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Complete List of Authors:	Darling, Chad; University of Massachusetts Medical School, Emergency Medicine Sala Mercado, Javier; Instituto Modelo de Cardiologia Privado SRL, Quiroga-Castro, Walter; Instituto Modelo de Cardiologia Privado SRL, Tecco, Gabriel; Instituto Modelo de Cardiologia Privado SRL, Zelaya, Felix; Instituto Modelo de Cardiologia Privado SRL, Conci, Eduardo; Instituto Modelo de Cardiologia Privado SRL, Sala, Jose; Instituto Modelo de Cardiologia Privado SRL, Smith, Craig; University of Massachusetts Medical School, Cardiovascular Medicine Michelson, Alan; Harvard Medical School, Whittaker, Peter; Wayne State University School of Medicine, Cardiovascular Research Institute Welch, Rob; Wayne State University, ; Wayne State University School of Medicine, Cardiovascular Research Institute Przyklenk, Karin; Wayne State University School of Medicine, Cardiovascular Research Institute
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Point-of-Care Assessment of Platelet Reactivity in the Emergency Department May Facilitate Rapid Rule-Out of Acute Coronary Syndromes: A Prospective Cohort Pilot Feasibility Study

Chad E. Darling MD*, Javier A. Sala Mercado MD PhD[§‡**], Walter Quiroga-Castro MD**, Gabriel F. Tecco MD**, Felix R. Zelaya MD**, Eduardo C. Conci MD**, Jose P. Sala MD**, Craig S. Smith MD[£], Alan D. Michelson MD[†], Peter Whittaker PhD*[§¶], Robert D. Welch MD[§¶] and Karin Przyklenk PhD*[§¶‡]

Departments of *Emergency Medicine and [£] Cardiovascular Medicine, University of Massachusetts Medical School, Worcester, MA USA

[**] Division of Cardiology, Instituto Modelo de Cardiologia Privado SRL, Cordoba, Argentina

[†] Center for Platelet Research Studies, Division of Hematology/Oncology, Boston Children's Hospital, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, USA

[§] Cardiovascular Research Institute and Departments of [¶] Emergency Medicine and [‡] Physiology, Wayne State University School of Medicine Detroit, MI USA

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Correspondence: Karin Przyklenk, PhD
Cardiovascular Research Institute,
Wayne State University School of Medicine,
Elliman Building, Room 1107,
421 E. Canfield,
Detroit, MI 48201 USA

Telephone: 313-577-9047
E-mail: kprzykle@med.wayne.edu

ABSTRACT

Objective: Accurate, efficient and cost-effective disposition of patients presenting to emergency departments (EDs) with symptoms suggestive of acute coronary syndromes (ACS) is a growing priority. Platelet activation is an early feature in the pathogenesis of ACS; thus, we sought to obtain preliminary insight into whether point-of-care testing of platelet function: (i) is feasible in the ED; and (ii) may provide additional predictive value to assist in the rule-out of ACS.

Design: Prospective cohort feasibility study.

Setting: Two urban tertiary care sites, one located in the United States and the second in Argentina.

Participants: 509 adult patients presenting with symptoms of ACS as determined by the treating physician(s).

Intervention: none.

Main outcome measures: Platelet reactivity was quantified using the Platelet Function Analyzer (PFA)-100®, with closure time (i.e., time [seconds] required for blood, aspirated under high shear, to occlude a 150 μm aperture) serving as the primary endpoint. Closure times were categorized as 'normal' or 'prolonged', defined objectively as the 90th percentile of the distribution for all subjects enrolled in the study. Diagnosis of ACS was made using standard criteria.

Results: Closure times for the study population ranged from 47-300 seconds, with a 90th percentile value of 138 seconds. The proportion of patients with closure times $\geq$138 seconds was significantly higher in the ACS-negative group (41/330; 12.4%) *versus* the ACS-positive cohort (2/105 [1.9%]; p=0.0006). The specificity of 'prolonged' closure times ($\geq$ 138 seconds) for a diagnosis of ACS-negative was 98.1%, with a positive predictive power of 95.4%. Multivariate analysis revealed that closure time provided incremental, independent predictive value in the rule-out of ACS.

Conclusion: Point-of-care assessment of platelet reactivity is feasible in the Emergency Department and may facilitate the rapid rule-out of ACS in patients with prolonged closure times. Further large-scale multi-center investigation, incorporating risk/benefit analysis, is warranted.

ARTICLE SUMMARY

Article focus:

- The development of accurate, efficient and cost-effective strategies to assist in the rapid rule-out of acute coronary syndromes (ACS) in the Emergency Department is a growing priority.
- Platelet activation is an early feature in the pathogenesis of ACS.
- Our aims in this pilot feasibility study were to investigate whether point-of-care testing of platelet function, assessed by measuring 'closure time' using the Platelet Function Analyzer (PFA)-100®: (i) is logistically feasible in the ED; and (ii) provides additional predictive value, beyond that obtained via routine clinical assessment, to assist in the rule-out of ACS.

Key messages:

- Point-of-care testing of platelet reactivity is feasible in the ED.
- ACS-negative *versus* ACS-positive patients were distinguished by differences in the proportion of patients with prolonged closure times.
- Measurement of closure time provided incremental, independent predictive value in the rule-out of ACS.

Strengths and Limitations:

- The study suggests that a technically straightforward and cost-effective test, with minimal patient risk, may serve as a useful adjunct to current, standard ED practices for the rule-out of ACS. Importantly, the results are generalizable: the observation of a higher incidence of prolonged closure times in ACS-negative patients was seen in subjects from two distinct health care systems and populations.
- PFA testing will only contribute to the identification of a subset of ACS-negative patients, with both the size of the subset and potential value of the test dependent on the threshold used to define 'prolonged' closure times.

- Limitations of this pilot study include the enrollment of patients via convenience sampling, the fact that patient outcomes were not monitored beyond hospital discharge, and that a risk/benefit analysis was not included in the study design.

Accurate, efficient and cost-effective diagnosis of patients presenting to emergency departments (EDs) with symptoms suggestive of acute coronary syndromes (ACS) – and, in particular, the exclusion and early discharge of patients with non-cardiac chest pain – is a growing priority [1, 2]. In an effort to meet this challenge, interest has focused on the identification of new approaches to augment standard ED procedures and facilitate the timely triage of patients with suspected ACS. For example, there is recent evidence that coronary CT angiography (CCTA) combined with routine clinical assessment may provide added prognostic value in the management of chest pain patients in the ED [3-8]. Use of CCTA in low-to-intermediate risk patients is reportedly safe and reduces ED costs and hospital length of stay [3-5, 7, 8]. However, these benefits are accompanied by exposure to radiation and associated with increases in diagnostic testing and subsequent invasive procedures [5, 8].

Assessment of platelet activation, an early feature in the pathogenesis of ACS [9-14], has also been investigated as a possible benign strategy to expedite the diagnosis of ACS [15, 16]. Application of flow cytometry, the 'gold standard' for the quantitation of molecular indices of platelet activation, is, however, impractical for routine use under emergent conditions [11, 12, 17, 18]. More importantly, classic molecular indices of platelet activation have not provided added benefit in the risk stratification of undifferentiated chest pain patients [15, 16].

We hypothesized that rapid assessment of platelet reactivity using a technically straightforward point-of-care device – specifically, the Platelet Function Analyzer (PFA)-100® (Siemens) – may represent a more feasible strategy to assist in the timely rule- out of ACS in the ED. Rather than quantifying molecular markers of platelet activation-aggregation, the output of the PFA-100® is 'closure time': that is, the time required for whole blood, aspirated under high shear, to occlude a small, 150 μm aperture in a membrane coated with standard platelet agonists (collagen-adenosine diphosphate [ADP] or collagen-epinephrine). Although the PFA-100® is typically utilized to investigate the responsiveness of patients to aspirin and other anti-platelet therapies and aid in the detection of platelet dysfunction [11, 12, 18-22], there is

evidence to suggest that shortened closure times may be a marker of the acuity of coronary disease [10]. Accordingly, our primary aims in this pilot study were to determine whether: (i) point-of-care testing of platelet function is logistically feasible in an emergent setting; (ii) assessment of closure times provides a specific test, with robust positive predictive value, for the rule-out of ACS, and (iii) closure time yields additional predictive value, beyond that obtained via routine clinical assessment, that may assist in the identification of ACS-negative *versus* ACS-positive patients. In addition, as a secondary analysis, we investigated whether closure time may be of value in discriminating among ST-elevation myocardial infarction (STEMI), non-ST-elevation myocardial infarction (NSTEMI) and unstable angina (UA).

METHODS

We conducted a prospective cohort feasibility study of patients presenting with potential ACS at two urban tertiary care sites: the Emergency Department at the University of Massachusetts (UMASS)-Memorial Medical Center, University Campus, Worcester, MA USA, and the Cardiology Department, Instituto Modelo de Cardiologia Privado SRL, Cordoba, Argentina. The enrollment period was from January 2007 to December 2010, with patients entered on a convenience basis (i.e., enrollments were not consecutive but, rather, coincided with the work schedules of the study investigators). The protocol was reviewed and approved by the respective Institutional Review Boards at both institutions.

Patient Population

Patients ≥18 years of age with a potential diagnosis of ACS (at the discretion of the treating physician and reflected by orders for an electrocardiogram (ECG) and cardiac biomarkers) were considered for enrollment in the study. Prospective exclusion criteria were pregnancy, renal insufficiency (defined as serum creatine levels ≥ 1.5 mg/dl), anemia

(hematocrit <30%), platelet count <100,000/μL, major bleeding, any gastrointestinal bleeding, trauma, or inability to provide informed consent for any reason.

Study Enrollment and Protocol

As soon as possible after evaluation by ED staff, potential subjects were approached for enrollment into the study. If the patient was too ill or otherwise unable to provide written consent, then proxy consent was attempted. If consent was obtained by proxy and the patient later became cognizant, they were given the option to continue or withdraw their participation in the study. If consent was withdrawn, all collected data were discarded.

Blood used in the PFA-100® was drawn early in the ED stay, at the same time as cardiac biomarker testing, and closure time was measured within 3 hours of collection. All samples were obtained via peripheral venipuncture and drawn into tubes containing 3.2% sodium citrate. Immediately before analysis, each sample was gently inverted; a 900 μL aliquot of whole blood was then applied to a collagen-ADP test cartridge and closure time was quantified. Maximum test duration for the PFA-100® is 5 minutes; if the aperture is not occluded within this period, a closure time of '>300 seconds' is displayed.

Upon enrollment, patients were questioned regarding a history of bleeding or platelet function disorders and ingestion of known anti-platelet agents within the past 7 days (aspirin, clopidogrel, non-steroidal anti-inflammatory agents). In addition, the medical record was reviewed and standard cardiac risk factors (diabetes, hypertension, hypercholesterolemia, smoking, family history of heart disease) were recorded and used in the calculation of TIMI risk score [23].

Patient Diagnosis

Final diagnosis (ACS-negative *versus* ACS-positive) was established via standardized chart review. All reviews were conducted by physicians in a blinded manner, without knowledge of the closure time data. For subjects enrolled at the Instituto Modelo de Cardiologia Privado, all reviews were performed by cardiologists. At UMASS, each chart was initially reviewed by an

ED physician (CED). For patients with a definitive diagnosis of ACS-negative, no additional review was performed. However, for patients who: (i) had positive cardiac biomarkers during admission; (ii) underwent cardiac catheterization; (iii) had a positive provocative cardiovascular test (e.g., exercise stress test); and/or (iv) had ischemic ECG changes, the chart was reviewed by a cardiologist. In addition, in any case in which the diagnosis was uncertain, the decision was adjudicated by a cardiologist (CSS).

Patients considered to be ACS-positive were categorized into one of three groups: (i) STEMI, defined as 1 mm ST segment elevation in at least 2 contiguous ECG leads, a history consistent with ACS, and/or cardiac catheterization findings consistent with acute coronary occlusion; (ii) NSTEMI, defined as any ECG finding other than STEMI, history and inpatient cardiac testing consistent with ACS, and positive diagnostic cardiac markers within 24 hours of hospital arrival; or (iii) UA, defined as any ECG findings, a history consistent with ACS, non-diagnostic cardiac markers within 24 hours of arrival, and/or cardiac diagnostic testing (e.g., cardiac catheterization) consistent with ACS. Patients who did not meet these criteria (those with a diagnosis of non-cardiac chest pain/symptoms) were classified as ACS-negative.

Statistical Analysis

Our target sample size in this exploratory study was empiric. We reasoned that, as an approximate order of magnitude, ~100 patients would be required in the ACS-positive group in order to have a likelihood of discerning a potential difference in closure times between ACS-negative and ACS-positive groups. Assuming that 75% of patients enrolled in the study would be ACS-negative (and thus 25% would be ACS-positive), a total of approximately 400 patients would be required. To account for exclusions, target enrollment was set at ~500 subjects.

Univariate comparisons

Our primary analyses focused on the comparison of patients in the two main outcome groups: ACS-negative *versus* ACS-positive. All categorical data were compared using the Fisher's Exact Test and are reported as percentages. For continuous data, the D'Agostino and

Pearson Test was applied to determine normality and the appropriate parametric (t-test) or non-parametric alternative (Mann-Whitney) tests were utilized. Secondary analysis (comparison of closure times among ACS-negative, STEMI, NSTEMI and UA groups) was conducted using the Kruskal-Wallis test. Results are reported as mean ± standard deviation or medians with associated 10^{th} and 90^{th} percentile ranges.

Sensitivity, specificity and multivariate analysis

Closure time was categorized into "normal" and "prolonged" values based on the 90^{th} percentile of the distribution for all patients enrolled in the study. Utilizing the normal and prolonged values of closure time, the sensitivity, specificity, negative and positive predictive values for identifying ***ACS-negative*** patients were determined. Two reasons contributed to our choice of ACS negative (rather than ACS positive) as the main outcome of interest: (i) this approach is consistent with current guidelines for the improvement of diagnostic accuracy [1, 8]; and (ii) we reasoned that prolonged closure times may be associated with the absence of ACS, whereas shorter closure times may be manifest in either ACS negative or ACS positive patients. Sensitivity, specificity and predictive values are reported with associated 95% confidence intervals.

To determine if PFA closure time provides additional predictive value to standard clinical diagnostic information, a logistic regression was developed to assess its independent association with the main outcome of ACS-negative. Independent predictor variables considered to be mandatory in the model were platelet closure time (utilizing continuous rather than the categorized values; the main predictor of interest) and the TIMI risk score (representing standard clinical information to predict ACS outcomes). To account for potential differences between UMASS and Cordoba, study site was also added to the regression model. Other non-mandatory variables considered for inclusion were sex, a medical history of diabetes, hypertension, hypercholesterolemia, smoking, and clopidogrel use. As age and aspirin use are individually incorporated in the TIMI risk score, these variables were not considered for separate

inclusion in the model. Non-mandatory variables were left in the model only if they yielded an increase in predictive value as determined by ROC (receiver operating characteristic) analysis or were of additional importance based on the 2 log likelihood ratio test.

The reported estimate and adjusted odds ratio for closure time were calculated for an increase in platelet closure time of 10 seconds (rather than 1 second), a choice based on the premise that a 1-second increase would be of limited clinical usefulness. The final model was evaluated by c-statistic (area under the ROC curve) and the Hosmer and Lemeshow fit test. As a sensitivity test, the results were calculated using the general estimating equation (PROC GENMOD) with study site evaluated as a subject factor (cluster) rather than a term in the model.

Analyses were performed using GraphPad Prism Version 5.04 (San Diego CA) and SAS Version 9.3, (Carey NC).

RESULTS

Enrollment and exclusions (Figure 1)

A combined total of 509 patients were enrolled at the two study sites. Sixty-one subjects were excluded because closure time data were not available: reasons included technical errors (n=14), closure times >300 seconds (possibly due to mild thrombocytopenia, anemia, or inadequate mixing of the blood sample prior to testing; n=36), and failure to measure closure time despite obtaining consent (n=11). An additional 5 subjects were removed from analysis because exclusion criteria were identified after consent was obtained (n=4), or consent was revoked after the blood sample was collected (n=1). For the remaining 443 patients in whom closure time was quantified, 8 were diagnosed with thrombotic events that were non-cardiac in origin and thus were excluded from further analysis. Accordingly, results are reported for 435 subjects (324 enrolled at UMASS and 111 enrolled in Cordoba): 105 were diagnosed with ACS and 330 were ACS-negative patients.

Demographics (Table 1)

As expected [24], the ACS-positive group had higher TIMI scores, was older, and had a higher proportion of male patients when compared with the ACS-negative cohort. In addition, the incidence of hypercholesterolemia and use of clopidogrel were significantly higher in the ACS group. There were, however, no differences in the use of aspirin, incidence of hypertension or diabetes, proportion of smokers or reported family history of heart disease between the two groups.

Closure time (Figure 2 and Table 2)

There was a modest but significant difference in median closure times in the ACS-negative *versus* ACS-positive groups: 91 *versus* 87 seconds, respectively (p=0.0061; Figure 2A).

Of potentially greater importance, the two groups were distinguished by differences in the proportion of patients with prolonged closure times. We found that 41/330 (12.4%) of ACS-negative subjects had closure times $\geq$ 138 seconds (i.e., the 90th percentile of the distribution for all patients enrolled in the study) while, in contrast, 2/105 (1.9%) of patients in the ACS-positive group had closure times $\geq$ this value (p=0.0006; Figure 2B and Table 2A). The specificity and sensitivity of 'prolonged' closure times ($\geq$ 138 seconds) for a diagnosis of ACS-negative were 98.1% and 12.4%, respectively, with positive a positive predictive value of 95.4% and negative predictive value of 26.3% (Table 2B).

When the ACS-positive group was divided into STEMI, NSTEMI and UA cohorts, differences in closure time in the secondary 4-group analysis remained significant (p=0.0005; Figure 2C). However, post-hoc pairwise comparisons revealed no significant differences among STEMI, NSTEMI and UA groups.

Site Differences: UMASS versus Cordoba (Table 3)

Patients at both sites displayed the expected differences in TIMI score, age and sex between ACS-negative and ACS-positive groups (data not shown). However, subjects enrolled

in Cordoba were significantly younger, with a higher proportion of males and smokers but lower proportion of diabetics, when compared with UMASS (Table 3). In addition, and as expected [25], aspirin use was significantly lower among the cohort in Cordoba *versus* the UMASS population (40% *versus* 80%, p<0.0001, Table 3). Despite these demographic differences, a higher incidence of prolonged closure times in ACS-negative patients was observed at both sites: i.e., the proportion of subjects with closure times ≥ 138 seconds was 13.0% *versus* 10.0% in ACS-negative groups and 1.9% *versus* 2.0% in ACS-positive patients at UMASS *versus* Cordoba, respectively.

Closure time as an independent predictor of diagnosis (Table 4)

Logistic regression analysis was first performed with both mandatory variables (closure time, TIMI risk score) and non-mandatory variables (study site, sex, diabetes, hypertension, hypercholesterolemia, smoking, clopidogrel use) included in the regression model (data not shown). ROC analysis revealed that, among the non-mandatory variables, only study site contributed to an increase to the predictive value of the model for a diagnosis of ACS-negative. Accordingly, the final multivariate logistic regression model incorporated PFA closure time, TIMI risk score, and site (UMASS versus Cordoba; Table 4).

For every 10-second increase in closure time, the adjusted odds of a diagnosis of ACS-negative was 1.17 (95% confidence interval: 1.06 to 1.29). That is, when controlling for TIMI risk score and study site: for every 10 second increase, the prolonged closure time was associated with a 17% increase in the patient being ACS negative. These results demonstrate that, *irrespective of TIMI score and site*, closure time was associated with the diagnosis of ACS-negative, with *closure time providing additional, incremental predictive value beyond that obtained by TIMI score.* The model demonstrated good predictive characteristics (c=0.82) and model fit (χ^2=5.24, df=8; p=0.73). Finally, the sensitivity analysis that utilized a general estimating equation accounting for potential correlations among sites did not result in any

important changes in the direction of the effects (adjusted odds for every 10 second increase in closure time: 1.15 with 95% confidence interval 1.10 to 1.23) or conclusions.

DISCUSSION

In this pilot study, we demonstrate that point-of-care testing of platelet reactivity using the PFA-100® is feasible in the environment of the ED. Results of our primary analysis revealed differences in closure times between ACS-negative and ACS-positive cohorts, with the most notable, discriminating feature being the higher incidence of prolonged closure times in the ACS-negative group. Finally, the outcome of our multivariate analysis is consistent with the concept that assessment of closure time provides incremental, independent prognostic value beyond that obtained using the standard clinical predictor of TIMI score.

Assessment of platelet reactivity as a diagnostic tool for ACS in emergent settings

It is well-established that heightened platelet activity occurs in the setting of ACS [9-11, 13, 14, 26]. Indeed, measurement of platelet reactivity has been utilized in an effort to discern the stability of coronary disease [10], predict the future incidence and outcomes of major adverse cardiovascular events [11, 27-31], and, although results have been disappointing, guide the dosing of anti-platelet agents with the goal of improving outcomes [26, 32, 33]. Our current study differs from these previous reports, in that it focused on the assessment of platelet reactivity as an adjunct strategy to risk stratify-patients with potential ACS in emergent conditions. Accordingly, the novelty of our study lies in our expanded analysis of closure time, and the identification of a more practical and feasible application of these data in the prognosis of ACS.

Our observation of a modest but significant reduction in median/mean closure times in patients with ACS is consistent with the outcomes of two previous, small ED studies [34, 35]. However, this ~4 second difference is of limited practical significance given the broad and overlapping distributions of closure times for ACS-negative and ACS-positive cohorts (Figure 2).

Rather, we propose ***that the clinical utility of this test lies in the identification of patients with a prolonged closure time,*** a threshold that we objectively defined as the 90th percentile of the distribution of the study population. In this regard, we found that prolonged closure time (≥ 138 seconds) had a high specificity and positive predictive value for a diagnosis of ACS-negative.

Strengths and weaknesses

The results of our pilot study are consistent with the hypothesis that point-of-care testing of platelet reactivity may assist in the timely rule-out of ACS in the ED. Strengths of this approach include the fact that assessment of closure time is technically straightforward and cost-effective, with minimal patient risk (i.e., does not involve exposure to radiation or additional invasive testing). In addition, our data suggest that the concept is generalizable: the observation of a higher incidence of prolonged closure times in ACS-negative patients was seen in subjects from two distinct health care systems and populations (UMASS and Cordoba) that differ in terms of both demographics and the use of anti-platelet therapy [25].

There is, however, an important caveat to this strategy. PFA testing will only contribute to the identification of a subset of ACS-negative patients, with both the size of the subset and potential value of the test dependent on the threshold used to define 'prolonged' closure times (Figure 2 and Table 5). For example, the prospective criterion used in our analysis (the 90th percentile of closure times for all patients enrolled in the study) discerned an arguably modest, 12.4% of ACS-negative patients. However, expediting the discharge of even a small proportion of patients with a non-cardiac diagnosis would limit the costs, potential risks and patient stress associated with unneeded advanced diagnostic testing and possibly invasive procedures [1, 2]. Reducing the threshold (i.e., to the 80th percentile of the distribution) would increase the size of the subset identified as ACS-negative, with an accompanying (and increasingly unacceptable) loss in specificity because of the growing proportion of false-positives (ACS-positive patients with prolonged closure times: Figure 2 and Table 5). In contrast, increasing the threshold (i.e., to

the 95th percentile) would identify a diminishing proportion of ACS-negative patients with increasing specificity (Figure 2 and Table 5). The appropriate definition of 'prolonged' closure time will therefore require refinement based on risk/benefit analysis.

Finally, we emphasize that point-of-case assessment of platelet reactivity and identification of patients with 'prolonged' closure times clearly cannot function as a stand-alone test for the rule-out of ACS. Rather, measurement of PFA closure times may serve as an adjunct to current, standard ED practices. In support of this concept, our multivariate logistic regression model revealed that, irrespective of TIMI score and study site (and, thus, irrespective of differences in demographics and aspirin use between sites), closure time was an independent predictor of the diagnosis of ACS-negative.

Summary, limitations and future directions

We report that measurement of closure time using the PFA-100® provides additional and independent, incremental predictive value in the rule-out of ACS. Limitations of this pilot feasibility study include the enrollment of patients via convenience sampling, and differences in the logistics of the chart review process between the two sites. In addition, neither monitoring of patient outcomes beyond hospital discharge nor risk/benefit analysis of PFA testing was incorporated into the study design. We emphasize that point-of-care assessment of platelet reactivity cannot serve as a stand-alone test to either discern ACS-negative *versus* ACS-positive patients or distinguish among STEMI, NSTEMI and UA in the emergent setting – limitations that in all likelihood reflect the complex and multi-factorial pathophysiology of acute myocardial ischemia and infarction. Rather, our results suggest that assessment of closure times may provide benefit by augmenting standard ED diagnostic practices, a concept that warrants further large-scale multi-center investigation.

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Competing Interests: none

Contributor Statement:

Conception and design of the study: KP, CED, ADM

Analysis and interpretation of the data: all co-authors

Drafting of the manuscript or revising it critically for important intellectual content: KP, CED, RDW, PW, JASM, CSS, ADM

Final approval of the manuscript: all co-authors

All authors have had full access to all data and take responsibility for the integrity of the data and the accuracy of the analysis. KP is the guarantor.

Data sharing

There are no additional unpublished data.

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FIGURE LEGENDS

Figure 1: Inclusion flow-chart.

Figure 2: PFA closure time (seconds). **(A)** Median values with 10th, 25th, 75th and 90th percentiles: ACS-positive and ACS-negative cohorts. **(B)** Individual data points for all subjects: ACS-positive and ACS-negative cohorts. Lines denote the 80th, 90th and 95th percentiles of closure times for all patients enrolled in the study. **(C)** Individual data points for all subjects: STEMI, NSTEMI, UA and ACS-negative cohorts. Lines denote the 80th, 90th and 95th percentiles of closure times for all patients enrolled in the study.

Table 1.
Demographics: All Patients

	ACS-Negative (total n=330)	ACS-Positive (total n=105)	*p*-value
Age (years): mean ± SD	57 ± 14 (n=328)	61 ± 13 (n=104)	0.034
Male	65% (n=330)	80% (n=105)	0.004
TIMI score: mean ± SD	1.9 ± 1.4 (n=320)	3.1 ± 1.4 (n=104)	<0.0001
Aspirin	71% (n=321)	64% (n=104)	0.222 (ns)
Clopidogrel	11% (n=325)	20% (n=104)	0.031
Smoker	29% (n=322)	28% (n=105)	0.901(ns)
Hypertension	57% (n=322)	62% (n=105)	0.425 (ns)
Hypercholesterolemia	57% (n=322)	69% (n=105)	0.030
Diabetes	24% (n=322)	27% (n=105)	0.601 (ns)
Family History	40% (n=319)	41% (n=105)	0.909 (ns)

Table 2A.

Incidence of Prolonged Closure Time
(≥ 138 seconds, defined as the 90th percentile of the distribution of the study population)

	ACS-Negative: Yes	ACS-Negative: No	Total
Prolonged closure time: Yes	41	2	43
Prolonged closure time: No	289	103	392
Total	330	105	435

Table 2B.

Sensitivity, Specificity, Positive and Negative Predictive Values of Prolonged Closure Time for a Diagnosis of ACS-Negative

Sensitivity	12.4%	95% confidence interval: 9.1% to 16.5%
Specificity	98.1%	95% confidence interval: 93.3% to 99.8%
Positive predictive value	95.4%	95% confidence interval: 84.2% to 99.4%
Negative predictive value	26.3%	95% confidence interval: 22.0% to 30.9%

Table 3.
Demographics: UMASS vs Cordoba

	UMASS-All (total n=324)	Cordoba-All (total n=111)	*p*-value
Age (years): mean ± SD	59 ± 14 (n=324)	56 ± 12 (n=108)	0.036
Male	65% (n=324)	78% (n=111)	0.009
TIMI score: mean ± SD	2.3 ± 1.5 (n=313)	2.0 ± 1.4 (n=111)	0.142 (ns)
Aspirin	80% (n=314)	40% (n=104)	<0.0001
Clopidogrel	15% (n=319)	9% (n=111)	0.146 (ns)
Smoker	26% (n=316)	37% (n=111)	0.038
Hypertension	61% (n=316)	51% (n=111)	0.093 (ns)
Hypercholesterolemia	62% (n=316)	53% (n=111)	0.115 (ns)
Diabetes	28% (n=313)	14% (n=111)	0.005
Family History	44% (n=313)	31% (n=51)	0.014

Table 4.

Multivariable Logistic Regression Model
(outcome modeled: ACS-negative)

Predictor	Adjusted Odds Ratio	95% Confidence Interval
Closure Time	1.17	1.06 to 1.29
TIMI Risk Score	0.48	0.40 to 0.59
Study Site (UMASS *versus* Cordoba)	7.21	4.05 to 12.86

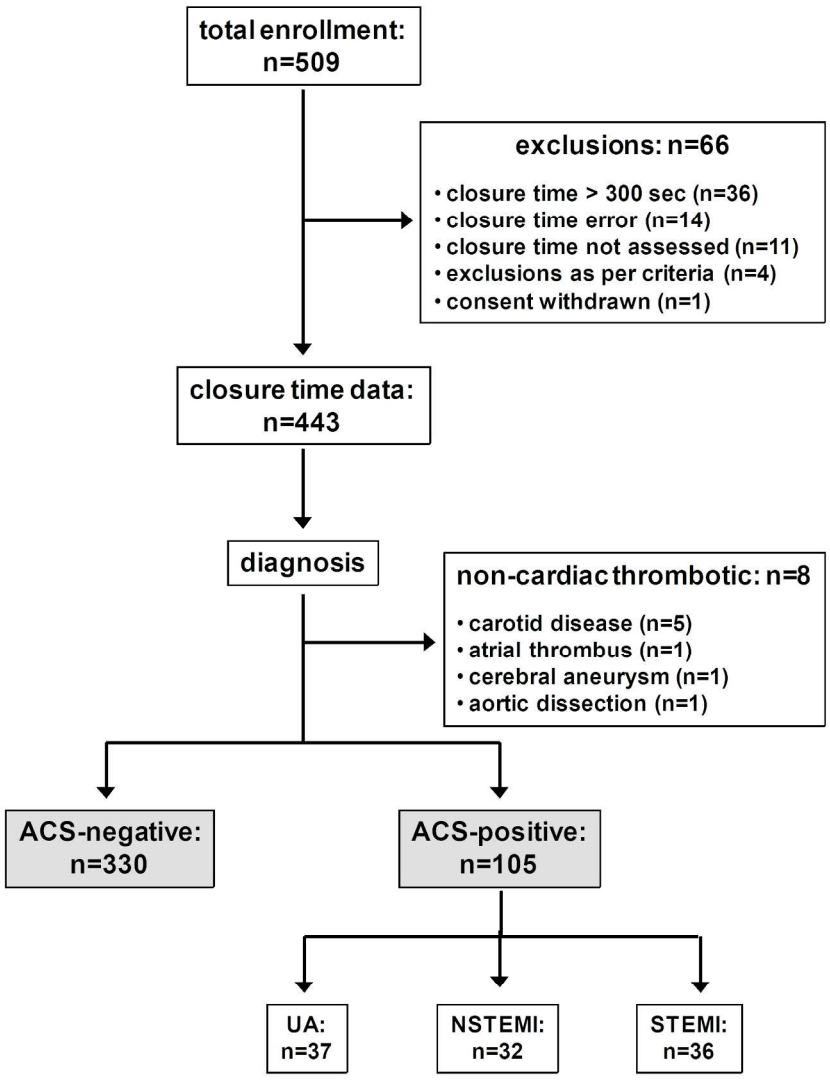
Table 5.

Effect of Definition of 'Prolonged' Closure Time on Specificity and Positive Predictive Value for a Diagnosis of ACS-Negative

Threshold	Specificity	Positive Predictive Value	% of ACS-Negative Patients Identified
95th percentile (≥ 160 seconds)	100% [96.6% to 100%]	100% [83.9% to 100%]	6.4% (21/330)
90th percentile (≥ 138 seconds)	98.1% [93.3% to 99.8%]	95.4% [84.2% to 99.4%]	12.4% (41/330)
80th percentile (≥ 117 seconds)	88.6% [80.9% to 94.0%]	86.2% [77.2% to 92.7%]	22.7% (75/330)

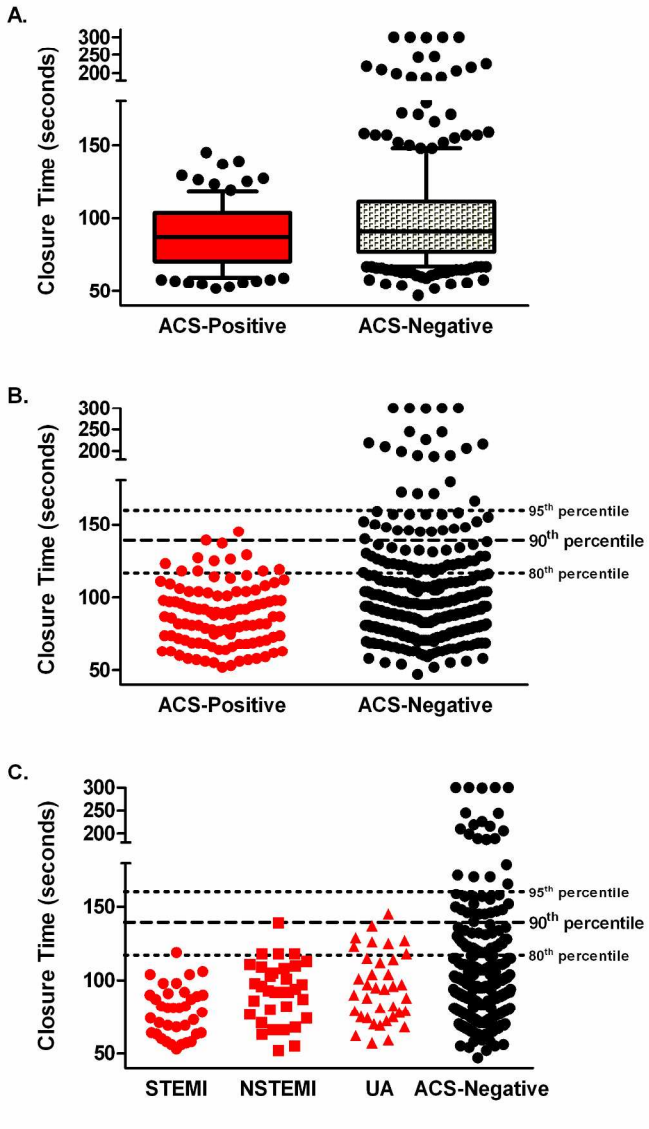
95% confidence intervals shown in square brackets

Figure 1.



191x253mm (300 x 300 DPI)

Figure 2.



194x270mm (300 x 300 DPI)

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Point-of-Care Assessment of Platelet Reactivity in the Emergency Department May Facilitate Rapid Rule-Out of Acute Coronary Syndromes: A Prospective Cohort Pilot Feasibility Study

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Point-of-Care Assessment of Platelet Reactivity in the Emergency Department May Facilitate Rapid Rule-Out of Acute Coronary Syndromes: A Prospective Cohort Pilot Feasibility Study

Chad E. Darling MD*, Javier A. Sala Mercado MD PhD[§‡**], Walter Quiroga-Castro MD**, Gabriel F. Tecco MD**, Felix R. Zelaya MD**, Eduardo C. Conci MD**, Jose P. Sala MD**, Craig S. Smith MD[£], Alan D. Michelson MD[†], Peter Whittaker PhD*[§¶], Robert D. Welch MD[§¶] and Karin Przyklenk PhD*[§¶‡]

Departments of *Emergency Medicine and [£] Cardiovascular Medicine, University of Massachusetts Medical School, Worcester, MA USA

[**] Division of Cardiology, Instituto Modelo de Cardiologia Privado SRL, Cordoba, Argentina

[†] Center for Platelet Research Studies, Division of Hematology/Oncology, Boston Children's Hospital, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, USA

[§] Cardiovascular Research Institute and Departments of [¶] Emergency Medicine and [‡] Physiology, Wayne State University School of Medicine Detroit, MI USA

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Correspondence: Karin Przyklenk, PhD
Cardiovascular Research Institute,
Wayne State University School of Medicine,
Elliman Building, Room 1107,
421 E. Canfield,
Detroit, MI 48201 USA

Telephone: 313-577-9047
E-mail: kprzykle@med.wayne.edu

ABSTRACT

Objective: Accurate, efficient and cost-effective disposition of patients presenting to emergency departments (EDs) with symptoms suggestive of acute coronary syndromes (ACS) is a growing priority. Platelet activation is an early feature in the pathogenesis of ACS; thus, we sought to obtain insight into whether point-of-care testing of platelet function: (i) may assist in the rule-out of ACS; (ii) may provide additional predictive value in identifying patients with non-cardiac symptoms *versus* ACS-positive patients; and (iii) is logistically feasible in the ED.

Design: Prospective cohort feasibility study.

Setting: Two urban tertiary care sites, one located in the United States and the second in Argentina.

Participants: 509 adult patients presenting with symptoms of ACS.

Intervention: none.

Main outcome measures: Platelet reactivity was quantified using the Platelet Function Analyzer (PFA)-100®, with closure time (seconds required for blood, aspirated under high shear, to occlude a 150 μm aperture) serving as the primary endpoint. Closure times were categorized as 'normal' or 'prolonged', defined objectively as the 90th percentile of the distribution for all subjects enrolled in the study. Diagnosis of ACS was made using standard criteria. The use of anti-platelet agents was not an exclusion criterion.

Results: Closure times for the study population ranged from 47-300 seconds, with a 90th percentile value of 138 seconds. The proportion of patients with closure times $\geq$138 seconds was significantly higher in patients with non-cardiac symptoms (41/330; 12.4%) *versus* the ACS-positive cohort (2/105 [1.9%]; p=0.0006). The specificity of 'prolonged' closure times ($\geq$ 138 seconds) for a diagnosis of non-cardiac symptoms was 98.1%, with a positive predictive power of 95.4%. Multivariate analysis revealed that closure time provided incremental, independent predictive value in the rule-out of ACS.

Conclusion: Point-of-care assessment of platelet reactivity is feasible in the ED and may facilitate the rapid rule-out of ACS in patients with prolonged closure times.

ARTICLE SUMMARY

Article focus:

- The development of accurate, efficient and cost-effective strategies to assist in the rapid rule-out of acute coronary syndromes (ACS) in the Emergency Department is a growing priority.
- Platelet activation is an early feature in the pathogenesis of ACS.
- Our aims in this pilot feasibility study were to investigate whether point-of-care testing of platelet function, assessed by measuring 'closure time' using the Platelet Function Analyzer (PFA)-100®: (i) may assist in the rule-out of ACS; (ii) may provide additional predictive value in identifying patients with non-cardiac symptoms *versus* ACS-positive patients; and (iii) is logistically feasible in the ED.

Key messages:

- Point-of-care testing of platelet reactivity is feasible in the ED.
- Patients with non-cardiac symptoms *versus* ACS-positive patients were distinguished by differences in the proportion of prolonged closure times.
- Measurement of closure time provided incremental, independent predictive value in the rule-out of ACS.

Strengths and Limitations:

- The study suggests that a technically straightforward and cost-effective test, with minimal patient risk, may serve as a useful adjunct to current, standard ED practices for the rule-out of ACS. Importantly, the results are generalizable: the observation of a higher incidence of prolonged closure times in patients with non-cardiac symptoms was seen in subjects from two distinct health care systems and populations.

- PFA testing will only contribute to the identification of a subset of patients with non-cardiac symptoms, with both the size of the subset and potential value of the test dependent on the threshold used to define ‘prolonged’ closure times.
- Limitations of this pilot study include the enrollment of patients via convenience sampling, the fact that patient outcomes were not monitored beyond hospital discharge, and that a risk/benefit analysis was not included in the study design.

Accurate, efficient and cost-effective diagnosis of patients presenting to emergency departments (EDs) with symptoms suggestive of acute coronary syndromes (ACS) – and, in particular, the exclusion and early discharge of patients with non-cardiac chest pain – is a growing priority [1, 2]. In an effort to meet this challenge, interest has focused on the identification of new approaches to augment standard ED procedures and facilitate the timely triage of patients with suspected ACS. For example, there is recent evidence that coronary CT angiography (CCTA) combined with routine clinical assessment may provide added prognostic value in the management of chest pain patients in the ED [3-8]. Use of CCTA in low-to-intermediate risk patients is reportedly safe and reduces ED costs and hospital length of stay [3-5, 7, 8]. However, these benefits are accompanied by exposure to radiation and associated with increases in diagnostic testing and subsequent invasive procedures [5, 8].

Assessment of platelet activation, an early feature in the pathogenesis of ACS [9-14], has also been investigated as a possible benign strategy to expedite the diagnosis of ACS [15, 16]. Application of flow cytometry, the 'gold standard' for the quantitation of molecular indices of platelet activation, is, however, impractical for routine use under emergent conditions [11, 12, 17, 18]. More importantly, classic molecular indices of platelet activation have not provided added benefit in the risk stratification of undifferentiated chest pain patients [15, 16].

We hypothesized that rapid assessment of platelet reactivity using a technically straightforward point-of-care device – specifically, the Platelet Function Analyzer (PFA)-100® (Siemens) – may represent a more feasible strategy to assist in the timely rule- out of ACS in the ED. Rather than quantifying molecular markers of platelet activation-aggregation, the output of the PFA-100® is 'closure time': that is, the time required for whole blood, aspirated under high shear, to occlude a small, 150 µm aperture in a membrane coated with standard platelet agonists (collagen-adenosine diphosphate [ADP] or collagen-epinephrine). Although the PFA-100® is typically utilized to investigate the responsiveness of patients to aspirin and other anti-platelet therapies and aid in the detection of platelet dysfunction [11, 12, 18-22], there is

evidence to suggest that shortened closure times may be a marker of the acuity of coronary disease [10]. Accordingly, our primary aims in this pilot study were to determine whether point-of-care testing of platelet function: (i) (may assist in the rule-out of ACS; (ii) may yield additional predictive value in identifying patients with non-cardiac symptoms *versus* ACS-positive patients; and (iii) is logistically feasible in an emergent setting. In addition, as a secondary analysis, we investigated whether closure time may be of value in discriminating between ST-elevation myocardial infarction (STEMI) and non-ST-elevation myocardial infarction (NSTEMI)/unstable angina (UA).

METHODS

We conducted a prospective cohort feasibility study of patients presenting with potential ACS at two urban tertiary care sites: the Emergency Department at the University of Massachusetts (UMASS)-Memorial Medical Center, University Campus, Worcester, MA USA, and the Cardiology Department, Instituto Modelo de Cardiologia Privado SRL, Cordoba, Argentina. The enrollment period was from January 2007 to December 2010. At each site, patients were entered on a convenience basis: i.e., enrollments were not consecutive but, rather, coincided with the work schedules of the study investigators. The protocol was reviewed and approved by the respective Institutional Review Boards at both institutions.

Patient Population

Patients $\geq$18 years of age with a potential diagnosis of ACS (at the discretion of the treating physician and reflected by orders for an electrocardiogram (ECG) and cardiac biomarkers) were considered for enrollment in the study. Prospective exclusion criteria were pregnancy, renal insufficiency (defined as serum creatine levels $\geq$ 1.5 mg/dl), anemia (hematocrit <30%), platelet count <100,000/µL, major bleeding, any gastrointestinal bleeding,

trauma, or inability to provide informed consent for any reason. The use of anti-platelet agents was not an exclusion criterion.

Study Enrollment and Protocol

As soon as possible after evaluation by ED staff, potential subjects were approached for enrollment into the study. If the patient was too ill or otherwise unable to provide written consent, then proxy consent was attempted. If consent was obtained by proxy and the patient later became cognizant, they were given the option to continue or withdraw their participation in the study. If consent was withdrawn, all collected data were discarded.

Blood used in the PFA-100® was drawn within <1 hour after presentation, at the same time as cardiac biomarker testing, and closure time was measured within 3 hours of collection. All samples were obtained via peripheral venipuncture and drawn into tubes containing 3.2% sodium citrate. Immediately before analysis, each sample was gently inverted; a 900 µL aliquot of whole blood was then applied to a collagen-ADP test cartridge and closure time was quantified. Maximum test duration for the PFA-100® is 5 minutes; if the aperture is not occluded within this period, a closure time of '>300 seconds' is displayed.

Upon enrollment, patients were questioned regarding a history of bleeding or platelet function disorders and ingestion of known anti-platelet agents within the past 7 days (aspirin, clopidogrel, non-steroidal anti-inflammatory agents). In addition, the medical record was reviewed and standard cardiac risk factors (diabetes, hypertension, hypercholesterolemia, smoking, family history of heart disease) were recorded and used in the calculation of TIMI risk score [23].

Patient Diagnosis

Final diagnosis (non-cardiac symptoms *versus* ACS-positive) was established via standardized chart review. All reviews were conducted by physicians in a blinded manner, without knowledge of the closure time data. For subjects enrolled at the Instituto Modelo de Cardiologia Privado, patients were seen by a cardiologist at the time of hospital presentation,

and all reviews were performed by a team of cardiologists (WQC, GFT, FRZ, ECC, JPS). At UMASS, each patient was first seen by an Emergency Medicine physician at the time of hospital presentation, and each chart was reviewed by an ED physician (CED). For patients with a definitive diagnosis of non-cardiac symptoms (that is, patients with no ischemic ECG changes, no positive markers or provocative cardiovascular test results, and no history of a diagnosis of ACS of any type in the chart), no additional review was performed. However, for patients who: (i) had positive cardiac biomarkers during admission; (ii) underwent cardiac catheterization; (iii) had a positive provocative cardiovascular test (e.g., exercise stress test); or (iv) had ischemic ECG changes, the chart was reviewed by both an ED physician and a cardiologist. In any case in which the diagnosis was uncertain, the decision was adjudicated by a cardiologist (CSS).

Patients considered to be ACS-positive were categorized into one of two groups, STEMI or NSTEMI/UA, in accordance with standard guidelines [24-26]. Patients who did not meet these criteria were classified as having a diagnosis of non-cardiac chest pain/symptoms.

Statistical Analysis

Our target sample size in this exploratory study was empiric. We reasoned that, as an approximate order of magnitude, ~100 patients would be required in the ACS-positive group in order to have a likelihood of discerning a potential difference in closure times between patients with non-cardiac symptoms and the ACS-positive cohort. Assuming that 75% of patients enrolled in the study would have a diagnosis of non-cardiac chest pain (and thus 25% would be ACS-positive), a total of approximately 400 patients would be required. To account for exclusions, target enrollment was set at ~500 subjects.

Univariate comparisons

Our primary analyses focused on the comparison of patients in the two main outcome groups: non-cardiac symptoms *versus* ACS-positive. All categorical data were compared using the Fisher's Exact Test and are reported as percentages. For continuous data, the D'Agostino and Pearson Test was applied to determine normality and the appropriate parametric (t-test) or

non-parametric alternative (Mann-Whitney) tests were utilized. Secondary analysis (comparison of closure times among non-cardiac chest pain, STEMI and NSTEMI/UA groups) was conducted using the Kruskal-Wallis test. Results are reported as mean ± standard deviation or medians with associated 10th and 90th percentile ranges.

Sensitivity, specificity and multivariate analysis

We made the prospective and arbitrary decision to categorize closure time into "normal" and "prolonged" values based on the 90th percentile of the distribution for all patients enrolled in the study. Utilizing the normal and prolonged values of closure time, the sensitivity, specificity, negative and positive predictive values and likelihood ratio for identifying patients with ***non-cardiac symptoms*** were determined. Two reasons contributed to our choice of non-cardiac chest pain (rather than ACS positive) as the main outcome of interest: (i) this approach is consistent with current guidelines for the improvement of diagnostic accuracy [1, 8]; and (ii) we reasoned that prolonged closure times may be associated with the absence of ACS, whereas shorter closure times may be manifest in either group of patients. Sensitivity, specificity, predictive values and the likelihood ratio are reported with associated 95% confidence intervals.

To determine if PFA closure time provides additional predictive value to standard clinical diagnostic information, a logistic regression was developed to assess its independent association with the main outcome of non-cardiac symptoms. Independent predictor variables considered to be mandatory in the model were closure time (utilizing continuous rather than the categorized values; the main predictor of interest) and the TIMI risk score (representing standard clinical information to predict ACS outcomes). To account for potential differences between UMASS and Cordoba, study site was also added to the regression model. Other non-mandatory variables considered for inclusion were sex, a medical history of diabetes, hypertension, hypercholesterolemia, smoking, and clopidogrel use. As age and aspirin use are individually incorporated in the TIMI risk score, these variables were not considered for separate inclusion in the model. Non-mandatory variables were left in the model only if they yielded an

increase in predictive value as determined by ROC (receiver operating characteristic) analysis or were of additional importance based on the 2 log likelihood ratio test.

The reported estimate and adjusted odds ratio for closure time were calculated for an increase in platelet closure time of 10 seconds (rather than 1 second), a choice based on the premise that a 1-second increase would be of limited clinical usefulness. The final model was evaluated by c-statistic (area under the ROC curve) and the Hosmer and Lemeshow fit test. As a sensitivity test, the results were calculated using the general estimating equation (PROC GENMOD) with study site evaluated as a subject factor (cluster) rather than a term in the model. The Net Reclassification Index (NRI) and Integrated Discrimination Improvement (IDI) statistic were not included in the analyses, given the skepticism and criticisms that have been raised concerning their value in predicting the potential, incremental prognostic impact of novel biomarkers [27, 28].

Analyses were performed using GraphPad Prism Version 5.04 (San Diego CA) and SAS Version 9.3, (Carey NC).

RESULTS

Enrollment and exclusions (Figure 1)

A combined total of 509 patients were enrolled at the two study sites. Sixty-one subjects were excluded because closure time data were not available: reasons included technical errors (n=14), closure times >300 seconds (possibly due to mild thrombocytopenia, anemia, or inadequate mixing of the blood sample prior to testing; n=36), and failure to measure closure time despite obtaining consent (n=11). An additional 5 subjects were removed from analysis because exclusion criteria were identified after consent was obtained (n=4), or consent was revoked after the blood sample was collected (n=1). For the remaining 443 patients in whom closure time was quantified, 8 were diagnosed with thrombotic events that were non-cardiac in origin and thus were excluded from further analysis. Accordingly, results are reported for 435

subjects (324 enrolled at UMASS and 111 enrolled in Cordoba): 105 were diagnosed with ACS and 330 had non-cardiac symptoms.

Demographics (Table 1)

As expected [29], the ACS-positive group had higher TIMI scores, was older, and had a higher proportion of male subjects *versus* patients with non-cardiac symptoms. In addition, the incidence of hypercholesterolemia and use of clopidogrel were significantly higher in the ACS group. There were, however, no differences in the use of aspirin, incidence of hypertension or diabetes, proportion of smokers or reported family history of heart disease between the two groups.

Closure time (Figure 2 and Table 2)

There was a modest but significant difference in median closure times in patients with non-cardiac symptoms *versus* the ACS-positive group: 91 *versus* 87 seconds, respectively (p=0.0061; Figure 2A). When the ACS-positive group was divided into STEMI and combined NSTEMI/UA cohorts, the modest differences in closure time in the secondary 3-group analysis remained significant (p=0.0001; Figure 2C).

Of potentially greater importance, patients with non-cardiac symptoms *versus* ACS-positive patients were distinguished by differences in the proportion of prolonged closure times. We found that 41/330 (12.4%) of patients with non-cardiac symptoms had closure times $\geq$ 138 seconds (i.e., the 90th percentile of the distribution for all patients enrolled in the study) while, in contrast, 2/105 (1.9%) of patients in the ACS-positive group had closure times $\geq$ this value (p=0.0006; Figure 2B and Table 2A). The specificity and sensitivity of 'prolonged' closure times ($\geq$ 138 seconds) for a diagnosis of non-cardiac symptoms were 98.1% and 12.4%, respectively, with positive a positive predictive value of 95.4%, negative predictive value of 26.3%, and likelihood ratio of 6.52 (Table 2B).

Site Differences: UMASS versus Cordoba (Table 3)

Patients at both sites displayed the expected differences in TIMI score, age and sex between non-cardiac chest pain and ACS-positive groups (data not shown). However, subjects enrolled in Cordoba were significantly younger, with a higher proportion of males and smokers but lower proportion of diabetics, when compared with UMASS (Table 3). In addition, and as expected [30, 31], aspirin use was significantly lower among the cohort in Cordoba *versus* the UMASS population (40% *versus* 80%, p<0.0001, Table 3). Despite these demographic differences, a higher incidence of prolonged closure times in patients with non-cardiac symptoms was observed at both sites: i.e., the proportion of subjects with closure times $\geq$ 138 seconds was 13.0% *versus* 10.0% in patients with non-cardiac chest pain and 1.9% *versus* 2.0% in ACS-positive patients at UMASS *versus* Cordoba, respectively.

Closure time as an independent predictor of diagnosis (Table 4)

Logistic regression analysis was first performed with both mandatory variables (closure time, TIMI risk score) and non-mandatory variables (study site, sex, diabetes, hypertension, hypercholesterolemia, smoking, clopidogrel use) included in the regression model (data not shown). ROC analysis revealed that, among the non-mandatory variables, only study site (UMASS *versus* Cordoba) contributed to an increase to the predictive value of the model for a diagnosis of non-cardiac symptoms. Comparison of ROC curves obtained by including closure time, TIMI score and site in the model *versus* TIMI score and site alone showed a significant, incremental increase in area under the curve with the addition of closure time (0.818 versus 0.795; p=0.009; Figure 3). Accordingly, the final multivariate logistic regression model incorporated these three variables (Table 4).

For every 10-second increase in closure time, the adjusted odds of a diagnosis of non-cardiac symptoms was 1.17 (95% confidence interval: 1.06 to 1.29). That is, when controlling for TIMI risk score and study site: for every 10 second increase, the prolonged closure time was associated with a 17% increase in the patient having non-cardiac chest pain. These results

demonstrate that, *irrespective of TIMI score and site*, closure time was associated with the diagnosis of non-cardiac symptoms, with *closure time providing additional, incremental predictive value beyond that obtained by TIMI score.* The model demonstrated good predictive characteristics (c=0.82) and model fit (χ^2=5.24, df=8; p=0.73). Finally, the sensitivity analysis that utilized a general estimating equation accounting for potential correlations among sites did not result in any important changes in the direction of the effects (adjusted odds for every 10 second increase in closure time: 1.15 with 95% confidence interval 1.10 to 1.23) or conclusions.

DISCUSSION

In this study, we demonstrate that point-of-care testing of platelet reactivity using the PFA-100® is feasible in the environment of the ED. Results of our primary analysis revealed differences in closure times between patients with non-cardiac symptoms *versus* ACS-positive cohorts, with the most notable, discriminating feature being the higher incidence of prolonged closure times in the group with non-cardiac chest pain. Finally, the outcome of our multivariate analysis is consistent with the concept that assessment of closure time provides incremental, independent prognostic value beyond that obtained using the standard clinical predictor of TIMI score.

Assessment of platelet reactivity as a diagnostic tool for ACS in emergent settings

It is well-established that heightened platelet activity occurs in the setting of ACS [9-11, 13, 14, 32]. Indeed, measurement of platelet reactivity has been utilized in an effort to discern the stability of coronary disease [10], predict the future incidence and outcomes of major adverse cardiovascular events [11, 33-37], and, although results have been disappointing, guide the dosing of anti-platelet agents with the goal of improving outcomes [32, 38, 39]. Our current study differs from these previous reports, in that it focused on the assessment of platelet reactivity as an adjunct strategy to risk stratify-patients with potential ACS in emergent

conditions. Accordingly, the novelty of our study lies in our expanded analysis of closure time, and the identification of a more practical and feasible application of these data in the prognosis of ACS.

Our observation of a modest but significant reduction in median/mean closure times in patients with ACS is consistent with the outcomes of two previous, small ED studies [40, 41]. However, this ~4 second difference is of limited practical significance given the broad and overlapping distributions of closure times for patients with non-cardiac symptoms and the ACS-positive cohort (Figure 2). Rather, we propose ***that the clinical utility of this test lies in the identification of patients with a prolonged closure time,*** a threshold that we objectively defined as the 90th percentile of the distribution of the study population. In this regard, we found that prolonged closure time ($\geq$ 138 seconds) had a high specificity, positive predictive value and likelihood ratio for a diagnosis of non-cardiac chest pain.

Strengths and weaknesses

The results of our pilot study are consistent with the hypothesis that point-of-care testing of platelet reactivity may assist in the timely rule-out of ACS in the ED. Strengths of this approach include the fact that assessment of closure time is technically straightforward and cost-effective, with minimal patient risk (i.e., does not involve exposure to radiation or additional invasive testing). In addition, our data suggest that the concept is generalizable. We observed significant differences in demographics (age and gender), the prevalence of specific risk factors (diabetes, smoking) and the use of anti-platelet therapy (aspirin: Table 3) between UMASS and Cordoba – findings that are in agreement with published reports focused on Argentine cohorts [30, 31, 42], and may underlie the robust contribution of study site as a predictor of outcome in our logistic regression model. Nonetheless, our results, obtained from two distinct health care systems and populations, consistently revealed a higher proportion of prolonged closure times in patients with non-cardiac symptoms.

There is, however, an important caveat to this strategy. PFA testing will only contribute to the identification of a subset of patients with non-cardiac symptoms, with both the size of the subset and potential value of the test dependent on the threshold used to define 'prolonged' closure times (Figure 2 and Table 5). For example, the prospective and arbitrary criterion used in our analysis (the 90th percentile of closure times for all patients enrolled in the study) discerned an arguably modest, 12.4% of patients with a diagnosis of non-cardiac symptoms. However, expediting the discharge of even a small proportion of patients with a non-cardiac diagnosis would limit the costs, potential risks and patient stress associated with unneeded advanced diagnostic testing and possibly invasive procedures [1, 2]. Reducing the threshold (i.e., to the 80th percentile of the distribution) would increase the size of the subset identified as ACS-negative, with an accompanying (and increasingly unacceptable) loss in specificity because of the growing proportion of false-positives (ACS-positive patients with prolonged closure times: Figure 2 and Table 5). In contrast, increasing the threshold (i.e., to the 95th percentile) would identify a diminishing proportion of patients with non-cardiac symptoms with increasing specificity (Figure 2 and Table 5). The appropriate definition of 'prolonged' closure time will therefore require refinement based on risk/benefit analysis.

Finally, we emphasize that point-of-case assessment of platelet reactivity and identification of patients with 'prolonged' closure times clearly cannot function as a stand-alone test for the rule-out of ACS. Rather, measurement of PFA closure times may serve as an adjunct to current, standard ED practices. In support of this concept, our multivariate logistic regression model revealed that, irrespective of TIMI score and study site (and, thus, irrespective of differences in demographics and aspirin use between sites), closure time was an independent predictor of the diagnosis of non-cardiac symptoms.

Summary, limitations and future directions

We report that measurement of closure time using the PFA-100® provides additional and independent, incremental predictive value in the rule-out of ACS. Limitations of this pilot

feasibility study include the enrollment of patients via convenience sampling, and differences in the logistics of the chart review process between the two sites. In addition, neither monitoring of patient outcomes beyond hospital discharge (raising the possibility of potential misclassification of some patients) nor risk/benefit analysis of PFA testing was incorporated into the study design. We emphasize that point-of-care assessment of platelet reactivity cannot serve as a stand-alone test to either discern patients with non-cardiac symptoms *versus* ACS-positive patients or distinguish between STEMI versus NSTEMI/UA in the emergent setting – limitations that in all likelihood reflect the complex and multi-factorial pathophysiology of acute myocardial ischemia and infarction. Rather, our results suggest that assessment of closure times may provide benefit by augmenting standard ED diagnostic practices, a concept that warrants further large-scale multi-center investigation.

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Contributor Statement:

Conception and design of the study: KP, CED, ADM

Analysis and interpretation of the data: all co-authors

Drafting of the manuscript or revising it critically for important intellectual content: KP, CED, RDW, PW, JASM, CSS, ADM

Final approval of the manuscript: all co-authors

All authors have had full access to all data and take responsibility for the integrity of the data and the accuracy of the analysis. KP is the guarantor.

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FIGURE LEGENDS

Figure 1: Inclusion flow-chart.

Figure 2: PFA closure time (seconds). **(A)** Median values with 10th, 25th, 75th and 90th percentiles: ACS-positive patients and patients with non-cardiac symptoms. **(B)** Individual data points for all subjects: ACS-positive patients and patients with non-cardiac symptoms. Lines denote the 80th, 90th and 95th percentiles of closure times for all patients enrolled in the study. **(C)** Individual data points for all subjects: STEMI, NSTEMI/UA cohorts and patients with non-cardiac symptoms. Lines denote the 80th, 90th and 95th percentiles of closure times for all patients enrolled in the study.

Figure 3: ROC analysis. Comparison of ROC curves obtained by including closure time, TIMI score and study site in the regression model *versus* TIMI score and site alone showed a significant, incremental increase in area under the curve with the addition of closure time (0.818 *versus* 0.795; p=0.009).

Table 1.
Demographics: All Patients

	Non-Cardiac Symptoms (total n=330)	ACS-Positive (total n=105)	*p*-value
Age (years): mean ± SD	57 ± 14 (n=328)	61 ± 13 (n=104)	0.034
Male	65% (n=330)	80% (n=105)	0.004
TIMI score: mean ± SD	1.9 ± 1.4 (n=320)	3.1 ± 1.4 (n=104)	<0.0001
Aspirin	71% (n=321)	64% (n=104)	0.222 (ns)
Clopidogrel	11% (n=325)	20% (n=104)	0.031
Smoker	29% (n=322)	28% (n=105)	0.901(ns)
Hypertension	57% (n=322)	62% (n=105)	0.425 (ns)
Hypercholesterolemia	57% (n=322)	69% (n=105)	0.030
Diabetes	24% (n=322)	27% (n=105)	0.601 (ns)
Family History	40% (n=319)	41% (n=105)	0.909 (ns)

Table 2A.

Incidence of Prolonged Closure Time
(≥ 138 seconds, defined as the 90th percentile of the distribution of the study population)

	Non-Cardiac Symptoms	ACS-Positive	Total
Prolonged closure time: Yes	41	2	43
Prolonged closure time: No	289	103	392
Total	330	105	435

Table 2B.

Sensitivity, Specificity, Positive and Negative Predictive Values and Likelihood Ratio of Prolonged Closure Time for a Diagnosis of Non-Cardiac Symptoms

Sensitivity	12.4%	95% confidence interval: 9.1% to 16.5%
Specificity	98.1%	95% confidence interval: 93.3% to 99.8%
Positive predictive value	95.4%	95% confidence interval: 84.2% to 99.4%
Negative predictive value	26.3%	95% confidence interval: 22.0% to 30.9%
Likelihood ratio:	6.52	95% confidence interval: 1.61 to 26.51

Table 3.
Demographics: UMASS vs Cordoba

	UMASS-All (total n=324)	Cordoba-All (total n=111)	*p*-value
Age (years): mean ± SD	59 ± 14 (n=324)	56 ± 12 (n=108)	0.036
Male	65% (n=324)	78% (n=111)	0.009
TIMI score: mean ± SD	2.3 ± 1.5 (n=313)	2.0 ± 1.4 (n=111)	0.142 (ns)
Aspirin	80% (n=314)	40% (n=104)	<0.0001
Clopidogrel	15% (n=319)	9% (n=111)	0.146 (ns)
Smoker	26% (n=316)	37% (n=111)	0.038
Hypertension	61% (n=316)	51% (n=111)	0.093 (ns)
Hypercholesterolemia	62% (n=316)	53% (n=111)	0.115 (ns)
Diabetes	28% (n=313)	14% (n=111)	0.005
Family History	44% (n=313)	31% (n=51)	0.014

Table 4.

Multivariable Logistic Regression Model
(outcome modeled: Non-Cardiac Symptoms)

Predictor	Adjusted Odds Ratio	95% Confidence Interval
Closure Time	1.17	1.06 to 1.29
TIMI Risk Score	0.48	0.40 to 0.59
Study Site (UMASS *versus* Cordoba)	7.21	4.05 to 12.86

Table 5.

Effect of Definition of 'Prolonged' Closure Time on Specificity and Positive Predictive Value for a Diagnosis of Non-Cardiac Symptoms

Threshold	Specificity	Positive Predictive Value	% of Patients with Non-Cardiac Symptoms Identified
95th percentile (≥ 160 seconds)	100% [96.6% to 100%]	100% [83.9% to 100%]	6.4% (21/330)
90th percentile (≥ 138 seconds)	98.1% [93.3% to 99.8%]	95.4% [84.2% to 99.4%]	12.4% (41/330)
80th percentile (≥ 117 seconds)	88.6% [80.9% to 94.0%]	86.2% [77.2% to 92.7%]	22.7% (75/330)

95% confidence intervals shown in square brackets

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Point-of-Care Assessment of Platelet Reactivity in the Emergency Department May Facilitate Rapid Rule-Out of Acute Coronary Syndromes: A Prospective Cohort Pilot Feasibility Study

Chad E. Darling MD*, Javier A. Sala Mercado MD PhD[§‡**], Walter Quiroga-Castro MD**, Gabriel F. Tecco MD**, Felix R. Zelaya MD**, Eduardo C. Conci MD**, Jose P. Sala MD**, Craig S. Smith MD[£], Alan D. Michelson MD[†], Peter Whittaker PhD*[§¶], Robert D. Welch MD[§¶] and Karin Przyklenk PhD*[§¶‡]

Departments of *Emergency Medicine and [£] Cardiovascular Medicine, University of Massachusetts Medical School, Worcester, MA USA

[**] Division of Cardiology, Instituto Modelo de Cardiologia Privado SRL, Cordoba, Argentina

[†] Center for Platelet Research Studies, Division of Hematology/Oncology, Boston Children's Hospital, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, USA

[§] Cardiovascular Research Institute and Departments of [¶] Emergency Medicine and [‡] Physiology, Wayne State University School of Medicine Detroit, MI USA

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Key words: acute coronary syndromes, platelet, emergency department:

Correspondence: Karin Przyklenk, PhD
Cardiovascular Research Institute,
Wayne State University School of Medicine,
Elliman Building, Room 1107,
421 E. Canfield,
Detroit, MI 48201 USA

Telephone: 313-577-9047
E-mail: kprzykle@med.wayne.edu

ABSTRACT

Objective: Accurate, efficient and cost-effective disposition of patients presenting to emergency departments (EDs) with symptoms suggestive of acute coronary syndromes (ACS) is a growing priority. Platelet activation is an early feature in the pathogenesis of ACS; thus, we sought to obtain insight into whether point-of-care testing of platelet function: (i) may assist in the rule-out of ACS; (ii) may provide additional predictive value in identifying patients with non-cardiac symptoms *versus* ACS-positive patients; and (iii) is logistically feasible in the ED.

Design: Prospective cohort feasibility study.

Setting: Two urban tertiary care sites, one located in the United States and the second in Argentina.

Participants: 509 adult patients presenting with symptoms of ACS.

Intervention: none.

Main outcome measures: Platelet reactivity was quantified using the Platelet Function Analyzer (PFA)-100®, with closure time (seconds required for blood, aspirated under high shear, to occlude a 150 μm aperture) serving as the primary endpoint. Closure times were categorized as 'normal' or 'prolonged', defined objectively as the 90th percentile of the distribution for all subjects enrolled in the study. Diagnosis of ACS was made using standard criteria. The use of anti-platelet agents was not an exclusion criterion.

Results: Closure times for the study population ranged from 47-300 seconds, with a 90th percentile value of 138 seconds. The proportion of patients with closure times ≥138 seconds was significantly higher in patients with non-cardiac symptoms (41/330; 12.4%) *versus* the ACS-positive cohort (2/105 [1.9%]; p=0.0006). The specificity of 'prolonged' closure times (≥ 138 seconds) for a diagnosis of non-cardiac symptoms was 98.1%, with a positive predictive power of 95.4%. Multivariate analysis revealed that closure time provided incremental, independent predictive value in the rule-out of ACS.

Conclusion: Point-of-care assessment of platelet reactivity is feasible in the ED and may facilitate the rapid rule-out of ACS in patients with prolonged closure times.

ARTICLE SUMMARY

Article focus:

- The development of accurate, efficient and cost-effective strategies to assist in the rapid rule-out of acute coronary syndromes (ACS) in the Emergency Department is a growing priority.
- Platelet activation is an early feature in the pathogenesis of ACS.
- Our aims in this pilot feasibility study were to investigate whether point-of-care testing of platelet function, assessed by measuring 'closure time' using the Platelet Function Analyzer (PFA)-100®: (i) may assist in the rule-out of ACS; (ii) may provide additional predictive value in identifying patients with non-cardiac symptoms *versus* ACS-positive patients; and (iii) is logistically feasible in the ED.

Key messages:

- Point-of-care testing of platelet reactivity is feasible in the ED.
- Patients with non-cardiac symptoms *versus* ACS-positive patients were distinguished by differences in the proportion of prolonged closure times.
- Measurement of closure time provided incremental, independent predictive value in the rule-out of ACS.

Strengths and Limitations:

- The study suggests that a technically straightforward and cost-effective test, with minimal patient risk, may serve as a useful adjunct to current, standard ED practices for the rule-out of ACS. Importantly, the results are generalizable: the observation of a higher incidence of prolonged closure times in patients with non-cardiac symptoms was seen in subjects from two distinct health care systems and populations.

- PFA testing will only contribute to the identification of a subset of patients with non-cardiac symptoms, with both the size of the subset and potential value of the test dependent on the threshold used to define ‘prolonged’ closure times.
- Limitations of this pilot study include the enrollment of patients via convenience sampling, the fact that patient outcomes were not monitored beyond hospital discharge, and that a risk/benefit analysis was not included in the study design.

Accurate, efficient and cost-effective diagnosis of patients presenting to emergency departments (EDs) with symptoms suggestive of acute coronary syndromes (ACS) – and, in particular, the exclusion and early discharge of patients with non-cardiac chest pain – is a growing priority [1, 2]. In an effort to meet this challenge, interest has focused on the identification of new approaches to augment standard ED procedures and facilitate the timely triage of patients with suspected ACS. For example, there is recent evidence that coronary CT angiography (CCTA) combined with routine clinical assessment may provide added prognostic value in the management of chest pain patients in the ED [3-8]. Use of CCTA in low-to-intermediate risk patients is reportedly safe and reduces ED costs and hospital length of stay [3-5, 7, 8]. However, these benefits are accompanied by exposure to radiation and associated with increases in diagnostic testing and subsequent invasive procedures [5, 8].

Assessment of platelet activation, an early feature in the pathogenesis of ACS [9-14], has also been investigated as a possible benign strategy to expedite the diagnosis of ACS [15, 16]. Application of flow cytometry, the 'gold standard' for the quantitation of molecular indices of platelet activation, is, however, impractical for routine use under emergent conditions [11, 12, 17, 18]. More importantly, classic molecular indices of platelet activation have not provided added benefit in the risk stratification of undifferentiated chest pain patients [15, 16].

We hypothesized that rapid assessment of platelet reactivity using a technically straightforward point-of-care device – specifically, the Platelet Function Analyzer (PFA)-100® (Siemens) – may represent a more feasible strategy to assist in the timely rule- out of ACS in the ED. Rather than quantifying molecular markers of platelet activation-aggregation, the output of the PFA-100® is 'closure time': that is, the time required for whole blood, aspirated under high shear, to occlude a small, 150 μm aperture in a membrane coated with standard platelet agonists (collagen-adenosine diphosphate [ADP] or collagen-epinephrine). Although the PFA-100® is typically utilized to investigate the responsiveness of patients to aspirin and other anti-platelet therapies and aid in the detection of platelet dysfunction [11, 12, 18-22], there is

evidence to suggest that shortened closure times may be a marker of the acuity of coronary disease [10]. Accordingly, our primary aims in this pilot study were to determine whether point-of-care testing of platelet function: (i) (may assist in the rule-out of ACS; (ii) may yield additional predictive value in identifying patients with non-cardiac symptoms *versus* ACS-positive patients; and (iii) is logistically feasible in an emergent setting. In addition, as a secondary analysis, we investigated whether closure time may be of value in discriminating between ST-elevation myocardial infarction (STEMI) and non-ST-elevation myocardial infarction (NSTEMI)/unstable angina (UA).

METHODS

We conducted a prospective cohort feasibility study of patients presenting with potential ACS at two urban tertiary care sites: the Emergency Department at the University of Massachusetts (UMASS)-Memorial Medical Center, University Campus, Worcester, MA USA, and the Cardiology Department, Instituto Modelo de Cardiologia Privado SRL, Cordoba, Argentina. The enrollment period was from January 2007 to December 2010. At each site, patients were entered on a convenience basis: i.e., enrollments were not consecutive but, rather, coincided with the work schedules of the study investigators. The protocol was reviewed and approved by the respective Institutional Review Boards at both institutions.

Patient Population

Patients ≥18 years of age with a potential diagnosis of ACS (at the discretion of the treating physician and reflected by orders for an electrocardiogram (ECG) and cardiac biomarkers) were considered for enrollment in the study. Prospective exclusion criteria were pregnancy, renal insufficiency (defined as serum creatine levels ≥ 1.5 mg/dl), anemia (hematocrit <30%), platelet count <100,000/μL, major bleeding, any gastrointestinal bleeding,

trauma, or inability to provide informed consent for any reason. The use of anti-platelet agents was not an exclusion criterion.

Study Enrollment and Protocol

As soon as possible after evaluation by ED staff, potential subjects were approached for enrollment into the study. If the patient was too ill or otherwise unable to provide written consent, then proxy consent was attempted. If consent was obtained by proxy and the patient later became cognizant, they were given the option to continue or withdraw their participation in the study. If consent was withdrawn, all collected data were discarded.

Blood used in the PFA-100® was drawn within <1 hour after presentation, at the same time as cardiac biomarker testing, and closure time was measured within 3 hours of collection. All samples were obtained via peripheral venipuncture and drawn into tubes containing 3.2% sodium citrate. Immediately before analysis, each sample was gently inverted; a 900 µL aliquot of whole blood was then applied to a collagen-ADP test cartridge and closure time was quantified. Maximum test duration for the PFA-100® is 5 minutes; if the aperture is not occluded within this period, a closure time of '>300 seconds' is displayed.

Upon enrollment, patients were questioned regarding a history of bleeding or platelet function disorders and ingestion of known anti-platelet agents within the past 7 days (aspirin, clopidogrel, non-steroidal anti-inflammatory agents). In addition, the medical record was reviewed and standard cardiac risk factors (diabetes, hypertension, hypercholesterolemia, smoking, family history of heart disease) were recorded and used in the calculation of TIMI risk score [23].

Patient Diagnosis

Final diagnosis (non-cardiac symptoms *versus* ACS-positive) was established via standardized chart review. All reviews were conducted by physicians in a blinded manner, without knowledge of the closure time data. For subjects enrolled at the Instituto Modelo de Cardiologia Privado, patients were seen by a cardiologist at the time of hospital presentation,

and all reviews were performed by a team of cardiologists (WQC, GFT, FRZ, ECC, JPS). At UMASS, each patient was first seen by an Emergency Medicine physician at the time of hospital presentation, and each chart was reviewed by an ED physician (CED). For patients with a definitive diagnosis of non-cardiac symptoms (that is, patients with no ischemic ECG changes, no positive markers or provocative cardiovascular test results, and no history of a diagnosis of ACS of any type in the chart), no additional review was performed. However, for patients who: (i) had positive cardiac biomarkers during admission; (ii) underwent cardiac catheterization; (iii) had a positive provocative cardiovascular test (e.g., exercise stress test); or (iv) had ischemic ECG changes, the chart was reviewed by both an ED physician and a cardiologist. In any case in which the diagnosis was uncertain, the decision was adjudicated by a cardiologist (CSS).

Patients considered to be ACS-positive were categorized into one of two groups, STEMI or NSTEMI/UA, in accordance with standard guidelines [24-26]. Patients who did not meet these criteria were classified as having a diagnosis of non-cardiac chest pain/symptoms.

Statistical Analysis

Our target sample size in this exploratory study was empiric. We reasoned that, as an approximate order of magnitude, ~100 patients would be required in the ACS-positive group in order to have a likelihood of discerning a potential difference in closure times between patients with non-cardiac symptoms and the ACS-positive cohort. Assuming that 75% of patients enrolled in the study would have a diagnosis of non-cardiac chest pain (and thus 25% would be ACS-positive), a total of approximately 400 patients would be required. To account for exclusions, target enrollment was set at ~500 subjects.

Univariate comparisons

Our primary analyses focused on the comparison of patients in the two main outcome groups: non-cardiac symptoms *versus* ACS-positive. All categorical data were compared using the Fisher's Exact Test and are reported as percentages. For continuous data, the D'Agostino and Pearson Test was applied to determine normality and the appropriate parametric (t-test) or

non-parametric alternative (Mann-Whitney) tests were utilized. Secondary analysis (comparison of closure times among non-cardiac chest pain, STEMI and NSTEMI/UA groups) was conducted using the Kruskal-Wallis test. Results are reported as mean ± standard deviation or medians with associated 10th and 90th percentile ranges.

Sensitivity, specificity and multivariate analysis

We made the prospective and arbitrary decision to categorize closure time into "normal" and "prolonged" values based on the 90th percentile of the distribution for all patients enrolled in the study. Utilizing the normal and prolonged values of closure time, the sensitivity, specificity, negative and positive predictive values and likelihood ratio for identifying patients with ***non-cardiac symptoms*** were determined. Two reasons contributed to our choice of non-cardiac chest pain (rather than ACS positive) as the main outcome of interest: (i) this approach is consistent with current guidelines for the improvement of diagnostic accuracy [1, 8]; and (ii) we reasoned that prolonged closure times may be associated with the absence of ACS, whereas shorter closure times may be manifest in either group of patients. Sensitivity, specificity, predictive values and the likelihood ratio are reported with associated 95% confidence intervals.

To determine if PFA closure time provides additional predictive value to standard clinical diagnostic information, a logistic regression was developed to assess its independent association with the main outcome of non-cardiac symptoms. Independent predictor variables considered to be mandatory in the model were closure time (utilizing continuous rather than the categorized values; the main predictor of interest) and the TIMI risk score (representing standard clinical information to predict ACS outcomes). To account for potential differences between UMASS and Cordoba, study site was also added to the regression model. Other non-mandatory variables considered for inclusion were sex, a medical history of diabetes, hypertension, hypercholesterolemia, smoking, and clopidogrel use. As age and aspirin use are individually incorporated in the TIMI risk score, these variables were not considered for separate inclusion in the model. Non-mandatory variables were left in the model only if they yielded an

increase in predictive value as determined by ROC (receiver operating characteristic) analysis or were of additional importance based on the 2 log likelihood ratio test.

The reported estimate and adjusted odds ratio for closure time were calculated for an increase in platelet closure time of 10 seconds (rather than 1 second), a choice based on the premise that a 1-second increase would be of limited clinical usefulness. The final model was evaluated by c-statistic (area under the ROC curve) and the Hosmer and Lemeshow fit test. As a sensitivity test, the results were calculated using the general estimating equation (PROC GENMOD) with study site evaluated as a subject factor (cluster) rather than a term in the model. The Net Reclassification Index (NRI) and Integrated Discrimination Improvement (IDI) statistic were not included in the analyses, given the skepticism and criticisms that have been raised concerning their value in predicting the potential, incremental prognostic impact of novel biomarkers [27, 28].

Analyses were performed using GraphPad Prism Version 5.04 (San Diego CA) and SAS Version 9.3, (Carey NC).

RESULTS

Enrollment and exclusions (Figure 1)

A combined total of 509 patients were enrolled at the two study sites. Sixty-one subjects were excluded because closure time data were not available: reasons included technical errors (n=14), closure times >300 seconds (possibly due to mild thrombocytopenia, anemia, or inadequate mixing of the blood sample prior to testing; n=36), and failure to measure closure time despite obtaining consent (n=11). An additional 5 subjects were removed from analysis because exclusion criteria were identified after consent was obtained (n=4), or consent was revoked after the blood sample was collected (n=1). For the remaining 443 patients in whom closure time was quantified, 8 were diagnosed with thrombotic events that were non-cardiac in origin and thus were excluded from further analysis. Accordingly, results are reported for 435

subjects (324 enrolled at UMASS and 111 enrolled in Cordoba): 105 were diagnosed with ACS and 330 had non-cardiac symptoms.

Demographics (Table 1)

As expected [29], the ACS-positive group had higher TIMI scores, was older, and had a higher proportion of male subjects *versus* patients with non-cardiac symptoms. In addition, the incidence of hypercholesterolemia and use of clopidogrel were significantly higher in the ACS group. There were, however, no differences in the use of aspirin, incidence of hypertension or diabetes, proportion of smokers or reported family history of heart disease between the two groups.

Closure time (Figure 2 and Table 2)

There was a modest but significant difference in median closure times in patients with non-cardiac symptoms *versus* the ACS-positive group: 91 *versus* 87 seconds, respectively (p=0.0061; Figure 2A). When the ACS-positive group was divided into STEMI and combined NSTEMI/UA cohorts, the modest differences in closure time in the secondary 3-group analysis remained significant (p=0.0001; Figure 2C).

Of potentially greater importance, patients with non-cardiac symptoms *versus* ACS-positive patients were distinguished by differences in the proportion of prolonged closure times. We found that 41/330 (12.4%) of patients with non-cardiac symptoms had closure times $\geq$ 138 seconds (i.e., the 90th percentile of the distribution for all patients enrolled in the study) while, in contrast, 2/105 (1.9%) of patients in the ACS-positive group had closure times $\geq$ this value (p=0.0006; Figure 2B and Table 2A). The specificity and sensitivity of 'prolonged' closure times ($\geq$ 138 seconds) for a diagnosis of non-cardiac symptoms were 98.1% and 12.4%, respectively, with positive a positive predictive value of 95.4%, negative predictive value of 26.3%, and likelihood ratio of 6.52 (Table 2B).

Site Differences: UMASS versus Cordoba (Table 3)

Patients at both sites displayed the expected differences in TIMI score, age and sex between non-cardiac chest pain and ACS-positive groups (data not shown). However, subjects enrolled in Cordoba were significantly younger, with a higher proportion of males and smokers but lower proportion of diabetics, when compared with UMASS (Table 3). In addition, and as expected [30, 31], aspirin use was significantly lower among the cohort in Cordoba *versus* the UMASS population (40% *versus* 80%, p<0.0001, Table 3). Despite these demographic differences, a higher incidence of prolonged closure times in patients with non-cardiac symptoms was observed at both sites: i.e., the proportion of subjects with closure times ≥ 138 seconds was 13.0% *versus* 10.0% in patients with non-cardiac chest pain and 1.9% *versus* 2.0% in ACS-positive patients at UMASS *versus* Cordoba, respectively.

Closure time as an independent predictor of diagnosis (Table 4)

Logistic regression analysis was first performed with both mandatory variables (closure time, TIMI risk score) and non-mandatory variables (study site, sex, diabetes, hypertension, hypercholesterolemia, smoking, clopidogrel use) included in the regression model (data not shown). ROC analysis revealed that, among the non-mandatory variables, only study site (UMASS *versus* Cordoba) contributed to an increase to the predictive value of the model for a diagnosis of non-cardiac symptoms. Comparison of ROC curves obtained by including closure time, TIMI score and site in the model *versus* TIMI score and site alone showed a significant, incremental increase in area under the curve with the addition of closure time (0.818 versus 0.795; p=0.009; Figure 3). Accordingly, the final multivariate logistic regression model incorporated these three variables (Table 4).

For every 10-second increase in closure time, the adjusted odds of a diagnosis of non-cardiac symptoms was 1.17 (95% confidence interval: 1.06 to 1.29). That is, when controlling for TIMI risk score and study site: for every 10 second increase, the prolonged closure time was associated with a 17% increase in the patient having non-cardiac chest pain. These results

demonstrate that, *irrespective of TIMI score and site*, closure time was associated with the diagnosis of non-cardiac symptoms, with *closure time providing additional, incremental predictive value beyond that obtained by TIMI score.* The model demonstrated good predictive characteristics (c=0.82) and model fit (χ^2=5.24, df=8; p=0.73). Finally, the sensitivity analysis that utilized a general estimating equation accounting for potential correlations among sites did not result in any important changes in the direction of the effects (adjusted odds for every 10 second increase in closure time: 1.15 with 95% confidence interval 1.10 to 1.23) or conclusions.

DISCUSSION

In this study, we demonstrate that point-of-care testing of platelet reactivity using the PFA-100® is feasible in the environment of the ED. Results of our primary analysis revealed differences in closure times between patients with non-cardiac symptoms *versus* ACS-positive cohorts, with the most notable, discriminating feature being the higher incidence of prolonged closure times in the group with non-cardiac chest pain. Finally, the outcome of our multivariate analysis is consistent with the concept that assessment of closure time provides incremental, independent prognostic value beyond that obtained using the standard clinical predictor of TIMI score.

Assessment of platelet reactivity as a diagnostic tool for ACS in emergent settings

It is well-established that heightened platelet activity occurs in the setting of ACS [9-11, 13, 14, 32]. Indeed, measurement of platelet reactivity has been utilized in an effort to discern the stability of coronary disease [10], predict the future incidence and outcomes of major adverse cardiovascular events [11, 33-37], and, although results have been disappointing, guide the dosing of anti-platelet agents with the goal of improving outcomes [32, 38, 39]. Our current study differs from these previous reports, in that it focused on the assessment of platelet reactivity as an adjunct strategy to risk stratify-patients with potential ACS in emergent

conditions. Accordingly, the novelty of our study lies in our expanded analysis of closure time, and the identification of a more practical and feasible application of these data in the prognosis of ACS.

Our observation of a modest but significant reduction in median/mean closure times in patients with ACS is consistent with the outcomes of two previous, small ED studies [40, 41]. However, this ~4 second difference is of limited practical significance given the broad and overlapping distributions of closure times for patients with non-cardiac symptoms and the ACS-positive cohort (Figure 2). Rather, we propose ***that the clinical utility of this test lies in the identification of patients with a prolonged closure time,*** a threshold that we objectively defined as the 90th percentile of the distribution of the study population. In this regard, we found that prolonged closure time ($\geq$ 138 seconds) had a high specificity, positive predictive value and likelihood ratio for a diagnosis of non-cardiac chest pain.

Strengths and weaknesses

The results of our pilot study are consistent with the hypothesis that point-of-care testing of platelet reactivity may assist in the timely rule-out of ACS in the ED. Strengths of this approach include the fact that assessment of closure time is technically straightforward and cost-effective, with minimal patient risk (i.e., does not involve exposure to radiation or additional invasive testing). In addition, our data suggest that the concept is generalizable. We observed significant differences in demographics (age and gender), the prevalence of specific risk factors (diabetes, smoking) and the use of anti-platelet therapy (aspirin: Table 3) between UMASS and Cordoba – findings that are in agreement with published reports focused on Argentine cohorts [30, 31, 42], and may underlie the robust contribution of study site as a predictor of outcome in our logistic regression model. Nonetheless, our results, obtained from two distinct health care systems and populations, consistently revealed a higher proportion of prolonged closure times in patients with non-cardiac symptoms.

There is, however, an important caveat to this strategy. PFA testing will only contribute to the identification of a subset of patients with non-cardiac symptoms, with both the size of the subset and potential value of the test dependent on the threshold used to define 'prolonged' closure times (Figure 2 and Table 5). For example, the prospective and arbitrary criterion used in our analysis (the 90th percentile of closure times for all patients enrolled in the study) discerned an arguably modest, 12.4% of patients with a diagnosis of non-cardiac symptoms. However, expediting the discharge of even a small proportion of patients with a non-cardiac diagnosis would limit the costs, potential risks and patient stress associated with unneeded advanced diagnostic testing and possibly invasive procedures [1, 2]. Reducing the threshold (i.e., to the 80th percentile of the distribution) would increase the size of the subset identified as ACS-negative, with an accompanying (and increasingly unacceptable) loss in specificity because of the growing proportion of false-positives (ACS-positive patients with prolonged closure times: Figure 2 and Table 5). In contrast, increasing the threshold (i.e., to the 95th percentile) would identify a diminishing proportion of patients with non-cardiac symptoms with increasing specificity (Figure 2 and Table 5). The appropriate definition of 'prolonged' closure time will therefore require refinement based on risk/benefit analysis.

Finally, we emphasize that point-of-case assessment of platelet reactivity and identification of patients with 'prolonged' closure times clearly cannot function as a stand-alone test for the rule-out of ACS. Rather, measurement of PFA closure times may serve as an adjunct to current, standard ED practices. In support of this concept, our multivariate logistic regression model revealed that, irrespective of TIMI score and study site (and, thus, irrespective of differences in demographics and aspirin use between sites), closure time was an independent predictor of the diagnosis of non-cardiac symptoms.

Summary, limitations and future directions

We report that measurement of closure time using the PFA-100® provides additional and independent, incremental predictive value in the rule-out of ACS. Limitations of this pilot

feasibility study include the enrollment of patients via convenience sampling, and differences in the logistics of the chart review process between the two sites. In addition, neither monitoring of patient outcomes beyond hospital discharge (raising the possibility of potential misclassification of some patients) nor risk/benefit analysis of PFA testing was incorporated into the study design. We emphasize that point-of-care assessment of platelet reactivity cannot serve as a stand-alone test to either discern patients with non-cardiac symptoms *versus* ACS-positive patients or distinguish between STEMI versus NSTEMI/UA in the emergent setting – limitations that in all likelihood reflect the complex and multi-factorial pathophysiology of acute myocardial ischemia and infarction. Rather, our results suggest that assessment of closure times may provide benefit by augmenting standard ED diagnostic practices, a concept that warrants further large-scale multi-center investigation.

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Contributor Statement:

Conception and design of the study: KP, CED, ADM

Analysis and interpretation of the data: all co-authors

Drafting of the manuscript or revising it critically for important intellectual content: KP, CED, RDW, PW, JASM, CSS, ADM

Final approval of the manuscript: all co-authors

All authors have had full access to all data and take responsibility for the integrity of the data and the accuracy of the analysis. KP is the guarantor.

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FIGURE LEGENDS

Figure 1: Inclusion flow-chart.

Figure 2: PFA closure time (seconds). **(A)** Median values with 10th, 25th, 75th and 90th percentiles: ACS-positive patients and patients with non-cardiac symptoms. **(B)** Individual data points for all subjects: ACS-positive patients and patients with non-cardiac symptoms. Lines denote the 80th, 90th and 95th percentiles of closure times for all patients enrolled in the study. **(C)** Individual data points for all subjects: STEMI, NSTEMI/UA cohorts and patients with non-cardiac symptoms. Lines denote the 80th, 90th and 95th percentiles of closure times for all patients enrolled in the study.

Figure 3: ROC analysis. Comparison of ROC curves obtained by including closure time, TIMI score and study site in the regression model *versus* TIMI score and site alone showed a significant, incremental increase in area under the curve with the addition of closure time (0.818 *versus* 0.795; p=0.009).

Table 1.
Demographics: All Patients

	Non-Cardiac Symptoms (total n=330)	ACS-Positive (total n=105)	*p*-value
Age (years): mean ± SD	57 ± 14 (n=328)	61 ± 13 (n=104)	0.034
Male	65% (n=330)	80% (n=105)	0.004
TIMI score: mean ± SD	1.9 ± 1.4 (n=320)	3.1 ± 1.4 (n=104)	<0.0001
Aspirin	71% (n=321)	64% (n=104)	0.222 (ns)
Clopidogrel	11% (n=325)	20% (n=104)	0.031
Smoker	29% (n=322)	28% (n=105)	0.901(ns)
Hypertension	57% (n=322)	62% (n=105)	0.425 (ns)
Hypercholesterolemia	57% (n=322)	69% (n=105)	0.030
Diabetes	24% (n=322)	27% (n=105)	0.601 (ns)
Family History	40% (n=319)	41% (n=105)	0.909 (ns)

Table 2A.

Incidence of Prolonged Closure Time
(≥ 138 seconds, defined as the 90th percentile of the distribution of the study population)

	Non-Cardiac Symptoms	ACS-Positive	Total
Prolonged closure time: Yes	41	2	43
Prolonged closure time: No	289	103	392
Total	330	105	435

Table 2B.

Sensitivity, Specificity, Positive and Negative Predictive Values and Likelihood Ratio of Prolonged Closure Time for a Diagnosis of Non-Cardiac Symptoms

Sensitivity	12.4%	95% confidence interval: 9.1% to 16.5%
Specificity	98.1%	95% confidence interval: 93.3% to 99.8%
Positive predictive value	95.4%	95% confidence interval: 84.2% to 99.4%
Negative predictive value	26.3%	95% confidence interval: 22.0% to 30.9%
Likelihood ratio:	6.52	95% confidence interval: 1.61 to 26.51

Table 3.
Demographics: UMASS vs Cordoba

	UMASS-All (total n=324)	Cordoba-All (total n=111)	*p*-value
Age (years): mean ± SD	59 ± 14 (n=324)	56 ± 12 (n=108)	0.036
Male	65% (n=324)	78% (n=111)	0.009
TIMI score: mean ± SD	2.3 ± 1.5 (n=313)	2.0 ± 1.4 (n=111)	0.142 (ns)
Aspirin	80% (n=314)	40% (n=104)	<0.0001
Clopidogrel	15% (n=319)	9% (n=111)	0.146 (ns)
Smoker	26% (n=316)	37% (n=111)	0.038
Hypertension	61% (n=316)	51% (n=111)	0.093 (ns)
Hypercholesterolemia	62% (n=316)	53% (n=111)	0.115 (ns)
Diabetes	28% (n=313)	14% (n=111)	0.005
Family History	44% (n=313)	31% (n=51)	0.014

Table 4.

Multivariable Logistic Regression Model
(outcome modeled: Non-Cardiac Symptoms)

Predictor	Adjusted Odds Ratio	95% Confidence Interval
Closure Time	1.17	1.06 to 1.29
TIMI Risk Score	0.48	0.40 to 0.59
Study Site (UMASS *versus* Cordoba)	7.21	4.05 to 12.86

Table 5.

Effect of Definition of 'Prolonged' Closure Time on Specificity and Positive Predictive Value for a Diagnosis of Non-Cardiac Symptoms

Threshold	Specificity	Positive Predictive Value	% of Patients with Non-Cardiac Symptoms Identified
95th percentile (≥ 160 seconds)	100% [96.6% to 100%]	100% [83.9% to 100%]	6.4% (21/330)
90th percentile (≥ 138 seconds)	98.1% [93.3% to 99.8%]	95.4% [84.2% to 99.4%]	12.4% (41/330)
80th percentile (≥ 117 seconds)	88.6% [80.9% to 94.0%]	86.2% [77.2% to 92.7%]	22.7% (75/330)

95% confidence intervals shown in square brackets

Figure 1.

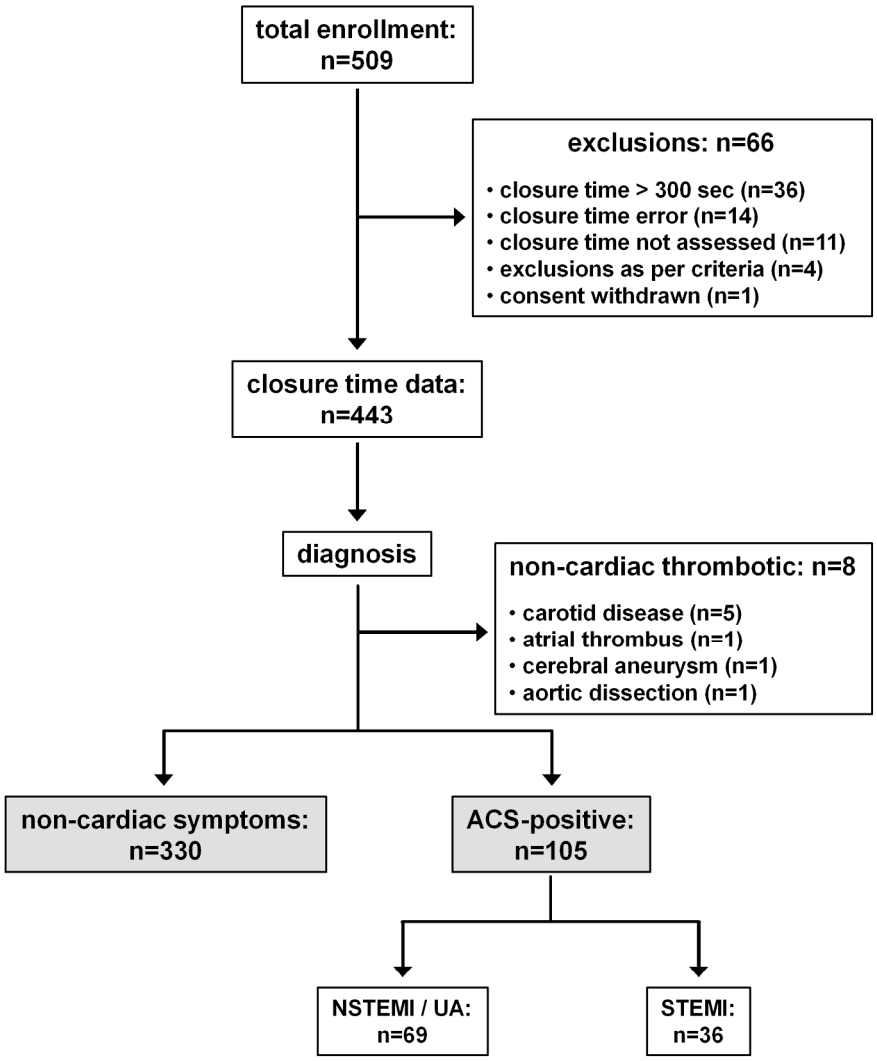

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Figure 2.

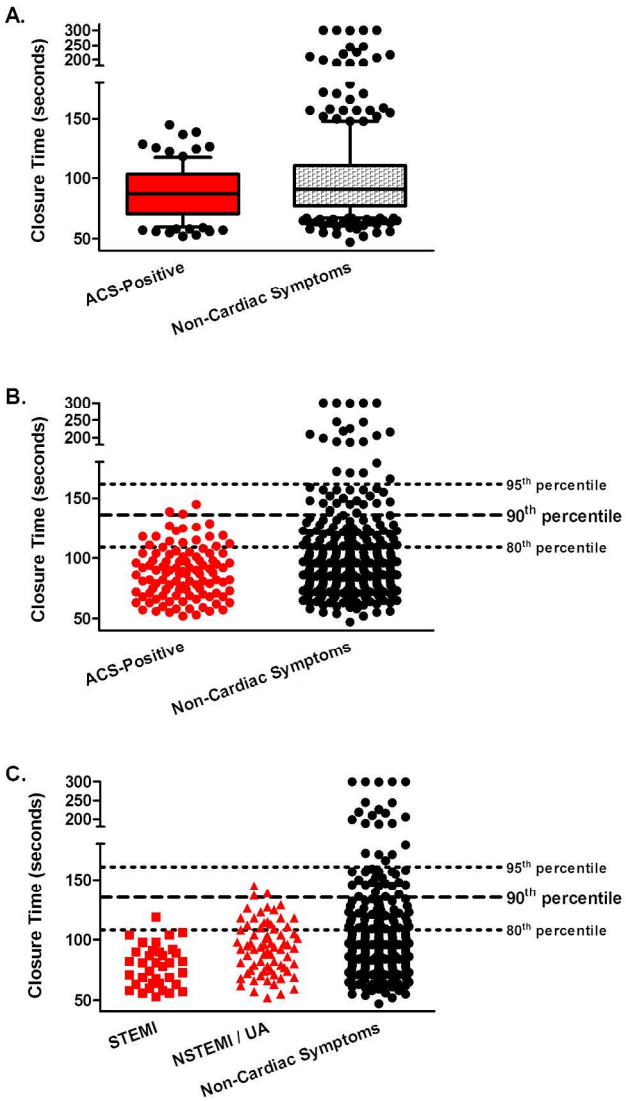

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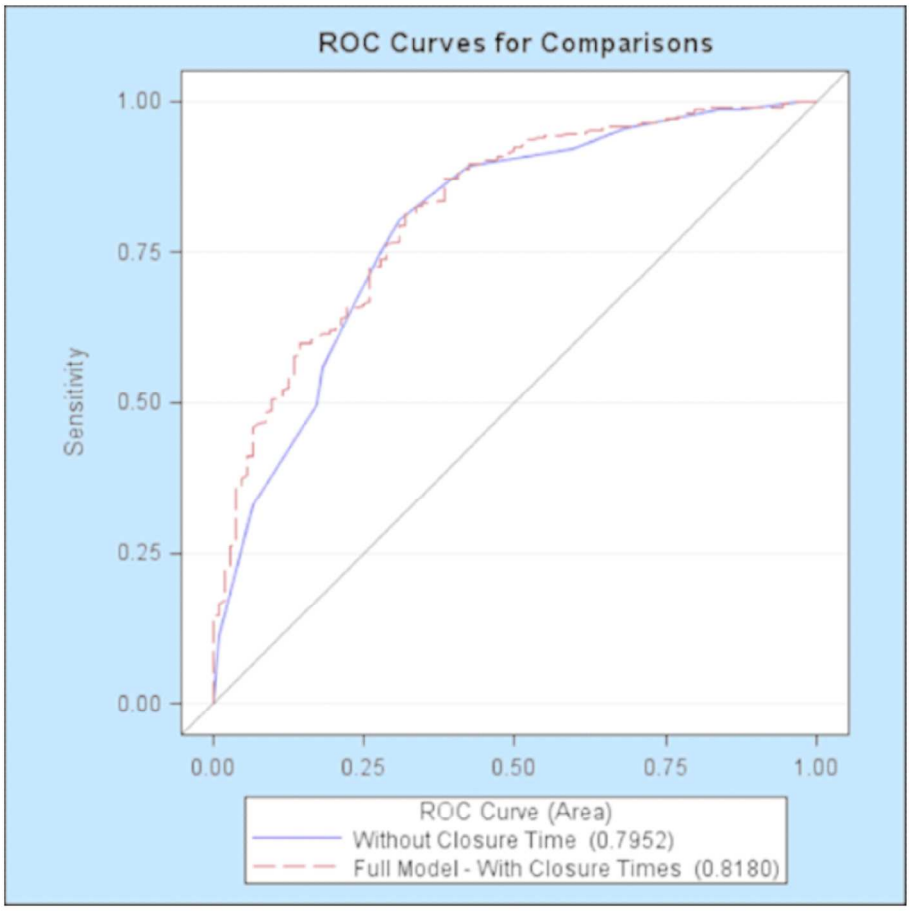
Figure 3.



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