

Bedside-Friendly Prediction for Presence of Post-Myocardial Infarction Systolic Dysfunction Using Multimarker Panel: Integrating Salivary Diagnostics into Clinical Practice

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Background and Objectives: We investigated if a combination of plasma or salivary interleukin-2 (IL-2), interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α), transforming growth factor-beta (TGF- β), and troponin can improve estimation of the pretest probability of the left ventricular systolic dysfunction (LVSD).

Subjects and Methods: Eighty patients with newly-diagnosed myocardial infarction (MI) were echocardiographically examined for LVSD (ejection fraction $\leq 40\%$). Measurements included traditional MI risk factors, plasma and salivary concentrations of troponin, IL-2, IL-6, TNF- α , and TGF- β . With the LVSD as the outcome variable, we developed logistic regression models, starting with a basic model incorporating traditional risk factors and consecutively adding salivary and plasma biomarkers. Models were compared using several criteria, including (but not limited to) C statistic (discrimination) and net reclassification improvement index (NRI).

Results: Apart from troponin, plasma, and salivary values of the biomarkers were correlated: spearman's ρ was 0.19 ($p=0.088$) for troponin, 0.36 ($p=0.001$) for IL-2, 0.74 ($p<0.001$) for IL-6, 0.61 ($p<0.001$) for TNF- α , and 0.65 ($p<0.001$) for TGF- β . The predictive performances of the basic model for estimating the pretest probability of the presence of LVSD considerably improved when cytokines were added (salivary added: C-statistic from 0.77 to 0.82 and NRI 77%; plasma added: C-statistic to 0.80 and NRI 134%).

Conclusion: Multiple biomarkers added diagnostic value to the standard risk factors for predicting the presence of post-MI LVSD. (Korean Circ J 2013;43:246-254)

KEY WORDS: Interleukins; Transforming growth factor-beta; Tumor necrosis factor-alpha; Saliva; Left ventricular dysfunction.

Introduction

Heart failure due to left ventricular systolic dysfunction (LVSD) is a common, costly, disabling, and life-threatening condition.¹⁾ Both

conditions are highly prevalent and carry worse prognosis than several common cancers.^{2,3)} Echocardiography is the key investigation in the diagnosis of LVSD, but access is limited and there are delays between referral and final diagnosis. As such, there continue to be unmet needs for diagnosis of heart failure. It is therefore prudent to develop a clinical prediction algorithm based on clinical findings and diagnostic tests. Implementation of such algorithm would help clinicians prioritize patients and improve targeted referral of patients to limited echocardiography services.²⁾

Accumulating evidence indicates that inflammatory cytokines, like interleukin-2 (IL-2), interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α), and transforming growth factor-beta (TGF- β), play a pathogenic role in heart failure, contributing to the continuous myocardial remodeling process.⁴⁻⁷⁾ In clinical standpoint, however, it is not clear if (pro- and anti-) inflammatory cytokines' levels have any diagnostic value for currently prevalent but yet undiagnosed heart fail-

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ure.⁸⁾ Furthermore, examining cytokines mandates obtaining samples from the body that could be quite challenging.⁹⁾ Saliva is a unique fluid and interest in it as a diagnostic medium has advanced exponentially in the last decade. The ability to measure and monitor a wide range of molecular components in saliva and compare them to plasma components has made it feasible to study microbes, chemicals, and immunologic markers.¹⁰⁾¹¹⁾ Using saliva in diagnosis rather than blood may offer many advantages (easy access, non-invasive collection, etc).

To the best of our knowledge, whether the multimarker panel of novel biomarkers troponin, IL-2, IL-6, TNF- α , and TGF- β can help better predict the presence of LVSD among patients with newly-diagnosed myocardial infarction (MI) has never been examined. It is also not clear if salivary levels of these biomarkers could achieve the same predictive ability as their levels in plasma.

We sought, thus, to investigate 1) If a multimarker panel consisting of novel biomarkers IL-2, IL-6, TNF- α , TGF- β , and troponin can help improve predictive ability, while estimating the probability of the presence of LVSD among patients with recent MI. 2) If the levels of novel markers as measured in salivary samples could be as informative as are their plasma counterparts for predicting the probability of the presence of the LVSD. As such, we hypothesized that a panel of salivary biomarkers can be used instead of its plasma counterpart.

Subjects and Methods

Study population

During 2011-2012, among patients who were referred to a specialty Heart Hospital of Ahvaz and were found to have newly-diagnosed MI, we consecutively recruited patients if they were not known to have cancer, any oral, dental, gingival, or rheumatologic disorders that might have affected their salivary glands, as well as any immunologic disorder that might have affected the levels of the biomarkers in the plasma or saliva. The final sample consisted of 80 patients (34 females) with age ranging from 29 to 88 years.

Measurements

History taking and physical examination

A trained interviewer collected information using a pretested questionnaire. The information obtained included demographic data, past medical history of cardiovascular disease (CVD), and smoking status, past medical history, and drug history. After a 15-minute rest in the sitting position, two measurements of blood pressure were taken, on the right arm, using a standardized mercury sphygmomanometer; the mean of the two measurements was considered as the

participant's blood pressure.

Laboratory measures

Blood samples were taken at arrival (troponin and creatine phospho kinase) and during the first 24 hours of MI after 8 to 12 hours of overnight fasting. Samples were then centrifuged within 15 minutes of collection. Saliva samples were noninvasively obtained. Immediately after arrival, each patient was given a sterile falcon tube to slowly drain 8-10 mL of their saliva. Samples were immediately extracted and stored at -20°C until further assay within 2 months.

All the blood analyses were undertaken at the Cardiovascular Research Center laboratory. Serum glucose and cholesterol were assayed using an automated machine (Furona, Japan, 2012). An enzyme-linked immunosorbent assay method was used to determine plasma concentrations of saliva and blood troponin and cytokines levels (ELIZA kits: CUSABIO, Germany). This assay employs the competitive inhibition enzyme immunoassay. Measurements were double checked. All samples were analyzed when internal quality control met the acceptable criteria. The intra- and inter-assay coefficients of variation were all <1.0%.

Echocardiography

Measurement of ejection fraction (EF) with the use of echocardiography, after the index MI, was ascertained from the medical records.

Definitions

The following criteria were used to make the diagnosis for an acute, evolving or recent MI: time-dependent changes in troponin and MB fraction of creatine kinase (CK-MB) activity {typical rise and gradual fall (troponin) or more rapid rise and fall (CK-MB) of biochemical markers of myocardial necrosis} with at least one of the following:

- 1) Ischemic symptoms;
- 2) Development of pathologic Q waves on the electrocardiography (ECG);
- 3) Electrocardiographic changes indicative of ischemia (ST segment elevation or depression); or
- 4) Coronary artery intervention (e.g., coronary angioplasty).

According to the published guidelines, EF was calculated using end-diastolic and end-systolic volume as:

$$\frac{(EDV-ESV)}{EDV} \times 100$$

Left ventricular systolic dysfunction was defined as EF of $\leq 40\%$.

The diagnosis of hypertension was made when the average of two diastolic blood measurements was ≥ 90 mm Hg or when the aver-

age of two systolic blood pressure was ≥ 140 mm Hg or when participants self-reported of taking blood pressure lowering medication(s). Participants were classified as having diabetes at the baseline, if they met at least one of these criteria: FPG ≥ 7 mmol \cdot l⁻¹, or taking anti-diabetic medication. Hyperlipidemia was defined as suggested in the Executive Summary of The Third Report from The National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). A participant was considered to be a smoker if he/she answered "yes" to the question of "Have you been smoking during the last year?"

Statistics

Data are presented as the mean (SD) {or median (IQR)} and frequency (%) for continuously- and categorically-distributed variables, respectively. Deviation from normal distribution was examined by calculating Skewness. Before being incorporated into linear regression models, biomarkers' values were naturally log-transformed to attain normality. Spearman's ρ and linear regression model (while controlling for age and sex) were used to examine how salivary concentration of a biomarkers contributed to its plasma concentration.

Multivariate analysis

We developed a series of logistic regression models with LVSD as the outcome variable. The basic model (Model 1) developed by introducing traditional coronary artery disease (CAD) risk factors plus troponin into a logistic model. Traditional risk factors included age, sex, hypertension, hyperlipidemia, diabetes, and smoking. Two other models were also developed: Model 2 was developed by further adding salivary biomarker (troponin, IL-2, IL-6, TNF- α , and TGF- β) to Model 1; and Model 3 was developed by adding plasma biomarkers (IL-2, IL-6, TNF- α , and TGF- β) to Model 2. To avoid over-parameterizing of models, while taking into account the effects of cytokines, we used a propensity score for multimarker panels. As such, for each participant, the probability of the presence of the LVSD was calculated by using the logistic regression model. We, naturally, logarithmically transformed these probabilities to obtain "multimarker scores." Two enhanced models were, then, developed by consecutively adding "multimarker scores" based on both salivary and plasma biomarkers to the basic model. This approach allowed us to perform diagnostic checks on the fit of the models more reliably than if there were many covariates included in the models. As such, we were able to compare diagnostic performances of the enhanced models with those of the basic model, incorporating traditional CAD risk factors. To assess the diagnostic performances of the models, we took several steps.

Assessment of model performance

We used several criteria to compare the overall diagnostic values of alternative models.

Goodness-of-fit

How effectively a model describes the outcome variable is referred to as its goodness-of-fit. Akaike information criterion (AIC) was used to account for complexity. Difference in AIC > 10 was considered significant.

Discrimination

Discrimination refers to the ability to distinguish high risk subjects from low risk subjects, and is commonly quantified by a measure of concordance, the c statistic. For binary outcomes, c is identical to the area under the receiver operating characteristic (ROC) curve. As a general rule, if ROC=0.5, it suggests no discriminatory power; if $0.70 \leq \text{ROC} < 0.80$, this is considered acceptable discriminatory power; if $0.80 \leq \text{ROC} < 0.90$, this is considered excellent discriminatory power; and if $\text{ROC} \geq 0.90$, this is considered outstanding discriminatory power.

Calibration

Calibration describes how closely predicted probabilities agree numerically with actual outcomes. We examined calibration using the Hosmer-Lemeshow test.

Added predictive ability

Traditionally, risk models have been evaluated by using the Harrell's c statistic, but this method has not insensitive enough in comparing models and is of little direct clinical relevance. New methods have recently been proposed to evaluate and compare the predictive risk models. These are based primarily on stratification into clinical categories on the basis of estimated probabilities and attempt to assess the ability of new models to more accurately reclassify individuals into higher or lower probability strata. Absolute and relative integrated discriminatory improvement index and cut-point-based and cut-point-free net reclassification improvement index were used as measures of predictive ability added to the traditional MI marker, troponin, by novel markers. Reclassification improvement is defined as an increase in the probability category for patients with the events and as a decrease for those without the event. Net reclassification improvement considers movement between categories in the wrong direction and applies different weights to events and nonevents.

Homogeneity in the strength of associations

Valid comparison of odds ratios (ORs) for different continuous measures requires that the units of both variables to be compara-

ble. We, thus, estimated ORs, with 95% confidence intervals (CIs) for the presence of LVSD for a one-unit increment in each "multimarker score". Wald tests of the linear hypotheses concerning the logistic regression models coefficients (paired homogeneity test) were performed to test the null hypotheses that the OR (effect size) for salivary levels of a biomarker was equal to that for its plasma counterpart.

We hereby certify that all applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during this research. Informed written consent was obtained from all participants and the Ethical Committee of the Ahvaz Jundishapur University of Medical science approved the design of this study.

The statistical significance level was set at a two-tailed type I error of 0.05. All statistical analyses were performed using STATA version 12 (STATA, College Station, TX, USA) and SAS 9.2 (SAS Institute Inc., Cary, NC, USA).

Results

Table 1 presents the baseline characteristics of the participants. The median (IQR) age of participants was 59 (20) for men and 62.5 (14) for women. As compared to men, diabetes, hyperlipidemia, and hypertension were more commonly observed among women. Smoking was more prevalent in men than women. Fifteen (18.8%) participants were found to have LVSD.

As shown in Table 2, the mean values of cytokines were not different among those with and without traditional CAD risk factors.

As shown in Table 3, log-transformed values of the salivary and plasma concentrations of different cytokines were not associated with log-transformed values of the plasma concentration of the troponin.

Apart from troponin, plasma and salivary values of the biomarkers under the investigation were correlated: spearman's ρ was 0.19 ($p=0.088$) for troponin, 0.36 ($p=0.001$) for IL-2, 0.74 ($p<0.001$) for IL-6, 0.61 ($p<0.001$) for TNF- α , and 0.65 ($p<0.001$) for TGF- β . As shown in Table 4, the fraction of variance in the log-transformed values of the plasma levels of biomarkers explained by their salivary counterparts was 29% for IL-2, 55% for IL-6, 38% for TGF- β and 41% for TNF- α .

Table 5 compares the predictive ability for estimating the pretest probability for the presence of LVSD between the basic model and the two enhanced model, one with salivary and the other with both salivary and plasma cytokines, in addition to the traditional risk factors.

The predictive performances of the basic model (Model 1) for estimating the pretest probability of the presence of LVSD consider-

Table 1. Baseline characteristics of participants

	Women (34)	Men (46)
Age (years)	62.5 (14)	59 (20)
Diabetes	20 (0.59)	13 (0.28)
Hypertension	16 (0.47)	12 (0.26)
Hyperlipidemia	14 (0.41)	9 (0.20)
Smoking	5 (0.15)	9 (0.20)
Salivary troponin (ng · mL ⁻¹)	85.08 (38.68)	103.77 (42.23)
Plasma troponin (ng · mL ⁻¹)	87.78 (34.58)	80.66 (40.03)
Salivary interleukin-2 (pg · mL ⁻¹)	49.73 (39.56)	38.02 (29.60)
Plasma interleukin-2 (pg · mL ⁻¹)	80.13 (74.57)	71.78 (63.57)
Salivary interleukin-6 (pg · mL ⁻¹)	5.91 (10.16)	6.24 (11.46)
Plasma interleukin-6 (pg · mL ⁻¹)	9.64 (23.29)	7.36 (26.86)
Salivary transforming growth factor (pg · mL ⁻¹)	8.73 (10.32)	11.30 (14.56)
Plasma transforming growth factor (pg · mL ⁻¹)	24.10 (44.53)	15.87 (50.56)
Salivary tumor necrotic factor (pg · mL ⁻¹)	52.41 (36.90)	48.37 (37.34)
Plasma tumor necrotic factor (pg · mL ⁻¹)	16.63 (11.05)	12.80 (16.07)

Figures are presented as either median (interquartile range) or number (%) for continuously- and categorically-distributed variables, respectively

ably improved when salivary biomarkers were added (Model 2). However, further adding serum biomarkers to model 2 did not consistently improve its predictive ability.

The multivariate-adjusted OR for the presence of the LVSD were 2.9 (95% CIs: 0.9-8.8) and 3.5 (95% CIs: 1.4-8.8) for a one unit increase in values of the salivary and plasma multimarker scores, respectively (Wald $\chi^2=0.24$, $p=0.622$).

Discussion

Main findings

In a population of patients with newly-diagnosed MI, we observed that a combination of the salivary levels of troponin, and (pro- and anti-) inflammatory cytokines IL-2, IL-6, TNF- α , and TGF- β alone, or in combination with their plasma counterparts, can statistically significantly and clinically meaningfully improve the prediction of having LVSD.

With advances in the treatment of CAD, most patients are surviving longer, and inasmuch as the CAD is the strongest risk factor for congestive heart failure, the number of patients at risk of congestive heart failure continues to grow.^{11,12,13} Current guidelines emphasize early identification of patients who are at risk for morbidity and mortality, which is important for the prevention or treatment for heart failure. Echocardiography has become one of the most com-

Table 2. The distribution of cytokines by cardiovascular risk factors

	Mean	SD	Mean	SD	p
Male sex (pg · mL ⁻¹)	No		Yes		
Salivary interleukin-2	56.74	27.01	63.02	95.64	0.711
Plasma interleukin-2	90.36	49.28	94.32	123.05	0.860
Salivary interleukin-6	10.02	11.30	12.96	16.73	0.378
Plasma interleukin-6	18.69	22.05	18.43	22.62	0.959
Salivary transforming growth factor	14.75	13.78	21.02	27.50	0.226
Plasma transforming growth factor	36.59	36.84	58.18	147.42	0.407
Salivary tumor necrotic factor	52.49	22.82	68.77	141.30	0.508
Plasma tumor necrotic factor	19.08	12.65	19.07	19.62	0.998
Smoking (pg · mL ⁻¹)	No		Yes		
Salivary interleukin-2	64.19	81.12	42.23	15.62	0.318
Plasma interleukin-2	97.12	106.07	71.51	41.91	0.379
Salivary interleukin-6	12.90	15.79	6.12	4.26	0.117
Plasma interleukin-6	17.89	20.85	21.63	28.63	0.571
Salivary transforming growth factor	18.92	24.45	15.70	12.75	0.635
Plasma transforming growth factor	50.78	124.48	40.61	42.46	0.764
Salivary tumor necrotic factor	52.22	29.14	107.24	252.79	0.083
Plasma tumor necrotic factor	19.10	14.12	18.97	27.22	0.980
Hypertension (pg · mL ⁻¹)	No		Yes		
Salivary interleukin-2	65.12	90.47	51.48	24.08	0.437
Plasma interleukin-2	99.35	117.18	80.17	45.03	0.408
Salivary interleukin-6	11.63	13.95	11.87	16.17	0.944
Plasma interleukin-6	15.91	17.84	23.42	28.43	0.151
Salivary transforming growth factor	19.29	23.93	16.61	20.86	0.619
Plasma transforming growth factor	56.55	138.81	34.99	38.80	0.424
Salivary tumor necrotic factor	69.41	132.63	47.81	23.76	0.397
Plasma tumor necrotic factor	18.53	18.63	20.09	13.41	0.698
Hyperlipidemia (pg · mL ⁻¹)	No		Yes		
Salivary interleukin-2	61.10	86.19	58.48	30.43	0.888
Plasma interleukin-2	96.49	113.20	83.09	43.28	0.584
Salivary interleukin-6	12.22	14.15	10.45	16.13	0.629
Plasma interleukin-6	16.86	18.62	22.70	29.47	0.291
Salivary transforming growth factor	17.31	18.33	20.94	31.66	0.523
Plasma transforming growth factor	36.95	39.22	78.87	204.15	0.139
Salivary tumor necrotic factor	66.09	127.04	51.32	24.74	0.583
Plasma tumor necrotic factor	17.57	16.02	22.82	18.80	0.211
Diabetes (pg · mL ⁻¹)	No		Yes		
Salivary interleukin-2	63.62	94.60	55.68	27.15	0.641
Plasma interleukin-2	100.49	121.93	81.46	47.07	0.397
Salivary interleukin-6	13.24	15.80	9.53	12.78	0.267
Plasma interleukin-6	18.83	20.21	18.14	25.17	0.982
Salivary transforming growth factor	18.65	19.51	17.93	27.14	0.890
Plasma transforming growth factor	38.72	41.00	63.65	171.61	0.400
Salivary tumor necrotic factor	70.86	139.28	49.01	24.56	0.376
Plasma tumor necrotic factor	17.76	17.30	20.96	16.42	0.408

Table 3. Correlation between plasma troponin and different cytokines

	β -coefficient	p	R-squared
Salivary interleukin-2 (ng · mL ⁻¹)	0.082	0.434	0.008
Plasma interleukin-2 (pg · mL ⁻¹)	0.015	0.874	0.000
Salivary interleukin-6 (pg · mL ⁻¹)	-0.009	0.870	0.000
Plasma interleukin-6 (pg · mL ⁻¹)	-0.033	0.513	0.006
Salivary transforming growth factor (pg · mL ⁻¹)	-0.040	0.558	0.004
Plasma transforming growth factor (pg · mL ⁻¹)	-0.046	0.374	0.010
Salivary tumor necrotic factor (pg · mL ⁻¹)	-0.047	0.581	0.004
Plasma tumor necrotic factor (pg · mL ⁻¹)	0.005	0.944	0.000

Table 4. Correlation between plasma and salivary levels of selected biomarkers

	β -coefficient	Standard error of β	p	R-squared
Salivary & Plasma interleukin-2	0.48	0.08	0.000	0.29
Salivary & Plasma interleukin-6	0.71	0.07	0.000	0.55
Salivary & Plasma TGF- β	0.47	0.07	0.000	0.38
Salivary & Plasma TNF- α	0.59	0.08	0.000	0.41
Salivary & Plasma troponin	0.18	0.13	0.178	0.01

Values have been naturally log-transformed before introducing into regression models and β -coefficients have been standardized. TGF- β : transforming growth factor-beta, TNF- α : tumor necrotic factor-alpha

monly used noninvasive modalities for assessment of ventricular volumes and function, and can provide prognostic information for the prediction of future heart failure events.¹³⁾

The post-MI LVSD can be partially attenuated by early administration of angiotensin converting enzyme (ACE) inhibitors and vasodilator therapy by reducing left ventricular loading conditions.¹⁴⁾ However, the period for which ACE inhibitor therapy should be continued and the group of patients for whom prolonged therapy is efficacious remain to be illustrated. Further studies are required to demonstrate the cost-effectiveness for prolonged therapy in diseases as prevalent as MI.

Thus, there is a growing need to develop a simple clinical algorithm to identify early patients at high risk for left ventricular dysfunction, who may be targeted for more aggressive treatment or for a prolonged period of time for survival benefit and to reduce adverse events and to discontinue therapy early in patients at low risk.¹⁵⁾

A number of studies have evaluated combinations of biomarkers for predicting CVD in the community-based setting with few having found substantial improvements in risk prediction with a multimarker approach.¹⁶⁻¹⁸⁾ The secondary prevention setting has been rarely focused upon, while evaluating a multimarker risk stratification approach. A combination of markers from independent pathophysiological pathways is likely to improve predictive information, as well as shedding light on potential novel targets for therapeutic interventions.¹⁹⁾

An exaggerated response of specific parts of the innate immune

system after MI causes additional injury among patients with MI leading to remodeling²⁰⁾ and quickening the appearance of LVSD, which on its own turn leads to congestive heart failure.²¹⁾ The cytokine hypothesis presently suggests that an excessive production of pro-inflammatory cytokines, such as TNF- α and IL-6, contributes to the pathogenesis of heart failure.²²⁾ In fact, many of the clinical hallmarks of heart failure, including LVSD, cardiomyopathy, and pulmonary edema can be explained by the known biological effects of TNF- α .²³⁾ Elevated values of circulating inflammatory markers, such as IL-6 commonly accompany CAD. Such elevations correlate with in-hospital and short-term adverse prognosis.²⁴⁾²⁵⁾ Elevated levels of several inflammatory cytokines among apparently healthy individuals have been proven to have predictive value for future vascular events.²⁴⁻²⁷⁾ It has become apparent that cytokines are expressed in the setting of heart failure.²²⁾ Furthermore, individuals with elevated levels of TNF- α were at increased risk for coronary death and recurrent MI. Thus, inflammation has a long-term prognostic value among apparently stable patients.²⁸⁾

Clinical implications

The incremental usefulness of adding multiple biomarkers from different disease pathways for predicting the risk of death from cardiovascular causes has been rarely examined.¹⁶⁾ A multi-marker risk prediction approach, which includes several newer biomarkers simultaneously, has been studied with the goal of improving the accuracy and clinical utility for prediction of cardiovascular mortality

Table 5. Predicting presence of low ejection fraction (<40%) using salivary cytokines

Model 1	
Akaike information criteria	67.0
Harrell's c index of discrimination	0.787 (0.651-0.922)
Hosmer-Lemeshow χ^2 (p)	2.43 (0.965)
Model 2	
Akaike information criteria	63.1
Harrell's c index of discrimination	0.832 (0.705-0.959)
Hosmer-Lemeshow χ^2 (p)	7.85 (0.448)
Model 3	
Akaike information criteria	57.9
Harrell's c index of discrimination	0.890 (0.806-0.954)
Hosmer-Lemeshow χ^2 (p)	4.4 (0.821)
Model 1 vs. Model 2	
Absolute IDI	0.08 (0.03-0.13)
Relative IDI	0.43 (0.12-0.74)
Cutpoint-based NRI	0.11 (-0.01-0.23)
Cutpoint-free NRI	0.90 (0.47-1.33)
Model 2 vs. Model 3	
Absolute IDI	0.06 (-0.01-0.13)
Relative IDI	0.24 (-0.06-0.53)
Cutpoint-based NRI	0.24 (0.03-0.45)
Cutpoint-free NRI	0.63 (0.15-1.10)

The basic model (Model 1) developed by introducing traditional CAD risk factors plus troponin into a logistic model. Traditional risk factors included age, sex, hypertension, hyperlipidemia, diabetes, and smoking. Two other models were also developed: Model 2 was developed by further adding salivary biomarker (troponin, IL-2, IL-6, TNF- α , and TGF- β) to the Model 1; and Model 3 was developed by adding plasma biomarkers (IL-2, IL-6, TNF- α , and TGF- β) to Model 2. 1) Odds ratios have been reported for a 1-SD change in each of covariates. 2) For cutpoint-based NRI, the cutpoints were set at 0.2 and 0.4. IDI: integrated discriminatory improvement index, NRI: net reclassification improvement index, CAD: cardiovascular disease, IL-2: interleukin-2, IL-6: interleukin-6, TNF- α : tumor necrosis factor-alpha, TGF- β : transforming growth factor-beta

and morbidity.¹⁶⁾

Since increased risk of LVSD with elevated levels of cytokine multi-marker scores were independent of traditional risk factor, current data supports the possibility that novel therapies designed to modulate the inflammatory response after MI may be a new horizon in the treatment of MI.²⁸⁾

Daniels and Maisel argued that established risk scores, such as the Framingham Risk Score, are appropriate for risk prediction in a community-based setting, and can help direct appropriate use of preventive lifestyle changes and medications. In higher risk populations and in secondary prevention populations, risk assessment with biomarkers may further improve stratification and could point to pathway-specific, targeted therapeutic interventions.¹⁹⁾ In the light of

the correlation between cytokines salivary and plasma levels the current finding that pro- and anti-inflammatory cytokines helped improve prediction of the presence of LVSD sparks a glimpse of hope for more feasibility to monitor post-MI patients for the development of heart failure. Further studies with longitudinal design will be required to examine the prognostic value for heart failure of the novel biomarkers as compared to that of echocardiographic findings.

Biomarkers are more sensitive, more specific and less costly than imaging techniques for the diagnosis of myocardial necrosis. Injury involving >20% of myocardial wall thickness is required before a segmental wall motion abnormality can be detected by echocardiography.²⁹⁾

One of the major therapeutic challenges in the arena of secondary prevention is to design strategies aimed at reducing myocardial tissue damage after MI. A prerequisite for wound healing after MI is the response to tissue injury by innate immune system, which orchestrates homeostatic responses. An exaggerated inflammatory reaction, however, countervails these beneficial effects and contributes to maladaptive tissue damage.²⁰⁾ We observed that IL-2 was inversely associated with the probability of the presence of LVSD. IL-2 might have played a role in balancing such an exaggerated immune response.

These new insights into the role of inflammation plays in atherosclerosis have practical clinical applications in risk stratification and targeting of therapy for this scourge of growing worldwide importance.²⁶⁾ However, many inflammatory molecules are involved in the atherothrombotic process, and some of them may reflect plaque-related activity more directly.²⁵⁾ Thus, other novel inflammatory markers may provide different information and thereby enhance risk stratification. We obtained biomarker levels before any data were available on the anatomy of the coronary arteries, and before starting any reperfusion treatment. That is, if confirmed in larger studies to be associated with LVSD, the multimarker panel suggested herein can be used in settings where access to angiography is limited and may help choosing a treatment modality.

Strength and limitations

The current findings should be interested in light of its limitations. Our study sample was not large enough to allow us to examine the homogeneity in the strength of association with LVSD of different cytokines. However, response to post-MI tissue damage issued by immune system involves many pro- and anti-inflammatory cytokines with complicated interplay, like the interaction between Reperfusion Injury Salvage Kinase pathway and a novel path, Survival Activating Factor Enhancement (SAFE) pathway. SAFE pathway is a pro-survival cardio-protective signaling pathway activated by pro-

inflammatory cytokines.³⁰⁾ Therefore, in order to save the statistical power, it is prudent to introduce a combination of cytokines as a risk score into regression models, while the aim of the study is to best predict an outcome rather than exploring the association with a single cytokine. Considering the cross-sectional nature of the study, we cannot clarify if these biological factors are markers or predictors of LVSD. Further studies with longitudinal designs will be required in the future to elucidate the causal relationship. In this paper, we used clinical parameters (age, sex, hypertension, hyperlipidemia, diabetes, and smoking) for the reference risk prediction model. Although some variables, such as age, may be considered as a risk for LVSD, most of these variables are related more to an increased risk of development of atherosclerotic vascular disease than increased risk for development of LVSD. The model incorporating such clinical variable might have not achieved a good predictive power for LVSD, in particular, among patients with acute MI. The poor predictive power of reference model can potentially render the predictive power of the multimarker panel biased toward over-estimation. Further studies will be needed in the future to examine if such multimarker panel could add predictive values to what could be achieved by cardiac enzyme, ECG, timing of reperfusion, presence of sign or symptom for heart failure, or vital signs. After MI, progressive LV enlargement (remodeling) is more important than echocardiographically estimated LV systolic dysfunction at the acute or subacute stage. Therefore, a cross sectional assessment of LV systolic function was not enough to evaluate the usefulness of salivary cytokine measurement. Finally, our study sample consisted of MI patients; the results obtained here, therefore, may not directly be applicable to asymptomatic patients.

In conclusion, multiple biomarkers of cardiovascular stress detectable in saliva add diagnostic value to the standard risk factors for predicting LVSD. The novel biomarkers can help improve the prediction for presence of LVSD in post-MI patients and may be useful in identifying high-risk patients who might benefit from aggressive interventions. The integration of salivary diagnostics into the clinical practice of cardiology can possibly be a new horizon to enable chair-side prognostication of post-MI mortality or morbidity.

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