A comparative study of biomarkers for risk prediction in acute coronary syndrome—Results of the SIESTA (Systemic Inflammation Evaluation in non-ST-elevation Acute coronary syndrome) study

Juan Carlos Kaski, Daniel J. Fernández-Bergés, Luciano Consuegra-Sánchez, José M. Cruz Fernández, Xavier García-Moll, José M. Mostaza, Rocío Toro Cebada, José Ramón González Juanatey, Gabriela Guzmán Martínez, Jaume Marrugat

Objective: We compared the 1-year predictive value of several inflammatory and non-inflammatory biomarkers in ACS patients.

Methods: In 610 patients (73.0% male) – 36.0% unstable angina (UA) and 64.0% NSTEMI – we assessed high-sensitivity C-reactive protein (hs-CRP), interleukins 6, 10 and 18, soluble CD40 ligand, P- and E-selectin, NT-proBNP, fibrinogen and cystatin C at hospital admission. Two outcomes at 1-year follow up were selected for analysis: (1) all-cause death, MI, UA, or coronary revascularization, and (2) all-cause death, and non-fatal MI. The effect of biomarker levels on endpoints was examined by the Cox proportional hazards model, and their discrimination ability with the C statistic (AUC).

Results: Of 549 patients (90.0%) who completed the 1-year follow up, 206 (37.5%) and 54 (8.9%) reached the first and second composite endpoints, respectively. None of the biomarkers studied improved prediction of the first endpoint. However, considered as continuous variables, and in combination, NT-proBNP and fibrinogen, increased the AUC from 0.64 (95% CI 0.55–0.72) to 0.73 (95% CI 0.64–0.81; p = 0.02) for prediction of the second endpoint. Cut-off values for NT-proBNP and fibrinogen, regarding best sensitivity and specificity for prediction of the secondary endpoint were examined by the Cox proportional hazards model, and their discrimination ability with the C statistic (AUC).

Results: Of 549 patients (90.0%) who completed the 1-year follow up, 206 (37.5%) and 54 (8.9%) reached the first and second composite endpoints, respectively. None of the biomarkers studied improved prediction of the first endpoint. However, considered as continuous variables, and in combination, NT-proBNP and fibrinogen, increased the AUC from 0.64 (95% CI 0.55–0.72) to 0.73 (95% CI 0.64–0.81; p = 0.02) for prediction of the second endpoint. Cut-off values for NT-proBNP and fibrinogen, regarding best sensitivity and specificity for prediction of the secondary endpoint were examined by the Cox proportional hazards model, and their discrimination ability with the C statistic (AUC).

Conclusion: In ACS patients, inflammatory biomarkers offer modest incremental information to that provided by clinical risk markers. Fibrinogen and NT-proBNP measurements, however, improve cardiovascular risk prediction.

© 2010 Elsevier Ireland Ltd. All rights reserved.

Abbreviations: ACS, Acute coronary syndrome; AMI, Acute myocardial infarction; UA, Unstable angina; CD, Cardiac death; CAD, Coronary artery disease; PCI, Percutaneous coronary intervention; hs-CRP, High-sensitivity C-reactive protein; TIMI, Thrombolysis in Myocardial Infarction; NSTE-ACS, Non-ST-segment elevation acute coronary syndrome; NSTEMI, Non-ST-segment elevation myocardial infarction; NT-proBNP, N-terminal prohormone brain natriuretic peptide.

* This paper was presented in part at the Scientific Sessions of the American Heart Association, New Orleans, Louisiana, November 2008.
* Corresponding author. Tel.: +44 208 725 5901/5939; fax: +44 208 725 3328.
E-mail addresses: jkaski@sghms.ac.uk, jkaski@sgul.ac.uk (J.C. Kaski).

1 On behalf of the SIESTA investigators.
1. Background

The acute coronary syndrome (ACS) (i.e. unstable angina (UA), non-ST elevation myocardial infarction (NSTEMI) and ST-segment elevation MI (STEMI)) is a common clinical presentation of coronary artery disease (CAD) that is associated with high mortality and morbidity [1,2]. ACS patient risk stratification, albeit helped by the use of biomarkers such as cardiac troponin and clinical risk scores (i.e. Thrombolysis in Myocardial Infarction (TIMI) risk score), is suboptimal at present [3].

Inflammation, with macrophage and T-cell activation, increased production of acute-phase proteins, cytokines and tissue factor, as well as platelet hyper-aggregability plays a major role in rapid CAD progression leading to ACS [4,5]. Therefore, markers of systemic and vascular inflammation have been proposed as potentially useful markers of cardiovascular risk in both apparently healthy subjects and patients with cardiovascular disease [6–8]. There is relatively little information, however, on the comparative prognostic role of inflammatory markers – particularly in patients of Mediterranean origin – in the ACS setting. In addition to acute-phase reactants such as C-reactive protein (CRP) and fibrinogen, and other pro-inflammatory molecules, substances such as brain (B-type) natriuretic peptide [9] and cystatin C are emerging as potentially useful markers of cardiovascular risk [10].

Here we present the main results of the SIESTA (Systemic Inflammation Evaluation in patients with non-ST-segment elevation Acute coronary syndromes) study, a prospective multicentre study aimed at assessing the comparative prognostic value of eight markers of inflammation and two other biomarkers of cardiovascular risk, measured at hospital admission, in patients admitted to hospital with NSTE-ACS.

2. Methods

2.1. Patients

SIESTA is a prospective cohort study of NSTE-ACS patients admitted to 25 hospitals in different Spanish geographical locations from April 2002 to June 2004. All patients had typical chest pain at rest >5 min, with onset of symptoms within 48 h of enrolment, plus at least one of the following: (1) electrocardiographic (ECG) signs of myocardial ischaemia, i.e. >1 mm horizontal or downsloping ST-segment depression, T-wave inversion, or both; (2) previously documented CAD and/or myocardial revascularisation with percutaneous coronary intervention (PCI) or by-pass surgery (CABG); (3) abnormal cardiac troponin levels.

We excluded patients with ST-segment elevation, left bundle branch block, moderate or severe aortic stenosis, hypertrophic or dilated cardiomyopathy, a previous history of congestive heart failure and/or heart failure at admission, acute MI or coronary revascularisation procedures within 12 weeks before admission, or other serious atherosclerotic events, i.e. stroke, complications of peripheral vascular disease (PVD) within 12 weeks before admission. Patients with uncontrolled systemic hypertension, anaemia, infectious diseases, thyrotoxicosis, local or systemic inflammatory conditions, end-stage renal disease, malignancies and other diseases that could have dramatically reduced life expectancy and/or triggered acute or chronic inflammatory responses, were also excluded.

The study was approved by the local ethics committees of the participating hospitals and all patients signed written informed consent prior to study entry.

Demographic and clinical variables obtained at study entry are summarized in Table 1. TIMI (Thrombolysis in Myocardial Infarction) Risk Score [3] was calculated in each subject. Characteristics of the chest pain and risk factors were carefully noted.

2.1.1. Patient management during the acute event and follow up

Patients received treatment in accordance to AHA/ACC [11] or European Society of Cardiology [12] recommendations. The managing physicians were blinded to the results of inflammatory markers assessed in the study. Patients received treatment with aspirin, beta blockers, heparin, GP IIb/IIIa inhibitors, clopidogrel, nitrates, lipid-lowering drugs, angiotensin converting enzyme (ACE) inhibitors, and angiotensin II blockers, as appropriate. Revascularization was recommended in accordance to guidelines [11,12].

Patients were followed for one year and assessed regularly in ad hoc out patient clinics to establish vital status and monitor the development of events.

Patients (n = 61, 10%) followed for less than one year did not differ from those who completed the 1-year follow up regarding baseline demographics, clinical and biochemical variables and angiographic characteristics. Median follow up for this subgroup was 30 days (range 7–180 days).

2.2. Serum biomarkers

Peripheral venous blood samples were obtained at study entry in every patient. The blood was centrifuged immediately and both plasma and serum aliquoted and stored at −80 °C for subsequent analysis at the core laboratories. The following biochemical variables, used for patient management, were assessed locally in the participating hospitals: CK-MB, cardiac troponin, white blood cell count (WBC) and lipid profile. Cardiac troponin levels were measured at each of the participating centres as part of their routine clinical management protocol. We did not centralize the measurement of troponin or attempted to standardize cTn measurements due to logistic reasons. We did, however, issue specific recommendations to the participating centres regarding blood specimen integrity, i.e. adequate mixing of the specimen and centrifugation times, to avoid false positive results (i.e. non-analyte reaction from red blood cells, platelets, clots or other debris) and false negative results due, for example, to the presence of bubbles in the sample. The following biochemical markers were measured at St George's Hospital MRC Protein Reference Unit (core lab): high-sensitivity CRP (hs-CRP), IL-6, IL-18, P- and E-selectin, soluble (s) CD40L, cystatin C, NT-proBNP, fibrinogen and the anti-inflammatory cytokine IL-10. In all patients who required revascularization during admission, blood samples were obtained before intervention.

hs-CRP measurements were performed with COBAS Integra (Roche Diagnostics Limited, Lewes, East Sussex, UK) using the hs-CRP-latex assay (analytical range 0.2–12 mg/L) and the normal application (analytical range 2–160 mg/L).

All samples were assayed in random order and blinded to outcome data.

Patient inclusion was from April 2002 to June 2004 and measurements were performed in December 2004, samples were stored for 6–32 months prior to measurement.

The biomarker concentrations were measured using enzyme-linked immunosorbent assays. Reagents for IL-6, IL-10, P-selectin, E-selectin and scCD40L were from R&D Systems (Abingdon, Oxon, United Kingdom) and reagents for IL18 were from MBL (Naka-ku Nagoya, Japan). The between batch CVs for the IL-10, P-selectin, E-selectin and CD40 ligand assays are <15.6%, <9.5%, <8.8% and <6.4%, respectively. Our inter-assay validation showed comparable assay performance for P-selectin (CV = 8.2% at 421.5 ng/ml), E-selectin (CV = 7.8% at 49.1 ng/ml and CD40L (CV = 13.9% at 7817 pg/ml). Our inter-assay validation for IL-10 showed a CV of 27% at a concentration of 5.6 pg/ml. The between batch CVs for the IL-18 assay are <11%. This was confirmed with our inter-assay validation using a
The presence of conjugated or unconjugated bilirubin (<200 mg/L), proANP(79–98), BNP32 or CNP32. The assay was unaffected by reactivity with ANP, NT-proANP(1–30), NT-proANP(31–67), NT-proANP(79–98), BNP32 or CNP32. The assay total precision measured by immunonephelometry on a Behring Nephelometer II was 3.4–5.6% in the concentration range 40.9–32,096 pg/mL. The analytical sensitivity of 10 pg/mL. There was no observed cross assay had a linear range from 20 pg/mL to >35,000 pg/mL with an ST-deviation, TIMI risk score, median (Q1–Q3) 3 (2–4) 4 (3–5) <0.001

Continuous variables are presented as mean values ± standard deviation or median (interquartile range Q1–Q3); categorical variables are presented as percentage.

ACE: angiotensin converting enzyme; AMI: acute myocardial infarction; ARB: angiotensin receptor blocker; BMI: body mass index; Bp: blood pressure; CABG: coronary artery by-pass graft; CAD: coronary artery disease; HDL: high-density lipoprotein; LDL: low-density lipoprotein; LV: left ventricle; MDRD: estimated creatinine clearance by modification of diet in renal disease method; NSTE-ACS: non-ST-segment elevation acute coronary syndrome; NSTEMI: non-ST-segment elevation myocardial infarction; PCI: percutaneous coronary intervention; TIMI: Thrombolysis in Myocardial Infarction; WBCC: white blood cell count.

Two outcomes were assessed for inclusion in the prediction analysis: first endpoint, a composite endpoint including all-cause death, non-fatal MI, UA, PCI and CABG at one year of follow up, and endpoint 2, comprising death of any cause and non-fatal MI.
Endpoint definitions used in the study were based on the 2001 AHA/ACC [13] guidelines for management of patients with ACS. MDRD (Modification of Diet in Renal Disease) was calculated using the following formula: eGFR (mL/min/1.73 m^2) = 186 × (serum creatinine (µmol/L) / 0.0113)^1.154 × age (years)^0.203 × (0.742, in females), to estimate glomerular filtration rate.

2.4. Statistical analysis

2.4.1. Sample size calculation

Event rates in UA/NSTEMI patients, as reported in different series, range from 15% to 30% [14,15] Power calculations in the present study were based on information available for hs-CRP. We calculated that for a difference of 10% in CRP levels between groups with and without events, an alpha error (type 1 error) equal to 0.05, a beta error (type 2 error) <0.20 and a bilateral (two-tailed) hypothesis contrast, 610 patients (244 with events and 366 without events) would be required.

Results are presented as mean ± standard deviation (SD) for continuous normally distributed variables, as median (interquartile range) for non-normally distributed variables, and as percentages (proportions) for categorical data.

The association of the selected panel of biomarkers with the two study endpoints was assessed with standard univariate tests with biomarkers considered as continuous variables, and comparing the areas under the Receiver Operating Characteristic (ROC) curves (C statistic). We carried out Bonferroni's correction for 10 comparisons to prevent chance-findings.

The unadjusted incidence of composite study endpoints over time was analyzed by the Kaplan–Meier method, and differences were compared using the log-rank (Mantel–Cox) test. The adjusted survival function (for relevant covariates included in the final multivariable model) was also depicted for each biomarker.

Patients were subdivided a priori into two groups: subjects “with events” and those “without events” for comparison. We fitted a Cox proportional hazards models to estimate the adjusted hazard ratio of the first endpoint (1-year unstable angina, acute myocardial infarction, all-cause death and percutaneous coronary intervention/coronary artery by-pass graft after discharge), and the second endpoint (non-fatal MI and/or all-cause death) at 1-year follow up for each of the 10 biomarkers. Demographic characteristics, and clinical variables that showed at least marginally significant differences (p < 0.15) on univariate analysis for each biomarker, and in patients with and without events, were considered potential confounders. Heart failure, previous cardiovascular disease and TIMI Risk Score consistently met the requirements described above. The assumption of proportionality was assessed by Schoenfeld residuals test and visual log-log plots. The discrimination (ability to classify risk) of the clinical model with and without biomarkers was assessed with the C statistic (comparison of areas under the ROC curve (AUC)). A Hosmer–Lemeshow test was used to evaluate the goodness-of-fit (calibration) of the model with and without biomarkers.

We bootstrapped the database (3000 times) to estimate standard errors and confidence intervals of hazard ratios.

Cut-off values (C), i.e. best sensitivity and specificity for prediction of the secondary endpoint were calculated for NT-proBNP and fibrinogen according to the Jacobson and Truax method using the following equation: C = (SD0 × M1 + SD1 × M0)/(SD0 + SD1), where M0 and SD0 = mean and standard deviation for NT-proBNP or fibrinogen in patients without events, M1 and SD1 = mean and SD for NT-proBNP or fibrinogen in patients with events (secondary endpoint) during follow up. Statistical significance was defined as p-value <0.05. All p-values were two-sided.

Calculations were performed using the SPSS software version 13.0 (SPSS Inc., Chicago, IL, USA). Comparison between ROC curves was performed using the STATA software version 8.2 (Stata Corp., College Station, TX, USA).

3. Results

From April 2002 to June 2004, we recruited 610 patients (73.0% male); 217 (36.0%) had UA, and 393 (64.0%) NSTEMI. A total of 549 patients (90.0%) completed the 1-year follow-up. During this period, 206 patients (37.5%) developed cardiovascular events (UA requiring hospital admission, n = 143; non-fatal AMI, n = 27; cardiac death, n = 11; non-cardiac death, n = 3 and coronary revascularization, n = 22). On univariate analysis, the following variables correlated with the occurrence of the first composite endpoint: diastolic blood pressure, a previous history of CAD, PCI, peripheral arterial disease, type-2 diabetes mellitus, hypertension, current smoking, family history of CAD, TIMI risk score, ST-segment deviation, multivessel coronary disease, LV ejection fraction (EF), heart failure during admission and elevated cardiac troponin levels.

3.1. Serum biomarkers and first study endpoint

None of the biomarkers assessed in the study was significantly associated with the first study endpoint after performing the Bonferroni correction.

3.2. Biomarkers and incidence of second study endpoint

During the 1-year follow up, 54 patients (9.8%) reached the second study endpoint (non-fatal MI, 34, cardiac death, 17 and non-cardiac death, 3) (Table 1). Peripheral artery disease, LVEF, TIMI risk score, ST-deviation at presentation, heart failure during hospitalization, creatinine concentration and glomerular filtration rate (as assessed by the MDRD formula) were significantly associated with the second study endpoint. When considered as continuous variables – and both combined (Table 2), NT-proBNP and fibrinogen correlated significantly with the second study endpoint. Assess-
ment of the 10 biomarkers in a basic model, adjusted for TIMI risk score, heart failure and previous CAD, confirmed an independent, significant, predictive value of fibrinogen and NT-proBNP (Table 3) (HR 4.66 (95% CI 1.65–13.13, and HR 1.38, 95% CI 1.09–1.75, respectively).

Fig. 1 shows the AUC for the combined secondary endpoint (non-fatal MI and/or all-cause death), which increased from 0.64 (0.57–0.72) with heart failure, previous cardiovascular disease and TIMI alone to 0.68 (0.60–0.77; p = 0.25) when NT-ProBNP was added to this function, to 0.70 (0.63–0.78; p = 0.02) when fibrinogen alone was added, and to 0.73 (0.64–0.81; p = 0.02) when both markers were added.

No significant interactions with age, gender, or diagnosis at admission were found for the association of NT-proBNP and fibrinogen with the second study endpoint.

NT-proBNP showed significant weak correlations with CRP ($r = 0.180, p < 0.001$), cystatin C ($r = 0.273, p < 0.001$), E-selectin ($r = -0.097, p = 0.033$), fibrinogen ($r = 0.136, p = 0.004$) and cardiac troponin ($r = 0.086, p = 0.06$). Fibrinogen was significantly correlated with CRP ($r = 0.394, p < 0.001$) and cystatin C ($r = 0.359$).

### Table 3

Non-fatal MI or all-cause death (second composite study endpoint), Hazard ratio for 10 biomarkers (continuous variables), as adjusted for heart failure, previous cardiovascular disease and TIMI risk score.

<table>
<thead>
<tr>
<th>Secondary endpoint</th>
<th>HR</th>
<th>95% CI for HR</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-selectin</td>
<td>0.75</td>
<td>0.38</td>
</tr>
<tr>
<td>E-selectin</td>
<td>1.47</td>
<td>0.93</td>
</tr>
<tr>
<td>Soluble CD40L</td>
<td>0.93</td>
<td>0.80</td>
</tr>
<tr>
<td>IL18</td>
<td>0.98</td>
<td>0.74</td>
</tr>
<tr>
<td>IL10</td>
<td>0.98</td>
<td>0.81</td>
</tr>
<tr>
<td>IL-6</td>
<td>1.05</td>
<td>0.86</td>
</tr>
<tr>
<td>hs-CRP</td>
<td>1.16</td>
<td>0.94</td>
</tr>
<tr>
<td>Cystatin C</td>
<td>2.15</td>
<td>0.93</td>
</tr>
<tr>
<td>NT-proBNP</td>
<td>1.38</td>
<td>1.09</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>4.66</td>
<td>1.65</td>
</tr>
</tbody>
</table>

CI: confidence interval; HR: Hazard ratio.
The HR is the corresponding risk associated to a change of 1 unit of each listed biomarker.
All biomarkers were log (base e) transformed before being entered in the model.
Thus the HR represents the relative effect for multiplying per 2.718 the value of each biomarker.

Fig. 1. Comparative area under the receiver operating characteristic (ROC) curves of the 360-day second composite study endpoint (non-fatal MI and/or all-cause death) for the basic model adjusted for heart failure, previous cardiovascular disease and TIMI risk score and models further adjusted for NT-proBNP (Panel A) and fibrinogen (Panel B), and both (Panel C).
p < 0.001) but did not correlate with cardiac troponin results (r = 0.037, p = 0.37).

Cut-off values for NT-proBNP and fibrinogen, regarding the best sensitivity and specificity for prediction of the secondary endpoint, were 1043.9 ng/L and 4.47 mg/dL respectively. For the mentioned cut-off point NT-proBNP showed a sensitivity of 40.5%, a specificity of 83.3%, a positive predictive value of 18.8% and a negative predictive value of 93.5%. Fibrinogen showed a sensitivity of 59.5%, a specificity of 67.1%, a positive predictive value of 14.9% and a negative predictive value of 94.4% for the chosen cut-off point.

4. Discussion

Our study showed that among 10 biomarkers of cardiovascular risk assessed in the SIESTA study, NT-proBNP and fibrinogen were the only independent predictors of cardiovascular events at one year of follow up. Moreover, the addition of fibrinogen and NT-proBNP to the clinical risk score model evaluated in the present study provided incremental prognostic information.

4.1. Inflammatory biomarkers and prognosis

In the present study, markers such as hs-CRP, IL-6, IL-18; cystatin C, sCD40L; E- and P-selectin failed to provide significant independent incremental prognostic information at 1 year of follow up. These results contrast with previous reports [7,16–19] suggesting a predictive role of inflammatory markers in ACS patients. The lack of incremental prognostic value of CRP in our study could be explained by low statistical power and the fact that most of our patients were receiving treatment with statins, which lower CRP levels [20]. However, our results agree with other studies showing a limited utility of serum inflammatory markers for risk prediction in angina patients [21,22] and with data in two large studies in showing that multiple serum biomarkers added only modestly to the overall prediction of risk resulting from the assessment of conventional cardiovascular risk factors, as assessed by the C statistic [23,24].

4.2. NT-proBNP and fibrinogen

In the present study, high NT-proBNP, a counter-regulatory hormone that plays an active role in the response to ischaemic injury [25], identified patients who were at an increased risk of death and MI. Moreover, the inclusion of this variable in the clinical risk score model improved marginally the prognostic power of the score, as shown by an increase in the AUC of the ROC. Our findings are in agreement with previous studies in patients with cardiovascular conditions which have reported that NT-proBNP, a marker for neurohumoral activation, was independently associated with death and/or the occurrence of MI during both short [26] and long-term follow up [27]. Increased NT-proBNP concentrations were shown to be associated with impaired outcome in patients with ACS [26,27] and reported to occur as a result of both LV dysfunction and myocardial ischaemia [28,29]. Investigation of the mechanisms responsible for NT-proBNP elevations was beyond the scope of the study but it can be speculated that in the context of ACS, both mechanisms, i.e. LV dysfunction and myocardial ischaemia, could have played a role in NT-proBNP elevation. Of interest, the addition of fibrinogen to the model further improved the AUC (8.4% change; p = 0.02). Fibrinogen is known to be a good predictor of cardiovascular risk [28,29] and our results confirm and expand previous findings regarding fibrinogen as a marker of risk in ACS patients. In the FRISC study (Fragmin during Instability in Coronary Artery Disease) [30] elevated fibrinogen levels were associated both with short and long-term increased risk of death or new MI. Similar findings were reported by Arnau Vives et al. [31] in Spanish patients. In our study, involving patients with ACS, NT-proBNP and fibrinogen, combined, were independent predictors of cardiovascular risk and provided additional information over and above that of clinical risk scores.

We carried out net reclassification improvement (NRI) calculations. In all cases, the contribution of the biomarkers assessed in the study was marginal in this regard. The median change of the NRI for cystatin C with respect to the first endpoint was 1%, 0.09% for NT-proBNP and, regarding the second endpoint, 0.4% for fibrinogen and 0.6% for NT-proBNP and fibrinogen.

4.3. Limitations

The study has limitations that can affect the interpretation of our results, i.e. the first study endpoint (widely encompassing) was designed in accordance with practice current at the time. The sample size was calculated to provide power for CRP the most extensively studied biomarker in this setting but it might be poorly construed for the other biomarkers studied. The secondary endpoint however, holds substantially greater clinical validity as a measure of predictive “efficacy” but is poorly powered for the study. The predominantly neutral findings of this study, in relation to inflammatory markers, are not surprising and agree with previous reports in patients, albeit with different presentations of angina pectoris [21–24]. Our results provide insight into the relative prognostic contribution of the biomarkers studied, with respect to the first endpoint, but is perhaps underpowered to exclude a predictive value for these markers.

We did not assess the significance of the biomarkers for prognostication over time and this represents a limitation regarding a potential role of these markers as predictors of risk over time. Eggers et al. [32] showed in patients with NSTE-ACS that CRP exhibits an increasing predictive value when measurements are carried out during follow up. Statistical methods for estimating time-varying effects of prognostic factors have been developed, i.e. a piecewise constant penalized spline approach, incorporating time-varying coefficients. This allows for deviations from the proportional hazards assumption. Statistical modeling allowing for the potential existence of time-dependent effects may be necessary in future studies. An important finding however is that NT-proBNP and fibrinogen, in combination, were strong independent predictors of risk and provided incremental information regarding cardiovascular risk in these patients.

Another limitation is that albeit we strongly encouraged the inclusion of consecutive patients in the study we could not verify whether all patients were consecutively enrolled at all sites. Patients with history of heart failure or with signs of heart failure at hospital admission were not included in the study and this most likely resulted in a relatively low risk population. However, patients who developed heart failure during admission – as part of the clinical evolution of the ACS – were not excluded from analysis. Our results cannot thus be extrapolated to all patients with ACS.

Another limitation is that 77% of patients were receiving statin treatment, which are known to exert anti-inflammatory actions and could thus affect inflammatory markers levels. However, the data obtained in these subjects are of clinical relevance as our patients are representative of contemporary management strategies.

We did not specifically assess the long-term stability of the cardiovascular markers used in the study. However, although the literature is scarce regarding long-term stability of biomarkers in frozen blood samples, studies showed that stability of markers such as CRP, NT-proBNP, IL-6, IL-10 and others is good when handled and stored in conditions comparable to those in our study [33–40].
5. Conclusions

The results of our study involving low to moderate risk Mediterranean ACS patients suggest that markers of inflammation add modest prognostic information to that afforded by conventional risk factors and other clinical variables often included in clinical risk scores. However, the prognostic performance of multimarker panels including fibrinogen and NT-proBNP appear to provide incremental information that may be of clinical importance. These markers interrogate biological pathways other than just those associated with inflammation, which are implicated in coronary disease progression and the development of cardiovascular events.

Conflict of interest

We have no conflict of interest to disclose.

Funding sources

The SIESTA study was supported by unrestricted grants from Bristol Myers Squibb (Spain), and the Spanish Society of Cardiology. Daniel Fernández-Bergés was supported by the Ministerio de Sanidad y Consumo, Instituto de Salud Carlos III and Fundesalud (Grupo emergente GRIMEX/ Emer07/046). Jaime Marrugat was supported by the FEDER- Ministerio de Ciencia e Innovación, Instituto de Salud Carlos III (Red HERACLES RD06/009). Luciano Consuegra Sánchez was supported by grants from the Spanish Society of Cardiology and Fundación de Investigación del Hospital Clínico de Valencia.

Appendix A. SIESTA study collaborators

Virgen Macarena-Sevilla
J.M. Cruz Fernández, V. López
García-Amada, R. Cabrera, M. García de la Borbolla
Puerta del Mar-Cádiz
D. Fernández-Bergés, M.J. Zaro, R.
San Benito-Badajoz
M. Aznar, M. Vega, A. Cordoba
Santiago de Compostela
JR. González-Juanatey, L. Grigorian, P.
Bassante
La Paz-Madrid
M. Iñiguez, D. Jordauro, G. Guzmán Martínez
Clínico de Salamanca
C. Martín Luengo, P.L. Sánchez, J.L.
Morillo
Juan Canalejo-A Coruña
A. Castro Beiras, I. Mosquera
Cruces-Bilbao
P.M. Montes Orbe, I. Eguzki Aztobiza
Santa Cruz San Pau-Barcelona
X. García-Moll, M.M. Martí
Central de Asturias-Oviedo
V. Barriales Álvarez, Dae-Hyun Lee Hwang
Juan Ramón Jiménez-Huelva
F.J. Carrasco Sánchez, A. Tobaruela, J.M.
Santos, S. Delgado, C. Santos
Universitario de Guadalajara
R. Arroyo Estepuerro, J. Balaguer
Clínico de Valladolid
P.L. Sánchez
San Jorge-Huesca
M.T. Villarreal, A. Capdevila, C. Liñaná
Valle de los Pedroches-Córdoba
J.C. Castillo Dominguez
Arnaú de Vilanova-Valencia
J. Solito Marri, E. Dalí, M.L. Martinez, M.T.
Moreno
Royo Villanueva-Zaragoza
J.M. Cruz Fernández, V. López
Reina Sofia Córdoba
C. Solano, J.A. Sanz, D.
General de Castellón
L.J. Diago Torrent
San Pedro de Alcántara
G. Marcos Cómez
General de Alicante
F. Sogor Gari, E. Payá Mora
Marina Alta, Denia-Alicante
A. Valls
Gregorio Marañón
R. Muñoz Aguilera, J.A. Sevano Sanz, D.
Santa María Nai-Ourense
Pascal Hernández, J.A. García Robles
Laboratorios Carlos III Madrid
M. Pérez de Juan Romero

References


