Heart Rate n-Variability (HRnV) and Its Application to Risk Stratification of Chest Pain Patients in the Emergency Department

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Abstract

Background: Chest pain is one of the most common complaints among patients presenting to the emergency department (ED). Causes of chest pain can be benign or life threatening, making its accurate risk stratification a critical issue in the ED. In addition to the use of established clinical scores, some studies attempted to create predictive models with heart rate variability (HRV). In this study, we proposed heart rate n-variability (HRnV), an alternative representation of beat-to-beat-variation in electrocardiogram (ECG) and investigated its association with major adverse cardiac events (MACE) for ED patients with chest pain.

Methods: We conducted a retrospective analysis of data collected from the ED of a Singapore tertiary hospital between September 2010 and July 2015. Patients >20 years old who presented to the ED with chief complaint of chest pain were conveniently recruited. Five to six-minute single-lead (lead II) ECGs, demographics, medical history, troponin, and other required variables were collected. We developed the HRnV-Calc software to calculate the HRnV parameters. The primary outcome was 30-day MACE, including all-cause death, acute myocardial infarction, and revascularization. Univariable and multivariable logistic regression analyses were conducted to investigate individual risk factors, and to develop a HRnV prediction model, respectively. The receiver operating characteristic (ROC) analysis was performed to compare the HRnV model against other clinical scores in predicting 30-day MACE.

Results: A total of 795 patients were included in the analysis, of which 247 (31%) had MACE within 30 days. The MACE group was older and had a higher proportion of male patients. Twenty-one conventional HRV and 115 HRnV parameters were calculated. In univariable analysis, eleven HRV parameters and 48 HRnV parameters were significantly associated with 30-day MACE. The stepwise logistic regression selected 16 predictors to construct a multivariable prediction model, which consisted of one HRV, seven HRnV

parameters, troponin, ST segment changes, and several other factors. The HRnV model outperformed several clinical scores in the ROC analysis (area under the ROC curve of 0.917).

Conclusions: The novel HRnV representation demonstrated its value of augmenting HRV and traditional risk factors in designing a robust risk stratification tool for patients with chest pain at the ED.

Keywords: Heart rate variability (HRV), heart rate n-variability (HRnV), electrocardiogram, chest pain, risk stratification, emergency department.

Introduction

Chest pain is one of the most common presenting complaints in the emergency department (ED)[1, 2], which may be due to life-threatening myocardial infarction (MI) or benign musculoskeletal pain[3]. Majority of chest pain patients are subjected to extensive diagnostic tests to rule out acute coronary syndrome (ACS), resulting in oftentimes, unnecessary prolonged and costly ED admission, since only a small proportion of these patients will eventually receive a diagnosis of ACS[3]. Hence, early identification of chest pain patients at high-risk of developing adverse cardiac events has been a pressing issue to contend with in the ED. Several established clinical scores have been used for risk stratifying chest pain patients in the ED[4, 5], including the History, ECG, Age, Risk factors and Troponin (HEART)[6], the Thrombolysis in Myocardial Infarction (TIMI)[7], and the Global Registry of Acute Coronary Events (GRACE)[8] scores. Among these scores, the HEART score is the best performing one with its ability to identify low and high risk patients with only a small

percentage of mis-classification[5, 9-12]. Further research included the development of risk score-based clinical pathways for safe discharge of low-risk patients[1, 3, 13].

Reported in a recent review of clinical scores for ED patients with chest pain[5], heart rate variability (HRV) demonstrated its feasibility of creating an alternative approach to build predictive models for risk stratification[14-16]. As a widely adopted tool for evaluating changes in cardiac autonomic regulation, HRV is believed to be strongly associated with the autonomic nervous system (ANS)[17-19]. HRV analysis characterizes the beat-to-beat variation in an electrocardiogram (ECG) by utilizing time domain, frequency domain, and nonlinear analyses[18]. Reduced HRV was found to be a significant predictor of adverse outcomes[20], although the impact of the ANS on HRV remains controversial[19]. Given the complexity of quantifying HRV representation, several tools such as the PhysioNet Cardiovascular Signal Toolbox[21] and Kubios HRV[22] have been developed to standardize HRV analyses.

Based on the principle of parameter calculation on normal R-R intervals (RRIs; in this paper, RRIs are equivalent to normal-to-normal [NN] intervals, in which abnormal beats have been removed), HRV analysis generates only one set of parameters from a fixed length of ECG record. This limits the amount of information that can be extracted from raw ECG signals. In this paper, we proposed a novel representation of beat-to-beat variation, named as heart rate n-variability (HRnV) to characterize RRIs from a different perspective. With the use of HRnV measures, multiple sets of parameters were calculated from the same ECG record, which greatly boosted the amount of extracted information. Our study was the first clinical application of the HRnV representation, in which the value of this novel measure was evaluated in risk stratification of chest pain patients in the ED. With the hypothesis that HRnV is closely related to conventional HRV while providing supplementary information,

we aimed to explore its association with adverse cardiac complications, and to investigate the potential use of HRnV to develop an effective risk prediction tool.

Methods

Study Design and Setting

We conducted a retrospective analysis of data collected in our previous study on risk stratification of chest pain patients in the ED[9]. A convenience sample of patients was recruited at the ED of Singapore General Hospital between September 2010 and July 2015. This was a tertiary hospital with around-the-clock primary percutaneous coronary intervention capabilities and a median door-to-balloon time of 101 minutes[23]. Patients >20 years old who presented to the ED with chief complaint of chest pain were included in the study. Patients were excluded if they had ST-elevation myocardial infarction (STEMI) or an obvious non-cardiac etiology of chest pain diagnosed by the primary emergency physician. Patients were also excluded if their ECGs had high percentage of noise or they were in non-sinus rhythm; these criteria were applied to ensure the quality of HRV and HRnV analyses. The ethical approval was obtained from the Centralized Institutional Review Board (CIRB, Ref: 2014/584/C) of SingHealth, the largest public healthcare system in Singapore that includes the Singapore General Hospital as a key partner. In the ethical approval, patient consent was waived.

Data Collection

During the data collection period, five to six-minute single-lead (lead II) ECG tracings were retrieved from the X-Series Monitor (ZOLL Medical Corporation, Chelmsford, MA). The first set of vital signs and troponin values from the recruited patients were extracted from the

hospital's electronic health records (EHR). In this study, the cardiac troponin-T was used, and an abnormal value was defined as greater than the 99th percentile for the assay (0.03 ng/mL); it was further coded as 0 if the value was <99th percentile, 1 if the value was between 1 and 3 times 99th percentile, and 2 if the value was >3 times the 99th percentile. Additionally, the patients' first 12-lead ECG records were interpreted by two independent clinical reviewers; ST-elevation, ST-depression, T-wave inversions, and Q-waves were recorded. In addition, patient demographics, medical history, and information required for computing the HEART, TIMI, and GRACE scores were retrospectively reviewed and obtained from the hospital's EHR.

Proposed HRnV Representation of Beat-to-Beat Variation in ECG

<u>HR$_n$V: A Novel Measure with Non-Overlapped RRIs</u>

Prior to introducing the new HR$_n$V measure, we define a new type of RRI called RR$_n$I, where $1 \leq n \leq N$, and $N \ll \widehat{N}$. $\widehat{N}$ is the total number of RRIs. The definition of RR$_n$I is illustrated in Figure 1a. When $n = 1$, RR$_n$I becomes conventional RRI, that is, RR$_1$I is equal to RRI. When $n > 1$, every n adjacent RRI is connected to form a new sequence of RR$_n$Is. By using this strategy, we can create a maximum number of $(N - 1)$ new RR$_n$I sequences from conventional single RRI sequence. With these newly generated RR$_n$I sequences, the calculation of HR$_n$V parameters is straightforward and can be accomplished by applying established quantitative methods including time domain analysis, frequency domain analysis, and nonlinear analysis[17, 18]. In describing this new measure, we use the term "HR$_n$V" prior to parameter names to indicate that these parameters are calculated from RR$_n$I sequences. As noted in the above, HR$_n$V is a novel measure based on newly generated, non-overlapped RR$_n$Is. The computed HR$_n$V parameters include but are not limited to the following: the

average of RR_nIs (HR_nV mean NN), standard deviation of RR_nIs (HR_nV SDNN), square root of the mean squared differences between RR_nIs (HR_nV RMSSD), the number of times that the absolute difference between two successive RR_nIs exceeds 50 ms (HR_nV NN50), HR_nV NN50 divided by the total number of RR_nIs (HR_nV pNN50), the integral of the RR_nI histogram divided by the height of the histogram (HR_nV triangular index), low frequency power (HR_nV LF power), high frequency power (HR_nV HF power), approximate entropy (HR_nV ApEn), sample entropy (HR_nV SampEn), and detrended fluctuation analysis (HR_nV DFA), among others. Notably, two new parameters NN50n and pNN50n are created, where 50×n ms is set as the threshold to assess the difference between pairs of consecutive RR_nIs.

<u>HR_nV_m: A Novel Measure with Overlapped RRIs</u>

Like RR_nI that is used in HR_nV, to define the HR_nV_m measure we introduce another type of RRI called RR_nI_m, where $1 \leq n \leq N$, $1 \leq m \leq N-1$, and $N \ll \widehat{N}$. In the RR_nI_m sequence, m is used to indicate the level of overlap between consecutive RR_nI_m sequences. As illustrated in Figure 1b, $(n-m)$ RRIs form the overlapped portions. When $m = n$, RR_nI_m becomes RR_nI; therefore, the upper limit of m is $N-1$. By controlling the overlap among these newly generated RR_nI_m sequences, we can create a maximum number of $(N \times (N-1)/2)$ RR_nI_m sequences (excluding the RR_nI sequence) from conventional single RRI sequence. For each of the newly created RR_nI_m sequences, we apply time domain analysis, frequency domain analysis, and nonlinear analysis to calculate HR_nV_m parameters. We add the term "HR_nV_m" prior to the parameters to denote that they are computed from RR_nI_m sequences. For example, the average RR_nI_m intervals and the sample entropy are written as HR_nV_m mean NN and HR_nV_m SampEn, respectively. The HR_nV_m measure extracts additional information than HR_nV does, by adopting a strategy of controlling sequence overlap.

HRnV Analysis and Parameter Calculation

We developed the HRnV-Calc software suite (https://github.com/HRnV) to calculate HRnV parameters. The HRnV-Calc software integrates functions from the PhysioNet Cardiovascular Signal Toolbox[21] to perform standardized ECG signal processing and QRS complex detection. Given the short ECG records in this study, the upper limit of n was set as three; thus, six sets of parameters were calculated, namely HRV, HR_2V, HR_2V_1, HR_3V, HR_3V_1, and HR_3V_2.

Clinical Outcomes

The primary endpoint in this study was a composite outcome of major adverse cardiac events (MACE)[24], including all-cause death, acute myocardial infarction (AMI), and revascularization (coronary artery bypass graft [CABG] and percutaneous coronary intervention [PCI]) within 30 days of ED presentation.

Statistical Analysis

Continuous variables were presented in terms of mean and standard deviation, and also compared between the two categories of the primary outcome (MACE) using two-sample t-test. Categorical variables were presented in terms of frequency and percentage, and also compared between the two categories of the primary outcome (MACE) using chi-square test. A statistically significant difference was defined as $p<0.05$. To evaluate the HRnV parameters and other risk factors, we conducted univariable and multivariable analyses and subsequently developed a simple prediction model using traditional logistic regression analysis. In building the HRnV prediction model, we selected candidate variables with $p<0.2$ in the univariable analysis and fed them into a multivariable stepwise logistic regression model.

The receiver operating characteristic (ROC) analysis[25] was performed to compare prediction performances among the HRnV model, HEART, TIMI and GRACE scores. The area under the ROC curve (AUC), sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were reported as the predictive measures. Data preparation, descriptive analysis, and predictive model development were performed in R version 3.6.0 (R Foundation, Vienna, Austria), and the ROC analysis was conducted in MATLAB R2019a (MathWorks, Natick, MA).

Results

A total of 795 patients were selected from the originally recruited 922 patients[9], in which 28 were excluded for ECG recording issues, four were excluded for clearly non-cardiac chest pain, and 95 were excluded for irregular rhythm/artifacts. Among the included 795 patients, 247 (31%) met the primary outcome, i.e., 30-day MACE. Table 1 shows patient baseline characteristics. MACE group was older (mean age 61.1 years vs. 59.0 years, p=0.035) and had a higher proportion of male patients (76.1% vs. 64.6%, p=0.002). There were no statistical differences between MACE and non-MACE groups in terms of patient ethnicity. Factors such as history of diabetes and current smoking status showed statistical differences between the two outcome groups.

Descriptive analyses of HRV and HRnV parameters are tabulated in Table 2. In this clinical case study, N was set as 3, thus HR_2V, HR_2V_1, HR_2V, HR_3V_1 and HR_3V_2 parameters were calculated. Among time domain parameters such as mean NN, SDNN and RMSSD, the HR_nV and HR_nV_m values were generally incremental with an increase in n. Notably, HR_2V

NN50 and HR_3V NN50 were much lower than conventional HRV NN50. Moreover, NN50*n* and pNN50*n* are the parameters specifically applicable to the HRnV representation. Like time domain parameters, the same trend of changes in frequency domain parameters were observed. One exception was the normalized HF power, whose HR_nV and HR_nV_m parameters were smaller than that of HRV. In nonlinear analysis, the differences in Poincaré SD2 values were obvious between HRV and HRnV parameters. HR_2V SampEn and HR_3V SampEn were remarkably larger compared to SampEn parameters of HRV, HR_2V_1, HR_3V_1, and HR_3V_2, as the confidence interval of SampEn was wide when data points were less than 200[18], since our ECG recordings were only five to six-minute long. HR_2V_1, HR_3V_1 and HR_3V_2 were free from this issue as they were calculated from overlapping RR_nI_m sequences where more than 200 data points were available.

Tables 3 and 4 present the results of univariable analyses of HR_nV and HR_nV_m parameters, respectively. Eleven out of 21 conventional HRV parameters were statistically significant. Additionally, 13 HR_2V, six HR_3V, 11 HR_2V_1, seven HR_3V_1 and 11 HR_3V_2 parameters were also significant. Overall, additional 115 HRnV parameters were derived, among which 48 showed statistical significance between patients who had 30-day MACE and who did not. Among all HRV and HRnV parameters, mean NN, SDNN, RMSSD, NN50, pNN50, HF power, Poincaré SD1 and SD2 appeared statistically significant in at least five out of six measures (i.e., HRV, HR_2V, HR_2V_1, HR_3V, HR_3V_1, and HR_3V_2). Furthermore, skewness, LF power, SampEn, and ApEn that were not significant in conventional HRV analysis became statistically significant in HRnV representation.

Table 5 lists the 16 variables that were selected through stepwise logistic regression to build the prediction model for 30-day MACE. Among several statistically significant predictors, in

addition to traditional biomarkers such as ST segment changes and troponin, HR_2V ApEn (OR=0.095; 95% CI 0.014-0.628), HR_2V_1 ApEn (OR=19.700; 95% CI 2.942-131.900) and HR_3V skewness (1.560; 95% CI 1.116-2.181) also demonstrated strong predictive power in assessing the risk of 30-day MACE for chest pain patients in the ED. In the final prediction model, one HRV parameters and seven HRnV parameters were chosen as independent predictors. Table 6 presents the results of ROC analysis in evaluating the predictive performances by the HRnV model, HEART, TIMI, and GRACE scores. Our HRnV model achieved the highest AUC value and outperformed HEART, TIMI, and GRACE scores in terms of sensitivity, specificity, PPV, and NPV at the optimal cutoff scores, defined as the points nearest to the upper-left corner of the ROC curves.

Discussion

HRV has been well investigated in the past decades[17, 18, 26]. While the majority of efforts in HRV research were focused on development of advanced nonlinear techniques to derive novel parameters[27, 28], few investigated alternative approaches to analyze RRIs. Vollmer[29] used relative RRIs to describe the relative variation of consecutive RRIs, with which HRV parameters were calculated. Likewise, we proposed a novel HRnV representation, providing additional HRnV parameters than conventional HRV analysis. In this paper, we introduced two measures of HRnV, namely HR_nV and HR_nV_m. HR_nV is calculated based on non-overlapped RR_nI sequences, while HR_nV_m is computed from overlapped RR_nI_m sequences. HRnV is not developed to replace the conventional HRV; instead, this representation is a natural extension of HRV. It enables us to create additional parameters from raw ECGs, and thus empowers the extraction of supplementary information.

In our clinical study, we investigated the predictive values of HRnV parameters to assess the risk of 30-day MACE outcome for chest pain patients in the ED. In addition to 21 HRV parameters, 115 HRnV parameters were derived, of which 48 were found to be statistically significant in their associations with the outcome. Notably, even with a small n (three in our study), newly generated HRnV parameters have greatly boosted the number of candidate predictors. When longer ECG records are available, more HRnV parameters can be calculated. We also built a HRnV model with predictors such as HRnV parameters, HRV parameters, vital signs, and several established risk factors. The final HRnV model consisted of age, diastolic BP, pain score, ST-elevation, ST-depression, Q wave, cardiac history, troponin, one conventional HRV parameters and seven HRnV parameters. In addition to traditional risk factors like ST segment changes, HR_2V ApEn, HR_2V_1 ApEn, and HR_3V skewness were found as strong predictors for 30-day MACE. Comparing with the HEART, TIMI, and GRACE scores, the HRnV model presented superior discrimination performance in achieving the highest AUC, sensitivity, specificity, PPV, and NPV values. This study demonstrated a proof of concept that HRnV was clinically useful in determining the risk of 30-day MACE for ED patients with chest pain.

Due to the wide differential diagnosis for chest pain, accurate stratification of chest pain patients is vital, particularly for ruling-out low-risk patients to avert high medical expenses[3]. The TIMI and GRACE scores have been validated for risk prediction of patients with chest pain in the ED[4, 30, 31], although they were not originally developed for this cohort of patients. They have been reported to be flawed and inappropriate in their applications to undifferentiated chest pain cohorts in the ED[1]. In comparison, the HEART score was derived from a group of ED patients with chest pain, and has been extensively validated worldwide[10, 13, 24, 32]. It has proven its applicability in identifying both low-risk patients for possible early

discharge and high-risk patients for urgent intervention. Built upon established scores, many chest pain pathways[33-36] have been implemented and tested, particularly for the management of low-risk patients. Than et al.[36] evaluated a TIMI score-based accelerated diagnostic protocol (ADP) and reported a sensitivity of 99.3% and NPV of 99.1%. Similarly, reported in a systematic review by Laureano-Phillips et al.[37], the HEART score achieved both sensitivity and NPV of 100% in several validation studies. Furthermore, a cost-effectiveness study conducted in Brisbane, Australia reported economic benefits by adopting an ADP in the ED, with its associated reduction in expected cost and length of stay amongst patients with chest pain[38].

Most established clinical scores use conventional risk factors such as biomarkers, medical history, and presenting vital signs. Many factors are either subjective such as history of cardiac conditions or require significant amount of time to obtain, e.g., troponin. HRV, as a noninvasive measure, can be easily calculated from ECGs; it is an objective tool to assess the activities of the ANS[18]. It also has the advantage of requiring only five minutes to acquire (in our protocol), which is much faster than serum biomarkers. Over the past decades, HRV has been widely investigated in a broad range of clinical applications, particularly in cardiovascular research. HRV was found to be closely associated with sudden cardiac death[17]. It also showed significant correlations with clinical outcomes in prehospital setting[39] and with MACE outcomes in ED patients with chest pain[16]. HRV parameters have been integrated with other risk factors into machine learning algorithms to predict adverse outcomes[40, 41]. These promising results motivated the use of HRV to develop objective and computerized risk stratification tools for chest pain patients[42, 43]. In an updated review of clinical scores for chest pain, Liu et al.[5] summarized several studies which aimed to develop alternative techniques for risk stratification.

This study was the first clinical validation of the HRnV representation and its measures. Although the HRnV parameters showed promising performance in identifying high-risk chest pain patients, this study was not intended to create a ready-to-use clinical tool. Instead, we have demonstrated the feasibility of utilizing HRnV parameters to augment conventional HRV and risk factors in designing a powerful prediction tool/score. These HRnV parameters can be readily calculated without the collection of supplementary data. In this study, with five to six-minute ECG and $n = 3$, five-fold more HRnV parameters were calculated compared to HRV. When longer ECGs are available and parameter n is set as a larger number, more HRnV parameters can be derived. To build a HRnV-based risk stratification tool, a systematic approach is needed to derive a point-based, consistent score to ease its clinical application and practical implementation.

As a natural extension of conventional HRV, HRnV representation creates the opportunity to generate additional parameters. This representation could also serve as a signal smoother for RRIs, by filtering out unexpected spikes and sudden changes that are not due to abnormal heart beats. However, since HRnV is a novel representation of beat-to-beat variations in ECG, many technical issues need to be resolved in future research. For instance, as shown in Table 2, SampEn became larger when the available number of data points was less than 200[18], suggesting that more research is required to investigate its applicability on short ECG records. Moreover, parameters NN50*n* and pNN50*n* are newly introduced in HRnV representation only. They characterize the number of times that the absolute difference between two successive RR_nI sequences exceeds 50×*n* ms, by assuming that the absolute difference may be magnified when the corresponding RR_nI is n times longer than RRI. Thus, in-depth investigations and more evidence are needed in the selection of appropriate thresholds. More

importantly, physiological interpretations of the HRnV parameters and their norms are needed[26].

Beyond its use in risk stratification of ED patients with chest pain, HRnV foresees potentials in many clinical domains where conventional HRV has been extensively investigated[44-47]. With the augmented RR_nI and RR_nI_m sequences, HRnV could possibly capture more dynamic changes in cardiac rhythms than HRV. This capability enables the extraction of extra information from limited raw data, i.e., ECGs. This study utilized HRnV parameters as independent risk factors and analyzed them with traditional biostatistical methods. There are multiple ways to use HRnV parameters, e.g., each set of HRnV parameters can be analyzed individually and are subsequently combined with an ensemble learning[48] (a special type of machine learning algorithm[49]) architecture to reach a final decision. However, artificial intelligence and machine learning methods generally create black-box predictive models, making interpretation a challenge[50].

Limitations

This study has several limitations. First, the primary aim of this study was to demonstrate the feasibility of using HRnV parameters and common risk factors to build a prediction model for stratification of patients with chest pain in the ED. Therefore, no scoring tool was developed for practical clinical use. Second, the HRnV model built in this study was not validated on a separate patient cohort, which could have inflated the model's predictive performance. When a HRnV-based scoring tool is ready, it is necessary to conduct external validations on cohorts with diverse patient characteristics. Furthermore, properly designed clinical pathways are needed as well. Third, the patients included in this study were mainly from the high acuity group, resulting in a higher 30-day MACE rate (i.e., 31%) compared to

other similar studies[10, 37]. As a result, the generalizability of the HRnV model developed in this study may be uncertain in other patient cohorts. Fourth, the calculated HRnV and HRV parameters depended on the choice of tools and methods for ECG signal analysis. Thus, the values of these parameters may vary across studies. Lastly, the physiological interpretations of HRnV parameters are mostly unknown, which require future collaborative efforts between clinicians and scientists to address.

Conclusions

In this study, we proposed a novel HRnV representation and investigated the use of HRnV and established risk factors to develop a predictive model for risk stratification of patients with chest pain in the ED. Multiple HRnV parameters were found as statistically significant predictors, which effectively augmented conventional HRV, vital signs, troponin, and cardiac risk factors in building an effective model with good discrimination performance. The HRnV model outperformed the HEART, TIMI, and GRACE scores in the ROC analysis; it also demonstrated its capability in identifying low-risk patients, which can facilitate possible early discharge. Moving forward, we suggest further development of a point-based, ready-to-use HRnV risk stratification tool, and its subsequent external validations. Although some issues remain to be addressed, we hope to stimulate a new stream of research on HRnV. We believe that future endeavors in this field will lead to the possibility of in-depth evaluation of the associations between HRnV measures and various human diseases.

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Author Contributions

NL invented the HRnV representation, conceived the study, supervised the project, and wrote the first draft of the manuscript. NL, DG, ZXK, and FX performed the analyses. All authors contributed to evaluation of the HRnV measures, interpretation of the results, and revision of the manuscript.

References

1. Long B and Koyfman A. Best Clinical Practice: Current Controversies in the Evaluation of Low-Risk Chest Pain with Risk Stratification Aids. Part 2. *The Journal of emergency medicine*. 2017;52:43-51.

2. Long B and Koyfman A. Best Clinical Practice: Current Controversies in Evaluation of Low-Risk Chest Pain-Part 1. *The Journal of emergency medicine*. 2016;51:668-676.

3. Hollander JE, Than M and Mueller C. State-of-the-Art Evaluation of Emergency Department Patients Presenting With Potential Acute Coronary Syndromes. *Circulation*. 2016;134:547-64.

4. Backus BE, Six AJ, Kelder JH, Gibler WB, Moll FL and Doevendans PA. Risk scores for patients with chest pain: evaluation in the emergency department. *Curr Cardiol Rev*. 2011;7:2-8.

5. Liu N, Ng JCJ, Ting CE, Sakamoto JT, Ho AFW, Koh ZX, Pek PP, Lim SH and Ong MEH. Clinical scores for risk stratification of chest pain patients in the emergency department: an updated systematic review. *Journal of Emergency and Critical Care Medicine*. 2018;2.

6. Six AJ, Cullen L, Backus BE, Greenslade J, Parsonage W, Aldous S, Doevendans PA and Than M. The HEART score for the assessment of patients with chest pain in the emergency department: a multinational validation study. *Crit Pathw Cardiol*. 2013;12:121-6.

7. Antman EM, Cohen M, Bernink PJ, McCabe CH, Horacek T, Papuchis G, Mautner B, Corbalan R, Radley D and Braunwald E. The TIMI risk score for unstable angina/non-ST elevation MI: A method for prognostication and therapeutic decision making. *Jama*. 2000;284:835-42.

8. Granger CB, Goldberg RJ, Dabbous O, Pieper KS, Eagle KA, Cannon CP, Van De Werf F, Avezum A, Goodman SG, Flather MD, Fox KA and Global Registry of Acute Coronary Events I. Predictors of hospital mortality in the global registry of acute coronary events. *Arch Intern Med*. 2003;163:2345-53.

9. Sakamoto JT, Liu N, Koh ZX, Guo D, Heldeweg MLA, Ng JCJ and Ong MEH. Integrating heart rate variability, vital signs, electrocardiogram, and troponin to triage chest pain patients in the ED. *Am J Emerg Med*. 2017.

10. Poldervaart JM, Langedijk M, Backus BE, Dekker IMC, Six AJ, Doevendans PA, Hoes AW and Reitsma JB. Comparison of the GRACE, HEART and TIMI score to predict major adverse cardiac events in chest pain patients at the emergency department. *International journal of cardiology*. 2017;227:656-661.

11. Fernando SM, Tran A, Cheng W, Rochwerg B, Taljaard M, Thiruganasambandamoorthy V, Kyeremanteng K and Perry JJ. Prognostic Accuracy of the HEART Score for Prediction of Major Adverse Cardiac Events in Patients Presenting With Chest Pain: A Systematic Review and Meta-analysis. *Academic emergency medicine : official journal of the Society for Academic Emergency Medicine*. 2019;26:140-151.

12. Reaney PDW, Elliott HI, Noman A and Cooper JG. Risk stratifying chest pain patients in the emergency department using HEART, GRACE and TIMI scores, with a single contemporary troponin result, to predict major adverse cardiac events. *Emergency medicine journal : EMJ*. 2018;35:420-427.

13. Van Den Berg P and Body R. The HEART score for early rule out of acute coronary syndromes in the emergency department: a systematic review and meta-analysis. *European heart journal Acute cardiovascular care*. 2018;7:111-119.

14. Liu N, Lin Z, Cao J, Koh ZX, Zhang T, Huang G-B, Ser W and Ong MEH. An intelligent scoring system and its application to cardiac arrest prediction. *IEEE Trans Inf Technol Biomed*. 2012;16:1324-1331.

15. Liu N, Koh ZX, Chua ECP, Tan LML, Lin Z, Mirza B and Ong MEH. Risk scoring for prediction of acute cardiac complications from imbalanced clinical data. *IEEE J Biomed Health Inform*. 2014;18:1894-1902.

16. Ong MEH, Goh K, Fook-Chong S, Haaland B, Wai KL, Koh ZX, Shahidah N and Lin Z. Heart rate variability risk score for prediction of acute cardiac complications in ED patients with chest pain. *Am J Emerg Med*. 2013;31:1201-1207.

17. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Heart rate variability: standards of measurement, physiological interpretation and clinical use. *Circulation*. 1996;93:1043-65.

18. Rajendra Acharya U, Paul Joseph K, Kannathal N, Lim CM and Suri JS. Heart rate variability: a review. *Med Biol Eng Comput*. 2006;44:1031-51.

19. Billman GE. Heart rate variability - a historical perspective. *Front Physiol*. 2011;2:86.

20. Carpeggiani C, L'Abbate A, Landi P, Michelassi C, Raciti M, Macerata A and Emdin M. Early assessment of heart rate variability is predictive of in-hospital death and major complications after acute myocardial infarction. *International journal of cardiology*. 2004;96:361-8.

21. Vest AN, Da Poian G, Li Q, Liu C, Nemati S, Shah AJ and Clifford GD. An open source benchmarked toolbox for cardiovascular waveform and interval analysis. *Physiological measurement*. 2018;39:105004.

22. Tarvainen MP, Niskanen JP, Lipponen JA, Ranta-Aho PO and Karjalainen PA. Kubios HRV–heart rate variability analysis software. *Comput Methods Programs Biomed*. 2014;113.

23. Ho AF, Fook-Chong S, Pek PP, Ng YY, Wong AS and Ong ME. Prehospital presentation of patients with ST-segment elevation myocardial infarction in Singapore. *International journal of cardiology*. 2013;168:4273-6.

24. Sakamoto JT, Liu N, Koh ZX, Fung NX, Heldeweg ML, Ng JC and Ong ME. Comparing HEART, TIMI, and GRACE scores for prediction of 30-day major adverse cardiac events in high acuity chest pain patients in the emergency department. *International journal of cardiology*. 2016;221:759-64.

25. Fawcett T. An introduction to ROC analysis. *Pattern Recogn Lett*. 2006;27:861-874.

26. Shaffer F and Ginsberg JP. An Overview of Heart Rate Variability Metrics and Norms. *Frontiers in public health*. 2017;5:258.

27. Peng CK, Havlin S, Stanley HE and Goldberger AL. Quantification of scaling exponents and crossover phenomena in nonstationary heartbeat time series. *Chaos*. 1995;5:82-7.

28. Barbieri R, Matten EC, Alabi AA and Brown EN. A point-process model of human heartbeat intervals: new definitions of heart rate and heart rate variability. *American journal of physiology Heart and circulatory physiology*. 2005;288:H424-35.

29. Vollmer M. A robust, simple and reliable measure of heart rate variability using relative RR intervals. *Computing in Cardiology Conference (CinC)*. 2015:609-612.

30. Chase M, Robey JL, Zogby KE, Sease KL, Shofer FS and Hollander JE. Prospective validation of the Thrombolysis in Myocardial Infarction Risk Score in the emergency department chest pain population. *Annals of emergency medicine*. 2006;48:252-9.

31. Pollack CV, Sites FD, Shofer FS, Sease KL and Hollander JE. Application of the TIMI risk score for unstable angina and non-ST elevation acute coronary syndrome to an unselected emergency department chest pain population. *Academic emergency medicine : official journal of the Society for Academic Emergency Medicine*. 2006;13:13-18.

32. Marcoon S, Chang AM, Lee B, Salhi R and Hollander JE. HEART score to further risk stratify patients with low TIMI scores. *Crit Pathw Cardiol*. 2013;12:1-5.

33. Long B, Oliver J, Streitz M and Koyfman A. An end-user's guide to the HEART score and pathway. *Am J Emerg Med*. 2017;35:1350-1355.

34. Mahler SA, Riley RF, Hiestand BC, Russell GB, Hoekstra JW, Lefebvre CW, Nicks BA, Cline DM, Askew KL, Elliott SB, Herrington DM, Burke GL and Miller CD. The HEART Pathway randomized trial: identifying emergency department patients with acute chest pain for early discharge. *Circ Cardiovasc Qual Outcomes*. 2015;8:195-203.

35. Than MP, Pickering JW, Aldous SJ, Cullen L, Frampton CM, Peacock WF, Jaffe AS, Goodacre SW, Richards AM, Ardagh MW, Deely JM, Florkowski CM, George P, Hamilton GJ, Jardine DL, Troughton RW, van Wyk P, Young JM, Bannister L and Lord SJ. Effectiveness of EDACS Versus ADAPT Accelerated Diagnostic Pathways for Chest Pain: A Pragmatic Randomized Controlled Trial Embedded Within Practice. *Annals of emergency medicine*. 2016;68:93-102 e1.

36. Than M, Cullen L, Reid CM, Lim SH, Aldous S, Ardagh MW, Peacock WF, Parsonage WA, Ho HF, Ko HF, Kasliwal RR, Bansal M, Soerianata S, Hu D, Ding R, Hua Q, Seok-Min K, Sritara P, Sae-Lee R, Chiu TF, Tsai KC, Chu FY, Chen WK, Chang WH, Flaws DF, George PM and Richards AM. A 2-h diagnostic protocol to assess patients with chest pain symptoms in the Asia-Pacific region (ASPECT): a prospective observational validation study. *Lancet (London, England)*. 2011;377:1077-84.

37. Laureano-Phillips J, Robinson RD, Aryal S, Blair S, Wilson D, Boyd K, Schrader CD, Zenarosa NR and Wang H. HEART Score Risk Stratification of Low-Risk Chest Pain Patients in the Emergency Department: A Systematic Review and Meta-Analysis. *Annals of emergency medicine*. 2019;74:187-203.

38. Cheng Q, Greenslade JH, Parsonage WA, Barnett AG, Merollini K, Graves N, Peacock WF and Cullen L. Change to costs and lengths of stay in the emergency department and the Brisbane protocol: an observational study. *BMJ Open*. 2016;6:e009746.

39. Ong ME, Padmanabhan P, Chan YH, Lin Z, Overton J, Ward KR and Fei DY. An observational, prospective study exploring the use of heart rate variability as a predictor of clinical outcomes in pre-hospital ambulance patients. *Resuscitation*. 2008;78:289-97.

40. Liu N, Zhang Z, Ho AFW and Ong MEH. Artificial intelligence in emergency medicine. *Journal of Emergency and Critical Care Medicine*. 2018;2:82.

41. Chiew CJ, Liu N, Tagami T, Wong TH, Koh ZX and Ong MEH. Heart rate variability based machine learning models for risk prediction of suspected sepsis patients in the emergency department. *Medicine*. 2019;98:e14197.

42. Liu N, Sakamoto JT, Cao J, Koh ZX, Ho AFW, Lin Z and Ong MEH. Ensemble-Based Risk Scoring with Extreme Learning Machine for Prediction of Adverse Cardiac Events. *Cognitive Computation*. 2017;9:545-554.

43. Liu N, Koh ZX, Goh J, Lin Z, Haaland B, Ting BP and Ong MEH. Prediction of adverse cardiac events in emergency department patients with chest pain using machine learning for variable selection. *BMC Med Inform Decis Mak*. 2014;14:75.

44. Quintana DS, Alvares GA and Heathers JA. Guidelines for Reporting Articles on Psychiatry and Heart rate variability (GRAPH): recommendations to advance research communication. *Transl Psychiatry*. 2016;6:e803.

45. Kloter E, Barrueto K, Klein SD, Scholkmann F and Wolf U. Heart Rate Variability as a Prognostic Factor for Cancer Survival - A Systematic Review. *Front Physiol*. 2018;9:623.

46. Schroeder EB, Chambless LE, Liao D, Prineas RJ, Evans GW, Rosamond WD and Heiss G. Diabetes, glucose, insulin, and heart rate variability: the Atherosclerosis Risk in Communities (ARIC) study. *Diabetes Care*. 2005;28:668-74.

47. Buchman TG, Stein PK and Goldstein B. Heart rate variability in critical illness and critical care. *Curr Opin Crit Care*. 2002;8:311-5.

48. Polikar R. Ensemble based systems in decision making. *IEEE Circuits Syst Mag*. 2006;6:21-44.

49. Bishop CM. *Pattern Recognition and Machine Learning*: Springer-Verlag; 2006.

50. Lundberg SM, Nair B, Vavilala MS, Horibe M, Eisses MJ, Adams T, Liston DE, King-Wai Low D, Newman SF, Kim J and Lee SI. Explainable machine-learning predictions for the prevention of hypoxaemia during surgery. *Nature biomedical engineering*. 2018;2:749-760.

Table 1: Patient baseline characteristics.

	Total (n=795)	**MACE (n=247)**	**Non-MACE (n=548)**	**p-value**
Age, mean (SD)	59.63 (12.88)	61.06 (11.38)	58.99 (13.47)	0.035
Male gender, n (%)	542 (68.2)	188 (76.1)	354 (64.6)	0.002
Race, n (%)				0.623
Chinese	492 (61.9)	159 (64.4)	333 (60.8)	
Indian	129 (16.2)	34 (13.8)	95 (17.3)	
Malay	150 (18.9)	46 (18.6)	104 (19.0)	
Other	24 (3.0)	8 (3.2)	16 (2.9)	
Medical history, n (%)				
Ischemic heart disease	343 (43.1)	115 (46.6)	228 (41.6)	0.22
Diabetes	278 (35.0)	106 (42.9)	172 (31.4)	0.002
Hypertension	509 (64.0)	161 (65.2)	348 (63.5)	0.707
Hypercholesterolemia	476 (59.9)	151 (61.1)	325 (59.3)	0.683
Stroke	58 (7.3)	15 (6.1)	43 (7.8)	0.458
Cancer	29 (3.6)	7 (2.8)	22 (4.0)	0.537
Respiratory disease	31 (3.9)	5 (2.0)	26 (4.7)	0.102
Chronic kidney disease	87 (10.9)	26 (10.5)	61 (11.1)	0.32
Congestive heart failure	38 (4.8)	9 (3.6)	29 (5.3)	0.407
History of PCI	199 (25.0)	68 (27.5)	131 (23.9)	0.316
History of CABG	71 (8.9)	26 (10.5)	45 (8.2)	0.355
History of AMI	133 (16.7)	48 (19.4)	85 (15.5)	0.288
Active smoker	197 (24.8)	73 (29.6)	124 (22.6)	0.003

MACE, major adverse cardiac events; SD, standard deviation; PCI, percutaneous coronary intervention; CABG, coronary artery bypass graft; AMI, acute myocardial infarction.

Table 2: Descriptive analyses of heart rate variability (HRV) and heart rate n-variability (HRnV) parameters.

	HRV	**HR_2V**	**HR_2V_1**	**HR_3V**	**HR_3V_1**	**HR_3V_2**
Mean NN (s)	829.40 (169.49)	1656.65 (339.85)	1658.81 (338.99)	2484.80 (509.33)	2488.22 (508.50)	2485.02 (509.84)
SDNN (s)	38.16 (25.49)	62.28 (45.45)	68.81 (47.00)	82.06 (62.47)	97.79 (67.46)	87.77 (64.52)
RMSSD (s)	30.04 (23.07)	32.61 (26.68)	33.79 (25.67)	34.83 (28.86)	36.27 (26.50)	34.98 (27.43)
Skewness	-0.65 (2.34)	-0.41 (1.66)	-0.59 (1.95)	-0.29 (1.29)	-0.55 (1.69)	-0.38 (1.42)
Kurtosis	14.59 (26.83)	7.33 (13.58)	10.17 (17.90)	5.15 (8.13)	8.06 (12.92)	5.98 (9.75)
Triangular index	7.68 (4.19)	10.38 (5.10)	12.60 (6.45)	11.47 (5.29)	16.25 (7.94)	13.06 (6.04)
NN50 (count)	21.08 (33.98)	14.46 (20.35)	29.35 (40.03)	11.57 (15.05)	35.29 (44.34)	17.41 (22.51)
pNN50 (%)	6.31 (11.08)	8.66 (13.18)	8.75 (12.97)	10.31 (14.27)	10.38 (13.95)	10.28 (14.20)
NN50*n* (count)	-	4.16 (9.72)	8.45 (18.76)	1.37 (3.72)	4.37 (10.72)	2.08 (5.48)
pNN50*n* (%)	-	2.60 (6.67)	2.64 (6.47)	1.32 (3.95)	1.39 (3.86)	1.33 (3.87)
Total power (ms^2)	2518.30 (4797.05)	7797.46 (16947.44)	9156.26 (17970.75)	13904.78 (37182.24)	18714.67 (37620.26)	15706.11 (34845.52)
VLF power (ms^2)	985.18 (1991.52)	3401.42 (6569.37)	3922.74 (7987.46)	6503.53 (14205.11)	8772.26 (17986.63)	7567.79 (14666.32)
LF power (ms^2)	732.36 (1841.88)	2626.83 (7593.16)	2782.48 (7212.62)	5091.49 (18402.20)	5740.99 (15243.38)	5397.76 (16001.18)
HF power (ms^2)	527.27 (1232.69)	1328.86 (4033.96)	1361.53 (3433.55)	1661.69 (7237.55)	1762.45 (4851.11)	1761.05 (6477.63)
LF power norm (nu)	56.76 (19.20)	66.82 (18.17)	66.42 (17.35)	76.53 (15.32)	77.65 (14.55)	77.93 (14.95)
HF power norm (nu)	43.24 (19.20)	33.18 (18.17)	33.58 (17.35)	23.47 (15.32)	22.35 (14.55)	22.07 (14.95)
LF/HF	1.99 (1.93)	3.24 (2.95)	3.04 (2.73)	5.60 (5.21)	5.79 (4.99)	6.06 (5.18)
Poincaré SD1 (ms)	21.27 (16.34)	23.12 (18.93)	23.92 (18.18)	24.72 (20.50)	25.68 (18.77)	24.80 (19.46)
Poincaré SD2 (ms)	48.82 (33.29)	84.47 (62.15)	93.88 (64.58)	112.87 (86.62)	135.55 (94.02)	121.20 (89.72)
SampEn	1.57 (0.51)	83.84 (2324.24)	1.33 (0.48)	248.48 (4020.64)	1.06 (0.41)	1.14 (0.45)
ApEn	0.99 (0.20)	0.72 (0.18)	0.91 (0.17)	0.60 (0.15)	0.84 (0.17)	0.70 (0.15)
DFA, α1	0.99 (0.31)	1.24 (0.29)	1.23 (0.27)	1.41 (0.27)	1.42 (0.23)	1.42 (0.25)

DFA, α2	0.95 (0.22)	0.98 (0.35)	0.98 (0.22)	0.86 (0.65)	1.01 (0.22)	1.02 (0.36)

HRV, heart rate variability; mean NN, average of R-R intervals; SDNN, standard deviation of R-R intervals; RMSSD, square root of the mean squared differences between R-R intervals; NN50, the number of times that the absolute difference between 2 successive R-R intervals exceeds 50 ms; pNN50, NN50 divided by the total number of R-R intervals; NN50*n*, the number of times that the absolute difference between 2 successive RR_nI/RR_nI_m sequences exceeds 50×*n* ms; pNN50*n*, NN50*n* divided by the total number of RR_nI/RR_nI_m sequences; VLF, very low frequency; LF, low frequency; HF, high frequency; SD: standard deviation; SampEn, sample entropy; ApEn, approximate entropy; DFA: detrended fluctuation analysis.

Table 3: Univariable analysis of HR_nV parameters.

	HRV		**HR_2V**		**HR_3V**	
	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
Mean NN	0.999 (0.998-1.000)	0.023*	0.999 (0.999-1.000)	0.023*	1.000 (0.999-1.000)	0.023*
SDNN	0.992 (0.986-0.999)	0.023*	0.996 (0.992-1.000)	0.028*	0.997 (0.995-1.000)	0.060
RMSSD	0.990 (0.982-0.998)	0.010*	0.992 (0.985-0.998)	0.011*	0.994 (0.988-0.999)	0.030*
Skewness	1.059 (0.991-1.132)	0.088	1.079 (0.981-1.186)	0.118	1.139 (1.006-1.290)	0.040*
Kurtosis	1.006 (1.000-1.011)	0.038*	1.009 (0.998-1.019)	0.113	1.011 (0.993-1.029)	0.242
Triangular index	0.961 (0.925-0.998)	0.039*	0.967 (0.938-0.997)	0.032*	0.978 (0.950-1.007)	0.133
NN50	0.993 (0.987-0.998)	0.008*	0.989 (0.981-0.998)	0.012*	0.988 (0.977-0.999)	0.031*
pNN50	0.978 (0.962-0.995)	0.009*	0.984 (0.971-0.997)	0.014*	0.987 (0.976-0.999)	0.027*
NN50*n*	-	-	0.982 (0.964-1.001)	0.065	0.952 (0.905-1.002)	0.059
pNN50*n*	-	-	0.974 (0.946-1.002)	0.069	0.951 (0.903-1.001)	0.054
Total power	1.000 (1.000-1.000)	0.031*	1.000 (1.000-1.000)	0.021*	1.000 (1.000-1.000)	0.072
VLF power	1.000 (1.000-1.000)	0.132	1.000 (1.000-1.000)	0.070	1.000 (1.000-1.000)	0.133
LF power	1.000 (1.000-1.000)	0.077	1.000 (1.000-1.000)	0.023*	1.000 (1.000-1.000)	0.063
HF power	1.000 (0.999-1.000)	0.002*	1.000 (1.000-1.000)	0.014*	1.000 (1.000-1.000)	0.074
LF power norm	1.001 (0.994-1.009)	0.738	0.999 (0.99-1.007)	0.733	0.994 (0.985-1.004)	0.248
HF power norm	0.999 (0.991-1.007)	0.738	1.001 (0.993-1.01)	0.733	1.006 (0.996-1.015)	0.248
LF/HF	1.034 (0.959-1.116)	0.381	1.014 (0.964-1.066)	0.592	1.001 (0.973-1.031)	0.923
Poincaré SD1	0.986 (0.975-0.997)	0.010*	0.988 (0.979-0.997)	0.011*	0.991 (0.983-0.999)	0.029*
Poincaré SD2	0.995 (0.990-1.000)	0.032*	0.997 (0.994-1.000)	0.032*	0.998 (0.996-1.000)	0.063
SampEn	0.813 (0.604-1.095)	0.173	0.730 (0.545-0.977)	0.035*	1.000 (1.000-1.000)	0.932
ApEn	1.645 (0.752-3.598)	0.213	2.319 (1.003-5.357)	0.049*	1.241 (0.463-3.327)	0.667
DFA, α1	0.953 (0.585-1.552)	0.846	1.031 (0.611-1.741)	0.908	0.968 (0.560-1.672)	0.907
DFA, α2	1.532 (0.773-3.034)	0.221	1.202 (0.782-1.848)	0.401	1.184 (0.934-1.500)	0.163

HRV, heart rate variability; OR, odds ratio; CI, confidence interval; mean NN, average of R-R intervals; SDNN, standard deviation of R-R intervals; RMSSD, square root of the mean squared differences between R-R intervals; NN50, the number of times that the absolute difference between 2 successive R-R intervals exceeds 50 ms; pNN50, NN50 divided by the total number of R-R intervals; NN50*n*, the number of times that the absolute difference

between 2 successive RR_nI/RR_nI_m sequences exceeds 50×*n* ms; pNN50*n*, NN50*n* divided by the total number of RR_nI/RR_nI_m sequences; VLF, very low frequency; LF, low frequency; HF, high frequency; SD: standard deviation; SampEn, sample entropy; ApEn, approximate entropy; DFA: detrended fluctuation analysis.

Table 4: Univariable analysis of HR_nV_m parameters.

	HR_2V_1		**HR_3V_1**		**HR_3V_2**	
	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
Mean NN	0.999 (0.999-1.000)	0.023*	1.000 (0.999-1.000)	0.023*	1.000 (0.999-1.000)	0.023*
SDNN	0.996 (0.993-1.000)	0.034*	0.997 (0.995-1.000)	0.042*	0.997 (0.995-1.000)	0.034*
RMSSD	0.991 (0.984-0.998)	0.010*	0.992 (0.986-0.999)	0.016*	0.993 (0.986-0.999)	0.016*
Skewness	1.061 (0.980-1.149)	0.144	1.072 (0.978-1.176)	0.139	1.098 (0.982-1.227)	0.100
Kurtosis	1.007 (0.999-1.015)	0.082	1.006 (0.994-1.017)	0.333	1.010 (0.995-1.025)	0.195
Triangular index	0.981 (0.958-1.005)	0.119	0.982 (0.963-1.001)	0.065	0.974 (0.949-0.999)	0.040*
NN50	0.995 (0.991-0.999)	0.018*	0.996 (0.993-1.000)	0.052	0.992 (0.985-0.999)	0.035*
pNN50	0.984 (0.972-0.997)	0.020*	0.988 (0.977-1.000)	0.049*	0.988 (0.976-0.999)	0.035*
NN50*n*	0.989 (0.979-1.000)	0.043*	0.982 (0.964-1.000)	0.054	0.974 (0.943-1.007)	0.118
pNN50*n*	0.969 (0.939-0.999)	0.046*	0.947 (0.895-1.002)	0.058	0.960 (0.914-1.009)	0.109
Total power	1.000 (1.000-1.000)	0.048*	1.000 (1.000-1.000)	0.072	1.000 (1.000-1.000)	0.029*
VLF power	1.000 (1.000-1.000)	0.139	1.000 (1.000-1.000)	0.145	1.000 (1.000-1.000)	0.074
LF power	1.000 (1.000-1.000)	0.084	1.000 (1.000-1.000)	0.092	1.000 (1.000-1.000)	0.027*
HF power	1.000 (1.000-1.000)	0.005*	1.000 (1.000-1.000)	0.010*	1.000 (1.000-1.000)	0.022*
LF power norm	1.000 (0.991-1.008)	0.937	0.995 (0.985-1.006)	0.382	0.995 (0.986-1.005)	0.356
HF power norm	1.000 (0.992-1.009)	0.937	1.005 (0.994-1.015)	0.382	1.005 (0.995-1.015)	0.356
LF/HF	1.024 (0.970-1.080)	0.387	1.003 (0.973-1.033)	0.863	0.999 (0.971-1.029)	0.966
Poincaré SD1	0.987 (0.978-0.997)	0.010*	0.989 (0.980-0.998)	0.016*	0.989 (0.981-0.998)	0.016*
Poincaré SD2	0.997 (0.995-1.000)	0.039*	0.998 (0.996-1.000)	0.045*	0.998 (0.996-1.000)	0.037*
SampEn	0.854 (0.623-1.171)	0.328	0.802 (0.553-1.161)	0.242	0.709 (0.500-1.005)	0.053
ApEn	2.065 (0.842-5.064)	0.113	1.207 (0.499-2.922)	0.677	2.558 (0.906-7.222)	0.076
DFA, α1	0.888 (0.514-1.537)	0.672	1.039 (0.547-1.971)	0.907	1.004 (0.549-1.835)	0.991
DFA, α2	1.557 (0.782-3.098)	0.208	1.554 (0.780-3.093)	0.210	1.169 (0.764-1.789)	0.472

HRV, heart rate variability; OR, odds ratio; CI, confidence interval; mean NN, average of R-R intervals; SDNN, standard deviation of R-R intervals; RMSSD, square root of the mean squared differences between R-R intervals; NN50, the number of times that the absolute difference between 2 successive R-R intervals exceeds 50 ms; pNN50, NN50 divided by the total number of R-R intervals; NN50*n*, the number of times that the absolute difference

between 2 successive RR_nI/RR_nI_m sequences exceeds 50×*n* ms; pNN50*n*, NN50*n* divided by the total number of RR_nI/RR_nI_m sequences; VLF, very low frequency; LF, low frequency; HF, high frequency; SD: standard deviation; SampEn, sample entropy; ApEn, approximate entropy; DFA: detrended fluctuation analysis.

Table 5: The heart rate n-variability (HRnV) model built with multivariable logistic regression for prediction of 30-day major adverse cardiac events.

Variable	**Adjusted OR**	**95% CI**
Age	1.021	1.002-1.041
Diastolic BP	1.018	1.003-1.034
Pain score	1.082	1.003-1.168
ST-elevation	6.449	2.762-15.059
ST-depression	4.827	2.511-9.277
Q wave	3.383	1.668-6.860
Cardiac history	7.838	5.192-11.832
Troponin	4.406	3.218-6.033
HRV NN50	0.981	0.970-0.991
HR_2V skewness	0.806	0.622-1.045
HR_2V SampEn	0.600	0.348-1.035
HR_2V ApEn	0.095	0.014-0.628
HR_2V_1 ApEn	19.700	2.942-131.900
HR_3V RMSSD	1.024	1.008-1.040
HR_3V skewness	1.560	1.116-2.181
HR_3V_2 HF power	1.000	1.000-1.000

BP, blood pressure; HRV, heart rate variability; OR, odds ratio; CI, confidence interval; mean NN, average of R-R intervals; RMSSD, square root of the mean squared differences between R-R intervals; NN50, the number of times that the absolute difference between 2 successive R-R intervals exceeds 50 ms; LF, low frequency; HF, high frequency; SampEn, sample entropy; ApEn, approximate entropy.

Table 6: Comparison of performance of the HRnV model, HEART, TIMI, and GRACE scores in predicting 30-day major adverse cardiac events (MACE).

	AUC (95% CI)	Cut-off	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
HRnV Model	0.917 (0.892-0.941)	0.2896†	87.9% (83.8% - 91.9%)	79.9% (76.6% - 83.3%)	66.4% (61.2% - 71.5%)	93.6% (91.4% - 95.8%)
	-	0.0329	99.6% (98.8% - 100.0%)	38.0% (33.9% - 42.0%)	42.0% (38.0% - 46.0%)	99.5% (98.6% - 100.0%)
HEART	0.841 (0.808-0.874)	5†	78.9% (73.9% - 84.0%)	72.8% (69.1% - 76.5%)	56.7% (51.4% - 61.9%)	88.5% (85.5% - 91.4%)
	-	3	99.6% (98.8% - 100.0%)	35.8% (31.8% - 39.8%)	41.1% (37.2% - 45.1%)	99.5% (98.5% - 100.0%)
TIMI	0.681 (0.639-0.723)	2†	63.6% (57.6% - 69.6%)	58.4% (54.3% - 62.5%)	40.8% (35.9% - 45.7%)	78.0% (74.0% - 82.1%)
	-	0	98.4% (96.8% - 100.0%)	19.3% (16.0% - 22.7%)	35.5% (31.9% - 39.1%)	96.4% (92.9% - 99.9%)
GRACE	0.665 (0.623-0.707)	107†	64.0% (58.0% - 70.0%)	60.8% (56.7% - 64.9%)	42.4% (37.3% - 47.4%)	78.9% (75.0% - 82.8%)
	-	60	98.8% (97.4% - 100.0%)	8.0% (5.8% - 10.3%)	32.6% (29.3% - 36.0%)	93.6% (86.6% - 100.0%)

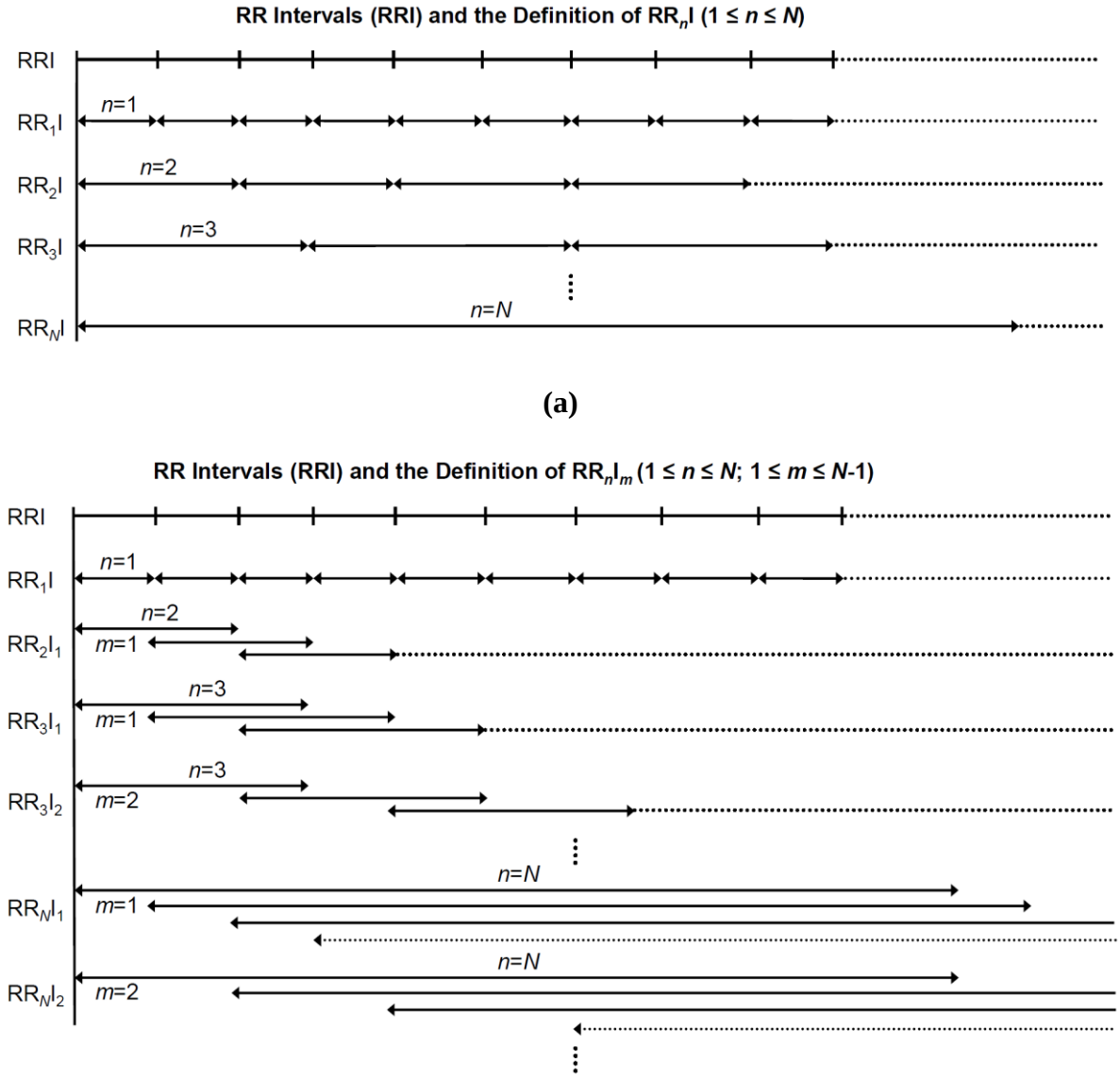
AUC, area under the curve; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value; HEART, History, ECG, Age, Risk factors and Troponin; TIMI, Thrombolysis in Myocardial Infarction; GRACE, Global Registry of Acute Coronary Events.

† Optimal cut-off values, defined as the points nearest to the upper-left corner on the ROC curves.

Figure Legends

Figure 1: **(a)** Illustration of R-R intervals (RRIs) and the definition of RR_nI where $1 \leq n \leq N$ and $N \ll \widehat{N}$. $\widehat{N}$ is the total number of RRIs; **(b)** Illustration of RRIs and the definition of RR_nI_m where $1 \leq n \leq N$, $1 \leq m \leq N - 1$, and $N \ll \widehat{N}$. $\widehat{N}$ is the total number of RRIs and m indicates the non-overlapped portion between two consecutive RR_nI_m sequences.

Figure 2: The receiver operating characteristic (ROC) curves produced by the heart rate n-variability (HRnV) model, the History, ECG, Age, Risk factors and Troponin (HEART) score, the Thrombolysis in Myocardial Infarction (TIMI) score, and the Global Registry of Acute Coronary Events (GRACE) score.



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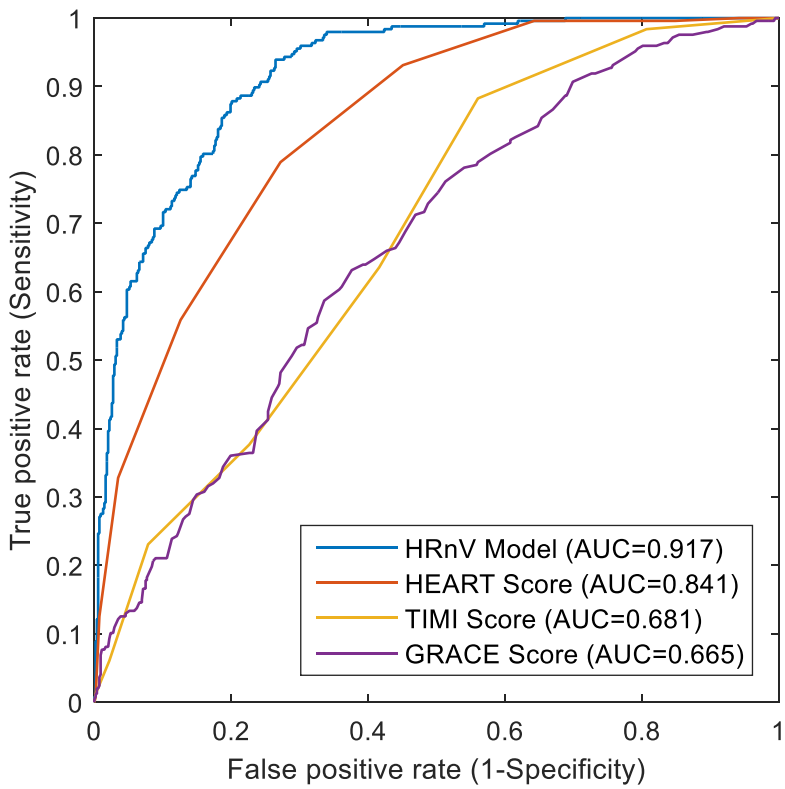

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