

Original article

Interleukin-6 and High-sensitivity C-reactive Protein for the Prediction of Outcomes in Non-ST-segment Elevation Acute Coronary Syndromes

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ABSTRACT

Introduction and objectives: High baseline levels of interleukin-6 and C-reactive protein confer an increased risk of mortality in non-ST-segment elevation acute coronary syndrome. The aim of the study was to determine whether serial measurements of interleukin-6 and high-sensitivity C-reactive protein provide additional information to baseline measurements for risk stratification of non-ST-segment elevation acute coronary syndrome.

Methods: Two hundred and sixteen consecutive patients with non-ST-segment elevation acute coronary syndrome were prospectively included. Blood samples were obtained within 24 h of hospital admission and at 30 days of follow-up. The endpoint was a composite of all-cause death, nonfatal myocardial infarction, or acute decompensated heart failure.

Results: Both interleukin-6 and high-sensitivity C-reactive protein levels decreased from day 1 to day 30, regardless of adverse events (both $P < .001$). Interleukin-6 levels at 2 time points (interleukin-6 day 1, per pg/mL; hazard ratio=1.006, 95% confidence interval, 1.002-1.010; $P=.002$ and interleukin-6 day 30, per pg/mL, hazard ratio=1.047, 95% confidence interval, 1.021-1.075, $P < .001$) were independent predictors of adverse events, whereas high-sensitivity C-reactive protein day 1 and high-sensitivity C-reactive protein day 30 levels were not. Patients with interleukin-6 day 1 ≤ 8.24 pg/mL and interleukin-6 day 30 ≤ 4.45 pg/mL had the lowest event rates (4.7%), whereas those with both above the median values had the highest event rates (35%). After addition of interleukin-6 day 30 to the multivariate model, C-index increased from 0.71 (95% confidence interval, 0.63-0.78) to 0.80 (95% confidence interval, 0.72-0.86), $P=.042$, and net reclassification improvement was 0.39 (95% confidence interval, 0.14-0.64; $P=.002$).

Conclusions: In this population, both interleukin-6 and high-sensitivity C-reactive protein concentrations decreased after the acute phase. Serial samples of interleukin-6 concentrations improved the prognostic risk stratification of these patients.

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Interleucina 6 y proteína C reactiva ultrasensible para la predicción de la evolución clínica en síndromes coronarios agudos sin elevación del segmento ST

RESUMEN

Introducción y objetivos: Las concentraciones basales de interleucina 6 y proteína C reactiva elevadas comportan un aumento del riesgo de muerte en el síndrome coronario agudo sin elevación del segmento ST. El objetivo del estudio es delucidar si las determinaciones seriadas de interleucina 6 y proteína C reactiva ultrasensible aportan información pronóstica adicional a las determinaciones basales para la estratificación del riesgo a largo plazo de los pacientes con síndrome coronario agudo sin elevación del segmento ST.

Métodos: Se incluyó prospectivamente en el estudio a 216 pacientes consecutivos con síndrome coronario agudo sin elevación del segmento ST. Se obtuvieron muestras de sangre en un plazo de 24 h tras el ingreso en el hospital y a los 30 días de seguimiento. La variable de valoración principal fue la combinación de muerte por todas las causas, infarto de miocardio no mortal e insuficiencia cardiaca aguda descompensada.

Resultados: Las concentraciones tanto de interleucina 6 como de proteína C reactiva ultrasensible se redujeron del día 1 al día 30, con independencia de los eventos adversos aparecidos ($p < 0,001$ en ambos casos). Los valores de interleucina 6 en los dos momentos de valoración (día 1, por pg/ml; *hazard ratio* = 1,006; intervalo de confianza del 95%, 1,002-1,010; $p = 0,002$; día 30, por pg/ml; *hazard ratio* = 1,047;

Palabras clave:

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intervalo de confianza del 95%, 1,021-1,075; $p < 0,001$) fueron predictores independientes de eventos adversos, pero no los de proteína C reactiva ultrasensible del día 1 y el día 30. Los pacientes con interleucina 6 el día 1 $\leq 8,24$ pg/ml y el día 30 $\leq 4,45$ pg/ml fueron los que presentaron la tasa de eventos adversos más baja (4,7%), mientras que los pacientes con valores superiores a la mediana de ambos parámetros fueron los que tuvieron la tasa de eventos adversos más alta (35%). Después de la adición de la interleucina 6 del día 30 al modelo multivariable, el índice C aumentó de 0,71 (intervalo de confianza del 95%, 0,63-0,78) a 0,80 (intervalo de confianza del 95%, 0,72-0,86; $p = 0,042$) y la mejora neta de la reclasificación fue de 0,39 (intervalo de confianza del 95%, 0,14-0,64; $p = 0,002$).

Conclusiones: En esta población, tanto la concentración de interleucina 6 como la de proteína C reactiva ultrasensible se reducen tras la fase aguda. La determinación de las concentraciones de interleucina 6 en muestras seriadas mejora la estratificación pronóstica del riesgo en estos pacientes.

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Abbreviations

hs-CRP: high-sensitivity C-reactive protein
 IL-6: interleukin-6
 NSTEMI: non-ST-segment elevation acute coronary syndromes

INTRODUCTION

Previous studies have shown high levels of both interleukin-6 (IL-6) and C-reactive protein confer an increased risk of long-term mortality in patients with non-ST-segment elevation acute coronary syndrome (NSTEMI).^{1–6} Moreover, early serial plasma level monitoring of these cytokines in acute coronary syndromes has also been found to be predictive of adverse outcomes,^{7–13} even in those patients with normal serum troponin T.¹⁴ However, most studies considered only early (24 h to 72 h) presenting values of these biomarkers, but the prognostic role of delayed serial measurements for long-term risk stratification in patients with NSTEMI remains uncertain.

Our objective was to evaluate the prognostic value of serial sampling of IL-6 in comparison to high-sensitivity C-reactive protein (hs-CRP), and to determine whether serial measurements provide additional information to isolated measurements in the long-term risk stratification of NSTEMI patients.

METHODS

Study Population and Design

This study was designed to determine the prognostic utility of serial measurements of IL-6 and hs-CRP in high-risk NSTEMI patients. From 1 September 2006 to 31 March 2008, 216 consecutive patients with high-risk unstable angina or non-ST-elevation myocardial infarction were prospectively enrolled. The diagnosis of high-risk NSTEMI was established on the basis of current criteria guidelines (defined as ischemic symptoms lasting ≥ 10 min, and occurring within 72 h before randomization, and either ST-segment deviation of ≥ 1 mm, deep symmetrical inversion of the T waves in the anterior chest leads, or elevated levels of a cardiac biomarker of necrosis). Patients with evidence of hepatic dysfunction (an alanine aminotransferase level that was more than twice the upper limit of the normal range); concomitant neoplastic, infectious, connective tissue, or inflammatory diseases; deep vein thrombosis or pulmonary embolism; and recent (< 1 month) surgery or trauma were excluded, as were patients taking immunosuppressant agents.

During the entire hospitalization period, baseline clinical characteristics were prospectively recorded and all patients

received standard management as recommended for NSTEMI.^{15,16} The clinical management decisions about each patient were decided by the cardiologist responsible who was unaware of the IL-6 or hs-CRP levels. After this, hospital discharged-patients were clinically followed up for at least 12 months—median 667 days [interquartile range, 397-944]—and adverse clinical events were obtained in all the patients. The study outcome was a composite of all cause death, nonfatal myocardial infarction, or acute decompensated heart failure during the follow-up period. Death was ascertained from available medical records and death certificates. Myocardial infarction was defined by detection of rise in cardiac biomarkers of necrosis (usually troponin T) with at least 1 value above the 99th percentile upper reference limit, together with evidence of myocardial ischemia with at least 1 of the following: electrocardiographic changes indicative of new ischemia (new ST-T changes or new left bundle branch block), new pathological Q waves in at least 2 contiguous leads, imaging evidence of new loss of viable myocardium, or new wall motion abnormality.^{17,18} Acute decompensated heart failure diagnosis was established on the basis of current criteria guidelines and defined as the rapid or gradual onset of signs and symptoms of heart failure, resulting in unplanned hospitalization.¹⁹ The study was approved by the local ethics committee, and informed consent was obtained from each patient at inclusion.

Biochemical Analysis

Blood samples were collected within 24 h of hospital admission and at 30 days of follow-up. At 30 days post acute coronary syndrome, 146 (68%) of the 216 patients recruited attended follow-up and underwent the blood test. All blood samples were obtained prior to coronary angiogram by venipuncture at 8:00 a.m. within 24 h of hospital admission and at 30 days of follow-up; aliquots of serum were stored at -80 °C until analyzed. Concentrations of IL-6 and hs-CRP were determined using Cobas® 6000 analyser (Roche Diagnostics; Mannheim, Germany). Detection limits were 1.5 pg/mL for IL-6 and 0.15 mg/dL for hs-CRP. Within-run and total coefficients of variation of assays were $< 2\%$ and 4.9% respectively for IL-6, and $< 2\%$ and 3.6% respectively for hs-CRP.

Statistical Analysis

Differences in population characteristics were compared using the analysis of variance or the Kruskal-Wallis test for continuous variables, and chi-square test or Fisher's exact test for categorical variables. IL-6 and hs-CRP concentrations were compared using the Wilcoxon test at the 2 time points assessed throughout the study protocol. Comparisons of both biomarkers between groups with and without events were performed by the Mann-Whitney U test. Correlations between both biomarkers and other clinical and analytical parameters were assessed by the Spearman rank correlation.

When analyzing the predictive value of both biomarker levels at day 30, events that occurred before 30 days were censored. Receiver operating characteristic curves analyses for predicting adverse clinical events were performed for both biomarkers. The best prognostic cut-off was defined as the highest product of sensitivity and specificity. To identify predictors of the composite study endpoint during the follow-up period, hazard ratios (HR) derived from the Cox regression analysis, were calculated. The independent effect of variables on the composite endpoint was calculated using a Cox multivariable regression analysis (forward stepwise), incorporating covariates with $P < .05$ in the univariate analysis and other covariates that are known to be associated with adverse outcomes. Log-cumulative hazard plots, time-dependent covariates, and Schoenfeld residuals were used to evaluate adherence of the Cox proportional hazard assumptions. Baseline IL-6 (IL-6_{day1}) and baseline hs-CRP (hs-CRP_{day1}) levels were first entered individually in the multivariable models. Subsequently, IL-6_{day1} were adjusted for IL-6 levels on day 30 (IL-6_{day30}) in a separate multivariable analysis.

To test the hypothesis that serial assessment of IL-6 would improve risk stratification, patients were categorized on the basis of the number of samples with “elevated IL-6”. IL-6 samples were defined as elevated if their levels were above their median values (>8.24 pg/mL for IL-6_{day1} and >4.45 pg/mL for IL-6_{day30}). A new ordinal variable was built on the basis of a combined IL-6 sample taking into account the presence of none, 1 or 2 samples with elevated IL-6 values. The IL-6 values were added separately and also as a combined biomarker score to a model containing clinical risk factors. Model performance was evaluated by C-indexes. Moreover, the improvement in predictive accuracy was evaluated by calculating the net reclassification improvement and integrated discrimination improvement, as described by Pencina et al.²⁰ All $P < .05$ were accepted as statistically significant. Statistical analysis was performed by the use of SPSS version 15.0 software (SPSS Inc.; Chicago, Illinois, United States).

RESULTS

The study population consisted of 216 hospitalized patients with high-risk NSTEMI/ACS (Tables 1 and 2). IL-6_{day1} were positively correlated with baseline hs-CRP (hs-CRP_{day1}) concentrations

($r=0.59$, $P < .001$). IL-6_{day1} weakly correlated with IL-6_{day30} ($r=0.25$; $P=.002$), age ($r=0.24$; $P < .001$); heart rate ($r=0.16$; $P=.022$), left ventricular ejection fraction ($r=-0.16$; $P=.019$); hemoglobin ($r=-0.24$; $P < .001$), leukocytes ($r=0.22$; $P=.001$), troponin T ($r=0.30$; $P < .001$), and GRACE (Global Registry for Acute Coronary Events) score ($r=0.26$; $P < .001$).

Over the study period, a total of 44 (20.3%) patients had the composite endpoint (16 deaths, 26 non-fatal myocardial infarctions and 15 acute decompensated heart failure hospitalizations). Of these, 23 (10.6%) patients had adverse events after the first 30 days (13 deaths, 21 non-fatal myocardial infarctions, and 11 acute decompensated heart failure). Median IL-6 levels were higher among patients with the composite endpoint than those without, both at presentation (15.38 [7.38-31.30] pg/mL vs 7.13 [4.29-16.35] pg/mL; $P < .001$) and at day 30 (8.59 [4.40-10.83] pg/mL vs 4.12 [2.67-5.80] pg/mL; $P=.001$). By contrast, only hs-CRP_{day1} levels (but not hs-CRP on day 30 [hs-CRP_{day30}]) were higher among those with the composite endpoint (1.50 [0.36-3.99] mg/dL vs 0.53 [0.23-1.28] mg/dL; $P=.009$ for hs-CRP_{day1}; and 0.39 [0.10-1.29] mg/dL vs 0.27 [0.13-0.44] mg/dL; $P=.220$ for hs-CRP_{day30}).

Figure 1 shows a significant decrease in concentrations of both biomarkers from presentation to day 30 regardless of adverse events, although those with events had higher concentrations of IL-6 at both times. Given the higher levels at presentation, the absolute decreases of IL-6 and hs-CRP were actually higher in patients with events (-5.87[-0.19 to -19.01] pg/mL vs -2.38[0.58 to -10.81] pg/mL; $P=.019$ for IL-6 and -0.72[-0.26 to -1.60] mg/dL vs -0.19[0.04 to -0.90] mg/dL; $P=.009$ for hs-CRP; respectively), whereas the relative changes of both biomarkers were similar in both groups (-46%[-6 to -65] vs -41%[8 to -76]; $P=.627$ for IL-6 and -61%[-49 to -87] vs -43%[15 to -83]; $P=.190$ for hs-CRP).

When receiver operating characteristic analyses were undertaken to evaluate the prognostic accuracy of both inflammatory biomarkers, IL-6_{day30} concentrations showed the best prognosis ability to discriminate between those patients with and without the composite endpoint (area under the curve [AUC]=0.72; 95% confidence interval [95%CI], 0.64-0.80), followed by IL-6_{day1} (AUC=0.67; 95%CI, 0.61-0.74), whilst hs-CRP_{day1} had overall lower prognostic accuracy (AUC=0.59; 95%CI, 0.52-0.66) (Table 3).

After multivariate Cox regression analyses (Table 4), IL-6 levels, as quantitative variables, were independent predictors of adverse

Table 1
Study Population Clinical Characteristics as a Function of Baseline Interleukin-6 Levels

Variables	Interleukin-6 day 1			P
	Tertile 1 (n=72)	Tertile 2 (n=71)	Tertile 3 (n=73)	
Age, years	63±11	69±11	70±12	.002
Male	47 (66)	47 (65)	46 (63)	.689
Body mass index, kg/m ²	27±4	29±4	29±5	.752
Systolic blood pressure, mmHg	139 [124-159]	140 [128-163]	140 [123-162]	.798
Heart rate, bpm	75 [65-85]	79 [68-93]	79 [69-94]	.096
Diabetes mellitus	28 (39)	40 (55)	33 (45)	.498
Hypertension	49 (69)	54 (75)	62 (85)	.025
Hyperlipidemia	35 (49)	40 (55)	37 (51)	.873
Current smoking	18 (25)	19 (26)	20 (27)	.820
Previous coronary artery bypass graft	4 (6)	13 (18)	2 (3)	.523
Previous percutaneous coronary intervention	19 (27)	26 (36)	20 (27)	.942
Previous non-ST elevation acute coronary syndromes	16 (22)	25 (35)	18 (25)	.787
Previous segment elevation myocardial infarction	15 (21)	11 (15)	11 (15)	.339
Left ventricular ejection fraction, %	60 [55-65]	58 [50-65]	55 [47-63]	.071
Previous stroke	8 (11)	5 (7)	7 (10)	.734

Data are expressed as no. (%), mean±standard deviation, or median [interquartile range].

Table 2
Clinical Characteristics, Laboratory, and Periprocedural Parameters as a Function of Baseline Interleukin-6 Levels

Variables	Interleukin-6 day 1			P
	Tertile 1 (n=72)	Tertile 2 (n=71)	Tertile 3 (n=73)	
Unstable angina/non ST elevation myocardial infarction	29 (41)/42 (59)	23 (32)/49 (68)	15 (20)/58 (79)	.009
ST segment depression	25 (35)	27 (37)	23 (31)	
Transient ST segment elevation	2 (3)	7 (10)	10 (14)	.056
T-wave inversion	12 (17)	15 (21)	13 (18)	
Creatinine, mg/dL	0.9 [0.8-1.0]	0.9 [0.8-1.1]	1.0 [0.8-1.2]	.125
Hemoglobin, g/dL	14.3±1.8	13.9±1.8	13.2±2.0	.002
Leucocytes, 10 ³ /μL	7.9 [6.1-10.4]	8.6 [7.0-10.2]	9.0 [7.6-11.2]	.011
High sensitivity C-reactive protein, mg/dL	0.27 [0.12-0.49]	0.57 [0.29-1.20]	1.76 [0.64-4.54]	<.001
Troponin T, ng/mL	0.12 [0.01-0.36]	0.36 [0.01-1.13]	0.58 [0.13-1.52]	<.001
GRACE risk score	116.2±27.2	132.6±36.2	143.2±46.9	<.001
1-/2-/3- or Left main disease vessel disease	20 (28)/14 (20)/16 (22)	20 (28)/17 (24)/20 (28)	20 (27)/23 (31)/15 (20)	.177
Revascularization: complete/incomplete/no	33 (46)/14 (20)/24 (34)	34 (48)/19 (26)/19 (26)	36 (49)/17 (23)/20 (28)	.411
Percutaneous coronary intervention/coronary bypass graft	40 (56)/7 (10)	45 (62)/8 (11)	51 (70)/2 (3)	.072
Drug eluting stent/bare metal stent/both	24 (34)/15 (21)/2 (3)	24 (33)/12 (17)/8 (11)	28 (38)/19 (26)/4 (5)	.692
Acetylsalicylic acid	67 (94)	69 (96)	67 (92)	.396
Clopidogrel	58 (82)	59 (82)	58 (79)	.710
β-blockers	61 (86)	65 (90)	71 (97)	.950
ACE inhibitors/ARB	61 (86)	64 (89)	56 (77)	.428
Statin	67 (94)	70 (97)	68 (93)	.135
Acenocoumarol	2 (3)	5 (7)	8 (11)	.044

ACE inhibitor; angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blockers; GRACE, Global Registry for Acute Coronary Events. Data are expressed as no. (%), mean±standard deviation, or median [interquartile range].

events at the 2 time points (IL-6_{day1} per pg/mL, HR=1.006; 95%CI, 1.002-1.010; *P*=.002; and IL-6_{day30} per pg/mL; HR=1.047; 95%CI, 1.021-1.075; *P*<.001). In addition, IL-6 levels above the median values both at presentation (>8.24 pg/mL; HR=1.290; 95%CI, 1.025-1.502; *P*=.027) and at day 30 (>4.45 pg/mL, HR=1.453; 95%CI, 1.059-1.994; *P*=.021) were significantly independent predictors of events (Fig. 2), as were its absolute changes (per Δ pg/mL; HR=1.050; 95%CI, 1.024-1.077; *P*<.001). However, both hs-CRP concentrations and the absolute changes in hs-CRP were not independently associated with adverse outcomes (Table 4).

The potential enhanced value from the combined use of serial measurements of IL-6 levels for prognosis risk stratification is shown in Figure 3. This figure shows that the lowest event rate was observed in those patients with IL-6 levels below the median values at presentation and at day 30 (n=43; 4.7%; score=0), whereas the event rate observed among patients who had IL-6 levels above the cut-off values at both time points assessed throughout the study protocol was considerably higher than in any other group (n=40; 35%; score=2; *P*<.001). In adjusted analysis, for each measure of elevated IL-6 levels in the combined score (from 0 to 2) a significantly higher risk of events was observed (per elevated measure, HR=1.35; 95%CI, 1.090-1.670; *P*=.005).

Table 3
Performance of Measures of Interleukin-6 Levels for Prediction of Adverse Events (all Cause Death, Nonfatal Myocardial Infarction, or Acute Decompensated Heart Failure)

Variables	AUC	95%CI	Cut-off	Sensitivity	Specificity	PPV	NPV
IL-6 day 1, pg/mL	0.67	0.61-0.74	8.24	0.75	0.56	0.30	0.90
IL-6 day 30, pg/mL	0.72	0.64-0.80	8.12	0.57	0.91	0.55	0.91
hs-CRP day 1, mg/dL	0.59	0.52-0.66	1.50	0.49	0.78	0.36	0.86
hs-CRP day 30, mg/dL	0.58	0.49-0.66	0.79	0.30	0.89	0.34	0.87

95%CI, 95% confidence interval; AUC, area under the curve; hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; NPV, negative predictive value; PPV, positive predictive value.

Lastly, the addition of IL-6_{day30} concentration to a multivariable model, adjusted for other risk factors (including IL-6_{day1} and GRACE risk score), was associated with a significant improvement in prediction performance and accuracy. The net reclassification improvement from the addition of IL-6_{day30} concentrations was 0.39 (95%CI, 0.14-0.64, *P*=.002), whereas the relative integrated discrimination improvement was 0.49 (95%CI, 0.12-0.66; *P*=.001). The probability of correctly predicting events and no events when IL-6_{day30} was added to the clinical multivariate model was reflected particularly in the percentage of non-events correctly reclassified (48%), while the percentage of events reclassified was -9%. The C-index increased from 0.71 (95%CI, 0.63-0.78) to 0.80 (95%CI, 0.72-0.86); *P*=.042, with the addition of IL-6_{day30} to the clinical multivariate model.

DISCUSSION

In the present study, we examined the prognostic value of serial measurements of circulating levels of IL-6 and hs-CRP in a hospitalized population with NSTEMI. Consistent with the results of earlier studies,^{2,21,22} we found high IL-6_{day1} to be associated with

Table 4
Cox Regression Risk Analysis for the Composite Endpoint (all Cause Death, Nonfatal Myocardial Infarction, or Acute Decompensated Heart Failure)

Variables	HR	P
<i>Model without inflammatory biomarkers (model 1)</i>		
Hemoglobin, per g/dL	0.747 (0.635-0.879)	<.001
Left ventricular ejection fraction	0.958 (0.934-0.982)	.001
<i>Model 1 plus both measurements of hs-CRP</i>		
hs-CRP day 1, per mg/dL	–	.108
hs-CRP day 30, per mg/dL	–	.102
<i>Model 1 plus both measurements of IL-6</i>		
IL-6 day 1, per pg/mL	1.006 (1.002-1.010)	.002
IL-6 day 30, per pg/mL	1.047 (1.021-1.075)	<.001
<i>Model 1 plus absolute changes in hs-CRP plus hs-CRP day 1</i>		
Absolute changes in hs-CRP, per Δ mg/dL	–	.106
<i>Model 1 plus absolute changes in IL-6 plus IL-6 day 1</i>		
Absolute changes in IL-6, per Δ pg/mL	1.044 (1.019-1.072)	<.001

HR, hazard ratio; hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin-6.

The model includes those variables showing statistical significance in univariate Cox regression analyses for the composite endpoint. Also adjusted by Global Registry for Acute Coronary Events risk score, systolic blood pressure, heart rate, sex, diabetes mellitus, hypertension, creatinine, troponin T, and revascularization.

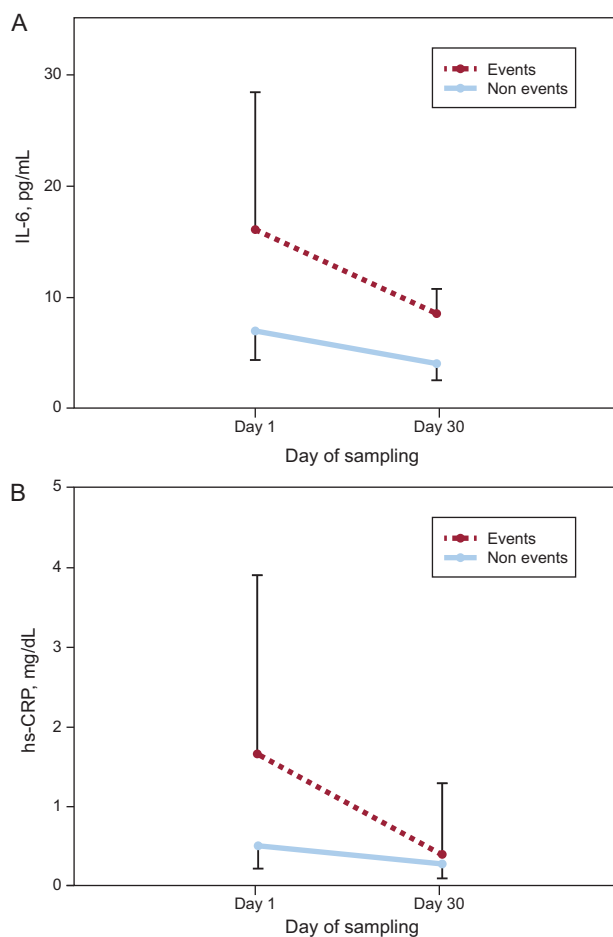


Figure 1. Change in A: interleukin-6. B: high-sensitivity C-reactive protein concentrations, from presentation to day 30 in subjects with and without adverse events. The median interleukin-6 and high-sensitivity C-reactive protein values and interquartile range (error bars) plotted against the day of sampling. hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin-6.

poor long-term outcomes. More importantly, to the best of our knowledge, we demonstrated for the first time, that serial sampling of IL-6_{day30} levels of NSTEMI presentation offers complementary information to its baseline levels and other clinical variables (including GRACE risk score) for long-term risk stratification. These results are novelty, considering, the use of contemporary reclassification analyses for the assessment of the prognostic value of serial measurements of IL-6 levels in this clinical scenario. Accordingly, our results support the use of repeated IL-6 testing in NSTEMI patients once the patients have been stabilized. A good strength of our study is that blood was sampled at a time when patients were considered to be clinically stabilized (30 days after presentation); yet IL-6 values were able to identify those patients who were at a lower risk for long-term adverse clinical events with high negative predictive values (>90%). In this sense, IL-6 monitoring may help us to identify

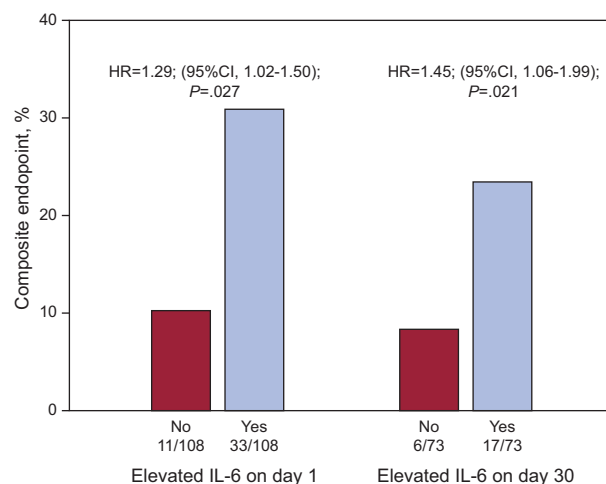


Figure 2. Cox regression risk dichotomized analysis for the composite endpoint (all cause death, nonfatal myocardial infarction, or acute decompensated heart failure) as a function of interleukin-6 levels above the median (>8.24 pg/mL for baseline interleukin-6 and >4.45 pg/mL for interleukin-6 levels on day 30). HR, hazard ratio; IL-6, interleukin-6.

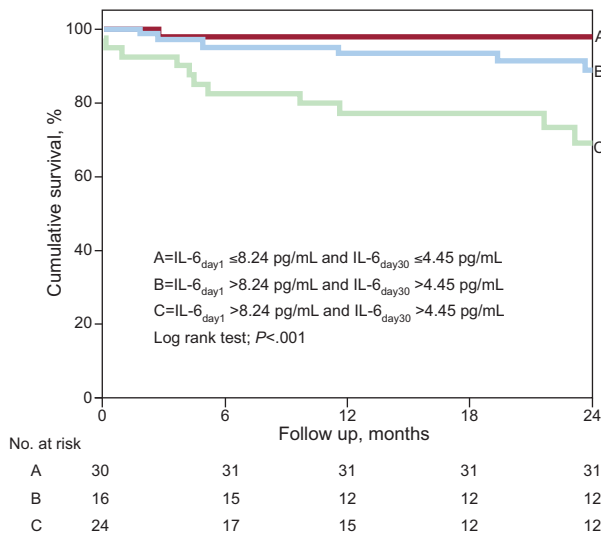


Figure 3. Adverse events rate according to the presence of none, 1 or, both measurements of interleukin-6 above the median values at presentation and at day 30. IL-6_{day1}, baseline interleukin-6; IL-6_{day30}, interleukin levels on day 30.

patients who might not benefit from more aggressive management and monitoring.

IL-6 is a ubiquitous cytokine with a crucial role in leukocyte and endothelial cell activation that promotes increased synthesis of a wide variety of acute phase reactant proteins, including C-reactive protein, and represents the main systemic mediator of the acute response to tissue damage. Furthermore, IL-6 is a key inflammatory cytokine in atherothrombotic disorders. IL-6 is expressed at the shoulder region of atherosclerotic plaques and acts locally to enhance recruitment of monocytes and T cells, with a consequent increase in plaque instability, and acts systemically to induce a pro-inflammatory and pro-thrombotic state.^{23–25} In this study, we found that IL-6_{day1} levels are closely related to long-term prognosis of patients with NSTEMACS, which reaffirms the prognostic significance of the pro-inflammatory status during the initial phase of an acute coronary syndrome.

Our results also emphasize the importance of monitoring “dynamic changes” in the inflammatory state of NSTEMACS patients. Previous studies have shown that patients with unstable angina who had a decrease in IL-6 levels within 48 h to 72 h of hospitalization, presented an uncomplicated in-hospital course, whereas patients with an increase in these levels had a higher rate of in-hospital¹⁴ or 30-day events.^{10,12} The delayed measurement of inflammatory biomarkers in assessing the long-term predictive value has 2 potential advantages. The first advantage is that we overcome the “problem” that is posed on prognosis by the easy access to revascularization, either during the hospital admission (urgently) or early after discharge (electively). This early invasive treatment in NSTEMACS patients may change the natural history of coronary artery disease and thus attenuate the long-term prognostic value of an inflammatory marker measured during the acute phase. Indeed, Ozer et al. demonstrated that early coronary angioplasty modifies levels of inflammatory biomarkers, including the type of stent used.²⁶ The second advantage is that, 4 weeks after the coronary event, the excess inflammatory response caused by the event should have decreased to the new steady state level or should be “normalized”, and inflammatory markers may then reflect the underlying chronic inflammatory process and thus, be better indicators of the severity of the disease. Consequently, a persistent elevation of inflammatory biomarkers beyond the acute phase suggests prolonged inflammation and could represent a subclinical hallmark of recurrent instability.²⁷

In our study, IL-6 levels showed a significant decrease at 30 days regardless of the occurrence of events but measurement of IL-6_{day30} levels provides additional information to isolated measurements in the long-term risk stratification of these patients. It supports the hypothesis that a persistent inflammatory activation, as indicated by higher levels of IL-6, is an important predictor of long-term prognosis in patients with NSTEMACS.

There are controversial results in the literature regarding the prognostic value of inflammatory markers. The SIESTA study (Systemic Inflammation Evaluation in patients with non-ST segment Elevation Acute Coronary Syndrome) evaluated the prognostic value of hs-CRP, IL-6, and other markers of inflammation in patients with low to moderate risk NSTEMACS.²⁸ In this study, inflammatory markers failed to provide independent prognostic information at 6 months and 12 months of follow-up. However, these apparently discrepant results may be attributed to differences in the population characteristics, including the higher risk profile and higher inflammatory burden of our patients (mainly reflected in higher levels of IL-6 in our population).^{29,30} A paper by Rallidis et al. also studied the prognostic value of cytokines monitoring after 6 weeks of presentation in patients with unstable angina, but IL-6 levels at 6 weeks were not independent predictors of long-term prognosis.¹³ One possible explanation could be the inclusion of the event revascularization because of new or worsening symptoms in the combined endpoint. Previous studies have failed to demonstrate an association between inflammatory markers and angina³¹ or inflammatory markers and late angiographic stent restenosis.³² Indeed, circulating biomarkers of inflammation may be more related to acute plaque rupture and thrombosis rather than to slowly progressive occlusive coronary atherosclerosis.³³

On the other hand, our composite endpoint also included acute decompensated heart failure. Previous studies have shown that elevated IL-6 concentrations in patients with coronary artery disease preceded progression to clinically overt heart failure.³⁴ Also IL-6 may by itself exacerbate the damage that results from minor myocardial necrosis/injury or may stimulate muscle atrophy and myocardial failure during acute coronary syndrome.³⁵ This may be 1 of the reasons explaining why circulating IL-6 levels have been implicated on acute coronary syndrome and heart failure, and their increase is associated independently, with long-term mortality/acute decompensated heart failure in an acute coronary syndrome population.²²

Our results may suggest that the prognostic value of serial IL-6 measurements may be superior to those of hs-CRP. The lack of predictive value of hs-CRP in our study contrasts with data in previous reports.^{3,36,37} This finding could be explained by the low statistical power of our study. However our results agree with other studies showing a limited role of hs-CRP for risk prediction. Thus, previous reports have showed that hs-CRP has added modest information to the overall prediction of risk resulting from the assessment of conventional cardiovascular risk factors.^{30,38} It probably suggests that hs-CRP predictive usefulness might not be universal. Further studies are needed to address this challenging issue in different populations.

Limitations

The small sample size of patients in a single center study and the relatively small number of events, especially after the 1st month, make it difficult to draw firm conclusions. Moreover, caution must be applied when interpreting our results, as they might not be transferable to all NSTEMACS patients/cases, and should be considered as hypothesis generating. Moreover, given the fact that patients with adverse clinical events within 30 days of

follow-up were censored, the survivorship bias in the acute phase may limit interpretation of the results.

Economic, biological, and analytical reasons, partially explain the limitations of the use of inflammatory biomarkers. The availability of high-sensitivity and relatively low-cost methods for IL-6 measurement could facilitate its routine use. In addition, although our samples were stored at -80°C , we cannot exclude the possibility of protein degradation. Such an effect is unlikely, however, because the distribution of IL-6 in our study was quite similar to that observed in studies that used fresh blood samples. Another consideration is the lack of standardized methods for IL-6 measurement, which would include the use of the same peptide preparation and the same units, references, and cut-off values. Nevertheless, since our detection limit is 1.5 pg/mL, our automatized system allows detection within the range of the most sensitive available commercial enzyme-linked immunosorbent assay kits (0.7–5 pg/mL), and avoids variations due to sample manipulation in manual protocols. Inflammatory indexes were measured in peripheral blood, and do not allow firm conclusions on the release of these factors within the coronary circulation. Medications may affect plasma levels of the measured inflammatory indexes,³⁹ however, patients with and without events, received similar pharmacological treatment. Another major limitation of determination of IL-6 is its low specificity, as the plasma concentrations of IL-6 are influenced by a variety of diseases. The circulating levels of IL-6 may also show a natural variability over the time-course of the disease. Indeed, IL-6 levels follow a circadian rhythm with a sharp nocturnal rise.⁴⁰ To minimize this problem, blood samples were always withdrawn at the same time (at 8 a.m.).

CONCLUSIONS

Among NSTEMI patients, serial sampling of IL-6 levels at 30 days provides complementary information for the long-term risk stratification, and may help to identify patients who might not benefit from more aggressive management and monitoring.

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CONFLICTS OF INTEREST

None declared.

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