Assay precision and risk of misclassification at rule-out cut-offs for high-sensitivity cardiac troponin

Running Title: Misclassification for rule-out

Title: **Assay precision and risk of misclassification at rule-out cut-offs for high-sensitivity cardiac troponin**

Peter A Kavsak PhD[a], Nicholas L Mills MD, PhD[b,c], Lorna Clark BSc[a], Dennis T. Ko MD, MSc[d], Sameer Sharif MD MSc[a], Annabel Chen-Tournoux MD[e], Steven M. Friedman MD, MPH[d], Emilie P Belley-Cote MD, PhD[a], Andrew Worster, MD, MSc[a], Jafna Cox MD[f], Venkatesh Thiruganasambandamoorthy MBBS, MSc[g], Amy Lou PhD[f], Jennifer Taher PhD[d], Frank Scheuermeyer MD MHSc[h], Chris McCudden PhD[g], Beth L. Abramson MD, MSc[d], Shaun Eintracht MD[e], Jennifer L. Shea PhD[i], Paul M. Yip PhD[d], Yun Huang PhD[j], Michael Chen MD[h], Albert K.Y. Tsui, PhD[k], Laurel Thorlacius,PhD[l], Kristin M Aakre MD, PhD[m,n], Joshua E Raizman PhD[k], Angela W.S. Fung PhD[h], Karin H Humphries MBA, DSc[h], Saranya Arnoldo PhD[d], Vipin Bhayana PhD[o], Rose Djiana PhD[e], Daniel R. Beriault PhD[d], Julie St-Cyr MD[e], Ronald A. Booth PhD[g], David W. Blank MD[e], Marco L.A. Sivilotti MD, MSc[j], Allan S. Jaffe MD[p]

[a] McMaster University, Hamilton, Ontario, Canada

[b] BHF/University Centre for Cardiovascular Science, University of Edinburgh, Edinburgh, UK

[c] Usher Institute, University of Edinburgh, Edinburgh, UK

[d] University of Toronto, Toronto, Ontario, Canada

[e] McGill University, Montréal, Quebec Canada

[f] Dalhousie University, Halifax, Nova Scotia, Canada

[g] University of Ottawa, Ottawa, Ontario, Canada

[h] University of British Columbia, Vancouver, British Columbia, Canada

[i] Department of Laboratory Medicine, Saint John Regional Hospital, Saint John, NB, Canada

[j] Queen's University, Kingston, Ontario, Canada

[k] University of Alberta, Edmonton, Alberta, Canada

[l] University of Manitoba, Winnipeg, Manitoba, Canada

[m] Department of Medical Biochemistry and Pharmacology & Department of Heart Disease, Haukeland University Hospital, Bergen, Norway.

[n] Department of Clinical Science, University of Bergen, Bergen, Norway

[o] Western University, London, Ontario, Canada

[p] Department of Cardiology and Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota

Corresponding Author: Dr. Kavsak, Juravinski Hospital and Cancer Centre, 711 Concession St, Hamilton, ON Canada L8V 1C3 | email: kavsakp@mcmaster.ca | Fax: 905-381-7066

Key words: Imprecision; high-sensitivity cardiac troponin; European Society of Cardiology; rule-out; misclassification, low cut-off

Research Letter

Clinical trials and guidelines support the use of very low high-sensitivity cardiac troponin (hs-cTn) results to rule-out a myocardial infarction (MI) (1). The International Federation of Clinical Chemistry and Laboratory Medicine Committee on Clinical Applications of Cardiac Biomarkers committee, through a modeling approach, suggests assays need to have a lower limit near 3 ng/L and an analytical variation of 10% below 7 ng/L if these low values are to perform consistently in practice (2). Our objectives for the present study were to assess: i) if any type of instrument or individual instrument could achieve a coefficient of variation (CV) of ≤10% at very low hs-cTn cut-offs (i.e., targets) recommended in clinical pathways; ii) the frequency of results at the hs-cTn target, above the target and below the target, with the latter group representing potential misclassification to the low risk group where the target level would in the intermediate risk range.

Briefly, frozen plasma aliquots were sent on dry ice to 8 Canadian provinces where monthly hs-cTn testing occurred at 35 hospital laboratories (65 instruments) (3). Imprecision (CV) was calculated for each instrument model [n=13 instrument models: Roche hs-cTnT e411 (n=9), e601 (n=4), e602 (n=14), e801(n=14); Beckman hs-cTnI Access2 (n=1), DxI 600 (n=3), DxI 800 (n=5); Abbott hs-cTnI ARCHITECT i1000 (n=1), i2000 (n=5), Alinity (n=2); Siemens hs-cTnI Atellica (n=4), Vista (n=2); and Ortho Vitros hs-cTnI (n=1)] using one decimal place (Figure 1) and whole numbers (ng/L) for the concentrations, with whole numbers being recommended for patient results (1,2). Also, we calculated for each individual instrument, the mean (whole number) standard deviation (SD) and CV for each instrument type. The target

concentrations evaluated for the single sample rule-out were as follows: Abbott hs-cTnI=5 ng/L, Beckman hs-cTnI=4 ng/L, Ortho hs-cTnI=1 ng/L, Roche hs-cTnT=6 ng/L, Siemens Atellica hs-cTnI=5 ng/L, and Siemens Dimension Vista hs-cTnI=9 ng/L (3). These target levels are the decision cut-offs for a single measurement rule-out for most of the assays used in different algorithms (i.e., results ≥ target would not be ruled-out).

There were no instrument model groups that achieved an imprecision ≤10% reporting concentrations with one decimal place (CV range:11-55%) or whole numbers (CV range:11%-60%) at the target concentrations (Figure 1). Only 27% of individual instruments (n=17) achieved a CV ≤10% (median=8.6%/range<1-10%) with the remainder of instruments (n=48) yielding a median (range) CV of 16% (11-60%) (Supplemental Table S1). The SDs for troponin on the instruments with CVs ≤10% were ≤0.9 ng/L whereas the SDs for the remaining instruments ranged from 0.5 to 2.5 ng/L. The overall frequency of reporting at, above, and below the target level ranged from 38% to 70%, 7% to 38%, and 19% to 50%, respectively (n=711 results, Chi-square p<0.001 and linear trend p=0.05) (Supplemental Figure S1). The overall misclassification to low risk (below target level) was 26.9% (95%CI: 23.2-31.0) (191 from 711). Misclassification to low risk using the very low cut-offs from the European Society of Cardiology 0/1-hour pathway for the Abbott, Beckman, Ortho and Roche hs-cTn assays was 8.3% (95%CI: 6.2-10.8) (53 from 640 results) (Supplemental Table S1). Overall, 20% (13/65) of instruments yielded no misclassifications: one Abbott ARCHITECT i2000SR (CV 8%), three Roche e411 (CVs 8%, 10%, 28%), one Roche e601(CV 9%), three Roche e602 (CVs 8%, 10%, 10%), four Roche e801 (CVs <1%, 8%, 11%, 8%), and one Siemens Atellica instrument (CV 8%).

The findings from this large analytical study indicates that less than one third of laboratories can achieve the 10% CV benchmark at concentration ranges advocated to rule-out MI on a single sample. Thus, some misclassification is inevitable and occurs in 8% to 27% of samples tested depending on how close the target value is to the decision threshold. The impact of imprecision on misclassification in practice was not evaluated here, but the frequency is likely to be much lower as only a very small proportion of patients with possible MI have troponin concentrations equivalent to the decision threshold. Recent modeling data of 1,000,000 simulated patients from clinical studies investigating patients with possible acute coronary syndrome in the emergency department (ED) with both Abbott hs-cTnI and Roche hs-cTnT suggests analytical variation up to 3 ng/L minimally impacted sensitivity but reduced the number of rule-out patients (4). However, in a study of a large population attending the ED with a low prevalence of MI (n=131,095), hs-cTn alone did not yield sufficient sensitivity and it is plausible that analytical variability may impact performance in this setting (5). These data reemphasize the need for clinical assessment of patients with possible MI in addition to using low troponin thresholds for risk stratification. Improvement in assay performance and laboratory monitoring is needed to minimise misclassification. In summary, clinicians should be aware of the possibility of risk misclassification at very low hs-cTn concentrations, particularly in females, who generally have lower hs-cTn values than do males.

Word count: 799 (maximum is 800)

Conflict of Interest Disclosures:

Dr. Kavsak has received grants/reagents/consultant/advisor/ honoraria from Abbott Laboratories, Abbott Point of Care, Beckman Coulter, Ortho Clinical Diagnostics, Quidel, Randox Laboratories, Roche Diagnostics, Siemens Healthcare Diagnostics, and Thermo Fisher Scientific. McMaster University has the following patent with Drs. Kavsak and Worster listed as inventors "METHOD OF DETERMINING RISK OF AN ADVERSE CARDIAC EVENT". McMaster University has also filed the following patent: "QUALITY CONTROL MATERIALS FOR CARDIAC TROPONIN TESTING" with Dr. Kavsak and Ms. Clark being listed as inventors.

Dr. Mills is supported by a Research Excellence Award (RE/24/130012) and a Chair Award (CH/F/21/90010) from the British Heart Foundation and reports research grants awarded to the University of Edinburgh from Abbott Diagnostics, Roche Diagnostics, and Siemens Healthineers outside the submitted work, and honoraria from Abbott Diagnostics, Siemens Healthineers, Roche Diagnostics, LumiraDx and Psyros Diagnostics.

Dr. Jaffe consults presently or has in the past consulted for most of the major diagnostic companies and Moderna. He also has equity in RCE Technologies.

Dr. Thiruganasambandamoorthy is supported through a Physicians' Services Incorporated Foundation Mid-Career Knowledge Translation Fellowship and University of Ottawa Tier-1 Clinical Research Chair in Cardiovascular Emergencies award.

Dr. Taher received in-kind funding from Roche for an independent and unrelated project.

Dr. Yip has received research support from Roche Diagnostics.

Dr. Aakre has served on advisory board for Roche Diagnostics and SpinChip, received consultant honoraria form CardiNor, lecturing honorarium from Siemens Healthineers and Snibe Diagnostics and research grants from Siemens Healthineers and Roche Diagnostics, she is Associate Editor of Clinical Biochemistry and Chair of the IFCC Committee of Clinical Application of Cardiac Bio-markers.

Funding/Support: Grant from the Canadian Institutes of Health Research (Kavsak). British Heart Foundation through a Chair Award (CH/F/21/90010), a Programme Grant (RG/20/10/34966) and a Research Excellence Award (RE/18/5/34216) (Mills).

Patient Consent Statements: The authors confirm that patient consent is not applicable to this article. This was a laboratory quality assurance study assessing imprecision at low hs-cTn concentrations using non-identifiable human material and research ethics board approval was not required.

Role of Sponsor: The funding organizations had no role in the design and conduct of the study; in the collection, analysis, and interpretation of the data, or in the final approval of the manuscript.

Acknowledgements: Special thanks for the following individuals who helped in this prospective study: Chantele Roy, Paul Malinowski, Tara Bryce (CRLB-GMEL, Hamilton Health Sciences, Hamilton, ON) Nadia Caruso (Juravinski Hospital and Cancer Centre, Hamilton ON), Vathany Kulasingam (University Health Network, Toronto, ON), Mark Cheung (Sunnybrook Hospital, Toronto, ON), Viktor Skihar (Regina General Hospital, Regina, SK), Joshua Buse (Regina General Hospital, Regina, SK), Janet Simons (St. Paul's Hospital, Vancouver, BC), Barry Hoffman ((Mount Sinai Hospital, Toronto, ON), Joël Lavoie (Institut de Cardiologie de

Montréal, Montréal, QC, Canada), Andrew Lyon (University of Saskatchewan, Saskatoon, Saskatchewan, Canada), Laura Burns (St. Paul's Hospital, Vancouver, BC), Allyse Hummelman (Mount Saint Joseph Hospital, Vancouver, BC), Jennifer Annand (Victoria General Hospital & Royal Jubilee Hospital, Victoria, BC), Robyn Dahl (Victoria General Hospital, Victoria, BC), Sabrina Hocken (Victoria General Hospital, Victoria, BC), Marisa Schneider (Royal Jubilee Hospital, Victoria, BC), Maryann Jiang (Royal Jubilee Hospital, Victoria, BC, Darryl Holden (Royal Jubilee Hospital, Victoria, BC), Krista McComb (University of Alberta Hospital, Edmonton, AB), Eric (Phong) Ly (University of Alberta Hospital, Edmonton, AB), Carmen Zimmer (University of Alberta Hospital, Edmonton, AB), Christina Gauvreau (University of Alberta Hospital, Edmonton, AB), Jan Feser (Regina General Hospital, Regina, SK), Rob Saunders (St. Paul's Hospital, Saskatoon, SK), Connie Neuberger (Royal University Hospital, Saskatoon, SK), Karen Nogier (Royal University Hospital, Saskatoon, SK), Kari Henwood (St. Boniface Hospital, Winnipeg, MB), Jeffrey Noel (St. Boniface Hospital, Winnipeg, MB), JoAnne Novakowski (St. Boniface Hospital, Winnipeg, MB), Debbie Kiel (Selkirk Regional Health Centre, Selkirk, MB), Meghan Rasmussen (Selkirk Regional Health Centre, Selkirk, MB), Katie Ndukwu (Health Sciences Centre, Winnipeg, MB), Steven Cong (Health Sciences Centre, Winnipeg, MB), Roy Augustin (Mount Sinai Hospital, Toronto, ON), Fari Rokhforooz (Mount Sinai Hospital, Toronto, ON), Bonny Lem Ragosnig (Sunnybrook Hospital, Toronto, ON), Liyan Ma (Sunnybrook Hospital, Toronto, ON), Sarah Lam (Toronto General Hospital (UHN), Toronto, ON), Julia Shao (St. Michael's Hospital, Toronto, ON), Robert Dean (Kingston General Hospital, Kingston, ON), Leanne Reilly (Kingston General Hospital, Kingston, ON), Emma Greenough (Ottawa Hospital - Civic Campus, Ottawa, ON), Sarah Cascanette (Ottawa Hospital - General Campus, Ottawa, ON), Janette Rutledge (Ottawa Hospital - General Campus,

Ottawa, ON), Isla Craig (Ottawa Hospital - Civic Campus, Ottawa, ON), Alan Burgess (Ottawa Hospital - Civic Campus, Ottawa, ON), Guylaine Imbeault (Ottawa Hospital - General Campus, Ottawa, ON), Sandy Bookalam (Ottawa Hospital - General Campus, Ottawa, ON), Purvi Patel (Brampton Civic Hospital, Brampton ON), Linda Stevenson (Etobicoke General Hospital, Etobicoke, ON), Lisa Brown (Peel Memorial Centre for Integrated Health and Wellness, Brampton, ON), Husam Abdoh (LHSC - Victoria Hospital, London, ON), Bill Richardson (LHSC - University Hospital, London, ON), Kylie McInerney (LHSC - University Hospital, London, ON), Rick Moore (LHSC - University Hospital, London, ON), Nathalie Payette (Montreal General Hospital, Montréal, QC), Gloria Marcaida (Montreal General Hospital, Montréal, QC), Kruno Lorkovic (Montreal General Hospital, Montréal, QC), Maryse Coderre (Montreal General Hospital, Montréal, QC), Lisa Murphy (McGill University Health Centre, Glen Site Montréal, QC), Maia Stallone (McGill University Health Centre, Glen Site, Montréal, QC), Ana Marcaida (McGill University Health Centre, Glen Site, Montréal, QC), Micheline Masangya (St. Mary's Hospital Center, Montréal, QC), Youcef Amimer (Institut de Cardiologie de Montréal, Montréal, QC), Rianne Madriaga (Jewish General Hospital, Montréal, QC), Oktavian Toka (Jewish General Hospital, Montréal, QC), Safiya Sweeney (Jewish General Hospital, Montréal, QC), Girupan Arasaratman (Jewish General Hospital, Montréal, QC), Ashley Stevens (Saint John Regional Hospital, Saint John, NB), Tiffany Moore (Saint John Regional Hospital, Saint John, NB), Christina Quondam Franks (Saint John Regional Hospital, Saint John, NB), Ian MacLean (Queen Elizabeth II Health Sciences Centre , Halifax, NS), Sandy Schlay (Queen Elizabeth II Health Sciences Centre , Halifax, NS), Kathleen Lawrence (Dartmouth General Hospital, Dartmouth, NS), Kelly Smith (Dartmouth General Hospital, Dartmouth, NS), Rose Bartlett (Cobequid Community Health Centre, Lower Sackville, NS), Craig Leblanc

(Cobequid Community Health Centre, Lower Sackville, NS), Kimberly Ross (Hants Community Hospital, Windsor, NS), Shelley Strong (Cobequid Community Health Centre, Lower Sackville, NS), Shannah Hynes (Cobequid Community Health Centre, Lower Sackville, NS), Jenilee Calbury (Hants Community Hospital, Windsor, NS), Rebecca Reid (Queen Elizabeth II Health Sciences Centre , Halifax, NS), Trish Jarvie (Hamilton General Hospital, Hamilton, ON), Jill Boreyko (Hamilton General Hospital, Hamilton, ON), Ronda Snowdy (Hamilton General Hospital, Hamilton, ON), Binal Parikhb (Hamilton General Hospital, Hamilton, ON), Trisha Bellamy (Hamilton General Hospital, Hamilton, ON), Ching Mark (Juravinski Hospital and Cancer Centre, Hamilton, ON), Elspeth Millar (Juravinski Hospital and Cancer Centre, Hamilton, ON), Brian Vandermey (Juravinski Hospital and Cancer Centre, Hamilton, ON), Leesa Lillie (Juravinski Hospital and Cancer Centre, Hamilton, ON), Matthew Hulett (West Lincoln Memorial Hospital, Grimsby, ON), Glen Edmunds (West Lincoln Memorial Hospital, Grimsby, ON) and the CODE-MI study.

References (5 max)

1) Jaffe AS, Body R, Mills NL, Aakre KM, Collinson PO, Saenger A, Hammarsten O, Wereski R, Omland T, Sandoval Y, Ordonez-Llanos J, Apple FS; IFCC Committee on Cardiac Bio-Markers. Single Troponin Measurement to Rule Out Myocardial Infarction: JACC Review Topic of the Week. J Am Coll Cardiol. 2023 Jul 4;82(1):60-69.
2) Aakre KM, Apple FS, Mills NL, Meex SJR, Collinson PO; International Federation of Clinical Chemistry Committee on Clinical Applications of Cardiac Biomarkers (IFCC C-CB). Lower Limits for Reporting High-Sensitivity Cardiac Troponin Assays and Impact of Analytical Performance on Patient Misclassification. Clin Chem. 2023 Dec 15:hvad185.
3) Kavsak PA, Clark L, Arnoldo S, Lou A, Shea JL, Eintracht S, et al. Analytic Result Variation for High-Sensitivity Cardiac Troponin: Interpretation and Consequences. Can J Cardiol. 2023 Jul;39(7):947-51.
4) Pickering JW, Kavsak P, Christenson RH, Troughton RW, Pemberton CJ, Richards MA, et al. Determination of clinically acceptable analytical variation of cardiac troponin at decision thresholds. Clin Chem. 2024; hvae059, https://doi.org/10.1093/clinchem/hvae059
5) Kavsak PA, Cerasuolo JO, Hewitt MK, Mondoux SE, Perez R, Seow H, et al. Identifying Very-Low-Risk Patients for Future Myocardial Infarction or Death. Can J Cardiol. 2023 Apr;39(4):527-530.

Figure 1. The distribution of results (reported to 1 decimal place) and their CVs for the 13 different instrument models: Roche hs-cTnT e411 (CV = 23% whole number), Roche e601 (CV = 17% whole number), Roche e602 (CV = 17% whole number), Roche e801 (CV = 18% whole number); Beckman hs-cTnI Access 2 (CV = 17% whole number), Beckman DxI 600 (CV = 20% whole number), Beckman DxI 800 (CV = 18% whole number); Abbott hs-cTnI ARCHITECT i1000 (CV = 14% whole number); i2000 (CV = 14% whole number), Alinity (CV = 13% whole number); Siemens Atellica (CV = 12% whole number), Vista (CV = 11% whole number); Ortho Vitros (CV = 60% whole number). Note there were 4 concentrations (one Roche <3 ng/L and three Ortho <1 ng/L) that were reported as undetectable, and for these values half the lower analytical limit (1.5 ng/L for Roche and 0.5 ng/L for Ortho) were used to calculate the CVs. The violin plot includes the median (square black box) and 25^{th}-75^{th} concentrations (black whiskers) with the dashed lines indicating the manufacturer listed 10%CV levels as whole numbers (see https://ifcc.org/ifcc-education-division/emd-committees/committee-on-clinical-applications-of-cardiac-bio-markers-c-cb/biomarkers-reference-tables/ accessed April 13, 2024).