Editor's Summary

Better Biomarkers for Predicting Coronary Artery Disease-Related Death

The symptoms of a heart attack––chest pain, sweating, etc.––can actually signal several serious coronary events, collectively termed acute coronary syndrome. Some of these put patients at high risk for death and must be treated aggressively; others are of relatively lower risk. Our ability to correctly assign patients to one of these groups quickly and reliably is inadequate for optimal care. Syed and his colleagues have extracted three biomarkers, by computational methods, from the continuous EKG readings obtained from these patients in the hospital. When added to existing predictors, the three derived computational biomarkers improve the classification of acute coronary syndrome patients by 7 to 13%.

The biomarkers derived from the EKG do not correspond readily to features easily recognizable by an observer in the tracings of the EKG. But they are clearly visible to a computer using time-series algorithms on those same tracings. The first, morphologic variability, quantifies energy differences between consecutive heart beats. The second, symbolic mismatch, quantifies the difference in the EKG of a particular patient from others with the same clinical course. The third, heart rate motifs, determines patterns of heart rate in the EKG that reveal autonomic functioning.

The power of this approach is that these biomarkers can be extracted from clinical data that are already continuously collected from the patient. It can be done in line, in real time with no overt change in the patients' clinical experience or the cost of care. This could lead to treatments that ultimately extend the lives of tens of thousands of patients.

CARDIOVASCULAR DISEASE

Computationally Generated Cardiac Biomarkers for Risk Stratification After Acute Coronary Syndrome

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The existing tools for estimating the risk of death in patients after they experience acute coronary syndrome are commonly based on echocardiography and clinical risk scores (for example, the TIMI risk score). These identify a small group of high-risk patients who account for only a minority of the deaths that occur in patients after acute coronary syndrome. Here, we investigated the use of three computationally generated cardiac biomarkers for risk stratification in this population: morphologic variability (MV), symbolic mismatch (SM), and heart rate motifs (HRM). We derived these biomarkers from time-series analyses of continuous electrocardiographic data collected from patients in the TIMI-DISPERSE2 clinical trial through machine learning and data mining methods designed to extract information that is difficult to visualize directly in these data. We evaluated these biomarkers in a blinded, prespecified, and fully automated study on more than 4500 patients in the MERLIN-TIMI36 (Metabolic Efficiency with Ranolazine for Less Ischemia in Non–ST-Elevation Acute Coronary Syndrome–Thrombolysis in Myocardial Infarction 36) clinical trial. Our results showed a strong association between all three computationally generated cardiac biomarkers and cardiovascular death in the MERLIN-TIMI36 trial over a 2-year period after acute coronary syndrome. Moreover, the information in each of these biomarkers was independent of the information in the others and independent of the information provided by existing clinical risk scores, electrocardiographic metrics, and echocardiography. The addition of MV, SM, and HRM to existing metrics significantly improved model discrimination, as well as the precision and recall of prediction rules based on left ventricular ejection fraction. These biomarkers can be extracted from data that are routinely captured from patients with acute coronary syndrome and will allow for more accurate risk stratification and potentially for better patient treatment.

INTRODUCTION

In 2010, about 1.25 million new and recurrent coronary attacks occurred in the United States, with an additional 195,000 estimated silent first heart attacks (*1*). These events caused nearly 1 of every 6 deaths, with more than 150,000 of these deaths taking place in patients less than 65 years old (*1*). Despite improvements in survival rates, one in four men and one in three women continue to die within a year of a first heart attack. This burden is similar in most of the developed world, and increasingly in the developing world, where it is estimated that 40% of all the deaths by the year 2020 will be attributable to cardiovascular disease (*2*).

The burden of coronary heart disease can be reduced, in part, by accurately identifying high-risk patients at early stages after an acute coronary syndrome (ACS) and by matching these patients to treatment and monitoring regimens appropriate for their individual risk. Indeed, early risk stratification is important because immediate coronary angiography and revascularization provide substantial benefit to high-risk patients who present with either ST-segment elevation ACS or non–ST-segment ACS (NSTEACS) (*3–6*). In addition, patients who fall into a high-risk subgroup warrant close clinical follow-up monitoring (*5*). Unfortunately, patients who present with ACS have a wide and highly variable spectrum of risk for cardiovascular death (CVD) (*7*), and accurately identifying those at the highest risk remains a persistent and challenging clinical dilemma.

All current practice guidelines for ACS emphasize risk stratification to appropriately gauge the intensity of therapy needed to reduce the risk of cardiovascular complications (*8*). Although the use of standard biochemical markers such as C-reactive protein (CRP) and brain natriuretic peptide (BNP) can identify high-risk patients in certain clinical settings, these markers do not identify all patients with an elevated risk of adverse cardiovascular events. In particular, the latest American College of Cardiology/American Heart Association guidelines for the management of non–ST-elevation myocardial infarction are not clear about how to use the existing set of biochemical markers in routine clinical practice (*8*). Additional studies are needed to more precisely define the exact role of these biomarkers in assessing patient risk on a routine basis after ACS (*9*, *10*).

Although the term biomarker is typically restricted to biochemical substances, such as CRP and BNP, that are correlated with adverse outcomes, a biomarker can be any metric obtained from a patient that is an index of increased risk. For example, a depressed left ventricular ejection fraction (LVEF) indicates an increased risk of congestive heart failure and death (*11*). Nevertheless, despite the availability of many parameters to risk stratify post-ACS patients, including patient demographics, comorbidity, laboratory, imaging, and electrocardiographic (ECG) data, finding biomarkers that provide accurate estimates of risk is challenging. For instance, although a depressed LVEF is a commonly used clinical marker of increased risk of CVD after ACS (*12–15*), there is no good biomarker for risk stratifying most of the patients with nondepressed LVEF (*16*).

Most biomarkers are derived from instantaneous data (for example, a single measurement of the concentration of a molecule like BNP in the blood) that are typically analyzed and combined in simple ways with limited or no computational aid. Supplementing these biomarkers with biomarkers generated by applying algorithms to physiological time series such as the ECG, which provide valuable longitudinal data

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related to cardiac health, can lead to a more accurate evaluation of the current state of a patient and the likely impact of various therapies. We refer to the information generated by such algorithms as computational biomarkers, which we define as a characteristic that is not directly measurable but that can be computationally derived from measurable data.

Our research leverages advanced analytical techniques from machine learning and data mining to extract potentially valuable information that is often overlooked in large volumes of cardiovascular data, but that may identify high-risk patients after ACS. We have developed three computational biomarkers [morphologic variability (MV), symbolic mismatch (SM), and heart rate motif (HRM)], using sophisticated machine learning and data mining techniques, that can be used to risk stratify post-ACS patients. Each metric is derived from long-term Holter ECG signals and its derivation is fully automated. Morphological variability (MV) assesses myocardial instability by quantifying low-amplitude probabilistic variability in the shape of the ECG waveform over long periods of time (17). SM quantifies the degree to which long-term ECG signals of individual patients are anomalous relative to those of other patients with a similar clinical history. It is based on detecting shifts in the morphology and dynamics of functional units of cardiac activity over long periods (18). HRM integrate the frequency with which high- or low-risk heart rate patterns reflecting autonomic function appear in a patient's ECG over long time periods (19). Each of these computational biomarkers uses time-series analytical techniques to extract new types of information that are presently unappreciated in large volumes of continuous cardiovascular data. These computational biomarkers measure subtle features of long-term ECG data that are outside the scope of human visualization but are consistently associated with future risk.

Here, we assess the prognostic ability of these biomarkers in a blinded, prespecified, and fully automated study on more than 4500 patients in the MERLIN-TIMI36 (Metabolic Efficiency with Ranolazine for Less Ischemia in Non–ST-Elevation Acute Coronary Syndrome–Thrombolysis in Myocardial Infarction 36) trial. We evaluate MV, SM, and HRM in a population with detailed follow-up data and rich clinical metadata for all patients. This allows us to compare the ability of these computationally generated biomarkers to that of other metrics such as the TIMI risk score (TRS) (20), LVEF, and several other metrics that are based on long-term ECG time series (described in more detail in the Supplementary Material): heart rate variability (HRV), heart rate turbulence (HRT), deceleration capacity (DC), severe autonomic failure (SAF), and a fully automated version of modified moving average T-wave alternans (TWA). We also study the incremental information provided by computational biomarkers relative to existing metrics through orthogonal statistical approaches to assess their effect on discrimination and reclassification of CVD after ACS.

RESULTS

Population characteristics
The study population comprised all patients in the MERLIN-TIMI36 trial for whom continuous ECG was available. The baseline clinical characteristics of these patients are presented in Table 1. The patients with available continuous ECG data did not have baseline characteristics that were significantly different from the entire MERLIN-TIMI36 population. One-half of the patients were diagnosed with a non–ST-elevation myocardial infarction, and almost three-quarters were at mod-
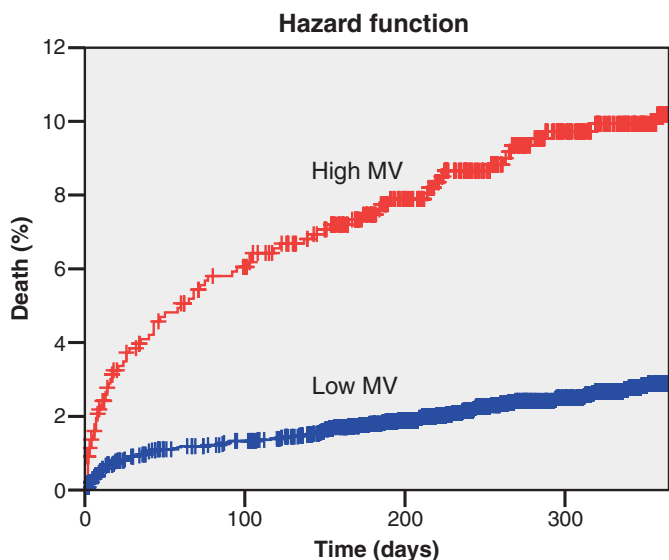
erate to high risk according to the TRS. The different HRV metrics, DC, TWA, and TRS were determined for all 4557 patients, whereas HRT and SAF were measured in all patients with premature ventricular contractions ($n = 3873$, 85%). Of the computationally generated biomarkers, MV and HRM were measured for all 4557 patients. SM was measured for 4033 (89%) of the patients and indeterminate in the rest (Supplementary Material). Three thousand seventy-one (67%) of the patients with continuous ECG data had LVEF assessed by the local investigator during the index hospitalization. Of the patients with available LVEF data, 2805 (91%) had LVEF $\geq$ 40%. There were a total of 195 cases of CVD during follow-up.

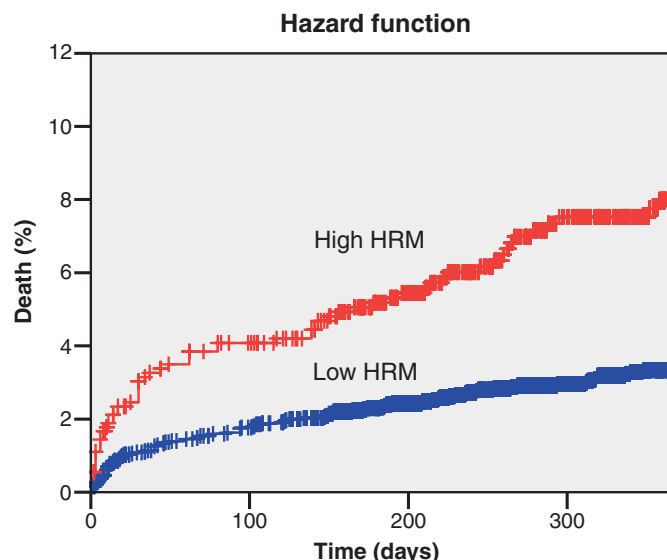### Univariate association of computationally generated biomarkers with CVD
We determined the Kaplan-Meier event curves for all three computationally generated cardiac biomarkers (Figs. 1 to 3). Each biomarker was strongly associated with CVD after non–ST-elevation ACS. The association was strongest for MV [hazard ratio (HR), 3.31; 95% confidence interval (CI), 2.49 to 4.40; $P < 0.001$] followed by SM (HR, 2.36; 95% CI, 1.73 to 3.22; $P < 0.001$) and HRM (HR, 2.21; 95% CI, 1.65 to 2.97; $P < 0.001$). Table S1 presents the univariate association of the other risk variables considered in this study with the endpoint of CVD after ACS.

Table 1. Baseline clinical characteristics for patients (none of the differences between the continuous ECG analysis cohort and the entire MERLIN-TIMI36 cohort were statistically significant at the 5% level). IQR, interquartile range; BMI, body mass index.

	Continuous ECG analysis cohort (n = 4557)	Entire MERLIN-TIMI36 cohort (n = 6560)
Age, years, median (IQR)	63 (55–72)	64 (55–72)
Age ≥75 years (%)	17	18
Female sex (%)	35	35
BMI, median (IQR)	29 (25–31)	28 (25–32)
Diabetes mellitus (%)	34	34
Hypertension (%)	73	74
Hyperlipidemia (%)	67	68
Current smoker (%)	26	26
Previous myocardial infarction (%)	33	34
Index event		
Unstable angina (%)	47	47
Myocardial infarction (%)	53	53
ST depression ≥1 mV (%)	36	35
TIMI risk score		
Low (1–2) (%)	27	27
Moderate (3–4) (%)	53	53
High (5–7) (%)	20	20
LVEF <40% (%)	9	9

Fig. 1. Association of MV with CVD in the MERLIN-TIMI36 trial (vertical lines denote censoring).

Fig. 3. Association of HRM with CVD in the MERLIN-TIMI36 trial (vertical lines denote censoring).
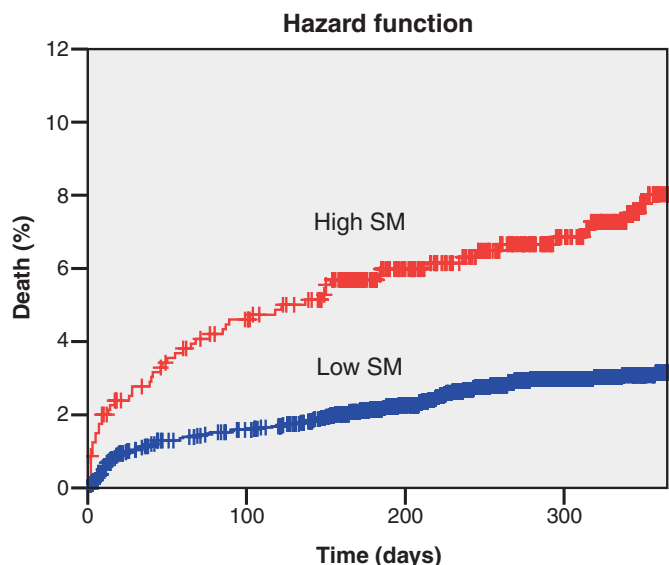
Fig. 2. Association of SM with CVD in the MERLIN-TIMI36 trial (vertical lines denote censoring).

Multivariate association of computationally generated biomarkers with CVD

Table 2 presents the correlation of MV, SM, and HRM with TRS, LVEF, and the other ECG-based risk metrics. All three computationally generated biomarkers had low correlation with TRS ($P = 0.059$ to 0.101) and LVEF ($P = 0.055$ to 0.139). MV showed low to moderate correlation with all of the other ECG-based metrics, whereas both SM and HRM had low correlation with the other ECG-based metrics.

The results of multivariate analysis are presented in Tables 3 and 4. MV, SM, and HRM were each independently associated with CVD

during follow-up even after being adjusted for TRS, LVEF, and all of the other ECG-based metrics (MV-adjusted HR, 1.67; 95% CI, 1.02 to 2.73; $P = 0.043$; SM-adjusted HR, 1.94; 95% CI, 1.27 to 2.98; $P = 0.002$; HRM-adjusted HR, 2.14; 95% CI, 1.40 to 2.36; $P < 0.001$) (Table 3). On stepwise backward elimination, only TRS, LVEF, and the three computationally generated cardiac biomarkers remained in the multivariate model (MV-adjusted HR, 2.04; 95% CI, 1.33 to 3.12; $P = 0.001$; SM-adjusted HR, 1.89; 95% CI, 1.24 to 2.86; $P = 0.003$; HRM-adjusted HR, 2.26; 95% CI, 1.51 to 3.39; $P < 0.001$) (Table 4). Identical results were obtained on stepwise forward selection with only TRS, LVEF, and the three computationally generated cardiac biomarkers selected in the multivariate model. The results of creating multivariate models with stepwise backward elimination and stepwise forward selection from only the different ECG-based metrics (including the computationally generated biomarkers) and LVEF, when TRS was excluded, are also presented in table S2.

Figure 4 presents the C-indices of the multivariate models produced by stepwise backward elimination as variables are removed. The C-index decreased from when all variables were used (0.808) to when only the variables retained by stepwise elimination were used for model construction (0.791). None of the multivariate models produced by stepwise elimination when the computationally generated cardiac biomarkers were excluded from the initial set achieved a C-index higher than 0.776.

Recall and precision combining LVEF and TRS with MV, SM, and HRM

Table 5 presents the recall and precision of predictive rules based on LVEF compared to predictive rules that combined LVEF with MV, SM, and HRM. The prediction rule of either LVEF < 40% OR (MV AND SM AND HRM) improved upon both the precision and recall of LVEF < 40% alone. The prediction rule of LVEF < 40% OR (MV AND HRM) increased the precision to almost 49% from 32% while maintaining roughly the same level of recall. The addition of

Table 2. Correlation of the computationally generated cardiac biomarkers with TRS, LVEF, and ECG-based risk metrics. Data are expressed as r values.

	MV	SM	HRM
HRV-SDNN	0.037	−0.021	0.053
HRV-SDANN	0.081	−0.015	0.022
HRV-ASDNN	0.041	0.036	0.147
HRV-RMSSD	−0.085	−0.017	0.11
HRV-PNN50	−0.135	−0.021	0.086
HRV-HRVI	−0.094	0.009	0.046
HRV-LFHF	0.369	0.069	0.112
HRT	0.211	0.044	0.133
DC	0.416	0.065	0.188
SAF	0.185	0.017	0.1
TWA	0.058	0.021	0.038
TRS	0.083	0.059	0.101
LVEF	0.139	0.059	0.055

Table 3. Multivariate model for computationally generated cardiac biomarkers, TRS, LVEF, and ECG-based metrics (single model with all variables). Computationally generated biomarkers are shown in bold. HR, hazard ratio; CI, confidence interval.

	Adjusted HR	95% CI	P
TRS 3	4.90	2.16–11.09	<0.001
LVEF	3.28	2.08–5.20	<0.001
HRM	2.14	1.40–2.36	<0.001
SM	1.94	1.27–2.98	0.002
TRS 2	2.48	1.11–5.55	0.027
MV	1.67	1.02–2.73	0.043
HRV-HRVI	0.64	0.33–1.22	0.171
HRV-ASDNN	1.45	0.80–2.62	0.226
HRV-SDANN	1.58	0.74–3.40	0.240
HRT 2	1.79	0.61–5.25	0.289
TWA	1.20	0.74–1.95	0.465
DC 2	1.24	0.63–2.47	0.533
HRT 1	1.14	0.70–1.86	0.587
HRV-SDNN	0.81	0.36–1.82	0.617
HRV-PNN50	0.85	0.43–1.71	0.657
DC 1	1.11	0.64–1.95	0.707
HRV-RMSSD	0.89	0.44–1.80	0.746
HRV-LFHF	1.07	0.64–1.80	0.793
SAF	0.91	0.28–2.98	0.875

Table 4. Multivariate model for computationally generated cardiac biomarkers, TRS, LVEF, and ECG-based metrics (single model constructed with stepwise backward elimination using the Wald statistic; identical results obtained using stepwise forward selection). Computationally generated biomarkers are shown in bold. HR, hazard ratio; CI, confidence interval.

	Adjusted HR	95% CI	P
TRS 3	5.60	2.50–12.53	<0.001
LVEF	3.60	2.33–5.56	<0.001
HRM	2.26	1.51–3.39	<0.001
MV	2.04	1.33–3.12	0.001
SM	1.89	1.24–2.86	0.003
TRS 2	2.64	1.19–5.86	0.017

Net reclassification improvement combining LVEF and TRS with MV, SM, and HRM

The addition of the computationally generated cardiac biomarkers provided net reclassification improvement (NRI) for both prediction rules based on only LVEF and on LVEF and TRS combined (Table 5). The NRI was greatest for the rule LVEF < 40% OR (MV AND HRM) (13.5% NRI; $P < 0.001$) and the related rule (LVEF < 40% AND TRS = 3) OR (MV AND HRM) (13.5% NRI; $P < 0.001$).

Computationally generated cardiac biomarkers in LVEF ≥ 40% population

Each of the computationally generated cardiac biomarkers showed a strong association with CVD in the LVEF ≥ 40% population (MV HR, 3.20; 95% CI, 2.07 to 4.95; $P < 0.001$; SM HR, 2.06; 95% CI, 1.28 to 3.34; $P = 0.003$; HRM HR, 2.71; 95% CI, 1.75 to 4.19; $P < 0.001$). On multivariate analysis, the biomarkers were independently associated with CVD in a model constructed using all the risk variables (Table 6). On stepwise backward elimination using the Wald statistic (Table 7A), all three computationally generated biomarkers were retained along with TRS and HRV-ASDNN. Both MV and HRM were independently associated with CVD in the stepwise multivariate model. Similar results were obtained using stepwise forward selection, although in this case only TRS and the three computationally generated biomarkers were selected (that is, without HRV-ASDNN in the model) (Table 7B).

Figure 5 presents the C-indices of the multivariate models produced by stepwise backward elimination as variables are removed. The C-index decreased from when all variables were used (0.786) to when only the variables retained by stepwise elimination were used for model construction (0.762). None of the multivariate models produced by stepwise elimination when the computationally generated cardiac biomarkers were excluded from the initial set achieved a C-index higher than 0.740.

Risk stratification in the placebo group of MERLIN-TIMI36 trial

The results of evaluating the computationally generated cardiac biomarkers among the placebo group patients in the MERLIN-TIMI36 are presented in tables S3 and S4. Similar results to those presented earlier for the entire patient cohort were obtained on multivariate analysis for MV and HRM in placebo group patients. Both these biomarkers were independently associated with CVD after ACS in the multivariate models

computationally generated cardiac biomarkers also improved both the recall and the precision of prediction based on the combination of LVEF < 40% and TRS = 3 (that is, high risk) (Table 5).

developed by using stepwise backward elimination or stepwise forward selection. In contrast, SM did not show a statistically significant association in multivariate models in this reduced patient population (roughly half of the patients from the analyses presented earlier).

When assessed in terms of improvements to existing prediction rules, the computationally generated biomarkers provided similar magnitudes of improvements to both precision and recall as the results reported earlier. The addition of MV, SM, and HRM led to a higher precision and recall, whereas MV and HRM achieved an even larger increase in recall while providing precision that was comparable to rules based on LVEF or the combination of LVEF and TRS. The highest NRI was obtained for the prediction rules combining MV and HRM with LVEF and the TRS. The results for the NRI achieved with the addition of MV and HRM showed statistical significance despite a reduction in the size of the patient population from earlier analyses.

Competing risk analysis for CVD and myocardial infarction
Table S6 presents the results of competing risks regression for CVD and the endpoint of myocardial infarction in the entire MERLIN-

Fig. 4. C-index of multivariate models at each iteration of stepwise elimination with the Wald statistic when all variables are used (blue top curve) and when all variables excluding the computationally generated cardiac biomarkers are used (red bottom curve). The elimination process is continued until all variables excluding TRS and LVEF are removed. The C-index of the multivariate model with only LVEF, TRS, and the computationally generated cardiac biomarkers is also shown (horizontal green line).

TIMI36 cohort. The results of this analysis closely parallel the findings reported earlier on survival analysis of the different computationally generated biomarkers (Table 4).

DISCUSSION

In this report, we explored the potential utility of computationally generated biomarkers to improve risk stratification after non–ST-elevation acute coronary syndrome (NSTEACS). We constructed these computationally generated biomarkers from time-series data using techniques drawn from machine learning and data mining. In particular, we focused on three technical ideas we have recently developed in preliminary investigations on patients in the TIMI-DISPERSE2 trial, and evaluated the clinical utility of these computational biomarkers in a fully automated, prespecified, and blinded study on patients in the MERLIN-TIMI36 trial.

The results of our investigation confirm the earlier promise shown by MV, SM, and HRM in smaller populations, both when evaluated separately and when evaluated in conjunction with an extensive set of other clinical variables (for example, LVEF). In our experiments, we consistently found that all three of the computationally generated cardiac biomarkers were strongly associated with CVD after NSTEACS. These biomarkers were independent of information in clinical risk scores such as the TRS, echocardiographic measurements such as LVEF, and an extensive panel of other ECG-based metrics. The use of computationally generated biomarkers simultaneously improved discrimination, net reclassification, precision, and recall over existing approaches to predicting the risk of CVD after NSTEACS. We investigated this impact through an evaluation of our biomarkers using complementary statistical methods on one of the largest long-term ECG data sets in existence while accounting for information provided by commonly used clinical variables.

Our results confirm the utility of TRS and LVEF, but indicate that optimal models to risk stratify patients should combine TRS and LVEF with complementary information in long-term ECG. We found, for example, that information in the shape of the long-term ECG signal, the dynamics of functional units in the ECG, and the rate of cardiac activity are all independently valuable in improving risk stratification.
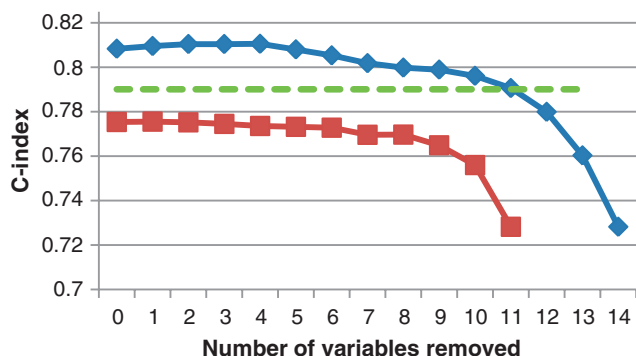
Table 5. Precision and recall of prediction rules combining LVEF with computationally generated cardiac biomarkers. The NRI and the associated significance of the improvement are also provided relative to LVEF < 40% or the rule LVEF < 40% AND TRS = 3 baseline predictive rules.

	Precision	Recall	NRI	NRI *P*
LVEF < 40%	31.7%	14.7%	—	—
LVEF < 40% OR (MV AND SM AND HRM)	38.7%	16.8%	7.6%	0.008
LVEF < 40% OR (MV AND SM)	42.5%	13.3%	7.9%	0.021
LVEF < 40% OR (MV AND HRM)	48.8%	14.5%	13.5%	<0.001
LVEF < 40% OR (SM AND HRM)	41.5%	13.9%	7.7%	0.019
(LVEF < 40% AND TRS = 3)	28.4%	15.5%	—	—
(LVEF < 40% AND TRS = 3) OR (MV AND SM AND HRM)	35.9%	18.0%	7.5%	0.008
(LVEF < 40% AND TRS = 3) OR (MV AND SM)	39.6%	13.7%	7.8%	0.023
(LVEF < 40% AND TRS = 3) OR (MV AND HRM)	45.5%	14.8%	13.5%	<0.001
(LVEF < 40% AND TRS = 3) OR (SM AND HRM)	39.6%	14.7%	8.6%	0.012

We believe this is because of the ability of this information to provide a holistic assessment of cardiovascular health related to factors external (for example, autonomic modulation) and internal (for example, impulse conduction) to the heart. We demonstrated that our three computationally generated biomarkers provide information that is complementary to each of the others, as well as to metrics that were previously proposed to extract information from ECG time series (a detailed discussion of these metrics is presented in the Supplementary Material).

We supplemented our analysis of the entire patient population by also examining patients with relatively preserved left ventricular function. We chose an ejection fraction of 40% as the cutoff because it is the most conservative of the cutoffs typically used in practice. The reported results suggest that the use of computationally generated cardiac biomarkers may help identify patients who are at high risk of CVD despite not being classified as at high risk on the basis of echocardiographic assessment of left ventricular systolic function. We did not separately evaluate the use of MV, SM, and HRM in patients with LVEF < 40% in the MERLIN-TIMI36 trial, because the small size of the group with available long-term ECG and measured LVEF (250 patients) did not provide sufficient strength to derive any statistically robust observations.

To consistently evaluate MV, SM, and HRM with previously proposed ECG metrics, we dichotomized each of our computational biomarkers to obtain a high-risk quintile of patients. (To eliminate the possibility that the use of quintiles in both the training and the validation sample biased our results inappropriately, we also present

results in the Supplementary Material where the biomarkers were recalculated using an alternative method where previously established quintiles from the TIMI-DISPERSE2 trial were used for dichotomization and then validated in the completely independent MERLIN-TIMI36 sample.) Our results show that the addition of MV, SM, and HRM as dichotomized variables significantly improves risk stratification after NSTEACS. We believe, however, that the eventual use of our computational biomarkers is not as dichotomized variables but as continuous features that may potentially be integrated by more sophisticated modeling approaches than the simple AND/OR predictive rules and the logistic regression multivariate models described here. Risk metrics that can be measured as continuous variables (for example, our computational biomarkers, BNP, and LVEF) can have greater utility and collectively achieve higher levels of precision and recall if used as continuous features and combined in more general ways. From this perspective, we believe that the results presented here, which focus exclusively on introducing the three computationally generated biomarkers and do not address the question of how best to construct models with these variables, represent a lower bound on the improvement possible in risk stratification after ACS. In this regard, there are a number of sophisticated methods that might be fruitfully applied to further improve our ability to identify high-risk subgroups. For example, given a large enough set of data, kernel-based methods (21) provide the ability to discover and model complex linear and nonlinear relationships among risk variables in a data-driven manner. These methods are becoming increasingly popular in a wide variety of biological applications (22) and offer an opportunity to improve the results reported here further.

Although our results are encouraging, our study has limitations. Continuous ECG data were available for only 4557 of the 6560 patients

Table 6. Multivariate model for computationally generated cardiac biomarkers, TRS, and ECG-based metrics (single model with all variables) in patients with LVEF ≥ 40%. Computationally generated biomarkers are shown in bold.

	Adjusted HR	95% CI	P
TRS 3	8.01	2.78–23.09	<0.001
TRS 2	3.44	1.21–9.80	0.021
HRM	1.80	1.07–3.04	0.027
MV	1.96	1.06–3.63	0.032
SM	1.73	1.02–2.93	0.041
HRV-ASDNN	1.79	0.87–3.68	0.111
HRV-PNN50	0.56	0.24–1.31	0.179
HRT 2	2.01	0.57–7.13	0.280
HRV-SDANN	1.64	0.64–4.18	0.302
HRV-HRVI	0.65	0.28–1.48	0.302
DC 2	1.53	0.65–3.62	0.334
HRV-SDNN	0.64	0.23–1.78	0.393
HRT 1	1.27	0.71–2.26	0.422
TWA	1.25	0.70–2.21	0.451
HRV-RMSSD	1.16	0.50–2.68	0.728
DC 1	1.10	0.54–2.24	0.800
HRV-LFHF	0.96	0.51–1.78	0.885
SAF	0.90	0.22–3.74	0.887

Table 7. Multivariate models for computationally generated cardiac biomarkers, TRS, and ECG-based metrics (models constructed with stepwise backward elimination and stepwise forward selection using the Wald statistic) in patients with LVEF ≥ 40%. Computationally generated biomarkers are shown in bold.

	Adjusted HR	95% CI	P
A. Stepwise backward elimination using Wald statistic			
TRS 3	8.98	3.15–25.60	<0.001
TRS 2	3.64	1.29–10.30	<0.001
MV	2.50	1.50–4.19	<0.001
HRM	1.81	1.08–3.02	0.024
SM	1.64	0.98–2.75	0.061
HRV-ASDNN	1.57	0.94–2.64	0.085
B. Stepwise forward selection using Wald statistic			
TRS 3	9.16	3.21–26.10	<0.001
MV	2.53	1.51–4.22	<0.001
HRM	1.98	1.20–3.27	0.007
TRS 2	3.61	1.28–10.23	0.016
SM	1.59	1.09–2.82	0.046
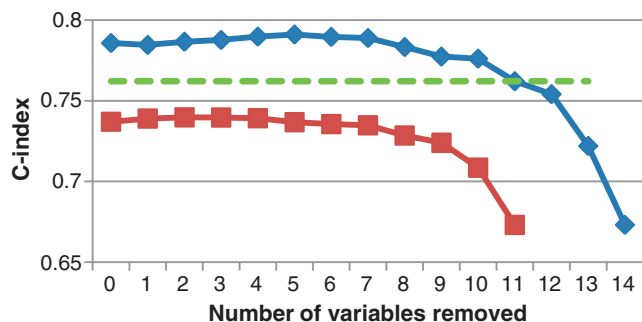
Fig. 5. C-index of multivariate models at each iteration of stepwise elimination with the Wald statistic in patients with LVEF $\geq$ 40% when all variables are used (blue top curve) and when all variables excluding the computationally generated cardiac biomarkers are used (red bottom curve). The elimination process is continued until all variables excluding TRS and LVEF are removed. The C-index of the multivariate model with only LVEF, TRS, and the computationally generated cardiac biomarkers is also shown (horizontal green line).

in the MERLIN-TIMI36 trial. Although we did not find any significant differences between patients with and without continuous ECG, the absence of continuous recordings reduced the size of our study population relative to the initial enrolment for the MERLIN-TIMI36 trial. LVEF, HRT, SAF, and SM values could also not be assessed in all patients in the MERLIN-TIMI36 trial, which reduced the size of multivariate models including these data. We further note that our study only included individuals who had experienced a recent NSTEACS. Although the computationally generated cardiac biomarkers might have utility in other populations such as those presenting with ST-segment elevation and in other patients with cardiac disease such as heart failure or even in the general population, this hypothesis needs to be evaluated in future work.

In conclusion, we believe that the computationally generated biomarkers discussed here could be readily integrated with clinical practice to improve both the precision and the recall of existing tools for risk stratification after NSTEACS. The addition of computationally generated biomarkers in our study increased by almost 50% the deaths predicted by echocardiography after NSTEACS while simultaneously improving precision. These improvements in precision and recall were backed by significant improvements assessed through a variety of other statistical measures. Each of the computationally generated biomarkers in our study can also be computed in a fully automated manner from Holter or telemetry data that are already routinely collected during hospitalization. By incorporating the algorithms to measure MV, SM, and HRM in monitoring devices by the bedside, or in systems where data from continuous monitoring may be uploaded, these computational biomarkers can be automatically assessed without imposing additional burden on either patients or caregivers. Ultimately, we envision a strategy for translating these methods to the clinic that involves measuring computationally generated biomarkers from inpatients after NSTEACS. These data could then be combined with more traditional indicators of high risk to effectively risk stratify patients in the post-NSTEACS setting, with the goal of using this information to decide how aggressively each patient should be treated and to tailor the frequency and extent of patient follow-up after discharge.

MATERIALS AND METHODS

Population
The MERLIN-TIMI36 trial (23, 24) was designed to compare the efficacy of ranolazine, an antianginal drug, to placebo in 6560 patients hospitalized with NSTEACS. Patients with moderate- to high-risk clinical features were enrolled within 48 hours of their last ischemic symptoms and received standard medical and interventional therapy according to local practice guidelines. Patients were followed for a median duration of 348 days. Continuous three-lead 128-Hz ECG recording (Lifecard CF, DelMark Reynolds/Spacelabs) was initiated at randomization (within 48 hours of the last ischemic discomfort) and continued for up to 7 days. A total of 4557 patients with available ECG data were included in this continuous ECG analysis. Full inclusion and exclusion criteria for the MERLIN-TIMI36 trial, as well as study procedures, have been published (23, 24).

ECG preprocessing
For all risk metrics, ECG signals were automatically segmented with the Physionet SQI package (25, 26). Ectopic and nonectopic beats were further distinguished with an automated beat classifier (EP Limited).

Computationally generated cardiac biomarkers
Each of the three computationally generated cardiac biomarkers was measured exactly as developed in earlier investigations on the TIMI-DISPERSE2 data and tested in a prespecific manner blinded to endpoints on the MERLIN-TIMI36 data.

Morphologic variability. MV was measured as described (17) by first using dynamic time warping (27) to quantify time-aligned energy differences between consecutive pairs of nonectopic heartbeats and by then measuring energy in the power spectrum of the resulting morphologic difference time series between 0.30 and 0.55 Hz (28). The Lomb-Scargle periodogram was used for frequency-domain measurements on the unevenly sampled time series resulting from the removal of ectopy (29, 30). MV was dichotomized at the highest quintile (51.84), consistent with the approach used in earlier studies, and used here for the various ECG metrics (31). The Supplementary Material details the process described in (17) through which MV was measured.

Symbolic mismatch. SM was measured as described (18). Long-term ECG signals for each patient were symbolized with iterative max-min clustering (32) with a dynamic time-warping distance metric (27), and the morphologies of the symbol centroids and dynamics of the symbols were compared between pairs of patients in the study population with the dissimilarity measure described (18). The resulting dissimilarity matrix was made positive semidefinite through spectrum clipping (33), and k-nearest neighbor-based anomaly detection was used to assign an anomaly score to each patient by measuring the dissimilarity relative to the closest 1% of individuals in the population. SM was dichotomized at the highest quintile (55.75). The Supplementary Material details the process described in (18) through which SM was measured.

Heart rate motifs. HRM was measured as described (19). The heart rate time series for each patient was reexpressed with symbolic aggregate approximation (SAX) (34), and the frequencies of high- and low-risk approximate patterns (that is, HRM) found in (19) were measured and used as input to a Cox proportional hazards regression model (35) trained on data from the TIMI-DISPERSE2 trial. HRM was dichotomized at the highest quintile (5.72). The Supplementary Material details the process described in (19) through which HRM was measured.

Existing risk metrics

The following existing risk metrics were assessed (details of the measurement of these metrics are provided in the Supplementary Material): HRV, HRT, DC, SAF, TWA, TRS, and LVEF.

Endpoint

A clinical events committee of certified cardiovascular specialists who were unaware of treatment assignment or continuous ECG results evaluated all deaths. Deaths were adjudicated as either cardiovascular or noncardiovascular. CVD was defined as any death for which there was no clearly documented noncardiovascular cause (24).

Statistical analyses

The risk of CVD associated with computationally generated biomarker was calculated with the HR and 95% CI estimated by use of a Cox proportional hazards regression model. Event rates are presented as Kaplan-Meier failure rates at 1 year. Each computationally generated biomarker was studied for univariate association with CVD and in two separate multivariate models. The first multivariate model included all other risk metrics, whereas the second was constructed with all metrics using stepwise backward elimination with the Wald statistic (36). This stepwise approach corresponded to creating an initial model with all the other risk metrics included, and then iteratively removing the least significant risk metric from this model as assessed by the probability of the Wald χ^2 statistic (with a removal testing threshold of 0.1). For the second model constructed with this stepwise approach, the C-index was also measured at each iteration of stepwise backward elimination as metrics were removed. This was done with the concordance statistic proposed by Harrell *et al.* (37), which measures the probability of concordance given the comparability of observations. A similar analysis with stepwise backward elimination was performed without any of the computationally generated biomarkers included. The correlation between the computationally generated biomarkers and all other metrics was also measured.

The increased discriminative value of the model with computationally generated biomarkers was further examined by the methods described by Pencina *et al.* (38). The NRI, which evaluated the degree of patients appropriately assigned to a higher or lower risk, was measured with Pencina's approach comparing groups for prediction rules. This approach measures the improvement in reclassification between two prediction rules A and B by separately computing the improvement in classifying patients who experienced events (that is, the fraction of these patients who are correctly moved to a higher-risk group by the prediction rule B versus the fraction of patients who are incorrectly moved to a low-risk group by the prediction rule B) and the improvement in classifying patients who remained event-free (that is, the fraction of these patients who are correctly moved to a lower-risk group by the prediction rule B versus the fraction of patients who are incorrectly moved to a higher-risk group by the prediction rule B). The improvements in patients who experienced events and remained event-free are then aggregated. In addition to the NRI, the ability of the computationally generated biomarkers to improve the precision and accuracy of prediction rules based on LVEF was also assessed.

We studied each of the risk metrics for an association with CVD in the entire population, as well as in the population with LVEF ≥ 40%. All analyses were performed in Stata 11 (Stata Corp.) and PASW/SPSS 18 (SPSS Inc.).

SUPPLEMENTARY MATERIAL

www.sciencetranslationalmedicine.org/cgi/content/full/3/102/102ra95/DC1
Materials and Methods
Table S1. Univariate association of existing ECG-based risk variables, TIMI risk score, and LVEF with CVD after ACS.
Table S2. Stepwise backward elimination and stepwise forward selection results with TIMI risk score excluded.
Table S3. Stepwise backward elimination and stepwise forward selection results in patients receiving placebo in the MERLIN-TIMI36 trial.
Table S4. Improvement in precision and recall with the addition of computational biomarkers in patients receiving placebo in the MERLIN-TIMI36 trial.
Table S5. Univariate association of premature ventricular contraction (PVC) frequency with CVD after ACS and results of stepwise backward elimination and stepwise forward selection when PVC frequency is included.
Table S6. Competing risk regression results for computationally generated cardiac biomarkers (with myocardial infarction as competing risk).
Table S7. List of acronyms used in the manuscript.
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