HIGH SENSITIVITY TROPONIN - ITS USE IN DIAGNOSIS OF CARDIAC DYSFUNCTION

A thesis submitted in the fulfilment of the requirements for the degree of Doctor of Philosophy
University of Canberra

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ABSTRACT

Troponins are regulatory proteins and part of the contractile apparatus that is integral to muscle contraction in skeletal and cardiac muscle but not smooth muscle and are important clinically because cardiac troponins (cTn) are sensitive indicators of myocyte injury and have become integral to the definition of myocardial infarction. There are several issues surrounding the significance of troponin and how it should be used, both for the assessment of cardiac disease and in settings of non-cardiac illness. This thesis examines a number of these areas of uncertainty.

This thesis focuses initially on the analytical validation of troponin assays and I offer guidelines for a standardised approach to undertaking the verification of these analytical characteristics. I report on these characteristics for 2 highly sensitive assays and their application to a cardio-healthy population.

In the second part of this thesis I focus on the physiology of troponin in the normal population. I describe studies undertaken with a cohort of healthy children and demonstrate the significance of population coning when determining the 99th percentile of the upper reference limit using 2 highly sensitive troponin assays.

The final part of this thesis investigates the significance of troponin in the acute coronary syndrome (ACS) and non ACS setting. I offer a hypothesis suggesting that bleb formation is a mechanism for troponin release. I describe how improvements in sensitivity of troponin T assays allow better prognostic information regarding all cause mortality in end stage renal disease patients, demonstrate troponin release after strenuous exercise in elite cyclists and I describe a cross-sectional study looking at troponin concentrations in subjects with non cardiac illness and the general community. Using data mining techniques I demonstrate how
the use of a new high sensitivity troponin I assay can offer greater assistance to the clinician in stratifying patients at risk of a major adverse cardiac event (MACE). I provide evidence that suggests the use of a multi-marker approach to identifying patients at risk is potentially viable.
ACKNOWLEDGEMENTS

Undertaking a PhD requires a lot from many people to achieve the ultimate outcome. It requires a lot of time, assistance, guidance, support and encouragement from mentors, colleagues and friend. It is almost impossible to thank everyone who helps in these ways but there are those who must be thanked for without the ongoing support and guidance the task of undertaking and completing this thesis would have been less enjoyable and far more onerous.

I would like to thank my supervisors and advisors Peter Hickman, Julia Potter, Brett Lidbury, Alice Richardson and Luby Simson. Their support, encouragement, advice and mentoring ultimately has allowed me to be able to submit this work.

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To my friends and clinical colleagues, Walter Abhyaratna, Girish Talaulikar, Daryl McGill and Louise Cullen thank you for access to the clinical samples and clinical outcome data used in these research studies.

Undertaking the many analyses performed in these studies would only have been possible with the technical assistance, access to analytical instrumentation, support and the gentle “encouragement” of staff in the clinical chemistry department of ACT Pathology - Jaya Canard, Suzi Apostoloska, Di Talsma, Carmen Oakman and Corrina Newman. Also to Peter Talsma, thanks for the continued supply of journal articles to read. Thank you all.

My colleagues, Nicole Chia and Kerrie Andriolo, both of whom are also undertaking post graduate studies, provided encouragement at times when it was most needed. It was and is very much appreciated.
DECLARATION

In this thesis I detail the findings from research carried out between July 2009 and August 2013. The research studies described in Chapters 3-5 were carried out in collaboration with my co-authors, the names of whom are listed at the start of each chapter. For each of these studies I took a lead role in the experimental design, subject recruitment, data collection and analysis, with all authors contributing to final submitted version of the manuscripts. I obtained assistance with these concepts from my supervisory panel members A/Professor Peter E Hickman, Professor Julia M Potter, A/Professor Brett Lidbury and Dr Alice Richardson.

I obtained assistance with and analysis with the mathematical approach to data mining from A/Professor Brett Lidbury and Dr Alice Richardson.

I obtained assistance with the administrative and scientific components of this thesis from A/Professor Luby Simson
DEDICATION

To my family, it was my mother’s wish to see her two sons receive “the floppy hat”. Unfortunately she passed away before both my brother, Paul, and I completed our studies. My dad will complete that wish for her.

To Anne, Liesel and Scott, thank you for putting up with the “Grumpy Gus” when he reared his head over the past few years and for the unconditional support.

*shukran kabeer.*
PUBLICATIONS AND PRESENTATIONS RELEVANT TO THIS THESIS

PEER REVIEWED JOURNAL ARTICLES


Tate JR, Panteghini M, **Koerbin G**, Hickman PE, Schneider HG, Jaffe A. Verification of the analytical characteristics of troponin assays in the laboratory – a how to guide. Clin Biochem Reviews Troponin Monograph 2012 69-85


Potter JM, Simpson A, Koerbin G, Kerrigan J, Southcott E, Hickman PE. Cardiac troponin and non-cardiac illness: high sensitivity cardiac troponins in a cross-sectional study in a general hospital and a community population. (submitted to Clin Chim Acta)
PEER REVIEWED CONFERENCE PROCEEDINGS


Hickman PE, Koerbin G, Potter JM, Talaulikar G, McGill D. 5 Year Outcomes in renal Dialysis Patients: New hsTnI assays are as informative as hsTnT. Clin Biochem Rev 2011;34:S26


SCIENTIFIC CONFERENCE AND MEETING PRESENTATIONS

2010
SW AIMS meeting, Canberra
“Troponin Past, Present and Future?”

2010
AACB NSW/ACT Branch Meeting
“Evaluation of the Roche hs-TnT Assay”

2010
AACB SES, Sydney
“hs-TnT which reference intervals?”

2010
AACB/AIMS Combined Annual Scientific Meeting, Perth
“Highly Sensitive TnT – An opening to a whole new world”

2011
Roche Cardiac Symposium, Heidelberg, Germany
“hs-Tn and Healthy Populations”

2011
Abbott New Zealand Architect User Symposium, Rotorua, NZ
“Highly Sensitive Troponin”

2012
Abbott Scientific Symposium. Sydney
“High Sensitivity troponin – its use in diagnosis of cardiac dysfunction

2012
Abbott Scientific Symposium. Melbourne
“High Sensitivity troponin – its use in diagnosis of cardiac dysfunction
AWARDS

The Roche Diagnostics Australia Award
“Best Poster Presentation prize for the 2012 AACB Scientific Conference, Melbourne 2012”
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<td>ACC</td>
<td>American College of Cardiology</td>
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<tr>
<td>ACS</td>
<td>Acute coronary syndrome</td>
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<tr>
<td>ADAPT</td>
<td>Accelerated Diagnostic protocol to Assess Patients with chest pain</td>
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<tr>
<td>ADP</td>
<td>Accelerated diagnostic protocol</td>
</tr>
<tr>
<td>ALP</td>
<td>Alkaline Phosphatase</td>
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<tr>
<td>ALT</td>
<td>Alanine transaminase</td>
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<td>AMI</td>
<td>Acute myocardial infarction</td>
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<td>APACE</td>
<td>Advantageous Predictors of Acute Coronary Syndromes Evaluation</td>
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<td>ApEn</td>
<td>Approximate entropy</td>
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<td>AUC</td>
<td>Area under curve</td>
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<td>BNP</td>
<td>B-type natriuretic peptide</td>
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<td>CAD</td>
<td>Coronary artery disease</td>
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<td>CHD</td>
<td>Coronary heart disease</td>
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<td>CK</td>
<td>Creatine Kinase</td>
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<td>CKD-EPI</td>
<td>Chronic Kidney Disease Epidemiological Collaboration</td>
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<td>CK-MB</td>
<td>Creatine Kinase MB isoenzyme</td>
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<td>CLSI</td>
<td>Clinical Laboratory Standards International</td>
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<tr>
<td>CRP</td>
<td>C-reactive protein</td>
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<td>cTn</td>
<td>Cardiac troponin</td>
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<tr>
<td>cTnI</td>
<td>Cardiac troponin I</td>
</tr>
<tr>
<td>cTnT</td>
<td>Cardiac troponin T</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of variation</td>
</tr>
<tr>
<td>CV\textsubscript{a}</td>
<td>Analytical variation (imprecision)</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
</tr>
<tr>
<td>CV\textsubscript{g}</td>
<td>Between individual variation</td>
</tr>
<tr>
<td>CV\textsubscript{i}</td>
<td>Within individual variation</td>
</tr>
<tr>
<td>CV\textsubscript{t}</td>
<td>Total variation</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>eGFR</td>
<td>Estimate of the glomerular filtration rate</td>
</tr>
<tr>
<td>EQA</td>
<td>External quality assessment</td>
</tr>
<tr>
<td>ESC</td>
<td>European Society of Cardiology</td>
</tr>
<tr>
<td>ESRD</td>
<td>End-stage renal disease</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
</tr>
<tr>
<td>g</td>
<td>Gram</td>
</tr>
<tr>
<td>GET</td>
<td>Gas exchange threshold</td>
</tr>
<tr>
<td>GGT</td>
<td>Gamma glutamyl transpeptidase</td>
</tr>
<tr>
<td>HA</td>
<td>Heterophile antibody</td>
</tr>
<tr>
<td>HAMA</td>
<td>Heterophilic anti mouse antibody</td>
</tr>
<tr>
<td>HbA1C</td>
<td>Haemoglobin A1C</td>
</tr>
<tr>
<td>HRV</td>
<td>Heart rate variability</td>
</tr>
<tr>
<td>hs-cTnI</td>
<td>High sensitivity troponin I</td>
</tr>
<tr>
<td>hs-cTnT</td>
<td>High sensitivity troponin T</td>
</tr>
<tr>
<td>IFCC</td>
<td>International Federation of Clinical Chemistry</td>
</tr>
<tr>
<td>II</td>
<td>Index of individuality</td>
</tr>
<tr>
<td>IQC</td>
<td>Internal Quality control</td>
</tr>
<tr>
<td>ISO</td>
<td>International organisation of standards</td>
</tr>
<tr>
<td>kDa</td>
<td>Kilo Dalton</td>
</tr>
<tr>
<td>L</td>
<td>Litre</td>
</tr>
<tr>
<td>Ln</td>
<td>Natural log</td>
</tr>
<tr>
<td>LoB</td>
<td>Limit of blank</td>
</tr>
<tr>
<td>LoD</td>
<td>Limit of detection</td>
</tr>
<tr>
<td>LOOK</td>
<td>Lifestyle Of Our Kids</td>
</tr>
<tr>
<td>LoQ</td>
<td>Limit of quantitation</td>
</tr>
<tr>
<td>LVEF</td>
<td>Left ventricular ejection fraction</td>
</tr>
<tr>
<td>m</td>
<td>Metre</td>
</tr>
<tr>
<td>MACE</td>
<td>Major adverse cardiac event</td>
</tr>
<tr>
<td>MDRD</td>
<td>Modification of Diet in Renal Diseased</td>
</tr>
<tr>
<td>MDS</td>
<td>Classical multidimensional scaling</td>
</tr>
<tr>
<td>mg</td>
<td>Milligram</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>MI</td>
<td>Myocardial infarction</td>
</tr>
<tr>
<td>MW</td>
<td>Molecular weight</td>
</tr>
<tr>
<td>ng</td>
<td>Nanograms</td>
</tr>
<tr>
<td>NPV</td>
<td>Negative predictive value</td>
</tr>
<tr>
<td>NSTEMI</td>
<td>non ST-elevation myocardial infarction</td>
</tr>
<tr>
<td>NTproBNP</td>
<td>N-terminal pro B type natriuretic peptide</td>
</tr>
<tr>
<td>p</td>
<td>Probability</td>
</tr>
<tr>
<td>PCA</td>
<td>Principal components analysis</td>
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<tr>
<td>PoCT</td>
<td>Point of Care Testing</td>
</tr>
<tr>
<td>PPV</td>
<td>Positive predictive value</td>
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<tr>
<td>PSE</td>
<td>Prolonged strenuous exercise</td>
</tr>
<tr>
<td>QC</td>
<td>Quality control</td>
</tr>
<tr>
<td>RCV</td>
<td>Reference Change Value</td>
</tr>
<tr>
<td>RMSSD</td>
<td>Root mean squares of successive differences</td>
</tr>
<tr>
<td>ROC</td>
<td>Receiver operator curve</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SE</td>
<td>Standard error</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard error mean</td>
</tr>
<tr>
<td>STEMI</td>
<td>ST-segment-elevation myocardial infarction</td>
</tr>
<tr>
<td>SVM</td>
<td>Support Vector Machine</td>
</tr>
<tr>
<td>TIMI</td>
<td>Thrombolysis in Myocardial Infarction</td>
</tr>
<tr>
<td>Tn</td>
<td>Troponin</td>
</tr>
<tr>
<td>TnC</td>
<td>Troponin C</td>
</tr>
<tr>
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<tr>
<td>U</td>
<td>Units</td>
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<tr>
<td>ug</td>
<td>Micrograms</td>
</tr>
<tr>
<td>URL</td>
<td>Upper reference limit</td>
</tr>
<tr>
<td>VO₂</td>
<td>Oxygen uptake</td>
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