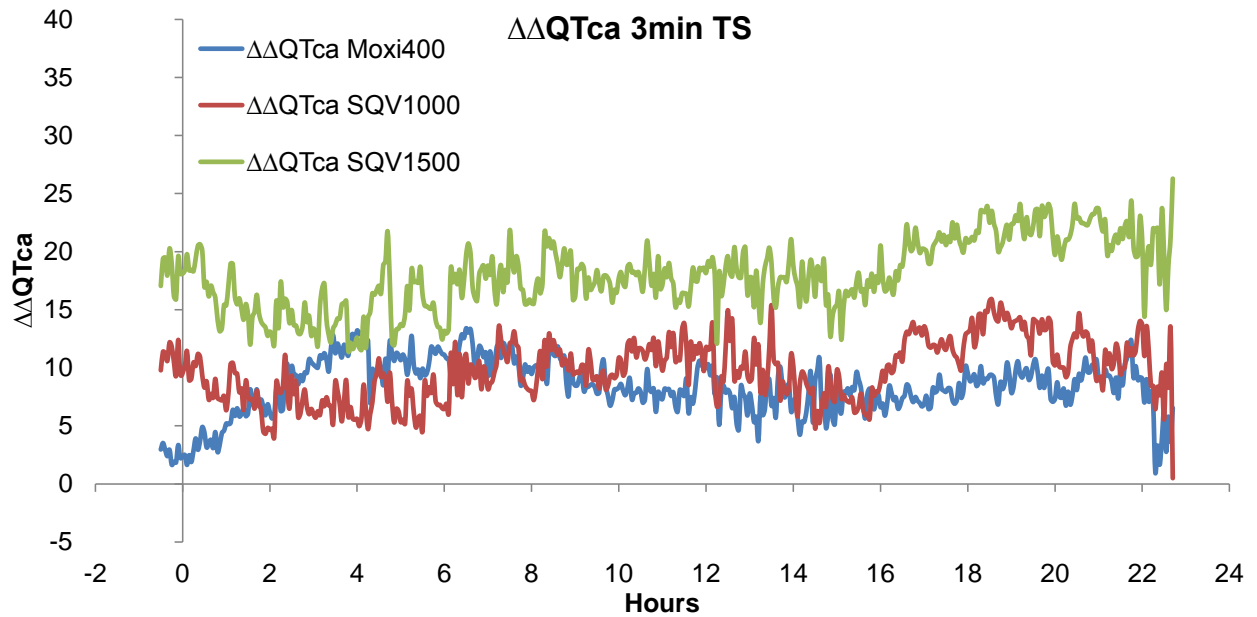


Figure 53C. PRO-derived $\Delta\Delta QTca$ for each treatment with 3 min time segments.

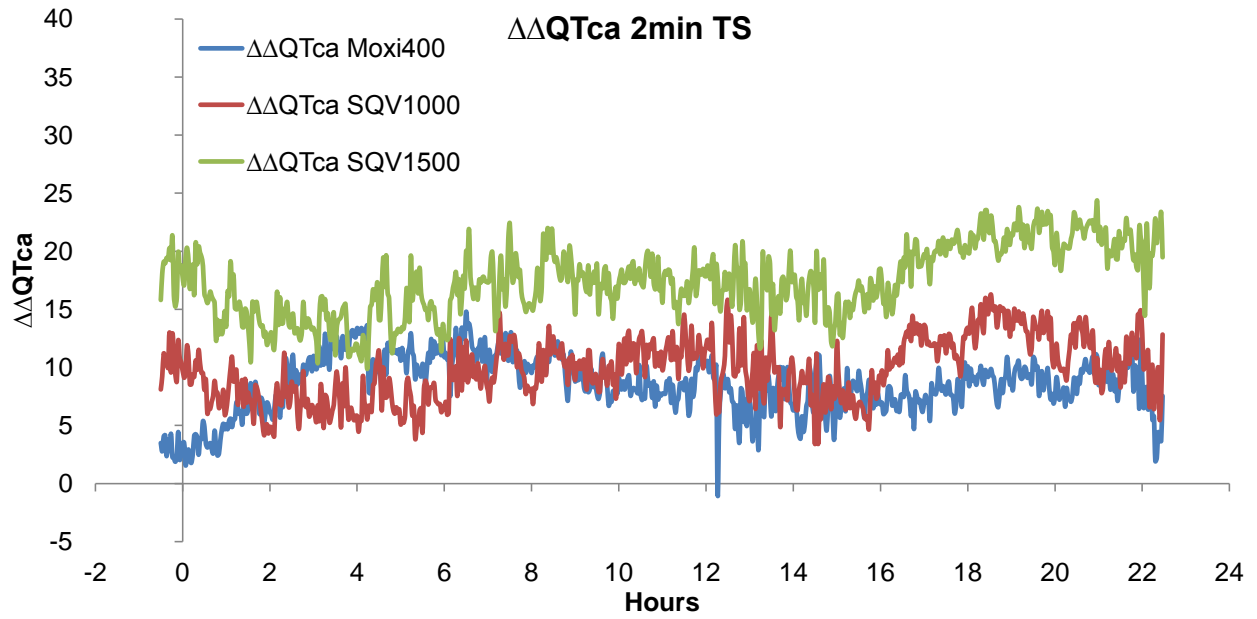


Figure 53D. PRO-derived $\Delta\Delta\text{QTca}$ for each treatment with 2 min time segments.

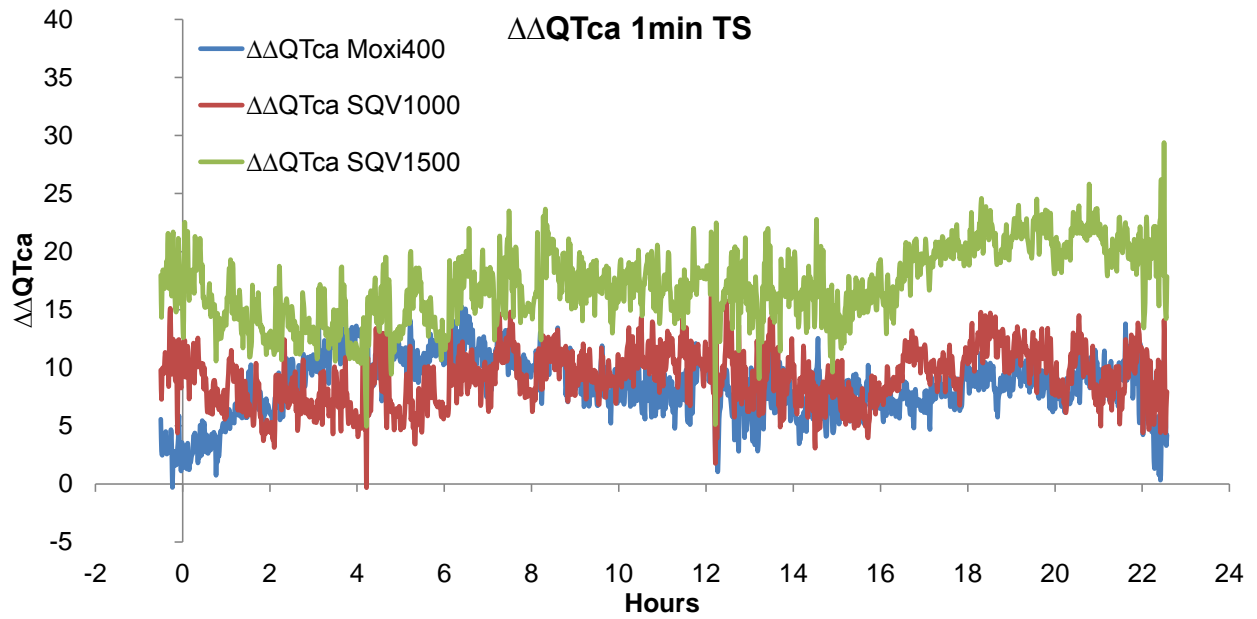


Figure 53E. PRO-derived $\Delta\Delta\text{QTca}$ for each treatment with 1 min time segments.

$\Delta\Delta\text{QTca}$ Moxifloxacin

Time Segment	n	mean effect	SD	CI
10 min	40.3 ± 4.2	8.0 ± 2.1	11.0 ± 1.2	3.5 ± 0.5
5 min	39.1 ± 4.5	8.0 ± 2.3	11.4 ± 1.4	3.7 ± 0.6
3 min	38.7 ± 5.2	8.5 ± 2.3	11.9 ± 1.5	3.9 ± 0.8
2 min	37.9 ± 5.3	8.6 ± 2.4	12.2 ± 1.5	4.1 ± 0.8
1 min	36.2 ± 5.9	8.5 ± 2.5	12.5 ± 1.7	4.3 ± 1.1

Table 9. Descriptive statistics of moxifloxacin-induced $\Delta\Delta\text{QTca}$ effects for time segment length. The 5-min time segment demonstrated the most appropriate balance between detailed information and variability.

3.4. QT/RR relationships

The application of individual heart rate correction factors to dissociate the effect of heart rate from QT is generally accepted as the most accurate and reliable correction method. However, individual heart rate-corrections are rather complex and the current absence of a gold standard precludes any firm conclusions about the accuracy of any derived QT/RR relationship β values. Indeed, a model can only be optimal for a specific set of data and it remains to be determined whether the use of all available data on baseline day, on-drug day, restricted to diurnal or moving periods, preceded or not by heart rate stability, with or without various levels of RR or QT averaging and hysteresis correction, or any combination of the above is optimal for individual QT correction. Accordingly, the effects of the circadian variations, the gender and the age on the QT/RR relationship were investigated.

3.4.1. Circadian variations and gender

The current analysis demonstrated QT/RR relationship differences between circadian periods and gender. For both male and female, the 24 h QT/RR relationship slope was steeper than the QT/RR relationship for the day or night alone (Table 10).

Period	Female		Male	
	n	β	n	β
24 h	74	0.300 ± 0.055	135	0.289 ± 0.059
Day	74	0.275 ± 0.062	135	0.261 ± 0.063
Night	74	0.243 ± 0.091	133	0.261 ± 0.116

Table 10. Mean β values between nycthemeral periods and gender - Baseline – SQR study. The β values obtained from 24 h data were increased compared to day or night data alone.

The differences between 24 h, day and night QT/RR relationships were further investigated and were attributed to the inclusion of circadian periods characterized by different sympatho-vagal influences. The combination within a 10-ms bin of QT and RR measurements in different autonomic states (from day and night) resulted in a shift of the 24 hours 10-ms bins towards an intermediary value between day and night (Figure 54). Indeed, the higher QT and RR values measured at night (increased vagal tone) are mixed in with the lower QT and RR values measured during the day (increased sympathetic tone) and result in mean values which are located between the day and night QT/RR relationships, thus shifting the QT/RR slope.

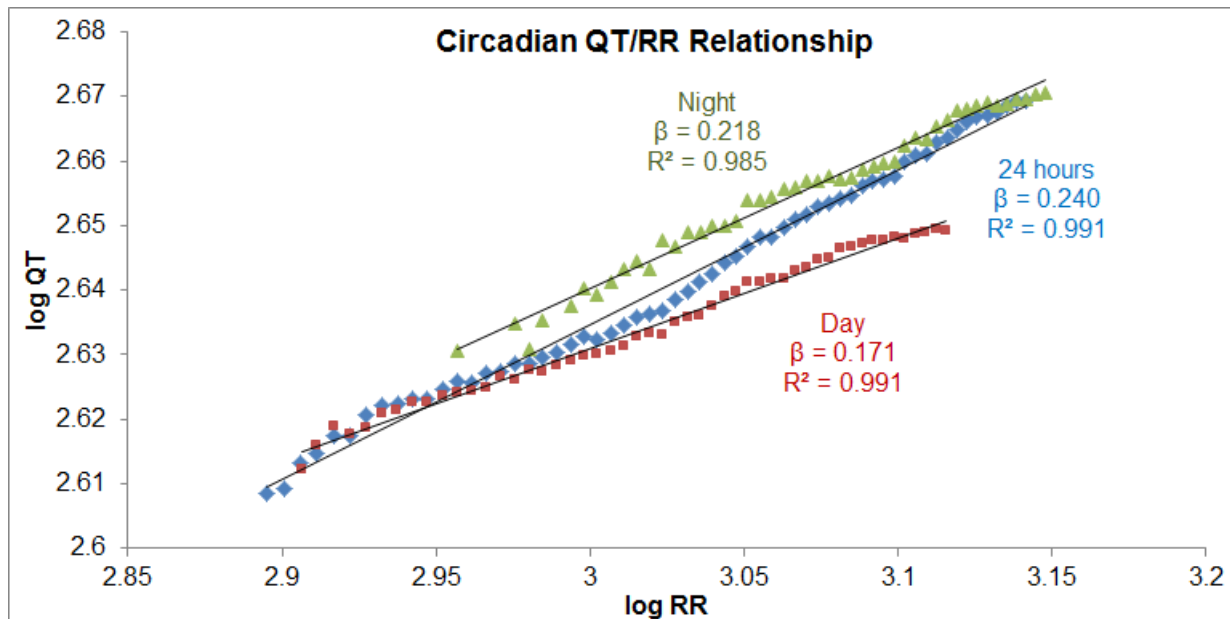


Figure 54. Example of circadian QT/RR relationship. Subject 1012 – Placebo – Day -1. 24h = All available data. Day = from 10 AM and 6 PM. Night from 0 AM to 6 AM. The mix of data obtained from day and night (periods characterized by different sympatho-vagal states) resulted in an increased slope.

This increase of the 24 h QT/RR relationship compared to day QT/RR relationship was consistently observed during baseline (Table 10) and treatments (Table 11) for SQR TQT study. The nocturnal QT/RR relationship was generally close to the day QT/RR relationship (Table 10). However, nocturnal QT/RR relationships were characterized by high variability, likely due to the reduced RR ranges and the limited period used for the derivation of the QT/RR relationship (6 hours).

Beta	Placebo			Moxi400		
	n	Mean value	Range	n	Mean value	Range
24 h Baseline	54	0.304 ± 0.059	0.198 - 0.410	56	0.288 ± 0.051	0.199 - 0.431
24 h On-Drug	54	0.313 ± 0.053	0.220 - 0.426	56	0.326 ± 0.064	0.206 - 0.473
Day Baseline	54	0.256 ± 0.063	0.101 - 0.430	56	0.245 ± 0.052	0.154 - 0.397
Day On-Drug	54	0.253 ± 0.059	0.134 - 0.394	56	0.276 ± 0.074	0.138 - 0.487

Beta	SQR1000			SQR1500		
	n	Mean value	Range	n	Mean value	Range
24 h Baseline	54	0.291 ± 0.059	0.192 - 0.451	52	0.290 ± 0.054	0.200 - 0.411
24 h On-Drug	54	0.363 ± 0.073	0.240 - 0.533	52	0.384 ± 0.086	0.223 - 0.596
Day Baseline	54	0.251 ± 0.062	0.161 - 0.446	52	0.249 ± 0.052	0.141 - 0.379
Day On-Drug	54	0.283 ± 0.069	0.114 - 0.486	52	0.293 ± 0.090	0.081 - 0.519

Table 11. Mean β values between on- and off-drug periods for each treatment – SQR study. The β value obtained using day data were quantitatively reduced compared to β obtained from 24 h data with all treatments.

Mean QT, RR, and QTca were compared between nycthemeral periods and gender (Table 12). As expected, QT, RR, and QTca were longer during the night period compared to the day period for both genders.

		Female			
Day -1	n	QT/RR pairs	RR	QT	QTca
24 h	74	74313	882 ± 71	401 ± 17	418 ± 14
Day	74	33715	855 ± 74	395 ± 17	416 ± 14
Night	74	17792	948 ± 74	417 ± 18	424 ± 14

		Male			
Day -1	n	QT/RR pairs	RR	QT	QTca
24 h	135	66174	929 ± 79	400 ± 23	411 ± 18
Day	135	30407	909 ± 85	397 ± 24	410 ± 18
Night	133	15145	999 ± 77	415 ± 24	417 ± 19

Table 12. Mean RR, QT, and QTca between nycthemeral periods and gender - Baseline - SQR study. RR, QT, and QTca intervals were increased at night compared to day.

With 24 h and day periods, female subjects demonstrated slightly higher QT-rate dependence (Table 10). This effect was also observed in BP22464 study where the β values were consistently reduced in males compared to females (Table 13).

Treatment	Female			Male		
	n	β	R^2	n	β	R^2
Moxi400	24	0.342 ± 0.074	0.977 ± 0.019	27	0.299 ± 0.063	0.974 ± 0.039
Placebo	24	0.340 ± 0.096	0.982 ± 0.018	28	0.300 ± 0.068	0.974 ± 0.025
SGLT-40	24	0.337 ± 0.087	0.978 ± 0.021	28	0.285 ± 0.071	0.981 ± 0.013
SGLT400	23	0.337 ± 0.091	0.975 ± 0.023	28	0.286 ± 0.058	0.973 ± 0.033

Table 13. Mean β values between genders for each treatment – SLGT2i study. The β values obtained in male subjects were quantitatively reduced compared to female subjects.

Compared to male subjects, female subjects also demonstrated increased mean QTca values (~7 ms; Table 12) during baseline period (Day -1) and a more pronounced SQR-induced effect (~14 vs. ~8 ms, female and male, respectively; Table 14).

		Female				
Pooled SQR						
	n	QT/RR pairs	RR	QT	QTca	
24 h	36	72180	829 ± 78	407 ± 20	432 ± 17	
Day	36	32103	804 ± 78	399 ± 21	427 ± 18	
Night	36	18826	884 ± 85	426 ± 22	443 ± 17	

		Male				
Pooled SQR						
	n	QT/RR pairs	RR	QT	QTca	
24 h	68	69425	863 ± 78	400 ± 25	418 ± 21	
Day	68	31553	839 ± 83	392 ± 27	414 ± 22	
Night	66	17103	938 ± 86	419 ± 27	428 ± 22	

Table 14. Mean RR, QT, and QTca between nycthemeral periods and gender – Pooled SQR - SQR study. The saquinavir-induced effects were quantitatively increased in female (~14 ms) compared to male (~8 ms).

3.4.2. Age and gender

The current analysis demonstrated QT, RR, QTca, and QT/RR relationship differences associated with the age of the subjects. The comparison of circadian QT/RR relationships based on the age yielded a trend for β values to decrease at night with increasing age. However, no β value differences were detected during the day period (Table 15). These results suggest that the parasympathetic nervous system, predominant during night periods, may be associated with age related changes in QT rate-dependence. However, the high variability observed between β measured during the night period precludes any firm conclusions, as the differences observed at night may have been driven by the measurement variability.

Age	Female				Male			
	n	β 24 h	β Day	β Night	n	β 24 h	β Day	β Night
18-24	13	0.306 ± 0.053	0.270 ± 0.064	0.309 ± 0.169	47	0.305 ± 0.052	0.267 ± 0.077	0.325 ± 0.135
25-34	26	0.316 ± 0.059	0.276 ± 0.070	0.265 ± 0.099	57	0.299 ± 0.068	0.267 ± 0.053	0.285 ± 0.097
35-44	25	0.297 ± 0.052	0.270 ± 0.063	0.198 ± 0.059	35	0.277 ± 0.051	0.257 ± 0.058	0.187 ± 0.055
45-54	28	0.288 ± 0.053	0.269 ± 0.052	0.239 ± 0.070	28	0.272 ± 0.053	0.258 ± 0.067	0.199 ± 0.083

Table 15. Mean β values between nycthemeral periods, gender, and age – Baseline – SQR study. The β values measured from the day data were highly consistent irrespective of the subject's age.

During BP22464 study, QT/RR relationships measured during baseline did not demonstrate the same pattern observed during NP21249 (Table 16). However, the number of off-drug 24 h periods was limited which may reduce the probative value of the results.

Age	Female		Male	
	n	β	n	β
18-24	3	0.287 ± 0.089	4	0.334 ± 0.097
25-34	7	0.286 ± 0.046	5	0.298 ± 0.032
35-44	6	0.384 ± 0.085	7	0.312 ± 0.026
45-54	4	0.337 ± 0.033	6	0.248 ± 0.026
>55	4	0.413 ± 0.167	6	0.319 ± 0.110

Table 16. Mean β values between gender, and age – Baseline – SGLT2i study. The β values measured from the 24 h data did not demonstrate any consistent trend between the age groups.

In both males and females, the mean QTca values increased with increasing age, especially after 45 years, irrespective of the nycthemeral period. In contrast, both the standard deviation of RR and QT decreased with increasing age (Table 17).

Age	Female				Male			
	n	QTca 24 h	QTca Day	QTca Night	n	QTca 24 h	QTca Day	QTca Night
18-24	13	412 ± 6	409 ± 7	424 ± 6	47	406 ± 13	405 ± 13	412 ± 18
25-34	26	410 ± 16	408 ± 15	418 ± 16	57	409 ± 20	408 ± 20	417 ± 21
35-44	25	419 ± 16	418 ± 16	425 ± 16	35	406 ± 15	404 ± 16	413 ± 13
45-54	28	426 ± 11	423 ± 11	432 ± 10	28	425 ± 16	424 ± 17	431 ± 15

Age	Female				Male			
	n	SD RR 24 h	SD RR Day	SD RR Night	n	SD RR 24 h	SD RR Day	SD RR Night
18-24	13	125.6 ± 22.2	110.6 ± 25.3	105.9 ± 13.9	47	118.8 ± 14.2	109.7 ± 16.3	107.8 ± 18.7
25-34	26	103.7 ± 20.5	89.1 ± 18.8	93.0 ± 19.6	57	114.1 ± 20.7	101.0 ± 26.4	94.7 ± 20.8
35-44	25	102.5 ± 12.7	85.4 ± 11.6	81.8 ± 13.3	35	110.4 ± 22.8	96.5 ± 22.9	90.5 ± 18.0
45-54	28	98.7 ± 14.9	86.9 ± 16.0	86.0 ± 17.6	28	104.5 ± 11.9	94.3 ± 11.9	84.0 ± 19.3

Age	Female				Male			
	n	SD QT 24 h	SD QT Day	SD QT Night	n	SD QT 24 h	SD QT Day	SD QT Night
18-24	13	22.0 ± 4.2	17.0 ± 4.3	14.6 ± 3.7	47	21.1 ± 3.5	17.6 ± 4.5	18.7 ± 5.1
25-34	26	19.3 ± 5.0	15.0 ± 4.9	14.8 ± 4.7	57	19.4 ± 5.5	15.4 ± 5.4	15.3 ± 4.7
35-44	25	17.7 ± 4.8	13.8 ± 3.9	10.2 ± 2.7	35	18.1 ± 4.8	14.7 ± 4.1	11.4 ± 4.2
45-54	28	16.3 ± 2.8	13.4 ± 2.5	12.3 ± 3.1	28	16.3 ± 2.2	13.8 ± 2.4	11.0 ± 3.0

Table 17. Mean parameters between nycthemeral periods, gender, and age – Baseline – SQR study. The QTca interval increased with increasing age and standard deviation of RR and QT decreased with increasing age, independently of the nycthemeral period.

3.4.3. Dataset

As previously demonstrated, QT/RR relationships measured on day period yielded reduced β values compared to the 24 h period. The 24 h on-drug QT/RR relationship was consistently increased with SQR compared to placebo (~0.05 to 0.07). Interestingly, when restricted to day on-drug QT/RR relationship, the SQR β values were quantitatively less increased compared to placebo (~0.03 to 0.04). These results suggest that the use of the 24 h period to derive on-drug QT-RR relationships may result in significant quantitative differences compared to the day period for the β value in presence of drug which prolongs the QT interval (Table 11).

The differences with on- and off-drug data for QT rate-correction were evaluated between 24 h and day periods. The treatment effects with $\Delta\Delta$ QTca derived from 24 h and day baseline periods were virtually superimposable (Figure 55, A and B) with a slightly increased variability for the day period rate-correction (Table 18). In contrast, treatment effects with $\Delta\Delta$ QTca derived from 24 h and day on-drug periods demonstrated significant differences (Figure 55, C and D; Table 18).

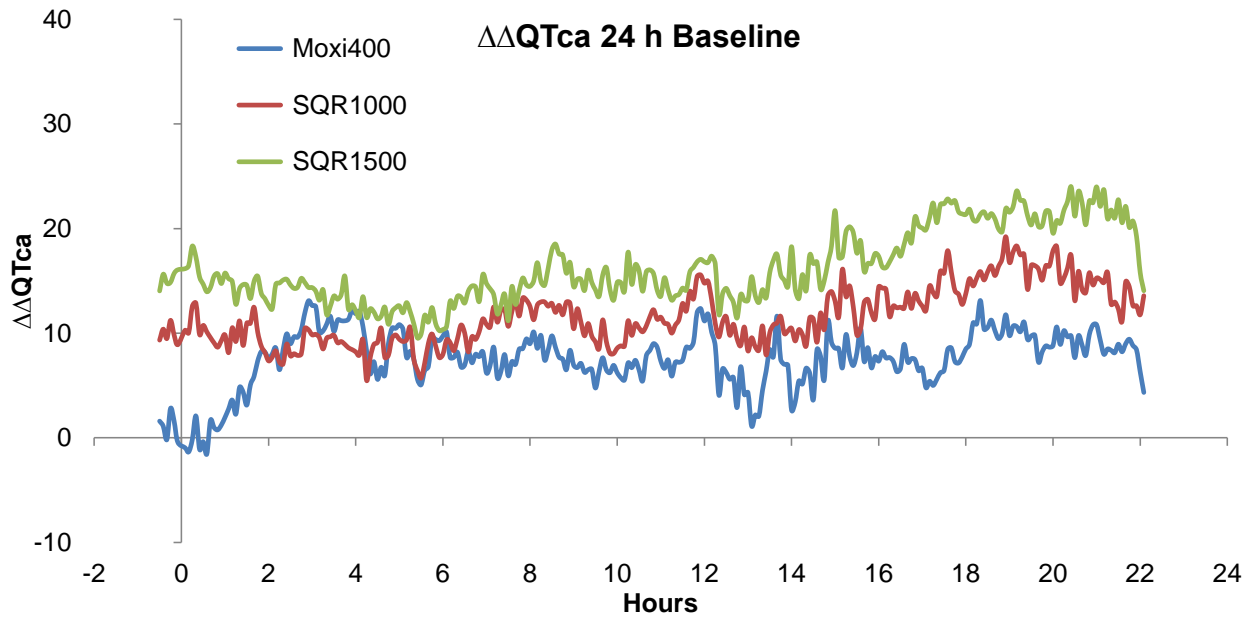


Figure 55A. PRO-derived $\Delta\Delta QTca$ for each treatment with 24 h baseline dataset

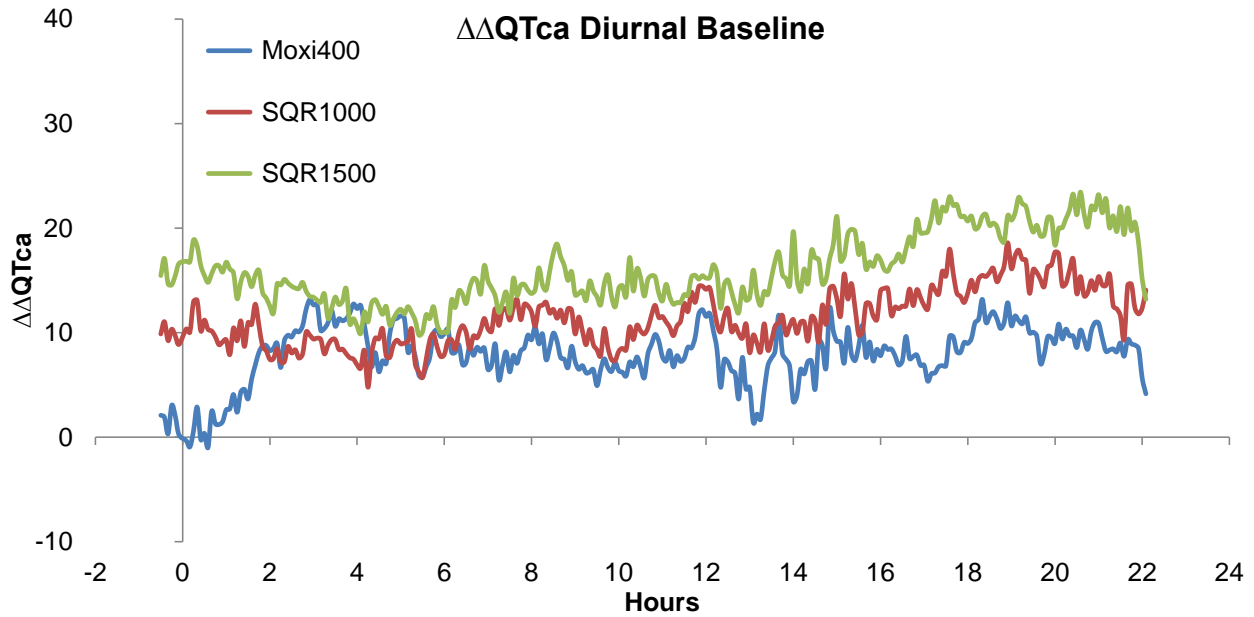


Figure 55B. PRO-derived $\Delta\Delta QTca$ for each treatment with day baseline dataset

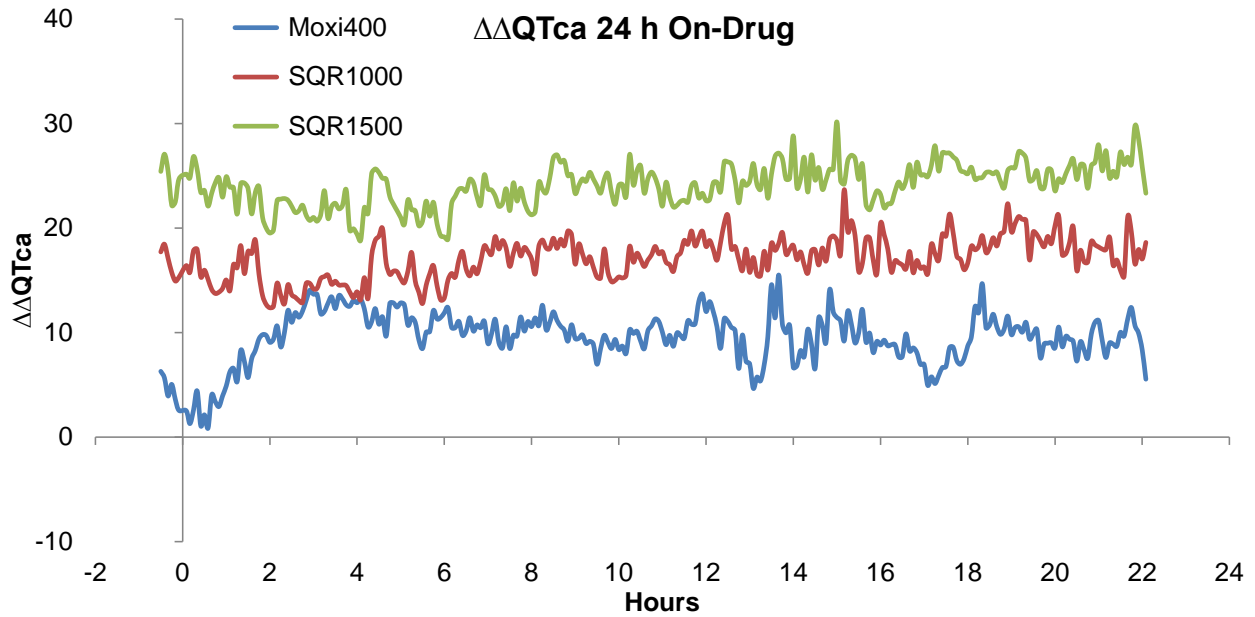


Figure 55C. PRO-derived $\Delta\Delta QTca$ for each treatment with 24 h on-drug dataset. The use of on-drug $\Delta\Delta QTca$ demonstrated quantitatively increased drug-effects with SQR, as well as a positive moxifloxacin effect during baseline.

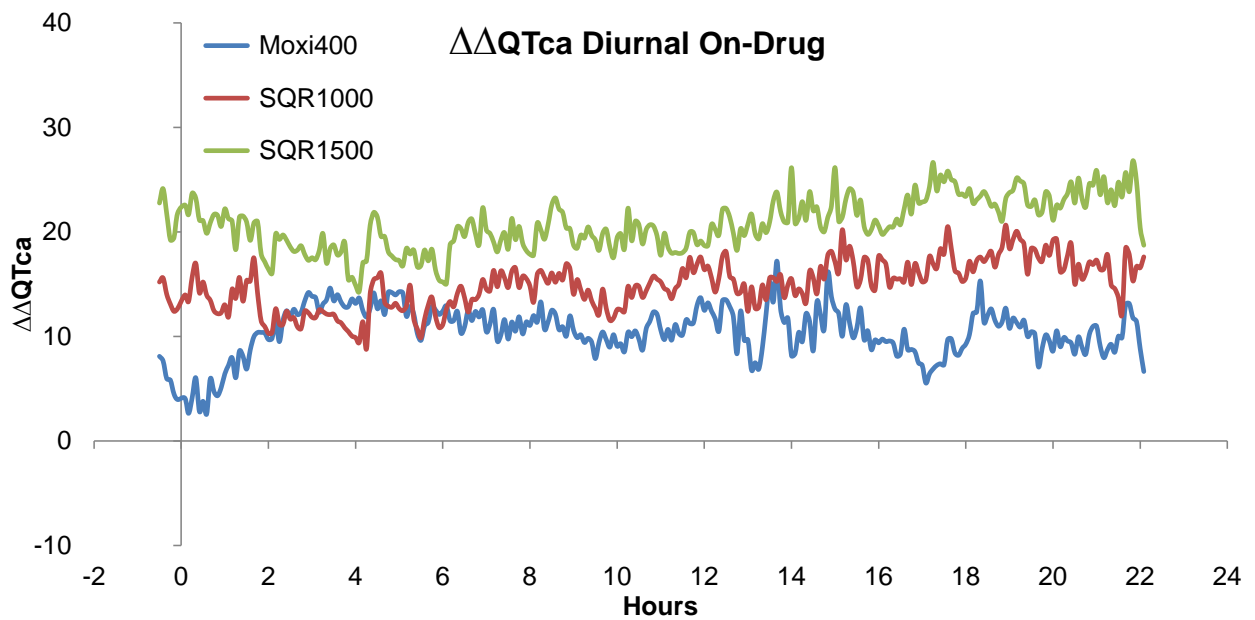


Figure 55D. PRO-derived $\Delta\Delta QTca$ for each treatment with day on-drug dataset. The use of on-drug $\Delta\Delta QTca$ demonstrated quantitatively increased drug-effects with SQR, as well as a positive moxifloxacin effect during baseline.

The use of on-drug QT/RR β values was associated with larger quantitative drug effects. The quantitative increase was proportional to the β value increase reported in Table 11, where a β increase of ~ 0.05 resulted in a QTca increase of ~ 6 ms (Table 18), and a β increase of ~ 0.07 resulted in a QTca increase of ~ 8 ms. When restricted to day data, the quantitative increase with SQR between off-drug and on-drug QT/RR correction was ~ 4 and 5 ms. For moxifloxacin, the overall drug effect was also increased by ~ 2 ms. However, when employing on-drug QT/RR correction, a QTca increase is detected even before dosing. This increase at predose is the result of an overestimation of the QTca effect. Indeed, applying a QT-rate correction derived from on-drug data to off-drug data is not appropriate and will likely result in over or under-estimation based on the drug specific effect. If the drug produces a β value increase, the QTca measurement at predose will be overestimated. Accordingly, for single dose administration, using on-drug rate-correction is not appropriate.

Treatment	$\Delta\Delta\text{QTca}$				$\Delta\Delta\text{QTca On-Drug}$			
	n	Mean effect	SD	CI	n	Mean effect	SD	CI
Moxi400	49.3 \pm 3.1	7.5 \pm 2.9	13.9 \pm 2.3	4.0 \pm 0.7	49.3 \pm 3.1	9.5 \pm 2.6	14.1 \pm 2.8	4.0 \pm 0.9
SQR1000	42.5 \pm 4.1	11.5 \pm 2.8	19.1 \pm 2.3	6.0 \pm 0.8	42.5 \pm 4.1	17.0 \pm 2.0	20.2 \pm 2.6	6.3 \pm 1.0
SQR1500	44.7 \pm 2.7	16.2 \pm 3.4	19.0 \pm 1.9	5.7 \pm 0.6	44.7 \pm 2.7	24.0 \pm 2.0	20.3 \pm 1.8	6.1 \pm 0.6

Treatment	$\Delta\Delta\text{QTca Day}$				$\Delta\Delta\text{QTca Day-On-Drug}$			
	n	Mean effect	SD	CI	n	Mean effect	SD	CI
Moxi400	49.3 \pm 3.1	7.9 \pm 2.9	15.4 \pm 2.4	4.4 \pm 0.7	49.3 \pm 3.1	10.4 \pm 2.5	15.4 \pm 2.5	4.4 \pm 0.8
SQR1000	42.5 \pm 4.1	11.3 \pm 2.7	19.8 \pm 2.3	6.2 \pm 0.9	42.5 \pm 4.1	14.9 \pm 2.3	20.8 \pm 2.1	6.5 \pm 0.8
SQR1500	44.7 \pm 2.7	15.9 \pm 3.3	20.4 \pm 2.0	6.1 \pm 0.7	44.7 \pm 2.7	20.7 \pm 2.4	21.6 \pm 1.7	6.5 \pm 0.6

Table 18. Descriptive statistics of $\Delta\Delta\text{QTca}$ for each treatment with different datasets. The use of an inappropriate rate-correction can result in increased drug effects.

3.5. RR averaging – Hysteresis

The QT interval does not immediately adapt to a RR change, but may take several minute to stabilize to the new RR interval. This phenomenon is called QT hysteresis. Fixed QTc formulae do not take this effect into account, which may result in inaccurate QTc measurements, thus increasing the QTc variability. To reduce this effect, it has been hypothesized that certain levels of RR averaging implemented into the QTc formula may further reduce the variability associated with QTc measurements based only on the precedent RR interval.

$$QTc_{30s} = QT / (RR_{30s})^\beta$$

$$QTc_{60s} = QT / (RR_{60s})^\beta$$

Where RR_{30s} is the mean RR over 30 seconds, and RR_{60s} is the mean RR over 60 seconds.

This analysis did not demonstrate any QTca difference at 5-min time points as demonstrated with Bland & Altman analysis (Table 19). Similarly, the $\Delta\Delta QTca$, $\Delta\Delta QTca_{30s}$ and $\Delta\Delta QTca_{60s}$ drug effects were identical.

Bland-Altman agreement	
QTca v QTca(30s)	-0.1 ± 1.2
QTca v QTca(60s)	-0.1 ± 2.2
QTca(30s) v QTca(60s)	-0.1 ± 1.7
n = 109578 time points	

Table 19. Bland-Altman comparisons between QTca, QTca_{30s} and QTca_{60s}. After the 5-min time segment creation, the use of QTca, QTca_{30s} or QTca_{60s} did not demonstrate any difference.

While it is expected that the intra-SD of QTca would be reduced compared to the intra-SD of QT after the application of a QT rate-correction formula, this was not the case when only the precedent RR was used for the derivation of the QTc measurement. The intra-individual intra-time point (5-min) standard deviation of the QTca was substantially reduced with QTca_{30s} and QTca_{60s} (Table 20).

Day	Treatment	Intra-individual intra-time point standard deviation			
		QT	QTca	QTca(30s)	QTca(60s)
D-1	Pooled	4.9 ± 3.6	7.8 ± 3.7	4.7 ± 3.1	4.3 ± 3.0
D3	Pooled	5.0 ± 3.8	7.2 ± 4.0	5.0 ± 3.6	4.6 ± 3.6
D3	Moxi400	5.1 ± 3.5	7.4 ± 3.4	4.9 ± 2.9	4.5 ± 2.8
	Placebo	5.0 ± 4.2	7.7 ± 4.2	4.9 ± 4.0	4.5 ± 3.9
	SQV1000	4.9 ± 3.5	7.0 ± 3.7	5.0 ± 3.4	4.7 ± 3.3
	SQV1500	5.0 ± 4.0	6.7 ± 4.4	5.1 ± 4.2	4.9 ± 4.2

Table 20. Intra-individual intra-time point standard deviation of QTca, QTca_{30s} and QTca_{60s}. The intra-SD of QTca was dramatically decreased with QTca_{30s} and QTca_{60s}.

These results suggest that applying a QTc formula which does not account for the hysteresis may not be the best available method for continuous ECG analysis. While the impact on the drug effect evaluation is negligible (Table 21) with a large datasets (such as those employed for continuous QT/QTc evaluation), the increased QTc variability when the fast RR variability is not minimized may substantially impact the QT/QTc evaluation when only few measurements are performed at each time point (which is why sparse ECG extractions are performed during periods of heart rate stability). Accordingly, the application of a QTc formula which minimizes the RR variability is important when analyzing continuous ECG data that are not restricted to periods of HR stability.

Moxi400				
RR average	n	Mean effect	SD	CI
$\Delta\Delta\text{QTca}$	39.1 ± 4.5	8 ± 2.3	11.4 ± 1.4	3.7 ± 0.6
$\Delta\Delta\text{QTca}(30\text{s})$	39.1 ± 4.5	8 ± 2.3	11.3 ± 1.3	3.7 ± 0.6
$\Delta\Delta\text{QTca}(60\text{s})$	39.1 ± 4.5	8.1 ± 2.3	11.3 ± 1.3	3.7 ± 0.6

SQR1000				
RR average	n	Mean effect	SD	CI
$\Delta\Delta\text{QTca}$	34.9 ± 4.8	9.6 ± 2.5	15.5 ± 1.6	5.4 ± 0.7
$\Delta\Delta\text{QTca}(30\text{s})$	34.9 ± 4.8	9.6 ± 2.5	15.4 ± 1.6	5.3 ± 0.7
$\Delta\Delta\text{QTca}(60\text{s})$	34.9 ± 4.8	9.5 ± 2.5	15.4 ± 1.5	5.4 ± 0.7

SQR1500				
RR average	n	Mean effect	SD	CI
$\Delta\Delta\text{QTca}$	40.7 ± 4.9	17.6 ± 2.7	19 ± 1.4	6.1 ± 0.7
$\Delta\Delta\text{QTca}(30\text{s})$	40.7 ± 4.9	17.6 ± 2.7	19 ± 1.4	6.1 ± 0.7
$\Delta\Delta\text{QTca}(60\text{s})$	40.7 ± 4.9	17.6 ± 2.7	19.1 ± 1.4	6.1 ± 0.7

Table 21. Descriptive statistics of $\Delta\Delta\text{QTca}$, $\Delta\Delta\text{QTca}_{30\text{s}}$ and $\Delta\Delta\text{QTca}_{60\text{s}}$ for each treatment. The use of QTca , $\text{QTca}_{30\text{s}}$ or $\text{QTca}_{60\text{s}}$ did not demonstrate any differences for the evaluation of drug effects.

3.6. Time segments representation

The minimum number of beats (n) required to deem a time segment “valid” is important as it directly affects the variability of the measurements. This number must be set to effectively exclude time segments represented by an inadequate (too low) number of ECG measurements without excluding time segments which are sufficiently represented and should be included in the analysis. In 2007, it was established that each time segment should be represented by a minimum number of 250 beats (Holzgrefe, Cavero et al. 2007). However, the higher the required minimum number of beats, the more likely it is that a whole time segment will be excluded from the analysis due to a short continuous measurement gap. Furthermore, with the implementation of advanced ECG measurement and review methods, it was hypothesized that reducing the minimum number of beats per time segment may improve the final analysis.

As expected, when all time segments were included (minimum n set to 0; n=0), the number of subjects per time point was the highest (43.5; Table 22). However, the standard deviation and the associated confidence intervals were increased compared to n=50 due to the inclusion of time segments represented by a limited number of beats. When using n=100 or n=150, while the standard deviation slightly decreased due to the exclusion of the time segments moderately represented (n<100), the associated number of subject per time point was also reduced, which resulted in increased confidence intervals. Accordingly, the use of n=50 was the most appropriate as it represented a good balance between variability and unnecessary exclusion of valid time segments.

$\Delta\Delta\text{QTca}$ Moxifloxacin

Filter TS	n (subject)	mean effect	SD	CI
n=0	43.5 ± 2.9	8.5 ± 2.4	12.4 ± 2.4	3.8 ± 0.8
n=50	39.1 ± 4.5	8.0 ± 2.3	11.4 ± 1.4	3.7 ± 0.6
n=100	35.6 ± 5.5	8.3 ± 2.2	11.2 ± 1.3	3.9 ± 0.8
n=150	32.2 ± 6.6	8.4 ± 2.4	11.2 ± 1.4	4.2 ± 1.2

Table 22. Descriptive statistics of moxifloxacin-induced $\Delta\Delta\text{QTca}$ for each time segment applied filter. The use of n=50 provided the most appropriate balance between number of subjects per time point and variability as it was associated with the most constrained confidence intervals.

3.7. TPE

A supplementary analysis evaluating possible drugs proarrhythmic effects was performed with Ponemah PRO which enabled to automatically mark and adjust T-peak fiducial marks on each 24 hour recording. This analysis was performed to evaluate the effects of SQR and moxifloxacin treatments on the Tp-e interval, measured as the $T_{\text{peak}} - T_{\text{end}}$. The Tp-e interval is a surrogate biomarker for the Transmural Dispersion of Repolarization, which has been described as a superior biomarker of proarrhythmic risk compared to QT prolongation due to its direct mechanistic link to TdP (Said, Wilson et al. 2012). It was thus hypothesized that the evaluation of the TPE interval during the SQR study might differentiate between proarrhythmic and non-proarrhythmic drugs.

Time-course profiles for the $\Delta\Delta\text{Tp-e}$ after SQR or Moxi administration are presented in figure 56 with the associated descriptive statistics in table 23. Interestingly, the moxifloxacin treatment was not associated with any $\Delta\Delta\text{Tp-e}$ increase (mean effect: 1.3 ± 1.1 ms). In contrast, SQR administration resulted in a significant dose-dependent increase in $\Delta\Delta\text{Tp-e}$ (mean effect: 7.0 ± 1.8 and 10.0 ± 1.7 ms, SQR1000 and SQR1500, respectively).

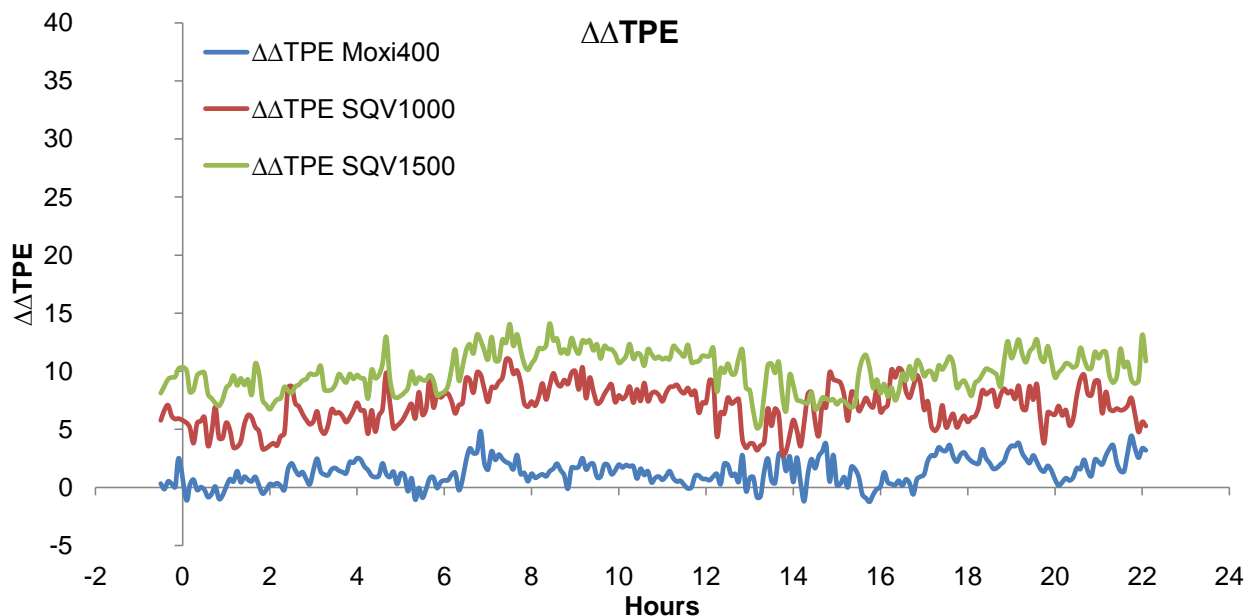


Figure 56. PRO-derived $\Delta\Delta\text{Tp-e}$ for each treatment. The administration of moxifloxacin did not result in a $\Delta\Delta\text{Tp-e}$ increase. In contrast, SQR administration resulted in a dose-dependent increase in $\Delta\Delta\text{Tp-e}$.

$\Delta\Delta$ TPE

Treatment	n (subject)	mean effect	SD	CI
Moxi	41.7 ± 4.8	1.3 ± 1.1	9.7 ± 1.7	3.1 ± 0.6
SQR1000	36.2 ± 4.9	7.0 ± 1.8	14.4 ± 2.1	4.9 ± 0.9
SQR1500	42.1 ± 5.1	10.0 ± 1.7	14.5 ± 1.9	4.5 ± 0.7

Table 23. Descriptive statistics of PRO-derived $\Delta\Delta$ Tp-e for each treatment. The administration of moxifloxacin did not result in a $\Delta\Delta$ Tp-e increase. In contrast, SQR administration resulted in a dose-dependent increase in $\Delta\Delta$ Tp-e.

These results confirm previously published preclinical analyses which demonstrated that Tp-e may provide supplementary information regarding proarrhythmic risk and suggest that the evaluation of Tp-e interval should be considered as an adjunct analysis during Thorough QT analysis. The current results suggest that the Tp-e interval, by detecting T-wave morphological changes, may provide valuable information for the differentiation of proarrhythmic from non-proarrhythmic drugs. However, this analysis was only based on a single study. Accordingly, further evaluation is necessary to provide firm conclusions.

3.8. Conclusion

Part III has demonstrated that several technical, methodological or analytical features have the potential to impact the end results of TQT study continuous analysis. The current analyses demonstrated similar results irrespective of the choice of the lead analyzed, where the only observable difference consisted in an increased variability when careful review and adjustment of the ECG measurements was omitted. Altogether, when analyzing ECG waveforms continuously, the lead to be analyzed must be chosen based on its ability to be consistently measured by a computerized platform. In other words, the selected lead must demonstrate limited signal-to-noise ratio and consistently high amplitude, well-inscribed R- and T-waves in order to facilitate the computerized measurements. In presence of poor quality signal (subject- or period-specific), another high quality lead (generally lead II or V2 to V5) can be used as a replacement as long as the replacement lead is consistently analyzed for each subject or period-specific measurements, depending on how the ΔQTc effect is measured. Similar conclusions were reached in a recent study which demonstrated that alternative leads could efficiently be used when the QT interval could not be suitably measured in the main lead (Salvi, Karnad et al. 2012).

When employing continuous ECG measurement platforms, careful review and adjustment of the automated waveform measurements should always be performed. Despite the fact that the current analyses demonstrated acceptable results with the fully-automated methods, the omission of the primordial review step will always lead to increased variability, and, in some cases, lead to consistent measurement errors which translate into incorrect assessment of drug effect, especially in presence of abnormal ECG waveforms.

The evaluation of QT/RR relationship and other ECG parameters depending on the gender, age, and nycthemeral period confirmed some of the previously published findings (Coumel, Fayn et al. 1994; Coumel, Maison-Blanche et al. 1995), such as the longer QT/QTc interval at night compared to day, the longer QTc interval (Smetana, Batchvarov et al. 2002), greater drug-induced QTc prolongation (Benton, Sale et al. 2000), and greater rate-dependence in women compared to men (Extramiana, Maison-Blanche et al. 1999; Sredniawa, Musialik-Lydka et al. 2005), and the decreased SD of RR with increasing age (Tsuji, Venditti et al. 1996). In contrast, the decreased QT/RR slope with increasing age during the day, or the decreased QT/RR slope at night compared to the day were not reproduced in the current analyses (Extramiana, Maison-Blanche et al. 1999), likely a consequence of the methodological differences between beat selection with preceding RR stability compared to distribution-based analysis. Studies

investigating the cardiac electrophysiology have frequently confirmed sex disparities, despite the lack of understanding of the physiological mechanisms responsible for these gender differences (Extramiana, Maison-Blanche et al. 1999; Bonnemeier, Richardt et al. 2003; Sredniawa, Musialik-Lydka et al. 2005; Smetana and Malik 2013). However, the clinical significance and possible impact of these known differences is yet to be determined. In light of these facts, the increased drug-induced QT prolongation observed in women after SQR administration suggests that TQT studies should include a balanced ratio of men and women, as including only one gender may over- or underestimate the drug-induced effects. Subjects ages from 18 to 60 can be included in clinical studies as the current analysis did not elicit any age-related effects that may substantially impact the QT prolongation assessment.

The derivation of the distribution-based individual QT/RR relationship should be performed on all available day QT-RR pairs. Indeed, while the day and night QT/RR slopes were generally similar, the inclusion of both day and night QT-RR pairs to derive the 24 h QT/RR relationship was associated with an artificially increased QT/RR slope due to the mixing of QT-RR pairs in different autonomic states into the 10-ms RR bins.

The effect of hysteresis did not demonstrate any impact on the continuous drug effect assessments ($\Delta\Delta\text{QTc}$) as the hysteresis-related QTc measurement variability was eliminated during the averaging process of thousands of beats into time segments. However, including levels of RR averaging (60 seconds is appropriate) in the QTc formula reduces the variability of the QTc measurement, which still provides a substantial improvement as the standard deviation of the QTc is generally used for the sample size calculation.

The length of the time segments for the depiction of the time-dependent changes in ECG parameters is important as longer time segments may mask rapid protocol-induced effects and shorter time segments may needlessly increase the variability. The current analysis demonstrated that 5-min segments provided the most appropriate balance of scientific information and limited variability.

In addition, while the current report focuses on the application and refinements of continuous evaluation of drug-induced QTc prolongation, the inclusion of other surrogate biomarkers and/or the adjunction of other methodologies (bin method, ECG restitution...) based on the same continuous ECG measurements may provide an integrated and superior evaluation of the risk of TdP, as demonstrated by the TPE analysis which demonstrated a positive signal with SQR which was not detected for moxifloxacin.

Discussion

1. Implication for drug development

1.1. Clinical studies

The ICH E14 guidance has been effective in terms of patient protection, as none of the accepted drug after 2005 have been removed from the market for TdP induction (Stockbridge, Zhang et al. 2012). However, a recent report has demonstrated that TQT studies were highly cost-ineffective due to the poor predictive value of QT prolongation as a surrogate biomarker of proarrhythmia and the rarity of the TdP events (Bouvy, Koopmanschap et al. 2012). Although estimates differ, the average cost of a TQT study is usually estimated to be approximately 1MM USD. The high TQT-associated costs, largely driven by the analysis of ECG recordings by ECG core laboratories (\$50/ECG measurement (Tyl, Kabbaj et al. 2009)), have several impacts on drug development. When a drug is suspected to increase the QT interval in human, it is likely that the drug development will be immediately stopped as a positive TQT study would require extensive ECG evaluation in phase II and III which would add millions to the drug development costs. Unless the indication of the drug is for a lethal disease or has no effective alternate treatment, it is likely that a sponsor will, after determining the risk/benefit ratio weighed against clinical development costs, discontinue potentially lives-saving drugs that may have been developed with less expensive ECG evaluations. Furthermore, while the current report demonstrates that high financial costs could be effectively reduced by the application of computerized continuous analysis, the widespread evolution of the methods will likely be restrained by the E14's "*faulty advice regarding the need for human overreading of ECGs*" (Stockbridge, Zhang et al. 2012), as well as by scientists which have a large financial interest in using only a few cardiologist for the currently mandated ECG overreads (Malik 2004). Accordingly, absent a revision of the current E14 guidance stating that advanced ECG measurements platforms are appropriate and reliable tools for cardiac repolarization assessments, it remains unlikely that any near term changes will occur regarding the current conduct of TQT study analysis. Nevertheless, as studies evaluating ECG measurement platforms and advanced methodologies continue to be performed and experience is gained, it can be expected that a consensus will develop and allow, in the foreseeable future, the widespread use of fully automated ECG measurement platforms and continuous QT analysis.

2. Assessment of TdP liability

Safety pharmacology in drug development is a discipline which has two purposes: fulfill the needs of regulatory authorities and provide scientific proofs of safety. In the absence of reliable tests to quantify and predict the risk of drug-induced TdP, the industry and the regulators have initiated working groups, of which the most important is the International Conference for Harmonization (ICH) which discusses both scientific and technical aspects of TdP liability assessment. The establishment of the ICH recommendations S7A, S7B, and E14 in 2005 has compelled the pharmaceutical companies to adapt to these texts for the conduct of both preclinical and clinical studies.

2.1. QT prolongation assessment vs. Torsadogenic potential

The use of a surrogate biomarker to predict a rare but possibly lethal adverse event such as Torsades de pointes is mandatory. By definition, TdP is associated with a prolongation of cardiac repolarization measured on the ECG as the QT interval. However, many evidences have shown that QT interval prolongation is an imperfect biomarker to evaluate the drug-mediated risk of inducing TdP. Indeed, not all drugs that prolong the QT interval have been associated with increased torsadogenic potential, which means that measuring drug-associated QT prolongation only serves to exclude a possible arrhythmic risk. Thus, a positive QT prolongation signal indicates that the evaluated drug might be proarrhythmic, but the differentiation between proarrhythmic and non-proarrhythmic drugs cannot be concluded. In other words, QT interval evaluations enable regulators to confidently identify safe drugs, and to uncertainly reject potentially unsafe drugs. To date, despite the limitations associated with the discontinuation of possibly safe drugs, QT interval prolongation remains the most reliable biomarker for increased TdP liability.

2.2. Preclinical studies vs. clinical studies

With the implementation of ICH guidance E14, the Thorough QT study quickly became well-standardized and has not seen substantial evolution over the past 8 years. The primary endpoint of the TQT study is to evaluate whether a drug induces a QT increase of 5-10 ms, considered to be the threshold for regulatory concern. In contrast, similar accepted preclinical criteria are not currently available and the difficulty to reliably predict human outcome from preclinical studies have resulted in a more homogeneous practice. While the *in-vitro* hERG assay and the *in-vivo*

dog study are most frequently used as a part of the 'core battery', there are several other appealing models and methods that have been and are being developed over time by different laboratories and researchers. The major problem associated with these preclinical methods is that none of them have demonstrated 100% specificity and selectivity for the proarrhythmic risk in man as high predictive value may only be obtained by conducting an integrated assessment of multiple methods and biomarkers. In the absence of a gold standard, the integrated assessment of preclinical methods remains the most scientifically relevant approach to evaluate possible drug-mediated TdP liability. Pharmaceutical companies are thus free to generate an array of preclinical assessments in order to demonstrate the safety of their drug in a convincing way to the regulatory authorities. Consequently, on the one hand, preclinical methods to evaluate cardiac repolarization are numerous and currently lack standardization and validation, and on the other hand, most of these methods are scientifically more pertinent than the sole evaluation of the QT interval prolongation performed during the well-standardized Thorough QT study (Holzgreffe, Ferber et al. 2013).

2.3. Regulatory issues vs. Scientific issues

In 2005, the important and unmet need for standardization of the cardiac repolarization assessments was fulfilled by the expert recommendations provided in the guidance E14. The experts presented a standardized method which was an elegant balance between scientific relevance, practicality, and technological advances available at the time. In order to move forward in the development of a new drug, pharmaceutical companies have been required to convince the regulatory agencies that the investigated drug is safe to use. To this end, they must provide the supporting data that is consistent with regulatory expectations. In other words, safety pharmacology studies are primarily designed to meet the regulatory requirements, and only secondarily to meet the scientific issues (Pugsley, Authier et al. 2008). For TdP liability in man, these requirements are well established which has resulted in a rapid standardization of the practice where the primary QT prolongation analysis is typically performed using semi-automated ECG measurements annotated by trained cardiologists. During the composition of the E14 guidance, the experts deliberately remained vague regarding possible methodological and technological advances that may develop in order to enable future methodological improvements to be implemented. However, the methods employed for TQT study analysis have remained virtually unchanged for the past 8 years, despite the fact that several scientific and technological advances have been made.

Based on non-clinical research conducted in *in-vitro* and *in-vivo* models, many scientists now consider other surrogate endpoints, such as TDR or T-wave morphology (Graff, Struijk et al. 2010), as relevant predictors that may improve the current assessment of TdP liability. Indeed, these biomarkers may provide important information to discriminate between proarrhythmic and non-proarrhythmic drugs. Despite the scientific advances, ground-breaking changes in the way proarrhythmic liabilities are evaluated are unlikely to occur in the next few years as a clear understanding of TdP mechanisms is yet to be established. In other words, the use of QT prolongation as a surrogate endpoint for the evaluation of proarrhythmic liability will likely remain the primary biomarker of proarrhythmia for the foreseeable future. However, with further experience and validation on other biomarkers, the evaluation of TdP liabilities would likely benefit from the integrative assessment of multiple biomarkers.

Regarding technical and methodological endpoints, the TQT study as recommended by the ICH E14 guidance displays several weak points which consist, among others, in the evaluation of sparse ECG extractions at specified time points which exclude ~99% of the available data, the lack of individual QT rate-correction, and the resource-intensive outsourcing of data analysis to ECG core labs. These methodological weaknesses were generally driven by practical considerations which can now be effectively addressed with technologies that were not available at the time of the guidance implementation.

3. Optimization of methods Continuous vs. sparse

3.1. Sparse sampling

The gold standard methodology for thorough QT studies prescribed by the E14 guidance document consists in the evaluation of the mean of several (3-9) semi-automatically measured beats extracted at sparse time points predefined in the study protocol. Due to the inherent physiological variability associated with cardiac repolarization (the so-called *probabilistic* nature of the QT interval (Holzgreffe, Cavero et al. 2007)), the QT intervals have been recommended to be extracted during periods of heart rate stability to reduce QT measurement variability by limiting the impact of hysteresis, variable autonomic states, and noise. In addition, a limited number of ECG extractions and time points are generally preferred as it reduces the overall financial cost of the TQT study analysis as each ECG complex reviewed by a cardiologist is charged to the pharmaceutical company (Natekar, Hingorani et al. 2010). While this practice

generally provides reliable measurements, it is important to note that SA methodology is mainly based on practical rather than scientific considerations. Indeed, SA methodology excludes 99% of the recorded data in order to reduce the variability and limit the cost of the TQT study analysis. However, some extent of the excluded variability consists of “real” physiological variability that may bear important information. Furthermore, discarding the major part of the data precludes the derivation of individual rate-corrections and dramatically reduces temporal resolution, resulting in possible QTc measurements errors and loss of information between the scheduled ECG extraction periods. The ICH E14 Statistic Group recommends the use a non-inferiority intersection-union test (IUT) implemented using ANCOVA models which prescribes that all time points must fulfill the condition (95% CI of $\Delta\Delta\text{QTc} > 10$ ms) in order to exclude H_0 and conclude the test as “negative” (Hutmacher, Chapel et al. 2008). As a consequence, correction for multiplicity is not needed but the power of the test goes down with the increased number of analyzed time points. Accordingly, sponsors generally tend to restrict to a limited number of time points in order to reduce the chance of type II error (false positive). In other words, it is implicitly recommended to restrict the TQT analysis to only few time points in order to obtain an improved statistical study power, which is scientifically counterintuitive as performing a more thorough analysis would be, in fact, penalizing for the sponsor.

The E14 recommendations were established at a time when continuous ECG analysis was not a preferable option due to the lack of confidence in fully-automated ECG measurement methods (threshold, tangent) which were generally associated with measurement inaccuracies in presence of abnormal ECG waveforms. However, the application of recently developed ECG measurement methods based on complex signal processing now provides reliable measurements devoid of algorithm-based errors for large volumes of ECG data. Accordingly, the recent availability of reliable automated ECG measurement platforms no longer justifies the analysis of sparse ECG extractions as continuous ECG analysis provides a more robust and scientifically relevant assessment of cardiac repolarization.

3.2. Beat-to-beat continuous ECG analysis

The main advantage of continuous assessment of cardiac repolarization is that every measurable ECG complex is included in the analysis, thus enabling the precise derivation of individual QT/RR relationships and a complete QTc evaluation over the 24 h period which enables the visualization of rapid protocol and/or drug-induced, rate-independent changes in the

QTc interval. Despite these advantages, continuous ECG analysis is only rarely used in clinical trials, likely due to its inherent complexity and current lack of standardization. Indeed, while the E14 guidance provides clear recommendations for the methodological, analytical, and statistical considerations of the sparse ECG analysis, there is no similar regulatory text currently available for continuous ECG analysis. Suggested methodological and analytical refinements were extensively described in Part III.

Regarding statistical considerations, the analysis of contiguous time points precludes the assumption of independence between time points and thus, the use of simple statistical models. Accordingly, the utilization of mixed effect models is required as they allow the inclusion of the effect of time into the model. While mixed effects models including the time as a fixed effect are the most appropriate approach for the evaluation of continuous ECG analysis, the use of such models may integrate an obviously misrepresented time point into the analysis when careful review of the input data is omitted. Indeed, in contrast to SA method which populates each sparse time point with ECG extraction measurements (3-9), continuous analysis measures each time segment (optimally of 5-min length) but excludes those which are not represented by a sufficient number of valid beats (>50). These missing individual time segments can sometimes result in poorly represented analysis time points (<10 subjects) which must be excluded from analysis before the application of the mixed effects model. Time points characterized by a limited number of subjects (<10) are generally encountered at the start and end of the 24 h recording, due to the time-matching process with variable subject-specific Holter starting times, or at protocol-specific events (e.g. meal, unrestrained physical activity) characterized by high signal-to-noise ratio. Accordingly, descriptive statistics including, for each contiguous time point, the number of subjects, the mean treatment effect, and the standard deviation and associated confidence intervals must be carefully reviewed before the application of a mixed effects model.

Another important advantage of continuous ECG analysis is its two-way portability. On the one hand, continuous analysis using distribution-based methodology can be effectively applied to other species for preclinical studies, and on the other hand, the reliable computerized ECG measurements which serve as the basis for the continuous ECG analysis can be easily exported for analysis with different methodologies or biomarkers, such as rate-binning analysis, TPE, QTbtb, ECG restitution, or 1-stage PK-PD modeling (Garnett, Zhu et al. 2012).

Indeed, very recently, a consortium of pharmaceutical research sites (note: To which I participated) have employed distribution-based analysis in dog, cynomolgus, marmoset, minipig, guinea pig, and man in order to elucidate any possible underlying mechanistic basis for the

current lack of preclinical-clinical concordance with *in-vivo* repolarization assays. Employing a common analytical scheme for all species, the consortium demonstrated that, when accurately modeled and evaluated, QT prolongation effects obtained after moxifloxacin exposure were directly transferable, not only between species, but also to man. In other words, uniform preclinical sensitivity to detect a repolarization delay with accurate and direct human translation has been confirmed across all major non-rodent species, establishing, for the first time, the requisite preclinical sensitivity to replicate the results obtained during a TQT study (Holzgreffe, Ferber et al. 2013).

Precise and accurate beat-to-beat ECG measurements are of great importance and represent the first and most critical step of any fully automated analytical method. While the current report focused on improved evaluation of the rate-corrected QT interval, the accepted regulatory endpoint, several other methods employ different approaches to evaluate the proarrhythmic risk. Alternative methods such as rate-binning or QTbtb do not rely on rate-correction, but compare on treatment data to drug-free data at similar RR intervals to eliminate potential source of errors associated with rate-correction functions. Indeed, QT rate-corrections are not devoid of limitations.

While individual QT rate-corrections are almost always established using off-drug data and subsequently applied to on-drug data, one may hypothesize that when a drug increases or decreases the QT-RR relationship, the application of an off-drug QT rate-correction may result in over- or under-corrected QTc values. Accordingly, it has been suggested that QT rate-correction derived from on-drug period may be more accurate when a drug modifies the QT-RR relationship. The major problem associated with this concept is the fact that clinical trials generally consist of a predose (off-drug) period, followed by the drug administration which results in variable drug concentration in the blood over time. These study protocols forbid the use of on-drug QT-rate corrections because it would inaccurately estimate the baseline QTc value. To solve this issue, one may suggest deriving mobile rate-corrections from short periods of time (~5-10 minutes) around the QT to be rate-corrected. However, short periods of time are generally associated with insufficient RR range and number of QT-RR pairs to reliably derive a QT-RR relationship. The method developed for BioQT consisted in applying mobile rate-correction factors measured over larger periods (4-6 hours) centered on the QT to be corrected. While BioQT provided reliable results, it must be emphasized that a QTc at 1h post dose will be measured using a rate-correction factor derived from 1h of drug-free data, plus 3h of rapidly (depending on the drug kinetics) changing drug concentration, which may possibly result in

inappropriate rate-correction factors. Altogether, unless stable drug concentration are administered for a long period of time, employing off-drug QT-rate correction is to be preferred as on-drug QT-RR relationship may confuse the interpretation of the results by over- or underestimating the baseline QTc values.

The 1-stage PK-PD modeling method supplements the raw QT and RR data with drug concentration data. This model treats the heart rate as a covariate in order to analyze drug products which modify the heart rate. Further alternative biomarkers of proarrhythmia, such as TPE or QT-TQ (ECG restitution) can be derived and analyzed from the beat-to-beat ECG measurements. Accordingly, computerized ECG measurement platforms facilitate the conduction of various repolarization analyses. Similar to the integrated risk assessment of cardiac repolarization in preclinical studies which has been associated with improved predictive value in man, it can be hypothesized that an integrated evaluation of various methods and biomarkers in man would very likely provide an improved assessment of the proarrhythmic potential of a drug compared to the sole evaluation of the QTc interval prolongation.

Several computerized ECG measurement platforms exist and employ various combinations of methodological and technical features. As characterized in the current report, each evaluated automated platform has demonstrated very similar results for the QTc evaluation after administration with moxifloxacin as well as with drugs with no impact on the QT interval. However, drugs associated with T-wave morphological changes resulted in quantitatively significant differences in the evaluation of drug-induced QTc prolongation between the automated methods. Although the magnitude of QTc prolongation obtained in TQT studies is not directly associated with the risk of proarrhythmia, it is still generally accepted that a large QTc prolongation (e.g. exceeding 20 ms) is more concerning than a smaller change (Garnett, Zhu et al. 2012). Accordingly, while the current results suggest that it remains very unlikely that any incorrect conclusions towards the QT-prolonging effect could be reached when employing continuous ECG analyses which included millions of beats, quantitative differences in QTc prolongation may, in rare cases, result in some uncertainty as to the discontinuance of drug development, or not. Importantly, despite the fact that SA analyses performed by ECG core laboratories are considered to be the gold standard, defining which method provides the true, accurate, optimal, and definite QTc evaluation cannot be achieved. Indeed, due to various method-specific features, computerized methods (including SA) do not evaluate the ECG traces exactly in same way. It is likely that the computation of median beats may result in slightly different ECG traces compared to beat-to-beat methods, or that a drug-induced change in the T-

wave descending slope may not be evaluated consistently with different algorithms. Collectively, each computerized method provided consistent conclusions and reproducible results with limited associated variability, suggesting that automated ECG measurement platforms now merit serious consideration as a replacement for sparse SA methodologies.

3.3. Median beats

While each automated method provided reproducible results with limited associated variability, the computation of median beats, unique to WinAtrec, resulted in the lowest standard deviation and confidence intervals, and highest numbers of subjects demonstrating a valid measurement for each time point. While this feature may not be appropriate to evaluate rapid beat-to-beat changes or QT and RR variability, WinAtrec presents itself as a powerful method to evaluate pharmacological effects such as in TQT studies. Indeed, the computation of 1-min median beats reduces the intrinsic rate-independent QT variability which both accelerates and facilitates the reproducible ECG measurements while keeping an appropriate time resolution. The current report suggests that a segment length of 5-min is optimal for continuous ECG analyses. Together with the multiple lead analyses and the well-designed user-interface, the current results support WinAtrec as the method of choice for the primary analysis of TQT studies, supplanting the current SA gold standard.

4. Research perspectives

While the current work focused on continuous ECG analysis employing different ECG measurement software and demonstrated that such methods provide a suitable alternative to the current SA gold standard methodology, several other methodologies have been developed to assess drug-induced QT prolongation effects. These methodologies include continuous and sparse individualized QT-RR correction, rate-binning analysis, dynamic QT_{btb}, and PK-PD modeling. Currently, it remains unclear whether one of these methods is superior to another, especially when evaluating drugs which impact waveform morphologies or heart rate. The parallel comparison of each methodology in TQT studies employing drugs which change the T-wave morphology and/or heart rate would provide very important information for an improved QT prolongation evaluation. However, extensively and systematically evaluating each method represents a huge amount of work that could only be performed by the collaboration of many pharmaceutical companies and research sites. Moreover, such work would require the release

of proprietary data in order to create large and accessible databases from which analyses could be performed employing each specific methodology.

Conclusions

The objectives of the current work were to evaluate whether the use of advanced computerized ECG measurement technologies could provide reliable continuous QT/QTc analyses for an improved assessment of proarrhythmic risks compared to traditional SA methods, during clinical studies.

The results obtained in Part I demonstrated that:

- Fully-automated algorithms are associated with measurement errors which are amplified in presence of T-wave morphology changes, and thus, require the combination with a review and adjustment feature.
- The implementation of a pattern recognition method effectively adjusted the algorithm-based measurement errors
- PRO decreased the QT measurement variability during baseline, in presence of T-wave morphology changes, and during periods of unrestrained physical activities, thus demonstrating that PRO is well suited for continuous ECG analysis.
- The application of distribution-based analysis on continuous data enabled the effective derivation of individual correction factors to dissociate the effect of heart rate.
- Compared to the gold standard SA method, PRO in conjunction with distribution-based analysis provided generally equivalent results and similar conclusions, but with greatly increased temporal resolution and reduced analytical costs.
- Altogether, Part I demonstrated that continuous ECG analyses enables a more detailed QT/QTc prolongation assessment through the use of an advanced computerized ECG measurement method (Ponemah PRO) in conjunction with an appropriate analysis methods (distribution-based analysis).

In Part II, four technologically different, commercially available ECG measurement methods (BioQT, ALG, PRO, WinAtrec, and SA) were evaluated to further assess the applicability of continuous ECG analysis and discover the possible impacts and differences related to unique

technologies and methodologies of QT prolongation measurement. The current results demonstrated that:

- Parallel comparison of all methods demonstrated similar moxifloxacin-induced effects and appropriate assay sensitivity.
- Positive SQR-induced and negative SGLT2i-induced effects were detected with each method, suggesting that computerized methods can supplant SA methods for primary TQT study analysis.
- Unique vendor-specific features resulted in differences in variability, confidence intervals, number of valid measurements, and sometimes quantitative QT prolongation effects, especially in presence of T-wave morphological changes. While each method demonstrated similar final analysis conclusions, the definite accuracy of each method cannot be ascertained.
- The Fridericia's QT correction (QTcF) can be reliably used as long as there are no substantial drug-induced RR effects.
- Collectively, the results obtained with the three advanced ECG measurement methods (BioQT, Ponemah PRO, WinAtrec) further support continuous ECG analysis as an effective alternative for the evaluation of TdP liabilities compared to the resource intensive semi-automated method.

Part III focused on providing recommendations regarding technical, methodological, and analytical aspects of continuous QT prolongation assessment:

- The choice of the lead to be analyzed must be chosen based on its ability to be consistently measured by a computerized platform and, when the selected lead present a poor-quality signal (for a whole subject or period), it can be effectively replaced by another high quality lead (generally lead II, or V2 to V5).
- Fully-automated ECG measurement methods which omit the careful review and adjustment processes are not recommended.
- The analysis of the influences of gender, age, and nycthemeral periods on the QT interval and the QT/RR relationship confirmed previously published results. These results further confirm that clinical study population should include both men and women of various ages.

- Determination of the individual correction factor with distribution-based analysis should preferentially be performed on day photoperiod data as mixing QT-RR pairs from different autonomic states may modify the QT-RR slope.
- The QT hysteresis did not demonstrate any impact on the drug effect assessment due to the inclusion of thousands of beats with continuous ECG analysis. However, including levels of RR averaging (60 seconds) in the corrected QT calculation formula reduces the variability of the QTc measurement.
- A 5 min time segments length is optimal for the depiction of the time-dependent changes of ECG intervals.
- Continuous ECG analysis facilitates the evaluation of other various surrogate biomarkers which, with broader experience, may provide an integrated and superior evaluation of the proarrhythmic risks.

In conclusion, the current work demonstrated that with technological advances, multiple tools are now available and enable to efficiently perform continuous ECG analysis which represents a more detailed alternative to the current gold standard semi-automated method.

References

- Ahnve, S. (1985). "Correction of the QT interval for heart rate: review of different formulas and the use of Bazett's formula in myocardial infarction." *Am Heart J* **109**(3 Pt 1): 568-574.
- Ahnve, S. and H. Vallin (1982). "Influence of heart rate and inhibition of autonomic tone on the QT interval." *Circulation* **65**(3): 435-439.
- Akiyama, T., J. Batchelder, et al. (1989). "Hypocalcemic Torsades de Pointes." *J Electrocardiol* **22**(1): 89-92.
- Ando, K., T. Hombo, et al. (2005). "QT PRODACT: in vivo QT assay with a conscious monkey for assessment of the potential for drug-induced QT interval prolongation." *J Pharmacol Sci* **99**(5): 487-500.
- Anson, B. D., J. G. Weaver, et al. (2005). "Blockade of HERG channels by HIV protease inhibitors." *Lancet* **365**(9460): 682-686.
- Antzelevitch, C. (2004). "Arrhythmogenic mechanisms of QT prolonging drugs: is QT prolongation really the problem?" *J Electrocardiol* **37** Suppl: 15-24.
- Antzelevitch, C. (2005). "Role of transmural dispersion of repolarization in the genesis of drug-induced torsades de pointes." *Heart Rhythm* **2**(2 Suppl): S9-15.
- Antzelevitch, C. (2007). "Role of spatial dispersion of repolarization in inherited and acquired sudden cardiac death syndromes." *Am J Physiol Heart Circ Physiol* **293**(4): H2024-2038.
- Antzelevitch, C. (2010). "M cells in the human heart." *Circ Res* **106**(5): 815-817.
- Antzelevitch, C., L. Belardinelli, et al. (2004). "Electrophysiologic properties and antiarrhythmic actions of a novel antianginal agent." *J Cardiovasc Pharmacol Ther* **9** Suppl 1: S65-83.
- Antzelevitch, C. and W. Shimizu (2002). "Cellular mechanisms underlying the long QT syndrome." *Curr Opin Cardiol* **17**(1): 43-51.
- Antzelevitch, C., W. Shimizu, et al. (1999). "The M cell: its contribution to the ECG and to normal and abnormal electrical function of the heart." *J Cardiovasc Electrophysiol* **10**(8): 1124-1152.
- Antzelevitch, C. and S. Sicouri (1994). "Clinical relevance of cardiac arrhythmias generated by afterdepolarizations. Role of M cells in the generation of U waves, triggered activity and torsade de pointes." *J Am Coll Cardiol* **23**(1): 259-277.
- Antzelevitch, C., S. Sicouri, et al. (1991). "Heterogeneity within the ventricular wall. Electrophysiology and pharmacology of epicardial, endocardial, and M cells." *Circ Res* **69**(6): 1427-1449.
- Arteyeva, N. V., S. L. Goshka, et al. (2013). "What does the T-T interval reflect? An experimental and model study." *J Electrocardiol*.
- AZCERT (2013). "Drugs That Prolong the QT Interval and/or Induce Torsades de Pointes." <http://www.torsades.org>.
- Babaeizadeh, S., R. E. Gregg, et al. (2009). "Improvements in atrial fibrillation detection for real-time monitoring." *J Electrocardiol* **42**(6): 522-526.
- Badilini, F. (2001). "The ISHNE Holter Standard Output File Format." <http://thw-project.org/papers/Badilini.ISHNE.Holter.Standard.pdf>.
- Badilini, F., P. Maison-Blanche, et al. (1999). "QT interval analysis on ambulatory electrocardiogram recordings: a selective beat averaging approach." *Med Biol Eng Comput* **37**(1): 71-79.
- Balse, E., D. F. Steele, et al. (2012). "Dynamic of ion channel expression at the plasma membrane of cardiomyocytes." *Physiol Rev* **92**(3): 1317-1358.
- Batchvarov, V. and M. Malik (2002). "Individual patterns of QT/RR relationship." *Card Electrophysiol Rev* **6**(3): 282-288.
- Bazett, H. C. (1920). "An analysis of the time relations of electrocardiograms." *Heart* **7**: 353-370.
- Bednar, M. M., E. P. Harrigan, et al. (2001). "The QT interval." *Prog Cardiovasc Dis* **43**(5 Suppl 1): 1-45.

- Belardinelli, L., C. Antzelevitch, et al. (2003). "Assessing predictors of drug-induced torsade de pointes." Trends Pharmacol Sci **24**(12): 619-625.
- Belardinelli, L., J. C. Shryock, et al. (2005). "Use of preclinical assays to predict risk of drug-induced torsades de pointes." Heart Rhythm **2**(2 Suppl): S16-22.
- Ben-David, J. and D. P. Zipes (1993). "Torsades de pointes and proarrhythmia." Lancet **341**(8860): 1578-1582.
- Benton, R. E., M. Sale, et al. (2000). "Greater quinidine-induced QTc interval prolongation in women." Clin Pharmacol Ther **67**(4): 413-418.
- Berger, E., K. Patel, et al. (2005). "Investigation of the effects of physiological and vasodilation-induced autonomic activation on the QTc Interval in healthy male subjects." Br J Clin Pharmacol **60**(1): 17-23.
- Bonnemeier, H., G. Richardt, et al. (2003). "Circadian profile of cardiac autonomic nervous modulation in healthy subjects: differing effects of aging and gender on heart rate variability." J Cardiovasc Electrophysiol **14**(8): 791-799.
- Bouvy, J. C., M. A. Koopmanschap, et al. (2011). "The Cost-Effectiveness of Drug Regulation: The Example of Thorough QT/QTc Studies." Clinical Pharmacology & Therapeutics **91**(2): 281-288.
- Bouvy, J. C., M. A. Koopmanschap, et al. (2012). "The cost-effectiveness of drug regulation: the example of thorough QT/QTc studies." Clin Pharmacol Ther **91**(2): 281-288.
- Braam, S. R., L. Tertoolen, et al. (2010). "Prediction of drug-induced cardiotoxicity using human embryonic stem cell-derived cardiomyocytes." Stem Cell Res **4**(2): 107-116.
- Browne, K. F., E. Prystowsky, et al. (1983). "Prolongation of the Q-T interval in man during sleep." Am J Cardiol **52**(1): 55-59.
- Browne, K. F., E. Prystowsky, et al. (1983). "Modulation of the Q-T interval by the autonomic nervous system." Pacing **6**(5 Pt 2): 1050-1056.
- Bufalari, A., D. Furbetta, et al. (1956). "Abnormality of the U wave and of the T-U segment of the electrocardiogram; the syndrome of the papillary muscles." Circulation **14**(6): 1129-1137.
- Carlsson, L. (2008). "The anaesthetised methoxamine-sensitised rabbit model of torsades de pointes." Pharmacol Ther **119**(2): 160-167.
- Carlsson, L., C. Abrahamsson, et al. (1993). "Proarrhythmic effects of the class III agent almokalant: importance of infusion rate, QT dispersion, and early afterdepolarisations." Cardiovascular Research **27**(12): 2186-2193.
- Cavero, I. (2012). "2011 Annual Meeting of the Safety Pharmacology Society: an overview." Expert Opin Drug Saf **11**(2): 341-353.
- Champeroux-Pascal, M.-E., Fowler-John-Sinclair-Lawrence, Maurin-Anne, Sola-Marie-Laure, Jude-Sebastien, Elamrani-Francine, Weyn-Andree-Anne, Laveissiere-Arnaud, Lala-Patricia, Richard-Serge. (2009). "Calculation of QT shift in non clinical safety pharmacology studies." Journal of Pharmacological and Toxicological Methods, {J-Pharmacol- Toxicol-Methods}, Mar-Apr 2009 (Epub: 10 Dec 2008), Vol. 59, No. 2, P. 73-85, Issn: 1873-488x.
- Champeroux, P., E. Martel, et al. (2013). "Power spectral analysis of heart rate variability in cynomolgus monkeys in safety pharmacology studies: Comparative study with beagle dogs." J Pharmacol Toxicol Methods.
- Champeroux, P., A. Ouille, et al. (2010). "Interferences of the autonomic nervous system with drug induced QT prolongation: a point to consider in non-clinical safety studies." J Pharmacol Toxicol Methods **61**(3): 251-263.
- Champeroux, P., K. Viaud, et al. (2005). "Prediction of the risk of Torsade de Pointes using the model of isolated canine Purkinje fibres." Br J Pharmacol **144**(3): 376-385.
- Chan, H. L., C. L. Wang, et al. (2010). "Recognition of ventricular extrasystoles over the reconstructed phase space of electrocardiogram." Ann Biomed Eng **38**(3): 813-823.

- Chen, J. and X. Zhao (2010). "A Bayesian measurement error approach to QT interval correction and prolongation." J Biopharm Stat **20**(3): 523-542.
- Chen, X., J. S. Cordes, et al. (2006). "Use of arterially perfused rabbit ventricular wedge in predicting arrhythmogenic potentials of drugs." J Pharmacol Toxicol Methods **54**(3): 261-272.
- Cheng, G., W. H. Litchenberg, et al. (1999). "Development of the cardiac conduction system involves recruitment within a multipotent cardiomyogenic lineage." Development **126**(22): 5041-5049.
- Cheng, H. C. and J. Incardona (2009). "Models of torsades de pointes: effects of FPL64176, DPI201106, dofetilide, and chromanol 293B in isolated rabbit and guinea pig hearts." J Pharmacol Toxicol Methods **60**(2): 174-184.
- Cordes, J. S., Z. Sun, et al. (2005). "Pentamidine reduces hERG expression to prolong the QT interval." Br J Pharmacol **145**(1): 15-23.
- Couderc, J., M. Zhou, et al. (2008). "Investigating the effect of sotalol on the repolarization intervals in healthy young individuals." Journal of Electrocardiology **41**(6): 595-602.
- Couderc, J. P. (2009). "Measurement and regulation of cardiac ventricular repolarization: from the QT interval to repolarization morphology." Philos Transact A Math Phys Eng Sci **367**(1892): 1283-1299.
- Couderc, J. P., C. Garnett, et al. (2011). "Highly automated QT measurement techniques in 7 thorough QT studies implemented under ICH E14 guidelines." Ann Noninvasive Electrocardiol **16**(1): 13-24.
- Coumel, P., J. Fayn, et al. (1994). "Clinical relevance of assessing QT dynamicity in Holter recordings." J Electrocardiol **27 Suppl**: 62-66.
- Coumel, P., P. Maison-Blanche, et al. (1995). "Different circadian behavior of the apex and the end of the T wave." J Electrocardiol **28 Suppl**: 138-142.
- Darpo, B. and C. Garnett (2012). "Early QT assessment - how can our confidence in the data be improved?" Br J Clin Pharmacol.
- Davidowski, T. A. and S. Wolf (1984). "The QT interval during reflex cardiovascular adaptation." Circulation **69**(1): 22-25.
- Dennis, A., L. Wang, et al. (2007). "hERG channel trafficking: novel targets in drug-induced long QT syndrome." Biochem Soc Trans **35**(Pt 5): 1060-1063.
- DiFrancesco, D. (2010). "The role of the funny current in pacemaker activity." Circ Res **106**(3): 434-446.
- Drew, B. J., M. J. Ackerman, et al. (2010). "Prevention of torsade de pointes in hospital settings: a scientific statement from the American Heart Association and the American College of Cardiology Foundation." Circulation **121**(8): 1047-1060.
- DSI (2010). "Template Analysis Manual (MU00279_Template Analysis Manual), Revision nn, Copyright Data Sciences International, 20nn."
- Eckardt, L., W. Haverkamp, et al. (1998). "Experimental models of torsade de pointes." Cardiovascular Research **39**(1): 178-193.
- Eckardt, L., W. Haverkamp, et al. (1998). "Experimental models of torsade de pointes." Cardiovasc Res **39**(1): 178-193.
- Elming, H., B. Brendorp, et al. (2002). "QTc interval in the assessment of cardiac risk." Card Electrophysiol Rev **6**(3): 289-294.
- Extramiana, F., F. Badilini, et al. (2007). "Contrasting Time- and Rate-Based Approaches for the Assessment of Drug-Induced QT Changes." The Journal of Clinical Pharmacology **47**(9): 1129-1137.
- Extramiana, F., P. Maison-Blanche, et al. (1999). "Circadian modulation of QT rate dependence in healthy volunteers: gender and age differences." J Electrocardiol **32**(1): 33-43.
- Extramiana, F., P. Maison-Blanche, et al. (2006). "Control of rapid heart rate changes for electrocardiographic analysis: implications for thorough QT studies." Clin Cardiol **29**(12): 534-539.

- Fayn, J. and P. Rubel (1988). "CAVIAR: a serial ECG processing system for the comparative analysis of VCGs and their interpretation with auto-reference to the patient." J Electrocardiol **21** **Suppl**: S173-176.
- FDA (2005). "Guidance for Industry: safety pharmacology studies for human pharmaceuticals (S7A). <http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm074959.pdf>."
- FDA (2005). "Guidance for Industry: The nonclinical evaluation of the potential for delayed ventricular repolarization by human pharmaceuticals (S7B). <http://www.fda.gov/OHRMS/DOCKETS/98fr/2004d-0378-gdl0001.pdf>."
- FDA (2012). "Guidance for Industry: Clinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential for Non-Antiarrhythmic Drugs (E14): Questions and Answers (R1) <http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm073161.pdf>."
- Fenichel, R. R., M. Malik, et al. (2004). "Drug-induced torsades de pointes and implications for drug development." J Cardiovasc Electrophysiol **15**(4): 475-495.
- Fiset, C., B. Drolet, et al. (1997). "Block of IKs by the diuretic agent indapamide modulates cardiac electrophysiological effects of the class III antiarrhythmic drug dl-sotalol." J Pharmacol Exp Ther **283**(1): 148-156.
- Fitzgerald, P. T. and M. J. Ackerman (2005). "Drug-induced torsades de pointes: the evolving role of pharmacogenetics." Heart Rhythm **2**(2 **Suppl**): S30-37.
- Fossa, A. A. (2004). "Dynamic Beat-to-Beat Modeling of the QT-RR Interval Relationship: Analysis of QT Prolongation during Alterations of Autonomic State versus Human Ether a-go-go-Related Gene Inhibition." Journal of Pharmacology and Experimental Therapeutics **312**(1): 1-11.
- Fossa, A. A., M. J. DePasquale, et al. (2002). "The relationship of clinical QT prolongation to outcome in the conscious dog using a beat-to-beat QT-RR interval assessment." J Pharmacol Exp Ther **302**(2): 828-833.
- Fossa, A. A., G. Langdon, et al. (2011). "The use of beat-to-beat electrocardiogram analysis to distinguish QT/QTc interval changes caused by moxifloxacin from those caused by vardenafil." Clin Pharmacol Ther **90**(3): 449-454.
- Fossa, A. A., T. Wisialowski, et al. (2006). "QT prolongation modifies dynamic restitution and hysteresis of the beat-to-beat QT-TQ interval relationship during normal sinus rhythm under varying states of repolarization." J Pharmacol Exp Ther **316**(2): 498-506.
- Fossa, A. A., T. Wisialowski, et al. (2007). "Analyses of dynamic beat-to-beat QT-TQ interval (ECG restitution) changes in humans under normal sinus rhythm and prior to an event of torsades de pointes during QT prolongation caused by sotalol." Ann Noninvasive Electrocardiol **12**(4): 338-348.
- Fossa, A. A., T. Wisialowski, et al. (2004). "Differential effect of HERG blocking agents on cardiac electrical alternans in the guinea pig." Eur J Pharmacol **486**(2): 209-221.
- Fosser, C., G. Duczynski, et al. (2009). "Comparison of manual and automated measurements of the QT interval in healthy volunteers: an analysis of five thorough QT studies." Clin Pharmacol Ther **86**(5): 503-506.
- Friedrichs, G. S., L. Patmore, et al. (2005). "Non-clinical evaluation of ventricular repolarization (ICH S7B): results of an interim survey of international pharmaceutical companies." J Pharmacol Toxicol Methods **52**(1): 6-11.
- Funck-Brentano, C. and P. Jaillon (1993). "Rate-corrected QT interval: techniques and limitations." Am J Cardiol **72**(6): 17B-22B.
- Gallacher, D. J., A. Van de Water, et al. (2007). "In vivo mechanisms precipitating torsades de pointes in a canine model of drug-induced long-QT1 syndrome." Cardiovascular Research **76**(2): 247-256.

- Garnett, C. E., H. Zhu, et al. (2012). "Methodologies to characterize the QT/corrected QT interval in the presence of drug-induced heart rate changes or other autonomic effects." Am Heart J **163**(6): 912-930.
- Glancy, J. M., P. J. Weston, et al. (1996). "Reproducibility and automatic measurement of QT dispersion." Eur Heart J **17**(7): 1035-1039.
- Graff, C., M. P. Andersen, et al. (2009). "Identifying drug-induced repolarization abnormalities from distinct ECG patterns in congenital long QT syndrome: a study of sotalol effects on T-wave morphology." Drug Saf **32**(7): 599-611.
- Graff, C., J. J. Struijk, et al. (2010). "Covariate analysis of QTc and T-wave morphology: new possibilities in the evaluation of drugs that affect cardiac repolarization." Clin Pharmacol Ther **88**(1): 88-94.
- Gralinski, M. R. (2003). "The dog's role in the preclinical assessment of QT interval prolongation." Toxicol Pathol **31 Suppl**: 11-16.
- Greaves, P. (1998). "Patterns of drug-induced cardiovascular pathology in the beagle dog: relevance for humans." Exp Toxicol Pathol **50**(4-6): 283-293.
- Green, C. L., P. Kligfield, et al. (2012). "Detection of QT prolongation using a novel electrocardiographic analysis algorithm applying intelligent automation: prospective blinded evaluation using the Cardiac Safety Research Consortium electrocardiographic database." Am Heart J **163**(3): 365-371.
- Guns, P. J., D. M. Johnson, et al. (2012). "Negative electro-mechanical windows are required for drug-induced Torsades de Pointes in the anesthetized guinea pig." J Pharmacol Toxicol Methods **66**(2): 125-134.
- Guo, D., J. Zhou, et al. (2008). "L-type calcium current recovery versus ventricular repolarization: preserved membrane-stabilizing mechanism for different QT intervals across species." Heart Rhythm **5**(2): 271-279.
- Guo, L., R. M. Abrams, et al. (2011). "Estimating the risk of drug-induced proarrhythmia using human induced pluripotent stem cell-derived cardiomyocytes." Toxicol Sci **123**(1): 281-289.
- Guth, B. D. (2007). "Preclinical cardiovascular risk assessment in modern drug development." Toxicol Sci **97**(1): 4-20.
- Haarmark, C., C. Graff, et al. (2010). "Reference values of electrocardiogram repolarization variables in a healthy population." J Electrocardiol **43**(1): 31-39.
- Hanson, L. A., A. S. Bass, et al. (2006). "ILSI-HESI cardiovascular safety subcommittee initiative: evaluation of three non-clinical models of QT prolongation." J Pharmacol Toxicol Methods **54**(2): 116-129.
- HESI (2012). "A HESI Consortium Approach to Assess the Human Predictive Value of Non-Clinical Repolarization Assays." http://www.hesiglobal.org/files/public/Committee%20Presentations/Cardiac%20Safety/SOT_03_1512_final.pdf.
- Hinterseer, M., B. M. Beckmann, et al. (2010). "Usefulness of short-term variability of QT intervals as a predictor for electrical remodeling and proarrhythmia in patients with nonischemic heart failure." Am J Cardiol **106**(2): 216-220.
- Hinterseer, M., M. B. Thomsen, et al. (2008). "Beat-to-beat variability of QT intervals is increased in patients with drug-induced long-QT syndrome: a case control pilot study." Eur Heart J **29**(2): 185-190.
- Hnatkova, K., Y. Gang, et al. (2006). "Precision of QT interval measurement by advanced electrocardiographic equipment." Pacing Clin Electrophysiol **29**(11): 1277-1284.
- Hnatkova, K. and M. Malik (1999). "'Optimum' formulae for heart rate correction of the QT interval." Pacing Clin Electrophysiol **22**(11): 1683-1687.
- Hoefen, R., M. Reumann, et al. (2012). "In silico cardiac risk assessment in patients with long QT syndrome: type 1: clinical predictability of cardiac models." J Am Coll Cardiol **60**(21): 2182-2191.

- Hoffman, B. F. (1962). "Electrophysiology of the conducting system of the heart." Trans N Y Acad Sci **24**: 886-890.
- Hoffmann, P. and B. Warner (2006). "Are hERG channel inhibition and QT interval prolongation all there is in drug-induced torsadogenesis? A review of emerging trends." J Pharmacol Toxicol Methods **53**(2): 87-105.
- Hofman, N., A. A. Wilde, et al. (2007). "Diagnostic criteria for congenital long QT syndrome in the era of molecular genetics: do we need a scoring system?" Eur Heart J **28**(5): 575-580.
- Holzgreffe, H., I. Cavero, et al. (2007). "Novel probabilistic method for precisely correcting the QT interval for heart rate in telemetered dogs and cynomolgus monkeys." Journal of Pharmacological and Toxicological Methods **55**(2): 159-175.
- Holzgreffe, H., G. Ferber, et al. (2013). "Preclinical QT Safety Assessment: Cross-Species Comparisons and Human Translation from an Industry Consortium." J Pharmacol Toxicol Methods.
- Holzgreffe, H. H., I. Cavero, et al. (2007). "Application of a probabilistic method for the determination of drug-induced QT prolongation in telemetered cynomolgus monkeys: Effects of moxifloxacin." Journal of Pharmacological and Toxicological Methods **55**(3): 244-254.
- Holzgreffe, H. H., I. Cavero, et al. (2007). "Application of a probabilistic method for the determination of drug-induced QT prolongation in telemetered cynomolgus monkeys: effects of moxifloxacin." Journal of Pharmacological and Toxicological Methods **55**(3): 227-237.
- Holzgreffe, H. H., I. Cavero, et al. (2007). "Analysis of the nonclinical telemetered ECG: impact of logging rate and RR bin width in the dog and cynomolgus monkey." Journal of Pharmacological and Toxicological Methods **56**(1): 34-42.
- Holzgreffe, H. H., G. Ferber, et al. (2012). "Characterization of the human QT interval: novel distribution-based assessment of the repolarization effects of moxifloxacin." J Clin Pharmacol **52**(8): 1222-1239.
- Honda, M., R. Komatsu, et al. (2010). "Application of probabilistic analysis for precisely correcting the QT interval for heart rate in telemetered common marmosets." Journal of Pharmacological and Toxicological Methods **61**(3): 264-270.
- Hondeghem, L. M. (2005). "TRLad: foundation for proarrhythmia (triangulation, reverse use dependence and instability)." Novartis Found Symp **266**: 235-244; discussion 244-250.
- Hondeghem, L. M. (2008). "Use and abuse of QT and TRLad in cardiac safety research: importance of study design and conduct." Eur J Pharmacol **584**(1): 1-9.
- Hondeghem, L. M., L. Carlsson, et al. (2001). "Instability and triangulation of the action potential predict serious proarrhythmia, but action potential duration prolongation is antiarrhythmic." Circulation **103**(15): 2004-2013.
- Hondeghem, L. M. and P. Hoffmann (2003). "Blinded test in isolated female rabbit heart reliably identifies action potential duration prolongation and proarrhythmic drugs: importance of triangulation, reverse use dependence, and instability." J Cardiovasc Pharmacol **41**(1): 14-24.
- Hopenfeld, B. and H. Ashikaga (2010). "Origin of the electrocardiographic U wave: effects of M cells and dynamic gap junction coupling." Ann Biomed Eng **38**(3): 1060-1070.
- Hoyt, R. H., M. L. Cohen, et al. (1989). "Distribution and three-dimensional structure of intercellular junctions in canine myocardium." Circ Res **64**(3): 563-574.
- Huh, D., G. A. Hamilton, et al. (2011). "From 3D cell culture to organs-on-chips." Trends Cell Biol **21**(12): 745-754.
- Huh, D., Y. S. Torisawa, et al. (2012). "Microengineered physiological biomimicry: organs-on-chips." Lab Chip **12**(12): 2156-2164.
- Hutmacher, M. M., S. Chapel, et al. (2008). "Performance characteristics for some typical QT study designs under the ICH E-14 guidance." J Clin Pharmacol **48**(2): 215-224.

- Ibrahim, M., J. Gorelik, et al. (2011). "The structure and function of cardiac t-tubules in health and disease." *Proc Biol Sci* **278**(1719): 2714-2723.
- Ince, T., S. Kiranyaz, et al. (2009). "A generic and robust system for automated patient-specific classification of ECG signals." *IEEE Trans Biomed Eng* **56**(5): 1415-1426.
- Izumi, D., M. Chinushi, et al. (2012). "The peak-to-end of the T wave in the limb ECG leads reflects total spatial rather than transmural dispersion of ventricular repolarization in an anthopleurin-A model of prolonged QT interval." *Heart Rhythm* **9**(5): 796-803.
- Jacobson, I., L. Carlsson, et al. (2011). "Beat-by-beat QT interval variability, but not QT prolongation per se, predicts drug-induced torsades de pointes in the anaesthetised methoxamine-sensitized rabbit." *J Pharmacol Toxicol Methods* **63**(1): 40-46.
- Jervell, A. and F. Lange-Nielsen (1957). "Congenital deaf-mutism, functional heart disease with prolongation of the Q-T interval and sudden death." *Am Heart J* **54**(1): 59-68.
- Johnson, D. M., J. Heijman, et al. (2012). "Diastolic Spontaneous Calcium Release from the Sarcoplasmic Reticulum Increases Beat-to-Beat Variability of Repolarization in Canine Ventricular Myocytes after beta-Adrenergic Stimulation." *Circ Res*.
- Joshi, A., T. Dimino, et al. (2004). "Preclinical strategies to assess QT liability and torsadogenic potential of new drugs: the role of experimental models." *J Electrocardiol* **37** Suppl: 7-14.
- Joung, B., H. Zhang, et al. (2011). "Delayed afterdepolarization in intact canine sinoatrial node as a novel mechanism for atrial arrhythmia." *J Cardiovasc Electrophysiol* **22**(4): 448-454.
- Kanters, J. K., S. Fanoë, et al. (2004). "T wave morphology analysis distinguishes between KvLQT1 and HERG mutations in long QT syndrome." *Heart Rhythm* **1**(3): 285-292.
- Katchman, A. N., J. Koerner, et al. (2006). "Comparative evaluation of HERG currents and QT intervals following challenge with suspected torsadogenic and nontorsadogenic drugs." *J Pharmacol Exp Ther* **316**(3): 1098-1106.
- Kautzner, J. (2002). "QT interval measurements." *Card Electrophysiol Rev* **6**(3): 273-277.
- Kawataki, M., T. Kashima, et al. (1984). "Relation between QT interval and heart rate. applications and limitations of Bazett's formula." *J Electrocardiol* **17**(4): 371-375.
- Kessler, R. C., P. Berglund, et al. (2003). "The epidemiology of major depressive disorder: results from the National Comorbidity Survey Replication (NCS-R)." *Jama* **289**(23): 3095-3105.
- Kessler, R. C., K. A. McGonagle, et al. (1994). "Lifetime and 12-month prevalence of DSM-III-R psychiatric disorders in the United States. Results from the National Comorbidity Survey." *Arch Gen Psychiatry* **51**(1): 8-19.
- Komatsu, R., M. Honda, et al. (2010). "Sensitivity of common marmosets to detect drug-induced QT interval prolongation: moxifloxacin case study." *Journal of Pharmacological and Toxicological Methods* **61**(3): 271-276.
- Kuryshv, Y. A., E. Ficker, et al. (2005). "Pentamidine-induced long QT syndrome and block of hERG trafficking." *J Pharmacol Exp Ther* **312**(1): 316-323.
- Lau, C. P., A. R. Freedman, et al. (1988). "Hysteresis of the ventricular paced QT interval in response to abrupt changes in pacing rate." *Cardiovascular Research* **22**(1): 67-72.
- Lawrence, C. L., M. H. Bridgland-Taylor, et al. (2006). "A rabbit Langendorff heart proarrhythmia model: predictive value for clinical identification of Torsades de Pointes." *Br J Pharmacol* **149**(7): 845-860.
- Lawrence, C. L., C. E. Pollard, et al. (2005). "Nonclinical proarrhythmia models: predicting Torsades de Pointes." *J Pharmacol Toxicol Methods* **52**(1): 46-59.
- LeGrice, I. J., B. H. Smaill, et al. (1995). "Laminar structure of the heart: ventricular myocyte arrangement and connective tissue architecture in the dog." *Am J Physiol* **269**(2 Pt 2): H571-582.

- Leishman, D. J., T. W. Beck, et al. (2011). "Best practice in the conduct of key nonclinical cardiovascular assessments in drug development: Current recommendations from the Safety Pharmacology Society." Journal of Pharmacological and Toxicological Methods.
- Leishman, D. J., T. W. Beck, et al. (2012). "Best practice in the conduct of key nonclinical cardiovascular assessments in drug development: current recommendations from the Safety Pharmacology Society." J Pharmacol Toxicol Methods **65**(3): 93-101.
- Liang, P., F. Lan, et al. (2013). "Drug Screening Using a Library of Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes Reveals Disease Specific Patterns of Cardiotoxicity." Circulation.
- Lindgren, S., A. S. Bass, et al. (2008). "Benchmarking safety pharmacology regulatory packages and best practice." J Pharmacol Toxicol Methods **58**(2): 99-109.
- Liu, T., B. S. Brown, et al. (2006). "Blinded validation of the isolated arterially perfused rabbit ventricular wedge in preclinical assessment of drug-induced proarrhythmias." Heart Rhythm **3**(8): 948-956.
- Liu, T., B. R. Choi, et al. (2005). "Sex modulates the arrhythmogenic substrate in prepubertal rabbit hearts with Long QT 2." J Cardiovasc Electrophysiol **16**(5): 516-524.
- Lodder, E. M. and A. A. Wilde (2012). "Clinical assessment of the pathogenicity of unknown variants in long-QT syndrome: does the pendulum swing back?" J Cardiovasc Electrophysiol **23**(6): 643-644.
- Ma, J., L. Guo, et al. (2011). "High purity human-induced pluripotent stem cell-derived cardiomyocytes: electrophysiological properties of action potentials and ionic currents." Am J Physiol Heart Circ Physiol **301**(5): H2006-2017.
- Magnano, A. R., S. Holleran, et al. (2002). "Autonomic nervous system influences on QT interval in normal subjects." J Am Coll Cardiol **39**(11): 1820-1826.
- Malik, M. (2001). "Problems of heart rate correction in assessment of drug-induced QT interval prolongation." J Cardiovasc Electrophysiol **12**(4): 411-420.
- Malik, M. (2004). "Errors and misconceptions in ECG measurement used for the detection of drug induced QT interval prolongation." Journal of Electrocardiology **37**: 25-33.
- Malik, M. and A. J. Camm (2001). "Evaluation of Drug-Induced QT Interval Prolongation: Implications for Drug Approval and Labelling." Drug Safety **24**(5): 323-351.
- Malik, M., K. Hnatkova, et al. (2004). "Differences between study-specific and subject-specific heart rate corrections of the QT interval in investigations of drug induced QTc prolongation." Pacing Clin Electrophysiol **27**(6 Pt 1): 791-800.
- Malik, M., K. Hnatkova, et al. (2008). "Near-Thorough QT Study as Part of a First-In-Man Study." The Journal of Clinical Pharmacology **48**(10): 1146-1157.
- Malik, M., K. Hnatkova, et al. (2012). "Importance of subject-specific QT/RR curvatures in the design of individual heart rate corrections of the QT interval." J Electrocardiol **45**(6): 571-581.
- Malik, M., K. Hnatkova, et al. (2008). "Subject-specific profiles of QT/RR hysteresis." Am J Physiol Heart Circ Physiol **295**(6): H2356-2363.
- Malik, M., K. Hnatkova, et al. (2008). "Accurately measured and properly heart-rate corrected QTc intervals show little daytime variability." Heart Rhythm **5**(10): 1424-1431.
- Malik, M., K. Hnatkova, et al. (2009). "Correction for QT/RR Hysteresis in the Assessment of Drug-Induced QTc Changes—Cardiac Safety of Gadobutrol." Annals of Noninvasive Electrocardiology **14**(3): 242-250.
- Markert, M., R. Shen, et al. (2011). "Heart rate correction models to detect QT interval prolongation in novel pharmaceutical development." J Pharmacol Toxicol Methods **64**(1): 25-41.
- Martignoni, M., G. Groothuis, et al. (2006). "Comparison of mouse and rat cytochrome P450-mediated metabolism in liver and intestine." Drug Metab Dispos **34**(6): 1047-1054.
- McLaughlin, N. B., R. W. Campbell, et al. (1996). "Accuracy of four automatic QT measurement techniques in cardiac patients and healthy subjects." Heart **76**(5): 422-426.

- Meurs, K. M., A. W. Spier, et al. (2001). "Use of ambulatory electrocardiography for detection of ventricular premature complexes in healthy dogs." J Am Vet Med Assoc **218**(8): 1291-1292.
- Meyer, J. H., A. A. Wilson, et al. (2004). "Serotonin transporter occupancy of five selective serotonin reuptake inhibitors at different doses: an [¹¹C]DASB positron emission tomography study." Am J Psychiatry **161**(5): 826-835.
- Meyer, O., G. Ferber, et al. (2012). "Pattern recognition analysis of digital ECGs: Decreased QT measurement error and improved precision compared to semi-automated methods." J Electrocardiol.
- Michalak, M. and M. Opas (2009). "Endoplasmic and sarcoplasmic reticulum in the heart." Trends Cell Biol **19**(6): 253-259.
- Milberg, P., E. Hilker, et al. (2007). "Proarrhythmia as a class effect of quinolones: increased dispersion of repolarization and triangulation of action potential predict torsades de pointes." J Cardiovasc Electrophysiol **18**(6): 647-654.
- Milberg, P., S. Ramtin, et al. (2004). "Comparison of the in vitro electrophysiologic and proarrhythmic effects of amiodarone and sotalol in a rabbit model of acute atrioventricular block." J Cardiovasc Pharmacol **44**(3): 278-286.
- Miyazaki, H., H. Watanabe, et al. (2005). "QT PRODACT: sensitivity and specificity of the canine telemetry assay for detecting drug-induced QT interval prolongation." J Pharmacol Sci **99**(5): 523-529.
- Morganroth, J. (2001). "Focus on issues in measuring and interpreting changes in the QTc interval duration." European Heart Journal Supplements **3**(K): K105-K111.
- Morganroth, J., J. P. Dimarco, et al. (2005). "A randomized trial comparing the cardiac rhythm safety of moxifloxacin vs levofloxacin in elderly patients hospitalized with community-acquired pneumonia." Chest **128**(5): 3398-3406.
- Moss, A. J., P. J. Schwartz, et al. (1991). "The long QT syndrome. Prospective longitudinal study of 328 families." Circulation **84**(3): 1136-1144.
- Nagy, D., R. DeMeersman, et al. (1997). "QTc interval (cardiac repolarization): lengthening after meals." Obes Res **5**(6): 531-537.
- Natekar, M., P. Hingorani, et al. (2010). "Effect of Number of Replicate Electrocardiographs Recorded at Each Time Point in a Thorough QT Study on Sample Size and Study Cost." The Journal of Clinical Pharmacology.
- Neyroud, N., P. Maison-Blanche, et al. (1998). "Diagnostic performance of QT interval variables from 24-h electrocardiography in the long QT syndrome." Eur Heart J **19**(1): 158-165.
- Obeyesekere, M. N., R. W. Sy, et al. (2012). "End-recovery QTc: a useful metric for assessing genetic variants of unknown significance in long-QT syndrome." J Cardiovasc Electrophysiol **23**(6): 637-642.
- Olshansky, B., H. N. Sabbah, et al. (2008). "Parasympathetic nervous system and heart failure: pathophysiology and potential implications for therapy." Circulation **118**(8): 863-871.
- Olson, H., G. Betton, et al. (2000). "Concordance of the toxicity of pharmaceuticals in humans and in animals." Regul Toxicol Pharmacol **32**(1): 56-67.
- Oros, A., J. D. Beekman, et al. (2008). "The canine model with chronic, complete atrio-ventricular block." Pharmacol Ther **119**(2): 168-178.
- Ozbyay, Y. (2009). "A new approach to detection of ECG arrhythmias: complex discrete wavelet transform based complex valued artificial neural network." J Med Syst **33**(6): 435-445.
- Panicker, G. K., D. R. Karnad, et al. (2009). "Intra- and interreader variability in QT interval measurement by tangent and threshold methods in a central electrocardiogram laboratory." J Electrocardiol **42**(4): 348-352.

- Pedersen, H. S., H. Elming, et al. (2007). "Risk factors and predictors of Torsade de pointes ventricular tachycardia in patients with left ventricular systolic dysfunction receiving Dofetilide." Am J Cardiol **100**(5): 876-880.
- Pelchovitz, D. J., J. Ng, et al. (2012). "QT-RR hysteresis is caused by differential autonomic states during exercise and recovery." Am J Physiol Heart Circ Physiol **302**(12): H2567-2573.
- Perez Riera, A. R., C. Ferreira, et al. (2008). "The enigmatic sixth wave of the electrocardiogram: the U wave." Cardiol J **15**(5): 408-421.
- Peters, N. S. and A. L. Wit (1998). "Myocardial architecture and ventricular arrhythmogenesis." Circulation **97**(17): 1746-1754.
- Pickham, D., E. Flowers, et al. (2013). "Hyperglycemia Is Associated With Corrected QT Prolongation and Mortality in Acutely Ill Patients." J Cardiovasc Nurs.
- Poelzing, S., F. G. Akar, et al. (2004). "Heterogeneous connexin43 expression produces electrophysiological heterogeneities across ventricular wall." Am J Physiol Heart Circ Physiol **286**(5): H2001-2009.
- Priori, S. G., C. Napolitano, et al. (1999). "Low penetrance in the long-QT syndrome: clinical impact." Circulation **99**(4): 529-533.
- Pueyo, E., P. Smetana, et al. (2004). "Characterization of QT interval adaptation to RR interval changes and its use as a risk-stratifier of arrhythmic mortality in amiodarone-treated survivors of acute myocardial infarction." IEEE Trans Biomed Eng **51**(9): 1511-1520.
- Pugsley, M. K., S. Authier, et al. (2008). "Principles of safety pharmacology." Br J Pharmacol **154**(7): 1382-1399.
- Pugsley, M. K., J. C. Hancox, et al. (2008). "Perception of validity of clinical and preclinical methods for assessment of torsades de pointes liability." Pharmacol Ther **119**(2): 115-117.
- Redfern, W. S., L. Carlsson, et al. (2003). "Relationships between preclinical cardiac electrophysiology, clinical QT interval prolongation and torsade de pointes for a broad range of drugs: evidence for a provisional safety margin in drug development." Cardiovascular Research **58**(1): 32-45.
- Roche, M., C. Renauleaud, et al. (2010). "The isolated rabbit heart and Purkinje fibers as models for identifying proarrhythmic liability." J Pharmacol Toxicol Methods **61**(3): 238-250.
- Roden, D. M. (1998). "Taking the "idio" out of "idiosyncratic": predicting torsades de pointes." Pacing Clin Electrophysiol **21**(5): 1029-1034.
- Roden, D. M. (2004). "Drug-induced prolongation of the QT interval." N Engl J Med **350**(10): 1013-1022.
- Roden, D. M. and D. H. Linsmith (1987). "Effects of low potassium or magnesium concentrations on isolated cardiac tissue." Am J Med **82**(3A): 18-23.
- Roden, D. M., R. Lazzara, et al. (1996). "Multiple mechanisms in the long-QT syndrome. Current knowledge, gaps, and future directions. The SADS Foundation Task Force on LQTS." Circulation **94**(8): 1996-2012.
- Rosca, M. G. and C. L. Hoppel (2010). "Mitochondria in heart failure." Cardiovascular Research **88**(1): 40-50.
- Rowan, W. H., 3rd, M. J. Campen, et al. (2007). "Heart rate variability in rodents: uses and caveats in toxicological studies." Cardiovasc Toxicol **7**(1): 28-51.
- Russel, W. M. S. and R. L. Burch (1959). "The principles of Humane Experimental Technique." (in press).
- Sager, P. T. (2008). "Key clinical considerations for demonstrating the utility of preclinical models to predict clinical drug-induced torsades de pointes." Br J Pharmacol **154**(7): 1544-1549.
- Sagie, A., M. G. Larson, et al. (1992). "An improved method for adjusting the QT interval for heart rate (the Framingham Heart Study)." Am J Cardiol **70**(7): 797-801.
- Said, T. H., L. D. Wilson, et al. (2012). "Transmural dispersion of repolarization as a preclinical marker of drug-induced proarrhythmia." J Cardiovasc Pharmacol **60**(2): 165-171.
- Salama, G. and B. London (2007). "Mouse models of long QT syndrome." J Physiol **578**(Pt 1): 43-53.

- Salvi, V., D. R. Karnad, et al. (2012). "Choice of an alternative lead for QT interval measurement in serial ECGs when Lead II is not suitable for analysis." Indian Heart J **64**(6): 535-540.
- Sanguinetti, M. C. and J. S. Mitcheson (2005). "Predicting drug-hERG channel interactions that cause acquired long QT syndrome." Trends Pharmacol Sci **26**(3): 119-124.
- Sarapa, N., I. Gussak, et al. (2009). "Comparison of QTinno, a fully automated electrocardiographic analysis program, to semiautomated electrocardiographic analysis methods in a drug safety study in healthy subjects." J Electrocardiol **42**(4): 358-366.
- Sarapa, N., D. J. Nickens, et al. (2008). "Ritonavir 100 mg does not cause QTc prolongation in healthy subjects: a possible role as CYP3A inhibitor in thorough QTc studies." Clin Pharmacol Ther **83**(1): 153-159.
- Schwartz, P. J., A. J. Moss, et al. (1993). "Diagnostic criteria for the long QT syndrome. An update." Circulation **88**(2): 782-784.
- Schwartz, P. J., S. G. Priori, et al. (2001). "Genotype-phenotype correlation in the long-QT syndrome: gene-specific triggers for life-threatening arrhythmias." Circulation **103**(1): 89-95.
- Schwartz, P. J., M. Stramba-Badiale, et al. (2009). "Prevalence of the congenital long-QT syndrome." Circulation **120**(18): 1761-1767.
- Scofield, M. A., J. D. Deupree, et al. (2000). "Construction of libraries for isolation of adrenergic receptor genes." Methods Mol Biol **126**: 3-35.
- Shah, R. R. (2002). "Drug-induced prolongation of the QT interval: regulatory dilemmas and implications for approval and labelling of a new chemical entity." Fundam Clin Pharmacol **16**(2): 147-156.
- Shah, R. R. (2008). "If a drug deemed 'safe' in nonclinical tests subsequently prolongs QT in phase 1 studies, how can its sponsor convince regulators to allow development to proceed?" Pharmacol Ther **119**(2): 215-221.
- Shah, S. A., J. Kluger, et al. (2007). "Monotherapy versus combination therapy with class III antiarrhythmic agents to attenuate transmural dispersion of repolarization: a potential risk factor for torsade de pointes." Pharmacotherapy **27**(9): 1297-1305.
- Shimizu, W. and M. Horie (2011). "Phenotypic manifestations of mutations in genes encoding subunits of cardiac potassium channels." Circ Res **109**(1): 97-109.
- Shimizu, W., B. McMahon, et al. (1999). "Sodium pentobarbital reduces transmural dispersion of repolarization and prevents torsades de Pointes in models of acquired and congenital long QT syndrome." J Cardiovasc Electrophysiol **10**(2): 154-164.
- Sicouri, S. and C. Antzelevitch (1991). "A subpopulation of cells with unique electrophysiological properties in the deep subepicardium of the canine ventricle. The M cell." Circ Res **68**(6): 1729-1741.
- Sicouri, S., S. Moro, et al. (1997). "Chronic amiodarone reduces transmural dispersion of repolarization in the canine heart." J Cardiovasc Electrophysiol **8**(11): 1269-1279.
- Smetana, P., V. Batchvarov, et al. (2003). "Circadian rhythm of the corrected QT interval: impact of different heart rate correction models." Pacing Clin Electrophysiol **26**(1 Pt 2): 383-386.
- Smetana, P., V. N. Batchvarov, et al. (2002). "Sex differences in repolarization homogeneity and its circadian pattern." Am J Physiol Heart Circ Physiol **282**(5): H1889-1897.
- Smetana, P. and M. Malik (2013). "Sex differences in cardiac autonomic regulation and in repolarisation electrocardiography." Pflugers Arch.
- Smetana, P., E. Pueyo, et al. (2004). "Individual Patterns of Dynamic QT/RR Relationship in Survivors of Acute Myocardial Infarction and Their Relationship to Antiarrhythmic Efficacy of Amiodarone." Journal of Cardiovascular Electrophysiology **15**(10): 1147-1154.
- Sredniawa, B., A. Musialik-Lydkka, et al. (2005). "Circadian and sex-dependent QT dynamics." Pacing Clin Electrophysiol **28 Suppl 1**: S211-216.

- Stein, P. K. and R. E. Kleiger (1999). "Insights from the study of heart rate variability." Annu Rev Med **50**: 249-261.
- Stockbridge, N., J. Zhang, et al. (2012). "Practice and challenges of thorough QT studies." J Electrocardiol **45**(6): 582-587.
- Strachan, I. G., N. P. Hughes, et al. (2009). "Automated QT analysis that learns from cardiologist annotations." Ann Noninvasive Electrocardiol **14 Suppl 1**: S9-21.
- Surawicz, B. (1998). "U wave: facts, hypotheses, misconceptions, and misnomers." J Cardiovasc Electrophysiol **9**(10): 1117-1128.
- Sy, R. W., C. van der Werf, et al. (2011). "Derivation and validation of a simple exercise-based algorithm for prediction of genetic testing in relatives of LQTS probands." Circulation **124**(20): 2187-2194.
- Taboureau, O. and F. S. Jorgensen (2011). "In silico predictions of hERG channel blockers in drug discovery: from ligand-based and target-based approaches to systems chemical biology." Comb Chem High Throughput Screen **14**(5): 375-387.
- Taccardi, B., B. B. Punske, et al. (1998). "Useful lessons from body surface mapping." J Cardiovasc Electrophysiol **9**(7): 773-786.
- Thomsen, M. B., J. Matz, et al. (2006). "Assessing the proarrhythmic potential of drugs: current status of models and surrogate parameters of torsades de pointes arrhythmias." Pharmacol Ther **112**(1): 150-170.
- Thomsen, M. B., S. C. Verduyn, et al. (2004). "Increased short-term variability of repolarization predicts d-sotalol-induced torsades de pointes in dogs." Circulation **110**(16): 2453-2459.
- Tian, H., W. Qiao, et al. (2010). "A comparison of several methods for analyzing data from thorough QT studies." J Biopharm Stat **20**(3): 632-640.
- Topilski, I., O. Rogowski, et al. (2007). "The morphology of the QT interval predicts torsade de pointes during acquired bradyarrhythmias." J Am Coll Cardiol **49**(3): 320-328.
- Toyoshima, S., A. Kanno, et al. (2005). "QT PRODACT: in vivo QT assay in the conscious dog for assessing the potential for QT interval prolongation by human pharmaceuticals." J Pharmacol Sci **99**(5): 459-471.
- Triposkiadis, F., G. Karayannis, et al. (2009). "The sympathetic nervous system in heart failure physiology, pathophysiology, and clinical implications." J Am Coll Cardiol **54**(19): 1747-1762.
- Tsuji, H., F. J. Venditti, Jr., et al. (1996). "Determinants of heart rate variability." J Am Coll Cardiol **28**(6): 1539-1546.
- Tyl, B., M. Kabbaj, et al. (2009). "Comparison of Semiautomated and Fully Automated Methods for QT Measurement During a Thorough QT/QTc Study: Variability and Sample Size Considerations." The Journal of Clinical Pharmacology **49**(8): 905-915.
- Vaglio, M., J. P. Couderc, et al. (2008). "A quantitative assessment of T-wave morphology in LQT1, LQT2, and healthy individuals based on Holter recording technology." Heart Rhythm **5**(1): 11-18.
- Valentin, J. P., P. Hoffmann, et al. (2004). "Review of the predictive value of the Langendorff heart model (Screenit system) in assessing the proarrhythmic potential of drugs." J Pharmacol Toxicol Methods **49**(3): 171-181.
- van de Borne, P., N. Montano, et al. (1997). "Relationship between repeated measures of hemodynamics, muscle sympathetic nerve activity, and their spectral oscillations." Circulation **96**(12): 4326-4332.
- van der Linde, H. J., B. Van Deuren, et al. (2010). "The Electro-Mechanical window: a risk marker for Torsade de Pointes in a canine model of drug induced arrhythmias." Br J Pharmacol **161**(7): 1444-1454.
- van der Meer, A. D. and A. van den Berg (2012). "Organs-on-chips: breaking the in vitro impasse." Integr Biol (Camb) **4**(5): 461-470.

- Vargas, H. M. (2010). "A new preclinical biomarker for risk of Torsades de Pointes: drug-induced reduction of the cardiac electromechanical window." British Journal of Pharmacology **161**(7): 1441-1443.
- Vijayakumar, L. (2005). "Suicide and mental disorders in Asia." Int Rev Psychiatry **17**(2): 109-114.
- Viskin, S. (1999). "Long QT syndromes and torsade de pointes." Lancet **354**(9190): 1625-1633.
- Viskin, S., U. Rosovski, et al. (2005). "Inaccurate electrocardiographic interpretation of long QT: The majority of physicians cannot recognize a long QT when they see one." Heart Rhythm **2**(6): 569-574.
- Vos, M. A., J. M. van Opstal, et al. (2001). "Electrophysiologic parameters and predisposing factors in the generation of drug-induced Torsade de Pointes arrhythmias." Pharmacol Ther **92**(2-3): 109-122.
- Wallis, R. M. (2010). "Integrated risk assessment and predictive value to humans of non-clinical repolarization assays." Br J Pharmacol **159**(1): 115-121.
- Webster, R., D. Leishman, et al. (2002). "Towards a drug concentration effect relationship for QT prolongation and torsades de pointes." Curr Opin Drug Discov Devel **5**(1): 116-126.
- Weiss, J. N., A. Garfinkel, et al. (2010). "Early afterdepolarizations and cardiac arrhythmias." Heart Rhythm **7**(12): 1891-1899.
- Weissenburger, J., J. M. Davy, et al. (1993). "Experimental models of torsades de pointes." Fundam Clin Pharmacol **7**(1): 29-38.
- Wild, S., G. Roglic, et al. (2004). "Global prevalence of diabetes: estimates for the year 2000 and projections for 2030." Diabetes Care **27**(5): 1047-1053.
- Wong, M. L. and J. Licinio (2001). "Research and treatment approaches to depression." Nat Rev Neurosci **2**(5): 343-351.
- Wong, M. L. and J. Licinio (2004). "From monoamines to genomic targets: a paradigm shift for drug discovery in depression." Nat Rev Drug Discov **3**(2): 136-151.
- Wymore, R. S., G. A. Gintant, et al. (1997). "Tissue and species distribution of mRNA for the IKr-like K⁺ channel, erg." Circ Res **80**(2): 261-268.
- Xia, Y., Y. Liang, et al. (2005). "In vivo validation of the coincidence of the peak and end of the T wave with full repolarization of the epicardium and endocardium in swine." Heart Rhythm **2**(2): 162-169.
- Xiao, L., J. Xiao, et al. (2008). "Feedback Remodeling of Cardiac Potassium Current Expression: A Novel Potential Mechanism for Control of Repolarization Reserve." Circulation **118**(10): 983-992.
- Xie, Y., D. Sato, et al. (2010). "So little source, so much sink: requirements for afterdepolarizations to propagate in tissue." Biophys J **99**(5): 1408-1415.
- Xu, X., S. J. Rials, et al. (2001). "Left ventricular hypertrophy decreases slowly but not rapidly activating delayed rectifier potassium currents of epicardial and endocardial myocytes in rabbits." Circulation **103**(11): 1585-1590.
- Xu, X., J. J. Salata, et al. (2002). "Increasing I(Ks) corrects abnormal repolarization in rabbit models of acquired LQT2 and ventricular hypertrophy." Am J Physiol Heart Circ Physiol **283**(2): H664-670.
- Yamada, K. A., E. M. Kanter, et al. (2004). "Transmural distribution of connexins in rodent hearts." J Cardiovasc Electrophysiol **15**(6): 710-715.
- Yan, G. X. and C. Antzelevitch (1996). "Cellular basis for the electrocardiographic J wave." Circulation **93**(2): 372-379.
- Yan, G. X., S. J. Rials, et al. (2001). "Ventricular hypertrophy amplifies transmural repolarization dispersion and induces early afterdepolarization." Am J Physiol Heart Circ Physiol **281**(5): H1968-1975.
- Yan, G. X., W. Shimizu, et al. (1998). "Characteristics and distribution of M cells in arterially perfused canine left ventricular wedge preparations." Circulation **98**(18): 1921-1927.

- Yan, G. X., Y. Wu, et al. (2001). "Phase 2 early afterdepolarization as a trigger of polymorphic ventricular tachycardia in acquired long-QT syndrome : direct evidence from intracellular recordings in the intact left ventricular wall." Circulation **103**(23): 2851-2856.
- Yan, L., J. Zhang, et al. (2010). "Statistical Characteristics of Moxifloxacin-Induced QTc Effect." Journal of Biopharmaceutical Statistics **20**(3): 497-507.
- Yap, Y. G. and A. J. Camm (2003). "Drug induced QT prolongation and torsades de pointes." Heart **89**(11): 1363-1372.
- Yasuda, M., Y. Nakazato, et al. (2006). "Clinical evaluation of adverse effects during bepridil administration for atrial fibrillation and flutter." Circ J **70**(6): 662-666.
- Yeragani, V. K., R. Pohl, et al. (2000). "Effect of Posture and Isoproterenol on Beat-to-Beat Heart Rate and QT Variability." Neuropsychobiology **41**(3): 113-123.
- Zabel, M. and M. Malik (2002). "Practical use of T wave morphology assessment." Card Electrophysiol Rev **6**(3): 316-322.
- Zareba, W., A. J. Moss, et al. (1998). "Influence of genotype on the clinical course of the long-QT syndrome. International Long-QT Syndrome Registry Research Group." N Engl J Med **339**(14): 960-965.
- Zeltser, D., D. Justo, et al. (2003). "Torsade de pointes due to noncardiac drugs: most patients have easily identifiable risk factors." Medicine (Baltimore) **82**(4): 282-290.
- Zhang, J. (2008). "Testing for positive control activity in a thorough QTc study." J Biopharm Stat **18**(3): 517-528.
- Zhou, X. and D. Wei (2011). "A multidifferentiator-based approach to the reliable determination of T-wave offset in electrocardiograms." Journal of Electrocardiology **44**(3): 330-339.

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Application des méthodes automatiques de mesure électrocardiographique continues pour l'évaluation des risques torsadogènes lors des essais cliniques : Une alternative fiable aux méthodes conventionnelles ?

Les médicaments qui provoquent un allongement de la repolarisation cardiaque, mesuré sur l'électrocardiogramme (ECG) par la prolongation de l'intervalle QT, ont été associés à une augmentation du risque pro-arythmique, et plus particulièrement à la survenue de Torsades de pointes, une tachycardie ventriculaire polymorphe potentiellement mortelle.

Les méthodes d'analyse du QT conventionnelles se restreignent à l'extraction de quelques complexes ECG. Cette pratique se traduit par de nombreuses limitations. L'inclusion de tous les battements enregistrés sur 24h et mesurés par des méthodes automatiques de mesure ECG a le potentiel de résoudre ces inconvénients.

Ce travail de thèse a démontré que les méthodes de mesure ECG automatisées et les analyses continues peuvent supplanter les méthodes conventionnelles pour l'analyse de la prolongation du QT lors des essais cliniques. Des recommandations ont été établies afin de permettre une utilisation optimale des méthodes d'analyse ECG continues.

Mots clef: Méthode automatique de mesure ECG; Analyse continue du QT; Prolongation du QT; Syndrome du QT long; Torsades de Pointes; Correction du QT; Etude "thorough" QT; Etude clinique

Drugs which induce a delay in cardiac repolarization measured as QT interval prolongation on the electrocardiogram (ECG) have been associated with a potential to increase the risk of arrhythmias, especially Torsades de pointes (TdP), a potentially lethal polymorphic ventricular tachycardia.

The analyses performed using conventional methods are restricted to the extraction of a few ECG complexes. This practice is associated with several limitations. In contrast, the inclusion of all beats measured by computerized methods from continuous 24 h recordings could resolve all of these deficiencies.

The current work demonstrated that automated ECG measurement methods employing continuous analysis can supplant conventional methods for the evaluation of QT prolongation in clinical studies. Recommendations have been established to provide an optimal use of continuous ECG analysis.

Key-words: Automated ECG measurement methods; Continuous QT analysis; QT prolongation; Long QT syndrome; Torsades de Pointes; QT correction; Thorough QT study; Clinical study