Usefulness of Soluble Fas Levels for Improving Diagnostic Accuracy and Prognosis for Acute Coronary Syndromes

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Although both inflammation and apoptosis occur in acute coronary syndromes (ACSs), previous studies have not tested the diagnostic and prognostic utility of an approach that measures circulating markers of these pathways. The aim of the present study was to assess whether measuring soluble Fas (sFas) and high-sensitivity C-reactive protein (hs-CRP), as markers of apoptosis and inflammation, improve ACS diagnostic and prognostic accuracy. In a prospective cohort of consecutive subjects admitted to the hospital for suspicion of ACS, we measured sFas, hs-CRP, and troponin T in those who had a final noncardiac chest pain diagnosis (n = 100), those who had an ACS diagnosis and experienced (n = 218) or did not experience (n = 170) recurrent cardiac events during 1 year of follow-up. sFas was strongly and independently associated with a discharge diagnosis of an ACS versus noncardiac chest pain during the index hospitalization (odds ratio 16.16 for the second vs first tertile, 95% confidence interval [CI] 7.07 to 36.91; and odds ratio 25.40 for the third vs first tertile, 95% CI 9.38 to 68.75). However, hs-CRP was not. sFas significantly improved the diagnostic accuracy for ACSs (C statistic increased from 0.85 to 0.93, difference +0.08, 95% CI for the difference 0.05 to 0.11). The sFas levels were high and did not vary with time in the subjects having early versus late measurements (β 0.00 ln pg/ml/hour, 95% CI −0.01 to 0.01). In contrast, troponin increased with time since the beginning of the symptoms (β 0.07 ln μg/L/hour, 95% CI 0.04 to 0.10). Baseline sFas and hs-CRP did not predict recurrent cardiac events. In conclusion, our results suggest that in suspected ACS cases, sFas, but not hs-CRP, helps to improve the diagnostic accuracy and timeliness over and above standard diagnostic criteria. **(Am J Cardiol 2010;105:797–803)**

The diagnosis of acute coronary syndromes (ACSs) remains challenging. Chest pain can be atypical, and myocardial necrosis marker levels are often normal at admission[1] and can remain negative in patients with unstable angina pectoris (UAP). Identifying the subjects who are at greatest risk of recurrence is also important for clinical management. A strategy that takes into account multiple relevant pathophysiologic pathways might help in improving both ACS diagnostic and prognostic accuracy. Hence, the aim of the present study was to assess whether a multimarker approach that includes soluble Fas (sFas), high-sensitivity C-reactive protein (hs-CRP), and troponin as markers of apoptosis, inflammation, and necrosis can improve the diagnostic accuracy and timeliness in patients with suspected ACS. We also wished to determine whether this approach could predict recurrent cardiac events in those who experienced an initial ACS.

Methods

This was a case-control study, which was nested within a prospective cohort, the *Récurrence et inflammation dans les syndromes coronariens aigus* (RISCA) study.[2] A nested case-control approach was chosen for efficiency motives and yielded unbiased estimates of the hazard ratio from a cohort study, provided that controls can become future cases.[3] The recruitment phase of the RISCA study began in 2000 and ended in early 2002. In all, 1,210 patients participated in the present study. To fully reflect the spectrum of practice patterns, 4 community and 4 tertiary participating centers were included. All consecutive patients hospitalized with an admitting diagnosis of an ACS (UAP or acute myocardial infarction [AMI]) were eligible for inclusion if they were approached within 24 hours of the end of ischemic symptoms. Clinical management was left to the discretion of the treating physicians. For inclusion in this cohort, AMI was defined as characteristic discomfort or pain with an elevation of creatine kinase-MB to ≥1.5 times

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the upper limit of normal and/or troponin I or T in the positive range according to each center's cutoff value diagnostic of AMI. The diagnosis of UAP required either one episode of typical chest pain at rest or with minimal exertion lasting ≥10 minutes or ≥2 episodes, each lasting ≥5 minutes. This could be either new-onset angina or an abrupt and significant change in the pattern of established angina, with creatine kinase-MB and/or cardiac troponin I or T levels remaining below the threshold for the diagnosis of AMI. No specific electrocardiographic criteria were necessary to be included in the RISCA study with an initial diagnosis of ACS.

From this cohort, we selected all patients who had experienced our primary outcome (recurrent AMI, UAP, or cardiac death within 1 year after their initial hospitalization) and randomly selected controls, matched for age (±5 years), gender, and follow-up duration (±0 days) in a 1:1 ratio. In a nested case-control approach, controls can serve more than once for different cases, the reason the control group could be smaller than the case group, even when matched in a 1:1 ratio.[3] Although the RISCA cohort was specifically selected to have a high probability of ACS (only hospitalized patients were approached), a small group of discharged patients had a final noncardiac chest pain diagnosis. Biomarkers were not measured in the whole cohort but only in patients with recurrence, their matched controls, and the noncardiac chest pain group. The institutional review committee of each of the participating hospitals approved the study, and all subjects gave informed consent.

Experimental blood samples were taken within 24 hours of the end of chest pain. After centrifugation, the plasma samples were distributed in aliquots of 2 ml each, stored locally at −70°C, and sent on dry ice to the central repository where they were stored at −80°C. Clinical data collection was performed prospectively by trained research nurses at each study site and systematically validated initially by the local principal investigator and finally by on-site chart review by the central investigators. The patients were followed up at 1 month after discharge (on-site visit) and 1 year after discharge (telephone interview), obtaining, as necessary, confirmatory hospital files.

Citrated plasma levels of sFas were measured at baseline using a commercially available enzyme-linked immunosorbent assay kit (Bender MedSystems, Vienna, Austria). The coefficient of variability among plaques was 6.33%. hs-CRP was measured using the N High-Sensitivity CRP mono assay using the BN ProsPec Nephelometer (Dade Behring, Deerfield, Illinois). In addition to each center measuring cardiac troponin T or I for clinical purposes, cardiac troponin T was measured centrally using a commercial assay (Roche, Mannheim, Germany) with a detection limit for myocardial injury of 0.1 μg/L.

Detailed clinical variables, as well as the baseline laboratory values for maximal creatine kinase, creatine kinase-MB, and creatinine (to estimate the glomerular filtration rate using the Modification of Diet in Renal Disease abbreviated equation[4]) were recorded prospectively. The admission electrocardiogram was centrally adjudicated for all patients. ST-segment elevation or depression ≥1 mm (not necessarily new or transient) in ≥2 contiguous leads was recorded.

Table 1
Baseline clinical characteristics (n = 488)

Baseline Characteristic	Noncardiac Chest Pain (n = 100)	Nonrecurrent ACS (n = 170)	Recurrent ACS (n = 218)
Age (years)	62 ± 10	65 ± 12	66 ± 12
Men	61 (61%)	121 (71%)	152 (69%)
Previous coronary artery disease	56 (56%)	73 (43%)	142 (65%)
Previous vascular disease	26 (26%)	26 (15%)	69 (32%)
Previous heart failure	2 (2%)	9 (5%)	29 (13%)
Hypertension	58 (58%)	87 (51%)	134 (61%)
Active smoker	26 (26%)	46 (27%)	60 (27%)
Past smoker	59 (59%)	87 (51%)	110 (50%)
Never smoked	15 (15%)	37 (22%)	49 (22%)
Dyslipidemia	72 (72%)	100 (59%)	152 (69%)
Diabetes mellitus	22 (22%)	24 (14%)	63 (29%)
Insulin-dependent diabetes	0	5 (3%)	25 (11%)
Recent infection or inflammation	16 (16%)	15 (8%)	29 (13%)
LV ejection fraction (%)			
Median	60	59	50
Interquartile range	55–65	50–62	42–60
Heart failure at baseline	2 (2%)	21 (10%)	39 (18%)
Per-hospital revascularization	1 (1%)	112 (51%)	94 (42%)
Mean body mass index (kg/m^2)	27 ± 4	26 ± 4	28 ± 8
Creatinine clearance (ml/min/1.73 m^2)	71 ± 18	67 ± 24	68 ± 31
Cardiac troponin T (μg/L)			
Median	0.01	0.48	0.17
Interquartile range	0.01–0.01	0.04–2.20	0.10–1.38
ST-segment deviation (baseline electrocardiogram)	3 (3%)	84 (49%)	97 (44%)

The baseline left ventricular ejection fraction was measured using echocardiography, scintigraphy, or angiography.

The initial discharge diagnoses of UAP, AMI, or noncardiac chest pain were made by the treating physicians and recorded from the medical chart. The principal investigator of the RISCA study (PB) reviewed all charts to ensure that these discharge diagnoses were consistent with the local cardiac enzyme and troponin definition limits, as well as the central laboratory troponin T measurements. When this was not the case, the diagnosis was adjudicated to reach conformity with these criteria. The diagnostic criteria for recurrent UAP and AMI were the same as those used for the initial diagnoses. All recurrent end points, with their relevant electrocardiograms, were centrally adjudicated independently by 2 investigators. In the case of disagreement, the final decision was reached by a third investigator. All diagnoses were made and fixed before the sFas and hs-CRP measurements were performed. The loss to follow-up at 1 year was minimal (0.3%).

The primary objectives were to determine: (1) the independent relation among sFas, hs-CRP, and an initial hospitalization discharge diagnosis of ACS (vs noncardiac chest pain); and (2) the independent relation among sFas, hs-CRP, and recurrent cardiac events and death during 1 year of follow-up. Normally distributed variables are presented as the mean ± SD, and non-normally distributed variables as the median with the interquartile range (twenty-fifth and seventy-fifth percentiles). Categorical variables are summarized using proportions. We performed initial bivariate analyses to identify potential confounders. Spearman correlation

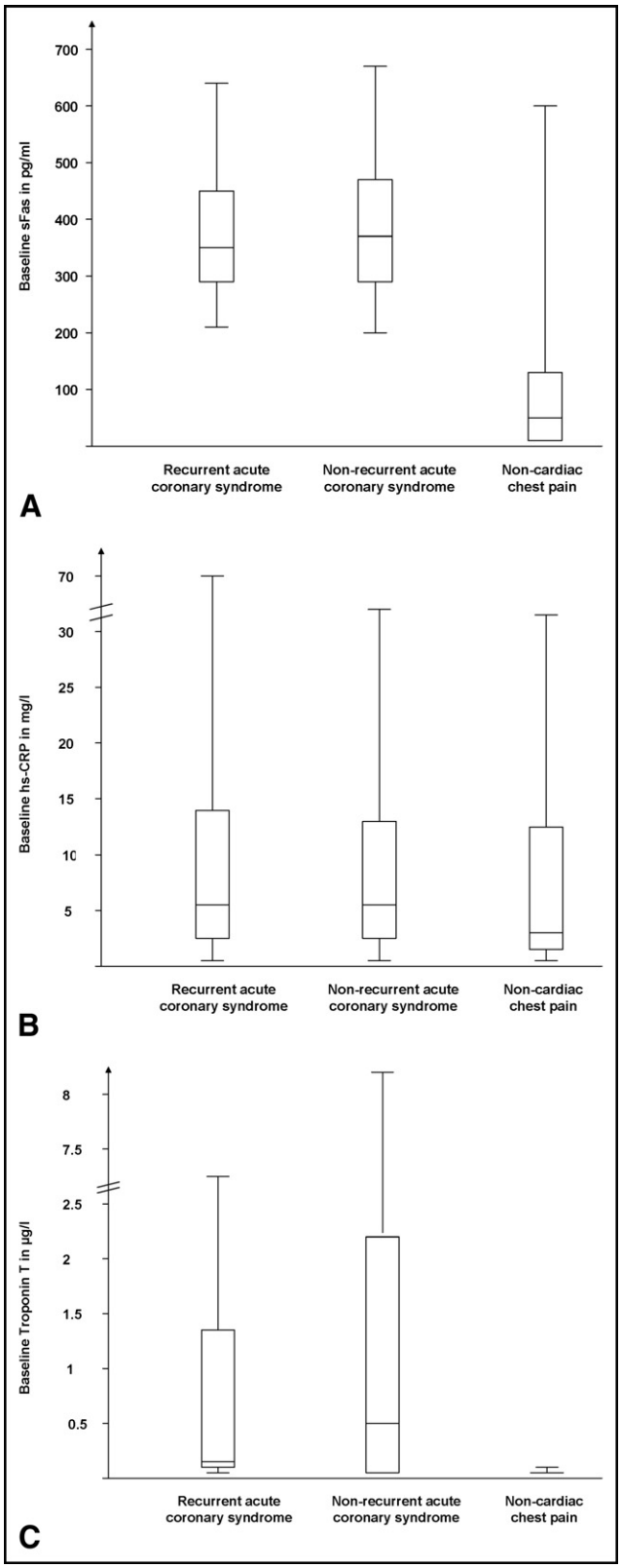

Figure 1. *(A)* sFas, *(B)* hs-CRP, and *(C)* troponin T levels in patients with discharge diagnosis of ACS who experienced recurrent cardiac events during 1 year of follow-up (recurrent ACS), those with a discharge diagnosis of ACS who did not experience recurrence during the 1-year follow-up (nonrecurrent ACS), and those whose initial discharge diagnosis was noncardiac chest pain. Boxplots represent medians, interquartile ranges, and fifth to ninety-fifth percentiles for distribution of biomarkers in 3 groups. sFas ($p = 0.001$), hs-CRP ($p = 0.01$), and troponin T ($p = 0.001$) levels were higher in subjects with ACS than in those with noncardiac chest pain (Wilcoxon rank sum test).

coefficients (continuous covariates) were also used to assess the relation among sFas, hs-CRP, and the covariates, including the necrosis markers. The association between the biomarker levels and the discharge diagnosis of ACS was assessed using standard logistic regression analysis, in univariate and multivariate models. Linearity on the logit scale was checked by graphing methods for each continuous variable. Because they were nonlinear, sFas and hs-CRP were categorized into tertiles and analyzed as categorical variables. The incremental value of the sFas measurement for the diagnosis of ACS was determined by comparing the area under the receiver operating curve[5] for models based on standard diagnostic criteria (electrocardiographic changes, elevated necrosis markers) with and without the addition of sFas and hs-CRP. We examined temporal trends in sFas and troponin T levels in patients with ACS using simple linear regression models in which the log transformed values of these markers were included as dependent variables and the interval since symptom onset was included as the independent variable. The normality assumption for residuals was verified visually and met. Associations among sFas, hs-CRP, and recurrent cardiac events were assessed using conditional logistic regression analysis in univariate and multivariate models. All statistical analyses were performed using Statistical Analysis Systems, version 9.1 (SAS Institute, Cary, North Carolina).

Results

To address the importance of apoptosis, inflammation, and necrosis biomarker levels in the diagnosis and prognosis of ACS, we measured sFas, hs-CRP, and troponin T in all patients with recurrent ACS ($n = 218$), their matched control ($n = 170$), and all subjects with a final noncardiac chest pain diagnosis ($n = 100$). The patients with a final discharge diagnosis of noncardiac chest pain were more likely to be younger and female and to have better cardiac and renal function and were less likely to have insulin-dependent diabetes (Table 1). By definition, their troponin levels were not elevated. Three subjects in this group had 1-mm ST-segment depression in ≥ 2 contiguous leads on their baseline electrocardiogram. However, these abnormalities were already present on previous electrocardiograms and judged to be nonevolutive. Among those who were discharged with a diagnosis of ACS, the subjects who experienced recurrent cardiac events were more likely to have a history of heart failure, diabetes, or coronary or peripheral vascular disease. Their mean body mass index was slightly greater, and they had experienced more heart failure episodes at baseline. They underwent revascularization slightly less often than subjects without recurrence. sFas and hs-CRP were weakly associated with each other, as well as with troponin, maximal creatine kinase, and a lower glomerular filtration rate.

Table 2
Regression models for diagnosis of acute coronary syndrome (ACS) (n = 488)

Baseline Characteristics	Crude OR (95% CI)	Adjusted OR (95% CI)	
		Model 1*	Model 2†
Age (per 10-year increase)	1.32 (1.09–1.60)	1.09 (0.78–1.51)	1.07 (0.73–1.58)
Heart failure	5.32 (1.26–22.44)	2.34 (0.41–13.46)	4.45 (0.71–29.98)
Creatinine clearance‡	1.95 (0.74–0.95)	1.12 (0.91–1.37)	1.14 (0.91–1.42)
LV ejection fraction (per 10% decrease)	1.54 (1.27–1.89)	1.33 (1.01–1.74)	0.91 (1.14–1.42)
Baseline soluble Fas			
First tertile (referent)	1.00	1.00	1.00
Second tertile	14.99 (7.56–29.75)	13.84 (6.70–28.61)	16.16 (7.07–36.91)
Third tertile	27.48 (11.50–75.72)	23.20 (9.33–57.66)	25.40 (9.38–68.75)
Baseline high-sensitivity C-reactive protein			
First tertile (referent)	1.00	1.00	1.00
Second tertile	3.18 (1.79–5.66)	2.40 (1.17–4.92)	2.31 (1.03–5.18)
Third tertile	1.93 (1.15–3.24)	1.59 (0.79–3.20)	0.90 (0.39–2.03)

* In addition to those listed, variables included in multivariate model 1 were gender, history of coronary artery disease, peripheral vascular disease, diabetes, hypertension, dyslipidemia, active smoking, and body mass index.

† In addition to those listed and in multivariate model 1, multivariate model 2 included ST-segment deviation and the presence of elevated traditional necrosis markers (locally measured cardiac troponin or creatine kinase-MB).

‡ Per 10-ml/min/1.73 m^2 decrease.

OR = odds ratio.

First, we evaluated the relation between a final ACS discharge diagnosis for the index hospitalization and the markers of apoptosis and inflammation. Both sFas and hs-CRP levels were higher in subjects who had ACS than in those with a discharge diagnosis of noncardiac chest pain (Figure 1). On univariate analysis, increasing age, previous heart failure, and a lower glomerular filtration rate and left ventricular ejection fraction were also associated with a final index ACS diagnosis. Two multivariate models are reported (Table 2). Model 1 included all potential confounders identified on bivariate analyses, including sFas and hs-CRP levels, but not the standard diagnostic criteria (ie, necrosis biomarkers creatine kinase and troponin or ST-segment deviation). sFas was strongly and independently associated with a discharge diagnosis of ACS after adjusting for all potential confounders. In contrast, we could not find a significant association between hs-CRP and ACS after adjusting for potential confounders. Model 2 also included the standard diagnostic criteria to evaluate whether sFas and hs-CRP measurement could have incremental value in the diagnosis of ACS beyond that supplied by the conventional necrosis biomarkers and ST-segment deviation. sFas remained strongly associated with a discharge diagnosis of ACS after adding the case definition variables and hs-CRP in the model, although hs-CRP did not (Table 2).

Because the diagnosis of ACSs can be more challenging in those who with negative necrosis biomarker levels and no ST-segment deviation, we performed a subgroup analysis of these patients (25 subjects with a final diagnosis of UAP and 94 with noncardiac chest pain). In this group, sFas was still very strongly associated with ACSs (odds ratio 6.32, 95% confidence interval [CI] 1.63 to 24.54 for the second vs first sFas tertile; and odds ratio 12.22, 95% CI 3.01 to 49.61 for the third vs the first sFas tertile).

To ascertain whether adding biomarkers of inflammation and apoptosis to the standard criteria for ACS added value in terms of diagnostic accuracy, we analyzed the C statistic for various logistic models. The C statistic of a model that included the traditional risk factors, ST-segment deviation and elevated necrosis markers (either high creatine kinase-MB or troponin) was 0.85 (95% CI 0.82 to 0.89). However, when sFas was added, the latter model, a significant improvement was found in the C statistic to 0.93 (+0.08, 95% CI for the difference 0.05 to 0.11), indicating that sFas improved the accuracy of the diagnosis of ACS in suspected cases over and above the standard criteria (Figure 2). When sFas alone was used to predict the diagnosis, the C statistic was comparable to that derived from the standard model (0.82, 95% CI 0.78 to 0.87). In contrast, adding hs-CRP values to the current criteria did not improve the diagnostic accuracy (C statistic 0.86 with and 0.85 without the hs-CRP values, difference +0.01, 95% CI −0.05 to 0.02).

Having observed an increase in diagnostic accuracy, we investigated whether sFas showed an early increase in ACS. We compared the troponin and sFas levels according to the interval elapsed since the beginning of the ischemic symptoms. In patients with a discharge diagnosis of ACS, the sFas levels were already elevated in subjects who had early versus late measurements, which was not the case for troponin (Table 3). As expected, in a linear regression analysis, the troponin levels increased with the interval since the beginning of symptoms (β 0.07 ln μg/L/hour, 95% CI 0.04 to 0.10, whereas the sFas levels did not (β 0.00 ln pg/ml/hour, 95% CI −0.01 to 0.01). These results suggest a precocious increase in sFas.

Among patients with a discharge diagnosis of ACS, the sFas and hs-CRP levels were similar in those who experienced recurrent cardiac events compared to those who did not (Figure 1). Previous coronary artery disease, peripheral vascular disease, diabetes, renal failure, and lower left ventricular ejection fraction were associated with recurrence on univariate analyses, but sFas, hs-CRP, and troponin T were not. Similar results were observed on multivariate analysis (Table 4), as well as when the outcome was redefined as recurrent AMI and cardiac death only (data not shown).

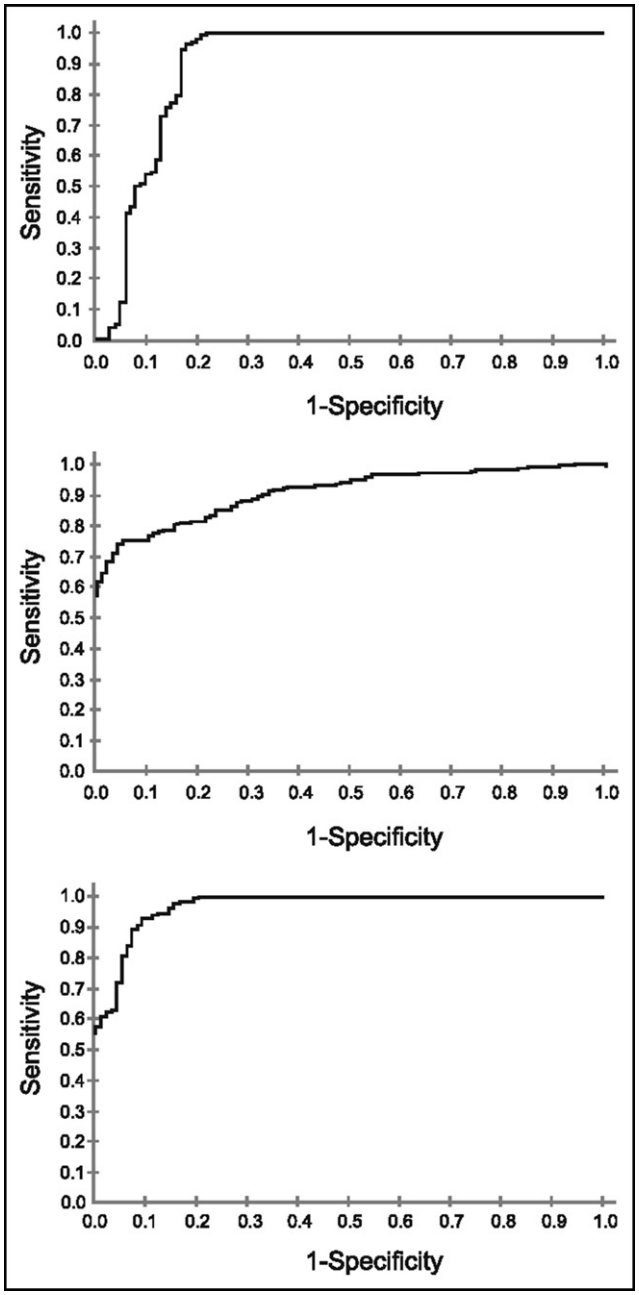

Figure 2. Receiver operating characteristic curves for diagnosis of ACS using various models. *(A)* Receiver operating characteristic curve for diagnosis of ACS using sFas alone. Area under curve was 0.82 (95% CI 0.78 to 0.87). *(B)* Receiver operating characteristic curve for diagnosis of ACS using variables included in multivariate model, including ST-segment deviation on electrocardiogram, and elevated traditional necrosis markers (either maximal creatine kinase-MB or cardiac troponin). Area under curve was 0.85 (95% CI 0.82 to 0.89). *(C)* Receiver operating characteristic curve for diagnosis of ACS using variables included in multivariate model, including sFas, ST-segment deviation on electrocardiogram, and elevated traditional necrosis markers (either maximal creatine kinase-MB or cardiac troponin). Area under curve was 0.93 (95% CI 0.91 to 0.96).

Table 3
Temporal trends in soluble Fas (sFas) and centrally measured cardiac T troponin levels in patients with final diagnosis of acute coronary syndrome (ACS) (n = 388)

Interval between symptoms and marker measurement (h)	Median sFas (pg/ml)	Median Troponin T (μg/L)
≤6	395 (314–539)	0.06 (0.01–0.26)
6–12	337 (273–437)	0.06 (0.01–0.62)
12–18	354 (290–473)	0.36 (0.02–1.79)
>18	361 (293–446)	0.47 (0.01–2.08)

Data in parentheses are interquartile range.

Table 4
Regression models for recurrent cardiovascular events in subjects with discharge diagnosis of acute coronary syndrome (ACS) (n = 388)

Baseline characteristic	Crude OR (95% CI)	Adjusted OR* (95% CI)
Previous coronary artery disease	1.47 (1.11–1.94)	1.33 (0.95–1.87)
Peripheral vascular disease	1.39 (1.04–1.85)	1.17 (0.85–1.61)
Diabetes mellitus	1.44 (1.07–1.93)	1.25 (0.91–1.71)
Renal failure†	1.67 (1.02–2.73)	1.25 (0.91–1.71)
Left ventricular ejection fraction‡	1.12 (1.01–1.23)	1.41 (0.81–2.46)
Baseline sFas		
First tertile (referent)	1.00	1.00
Second tertile	1.10 (0.80–1.50)	1.03 (0.74–1.43)
Third tertile	0.92 (0.66–1.29)	0.88 (0.62–1.24)
Baseline hs-CRP		
First tertile (referent)	1.00	1.00
Second tertile	0.97 (0.70–1.34)	0.95 (0.70–1.36)
Third tertile	0.99 (0.72–1.37)	0.97 (0.67–1.35)

* In addition to those listed, variables included in multivariate model were history of heart failure, heart failure at baseline, hypertension, dyslipidemia, smoking status, body mass index, baseline cardiac troponin T, and per-hospital revascularization; age and gender were not included because cases were matched with controls for these 2 variables.

† Estimated creatinine clearance ≤30 ml/min/1.73 m^2.

‡ Per 10% decrease.

Discussion

The present study reports on the usefulness of a multipurpose diagnostic approach that takes into account the different biologic pathways in attempting to refine the diagnosis of ACS and the prediction of recurrent events. In patients presenting with ischemic symptoms, we observed that the plasma sFas levels were strongly and independently associated with a discharge diagnosis of ACS. sFas measurement significantly improved the diagnostic accuracy in suspected cases of ACS, and its diagnostic levels increased more precociously than did those of troponin. In contrast, no added value was found in diagnostic accuracy when hs-CRP was measured. The baseline levels of sFas and hs-CRP were not associated with recurrent cardiac events during a 1-year follow-up period.

Evidence is extensive for the involvement of apoptotic cell death in ACS. In vitro, myocardial cells undergo apoptosis when exposed to hypoxic conditions.[6] Animal models of ischemia/reperfusion have revealed increased apoptotic myocardial cell death.[7] In humans, autopsy studies have shown high levels of apoptotic cardiomyocytes in the border zone of the infarct.[8] In this context, apoptosis is at least partly mediated by the upregulation of Fas that occurs during hypoxia.[9,10] A recent study showed that hypoxia induces the p53 protein, which, in turn, activates Fas transcription and promotes apoptosis.[9] To evaluate the indepen-

dent contribution of this pathway in ACS, we measured the soluble form of Fas, which is produced by alternative splicing.[11] The pathophysiologic significance of increased sFas levels during ACS is unclear and deserves additional study. However, in vitro data have suggested that sFas inhibits apoptosis in Fas-expressing cells, either by binding Fas-ligand, binding membrane-bound Fas, or interacting with other proteins expressed on these cells.[11] Increased production of sFas during ACS might represent a negative feedback mechanism aimed at preventing additional cell loss in the myocardium.

Our findings have extended earlier reports[12,13] by showing that the association between sFas and ACSs is not only strong, but also independent of circulating markers of other relevant biologic pathways such as inflammation and necrosis. Furthermore, because both heart failure and left ventricular ejection fraction were included in the multivariate models, our results suggest that the association between ACS and sFas is not due to confounding by heart failure. The latter is particularly important given the strong association between heart failure and both ACSs and high sFas levels.[14,15] In contrast, no independent association was observed between hs-CRP and ACSs.

The diagnosis of ACSs is often challenging because the chest pain can be atypical, electrocardiographic changes can be absent or nondiagnostic, and the necrosis marker levels can remain negative in cases of UAP. Our cohort was preselected to have a high probability of an ACS, because only hospital-admitted patients were eligible for recruitment. Even so, 100 patients were eventually discharged with a diagnosis of noncardiac chest pain. Given the important apoptotic signal we observed with ACSs, we examined whether adding sFas values to the actual diagnostic criteria could improve the diagnostic accuracy and found that it did so in a very clinically meaningful manner. sFas was also strongly associated with a diagnosis of an ACS in the harder-to-diagnose subgroup of patients with negative necrosis biomarker levels and no ST-segment deviation. Furthermore, although the sFas kinetics in ACSs remain to be formally defined by serial measurements, our data suggest that it increases precociously.

Current risk stratification scores after ACSs rely on clinical parameters and myocardial necrosis markers[16] and have good, but imperfect, discriminative ability.[17] Much research has focused on whether inflammatory biomarkers can help in refining the risk assessment after ACSs. hs-CRP has improved the prediction of recurrent events in some,[18,19] but not all, studies.[2] Apoptosis has been reported in endothelial cells, vascular smooth muscle cells, and macrophages in atheromatous lesions.[20] Furthermore, the expression of pro-apoptotic genes,[21] apoptotic vascular smooth muscle cells, and macrophages[22] is greater in unstable plaques than in stable ones, suggesting a role for apoptosis-related cell death in the pathogenesis of ACSs. Hence, we had hypothesized that in subjects with ACSs, those with increased ongoing apoptosis in atherosclerotic plaques would have greater sFas levels and an increased likelihood of recurrent cardiac events. However, we did not observe this to be true, at least for sFas values obtained acutely in ACSs. It is likely that the bulk of the apoptotic signal at that moment comes from the myocardium, which might not allow the detection of intravascular apoptotic events. sFas values measured in a stable state, at a distance from any acute event, might be a better reflection of apoptosis in the vascular wall. This has been supported by studies performed in stable hemodialysis patients, in whom sFas levels were strongly associated with future cardiovascular events.[23]

Our study had certain limitations. Because the potential clinical use of sFas measurements would be to enhance diagnostic accuracy in atypical or uncertain cases and to hasten the diagnosis or discharge, our findings need to be confirmed in larger, prospective validation studies before sFas measurement can be used clinically. Diagnostic tests are usually compared to a reference standard diagnosis. In the case of ACS, no such reference exists, especially for UAP. We chose to use the discharge treating clinician's diagnosis as the reference criterion to reproduce the real-life setting. However, some misclassification on the outcome might have occurred. Because the sFas levels were unknown to the clinicians making the final diagnoses, the misclassification should have been nondifferential and should have biased the results toward the null, not in the direction of an exaggerated effect. Finally, we used a standard troponin T assay. Because recent findings have shown improved and earlier diagnostic accuracy using high-sensitivity troponin,[24] future studies of the role of sFas in ACS should be compared to these new troponin measurements.

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