SUPPLEMENTAL MATERIAL

APPENDIX METHODS

Biochemical Data

Serum cardiac troponin I concentrations were assessed by two independent CLIA-approved laboratories at the University of Louisville (ULH) and KentuckyOne Health Jewish Hospitals (JH). The Ortho Vitros 5600 assay was used to assess cardiac troponin I in subjects enrolled at the University of Louisville Hospital. For this assay, the 99% cutoff level for a healthy population was 0.035 ng/mL and had a coefficient of variance below 10% at this level. Troponin levels <0.035 ng/mL were considered non-elevated. This assay's FDA-approved package insert further defined 0.12 ng/mL as the most efficient (more specific) cutoff point for the diagnosis of acute MI with this assay. Troponin levels >0.12 ng/mL were considered elevated, and levels from 0.035 – 0.12 ng/mL were considered non-diagnostic for the purposes of the present study.

The Beckman Access assay was used to assess cardiac troponin I in subjects enrolled at KentuckyOne Jewish Hospitals. For this assay, the 99% cutoff level for a healthy population was 0.04 ng/mL, but the results did not achieve a coefficient of variance below 10% until 0.06 ng/mL was reached. Troponin levels <0.04 ng/mL were considered non-elevated. This assay further defined 0.5 ng/mL as the most efficient (more specific) cutoff point for the diagnosis of acute MI. Troponin levels >0.5 ng/mL were considered elevated, and levels from 0.04 – 0.5 ng/mL were considered non-diagnostic for this study.

Statistical Analysis

Calibration of Measurements

If significant differences were found, a calibration methodology was to be developed. For this study, five healthy volunteers were recruited from whom 6 tubes of blood was drawn (approximately 18 ml blood). The tubes were randomized and single blinded. The approximate time from sample collection to analysis was 35 minutes. Three samples were evaluated at both hospitals for each of the five subjects in triplicate. For both MPV and platelet count separate two-way analysis of variance (ANOVA) was conducted to determine if measurements differed between ULH and JH after adjusting for the effect of subject. The effect of hospital was significant for both MPV and platelet count, necessitating calibration of both measurements. The interaction plots illustrating the trend of the differences in measurement of MPV and PLT count by hospitals is shown in (Appendix Figure 1).

Since the majority of samples were analyzed at JH, the ULH measurements were scaled to be consistent with the JH measurements. This was done by regression of mean values by subject at JH on the mean values by subject at ULH for both MPV and platelet counts. The coefficients from the regression models were then used to adjust the ULH measurements.

APPENDIX FIGURE LEGENDS

Appendix Figure 1. Trend of differences in measurement of MPV and PLT count by laboratories

Abbreviations:

Jewish – KentuckyOne Health Jewish Hospitals, ULH – University of Louisville

Figure 2. Study enrollment scheme

Abbreviations:

ASCVD – Atherosclerotic Cardiovascular Disease; CAD – Coronary Artery Disease; MI – Myocardial Infarction; STE – ST Segment Elevation on Electrocardiography

APPENDIX TABLES

Appendix Table 1. Enrollment criteria

Diagnoses	Definitions
Acute Myocardial Infarction (MI)	Must satisfy ALL: • Symptoms consistent with acute ischemia lasting >10 min in the last 24 hours • ST-segment depression/elevation in ≥2 continuous ECG leads –OR– Elevated cardiac troponin
Stable Coronary Artery Disease (CAD)	Must satisfy ALL: • Clinically stable • Presenting for an elective catheterization to evaluate for stable CAD • No recent revascularization attempts

Appendix Table 2: Study Phenotype Criteria

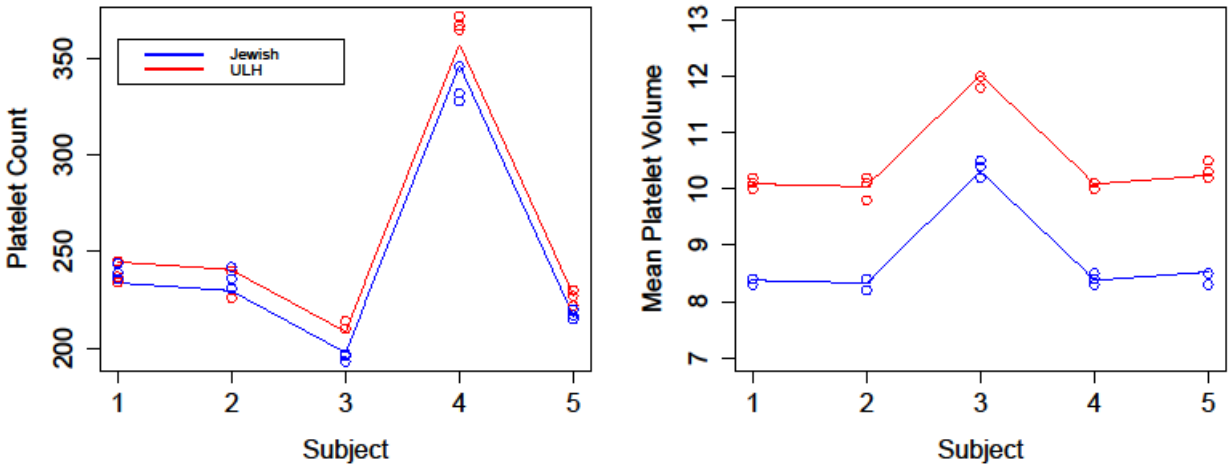
Study Phenotype	Criteria			
	Troponin (ng/ml)	**Histology**	**Presentation**	**Blinded Angiographic Assessment**
Stable CAD **(n=14)**	**Ortho Vitros 5600 Assay** "Peak" Troponin Level **<0.035** **-OR-** **Beckman Access** "Peak" Troponin Level **<0.04**	-	History of CVD* **-OR-** Angiographic findings -**AND**- Elective coronary angiogram	Satisfies **ALL** criteria below: 1. > 50% stenosis in one or greater epicardial vessel **(only required if no history of CVD)** 2. TIMI flow = 3 (all vessels) 3. TIMI MPG = 3 (all vessels)
Acute MI **(n=47)**	**Ortho Vitros 5600 Assay** "Peak" Troponin Level **>0.12** **-OR-** **Beckman Access** "Peak" Troponin Level **>0.5**	-	Non-elective coronary angiogram -**AND**- Clinical presentation consistent with WHF/ECC/ACC/AHA Universal definition of AMI	-
Thrombotic MI **(n=22)**	**Ortho Vitros 5600 Assay** "Peak" Troponin Level **>0.12** **-OR-** **Beckman Access** "Peak" Troponin Level **>0.5** **-AND-** >30% increase in troponin from T0 to T6	Histologically confirmed, 0-4 days old, coronary thrombus by blinded pathological assessment.	Non-elective coronary angiogram -**AND**- Clinical presentation consistent with WHF/ECC/ACC/AHA Universal definition of AMI	Stenosis of 50–100% in vessel from which thrombus recovered -**AND**- ST elevation in territory supplied by vessel from which thrombus recovered
non-Thrombotic MI **(n=12)**	**Ortho Vitros 5600 Assay** "Peak" Troponin Level **>0.12** **Beckman Access** "Peak" Troponin Level **>0.5**	No histologically confirmed thrombus recovered	Clinical presentation consistent with WHF/ECC/ACC/AHA Universal definition of AMI	Satisfies **ALL** criteria, **in all vessels**: 1. <50% stenosis 2. No filling defect 3. Simple Ambrose lesion morphology 4. TIMI flow = 3 5. TIMI MPG = 3

*CVD= CABG, PCI, CVA / TIA, CEA, PAD or AAA procedure

CAD = coronary artery disease, MI = myocardial infarction, TIMI = Thrombolysis In Myocardial Infarction, MPG = myocardial perfusion grade, CABG = coronary artery bypass grafting, PCI = percutaneous coronary intervention, CVA = cerebral vascular accident, TIA = transient ischemic attack, CEA = carotid endarterectomy, PAD = peripheral artery disease, AAA = abdominal aortic aneurysm

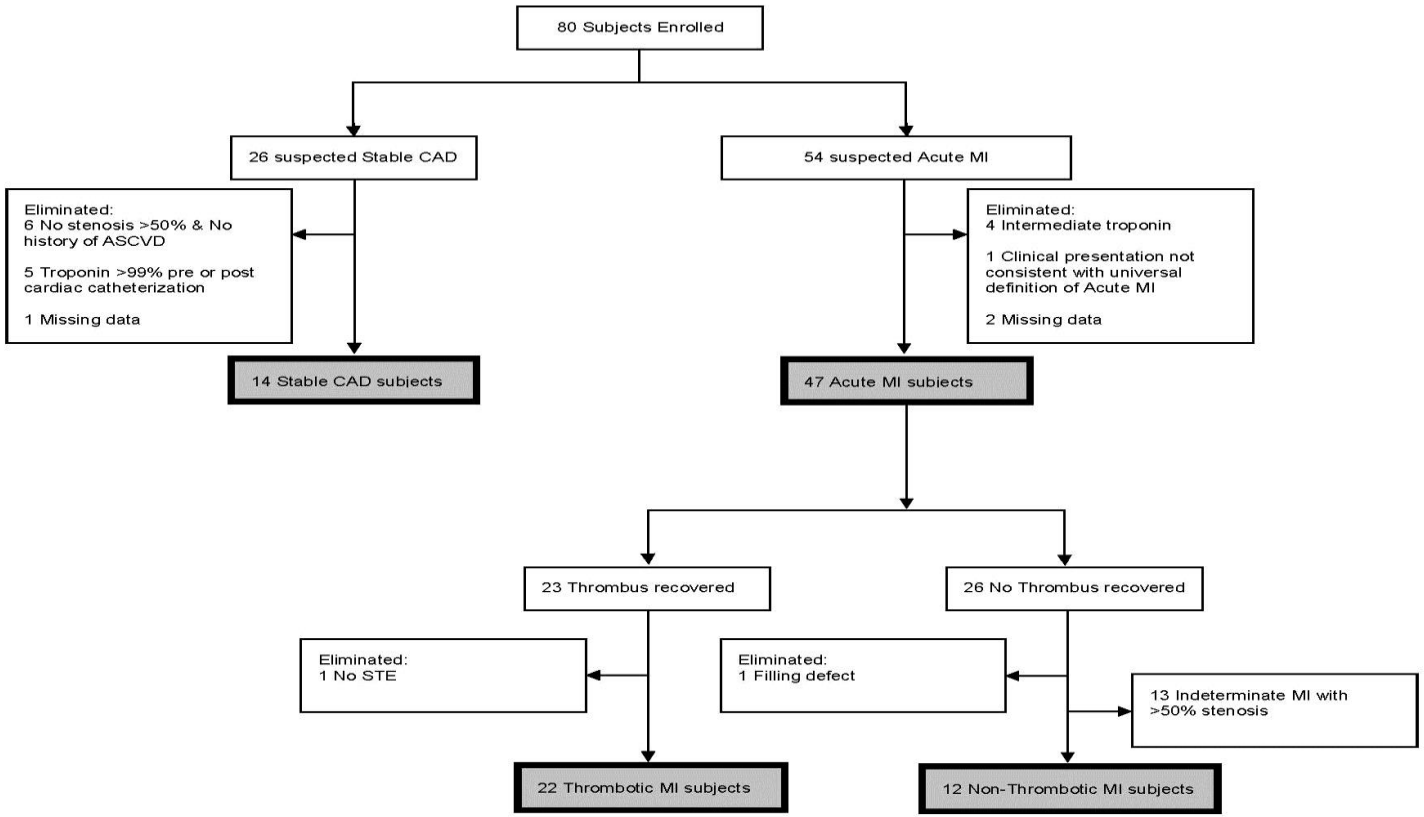
APPENDIX FIGURES

Appendix Figure 1. Trend of differences in measurement of MPV and PLT count by laboratories



Abbreviations:
Jewish – KentuckyOne Health Jewish Hospitals, ULH – University of Louisville

Appendix Figure 2. Study enrollment scheme



Abbreviations:
ASCVD – Atherosclerotic Cardiovascular Disease; CAD – Coronary Artery Disease; MI – Myocardial Infarction; STE – ST Segment Elevation on Electrocardiography