High-Sensitivity C-Reactive Protein Is Within Normal Levels at the Very Onset of First ST-Segment Elevation Acute Myocardial Infarction in 41% of Cases

A Multiethnic Case-Control Study

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Objectives

This study sought to assess the prevalence of normal levels of high sensitivity C-reactive protein (hsCRP) at the very onset of ST-segment elevation myocardial infarction (STEMI).

Background

Levels of hsCRP ≥ 2 mg/l identify individuals who benefit from lipid lowering and possibly anti-inflammatory agents, but how many patients develop infarction in spite of hsCRP levels < 2 mg/l and thus would be ineligible for these treatments?

Methods

We studied 887 patients with unequivocally documented STEMI as the first manifestation of coronary disease and 887 matched control subjects from urban areas of Italy, Scotland, and China. Blood samples were obtained before reperfusion strategies < 6 h from symptoms onset in order to limit acute event-related increases.

Results

hsCRP values were similar in samples obtained < 2 h, 2 to 4 h, and 4 to 6 h from symptoms onset in all ethnic groups, consistent with the delayed hsCRP elevation after myocardial necrosis and thus indicative of pre-infarction levels. Median hsCRP values were significantly higher in patients than in control subjects: 2.49 (interquartile range [IQR]: 1.18 to 5.55) mg/l versus 1.32 (IQR: 0.58 to 3.10) mg/l (p < 0.0001), which is consistent with previous findings. However, 41% of patients had hsCRP levels < 2 mg/l and conversely, 37% of control subjects had values ≥ 2 mg/l.

Conclusions

The measurement of hsCRP, with a 2 mg/l cutoff, would not have predicted 41% of unequivocally documented STEMIs in 3 ethnic groups without evidence of previous coronary disease, thus indicating both its limitations as an individual prognostic marker and as an indicator of a generalized inflammatory pathogenetic component of STEMI. New specific prognostic and therapeutic approaches should be found for such a large fraction of patients at risk. (J Am Coll Cardiol 2011;58:2654–61) © 2011 by the American College of Cardiology Foundation

Elevated circulating high-sensitivity C-reactive protein (hsCRP) was found to be predictive of acute coronary syndromes in secondary (1,2) as well as in primary prevention studies (3), nonprofit organization, and the Ministero dell’Istruzione dell’Università e della Ricerca (Italy) grant number: FIRB RBAA01FSA4 and Regione Friuli (Italy). The funding sources had no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication. The authors have reported that they have no relationships relevant to the contents of this paper to disclose. Drs. Cristell and Cianflone contributed equally to this paper.

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although with variable incremental values above that provided by established risk factors (RF) (4,5). In addition, healthy individuals with hsCRP ≥2 mg/l have been shown to benefit from high dose statin therapy, and trials with treatments specifically aimed to lower the inflammatory component suggested by elevated hsCRP are being considered (6). Therefore, a critical question becomes: What percentage of patients develops an acute myocardial infarction despite having hsCRP levels within normal limits?

The prevalence of normal hsCRP levels at the very onset of acute myocardial infarction indicates the proportion of patients who would not have been identified as at risk by baseline, pre-infarction measurements. This percentage could be underestimated if, in some patients, at admission, hsCRP was increased because of inflammatory pathogenetic mechanism of infarction, whereas the effects of necrosis are unlikely to be detectable in a blood sample taken within 6 h from the onset of symptoms (7).

We measured circulating levels of hsCRP in carefully selected patients with unequivocally documented ST-segment elevation myocardial infarction (STEMI) belonging to different ethnic groups with a 4-fold difference in coronary artery disease incidence (8): namely, Italians, Scottish, and Chinese from metropolitan areas, together with age- and sex-matched control subjects. To minimize the possible contribution of inflammatory components secondary to myocardial injury, we included only patients in whom a blood sample could be obtained within a maximum of 6 h from the onset of symptoms and prior to coronary reperfusion strategies. We also excluded patients with previous evidence of cardiovascular disease to reduce potential confounding effects of drug therapy and/or lifestyle modifications, as hsCRP levels were not found to have a predictive value in healthy individuals taking aspirin (9,10).

In addition, we measured interleukin (IL)-6 as an earlier marker of inflammatory response, which is also a putative predictor of cardiovascular risk (11). We correlated serum levels of hsCRP and IL-6 with the time of sampling after onset of symptoms, with troponin I levels, and with cardiovascular RF. Finally, we calculated the average incremental predictive value for STEMI of hsCRP over that of traditional RF.

**Methods**

**Study population.** In the FAMI (First Acute Myocardial Infarction) study, participants were recruited from urban areas of Italy, Scotland, and China (a total of 32 centers participated) (Online Appendix). These 3 countries were chosen based on data from the MONICA (Monitoring Trends and Determinants in Cardiovascular Disease) registry (8), which showed extreme differences in coronary artery event rates, with Scotland having 4 times higher incidence rate of myocardial infarction than China and Italy did in an intermediate position. All patients, irrespective of age, who arrived in hospital via the emergency room or admitted to the coronary care unit within 6 h of symptom onset of STEMI, were screened. Patients were eligible if they had electrocardiographic evidence of STEMI, no previous history of coronary artery disease, and reported symptom onset of <6 h. A blood sample was obtained before any reperfusion procedures.

One control subject matched for age (up to 5 years older or younger), sex, and environment (enrolled from the same city) was recruited for each index case. Inclusion criteria for control subjects included no previous diagnosis of coronary disease or history of exertional chest pain.

A total of 1,206 cases and 984 control subjects were enrolled between October 2002 and April 2007. Of these, 887 patients and 887 control subjects who met all entry and matching criteria were finally analyzed in this paper: Figure 1 shows the consort flow diagram, and Table 1 shows the distribution per country.

**Risk factor evaluation.** Each participating center received on-site training, either directly by a member of the coordinating center or by the local coordinator, for patient and control enrollment. A manual explaining all questions in the case report forms was distributed with the complete material for case enrollment. A standardized structured questionnaire was administered, and physical examinations were undertaken in the same manner in cases and control subjects. Information about demographic factors, socioeconomic factors (income, employment, level of education), lifestyle (physical activity during leisure and work time, dietary habits), psychosocial factors, and personal and family history of cardiovascular disease and traditional RF (smoking, diabetes, dyslipidemia, hypertension) was obtained. Height, weight, and abdominal circumference were determined by a standardized protocol (8). Self-reported hypertension and diabetes were used in the analysis of RF. We considered the ratio of apolipoprotein (Apo) B to ApoA1 for dyslipidemia because apolipoprotein concentrations are not appreciably affected by fasting status and have been shown to be superior to any of the traditional cholesterol ratios for estimating the risk of myocardial infarction (12,13). Threshold levels for the tertiles of the ApoB/ApoA1 ratio were derived from all control subjects (1).

Dyslipidemia was calculated from the ApoB/ApoA1 ratio and the threshold value was defined as the highest tertile of control subjects (≥1.14). We defined current smoking as individuals who currently smoked any tobacco or stopped smoking >1 year prior to the event. Former smokers were defined as individuals who stopped smoking more than 1 year earlier.
The case report forms were both paper and electronic. We developed a real-time, online framework for the collection, control, and verification of case report forms and study management forms, as well as for real-time study progress monitoring and quality control procedures.

Data on smoking were missing in 0.4% of participants, hypertension in 0.6%, diabetes in 0.5%, and abdominal circumference in 0.6%.

Nonfasting blood samples (50 ml) were drawn from each patient before treatment. After separation, microaliquots of serum, sodium-citrate plasma, heparin plasma, and whole blood were frozen immediately at −80°C. Fasting blood samples were drawn from each control subject and processed similarly. All samples were shipped by courier in dry ice to the core laboratory in Milan, Italy, where they were stored at −80°C (Polar 530V AHSI-Angelantoni freezer, Milan, Italy). Serum samples from all countries were analyzed at the Università Vita-Salute San Raffaele Core laboratory for ApoB, ApoA1, hsCRP, troponin I, and IL-6. Turbidimetric assays were used to measure ApoA1 and ApoB concentrations in serum samples (Cobas Mira Plus/Horiba ABX, Kyoto, Japan). High and low concentrations as control subjects (Horiba ABX) were analyzed in every run, and a pool control serum that had previously been tested in the core laboratory was measured for all analytes. IL-6 was measured using an enzyme-linked immunoassorbent assay.

The number of enrolled patients and controls is shown in Figure 1. The enrollment numbers and age and sex distribution for the overall population and for individual ethnic groups are detailed in Table 1. In the 3 ethnic groups, men were about 9 years older than women.
kit (R&D Systems, Minneapolis, Minnesota), and a pool of control serum was tested in all plates to determine interassay precision (detection limit: 0.16 to 50.0 pg/ml, and interassay variability, defined by a coefficient of variation of 11.3%). Immunochemical luminescent assays were used to measure hsCRP (detection limit: 0.10 to 150.0 mg/l, and interassay variability, defined by a coefficient of variation of 10.2%) and troponin I concentration in serum with Immulite 2000 (Medical Systems SpA, Genoa, Italy). High and low control concentrations were analyzed for each analyte. Every calibration kit (collected about every 1 or 2 weeks) and a pool control serum that had previously been tested in the core laboratory was measured for all analytes every run. Several aliquots for each case and control were saved for future analysis.

The FAMI Study was approved by appropriate regulatory and ethics committees in all participating centers in all 3 countries. All participants provided informed consent before taking part in the study.

**Statistical analysis.** Comparisons between cases and control subjects were performed by paired $t$ tests or Wilcoxon test and McNemar test as appropriate. A conditional logistic regression model for matched pairs was run. Univariate models for each single RF and a multivariate analysis were run. Interactions of each RF and a multivariate analysis were run. Interactions of each RF with ethnic groups were tested. In order to investigate the risk of STEMI associated with multiple RF, a model including the number of classical RF (diabetes mellitus, hypertension, dyslipidemia, smoking) was run and the predictive value of hsCRP $\geq 2$ mg/l for STEMI was tested, including hsCRP and its interaction with the number of RF. Estimates of odds ratios and accompanying 95% confidence intervals are presented for each RF.

Statistical analysis and graphics were produced with STATA (version 9.2, StataCorp, College Station, Texas), SPSS (version 13, IBM SPSS, Chicago, Illinois), and GraphPad Prism (version 5, GraphPad Software, La Jolla, California).

**Results**

A total of 887 patients with acute myocardial infarction occurring as their first manifestation of coronary artery disease and 887 matched control subjects were included in the study (Fig. 1). Overall, 76% of patients in the total population were men, and their mean age at presentation was 9 years lower than that of women ($68 \pm 11.9$ years vs. $59 \pm 11.7$ years) across all 3 ethnic groups (Table 1). There was a very low percentage of patients on cardiovascular drugs, as we eliminated those with a previous history of cardiovascular disease (Table 2). The diagnosis of infarction was documented unequivocally in all by the presence of ST-segment elevation on presentation and by the subsequent peak creatine phosphokinase: median 1,606 (interquartile range [IQR]: 770 to 3,037) U/l. In addition, 795 (89.6%) patients underwent emergency coronary reperfusion therapy. Overall, 34.4% of STEMI patients had 1 or more episodes of angina in the 48 h preceding admission prior to the incident that brought them to hospital admission.

In these patients with a definite STEMI, the interval between symptom onset and blood sampling was short enough not to have increased appreciably pre-existing hsCRP levels, as troponin I in the admission blood sample was $\leq 0.2$ ng/ml in 52% of patients and was only slightly elevated in the remaining patients: median 2.09 (IQR: 0.32 to 14.03) ng/ml. Indeed, there was no difference in hsCRP levels in patients sampled $\leq 2$, 2 to 4, or $\geq 4$ h from the onset of symptoms. Moreover, hsCRP levels were similar in patients with troponin I $\leq 0.2$ and $> 0.2$ ng/ml. Thus, these hsCRP values are likely to closely reflect those preceding the acute event.

Although these patients had an unequivocally documented acute STEMI, 40.9% had admission hsCRP values lower than the cutoff value of 2 mg/l; conversely, 37.0% of control subjects had values $\geq 2$ mg/l, with distribution of values similar across the 3 ethnic groups.

Median hsCRP levels in the admission blood sample in the whole study group were significantly higher than those of control subjects: median 2.49 (IQR: 1.18 to 5.55) mg/l versus median 1.32 (IQR: 0.58 to 3.10) mg/l, respectively ($p < 0.0001$), but with a very large overlap (Fig. 1). Indeed, 27.0% of STEMI patients had hsCRP values lower than the median value for control subjects and, conversely, 31.1% of control subjects had values higher than the median value for

| Table 2 Clinical Characteristics for the Overall Population and Separated by Country |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| **Symptoms time, min**        | 180 (110–270)               | 165 (100–261)               | 140 (93–228)               | 210 (130–300)               | $<0.0001$                 |
| **Troponin I, ng/ml**          | 0.62 (0.20–3.26)            | 0.74 (0.20–4.41)            | 0.39 (0.20–1.38)           | 0.73 (0.20–4.1)             | $<0.0001$                 |
| **Peak CPK, U/l**              | 1,606 (770–3,037)           | 1,355 (650–2,616)           | 1,745 (945–3,465)          | 1,760 (708–3,059)           | 0.005                     |
| **Therapy**                    |                            |                            |                            |                            |                          |
| Aspirin                      | 142 (16)                    | 67 (21)                     | 42 (20)                    | 32 (9)                      |                          |
| Beta-blocker                 | 62 (7)                      | 29 (9)                      | 25 (12)                    | 11 (3)                      |                          |
| ACE inhibitor/ARB            | 89 (10)                     | 48 (15)                     | 23 (11)                    | 18 (5)                      |                          |
| Statin                       | 44 (5)                      | 19 (6)                      | 23 (11)                    | 11 (3)                      |                          |
| Other lipid-lowering agents  | 6 (0.67)                    | 3 (1)                       | 1 (0.4)                    | 3 (0.8)                     |                          |

Values are median (interquartile range) or n (%). $p$ value refers to overall comparison among Italians, Scottish, and Chinese. ACE = angiotensin-converting enzyme; ARB = angiotensin receptor blocker; CPK = creatine phosphokinase; IQR = interquartile range.
STEMI patients. Thus, the calculated sensitivity, specificity, and overall accuracy for identifying the presence of STEMI with a 2 mg/l cutoff were 66%, 64%, and 65%, respectively.

There were no significant differences in hsCRP levels between patients with (34.4% of the cases) and without (65.6%) episodes of angina in the 48 h preceding admission: median 2.65 (IQR: 1.13 to 5.82) versus median 2.39 (IQR: 1.16 to 5.37), respectively, $p = \text{NS}$.

Interleukin 6 levels were also significantly higher in patients than in control subjects, with a large overlap: median 5.08 (IQR: 2.88 to 9.89) pg/ml versus median 1.38 (IQR: 0.71 to 2.67) pg/ml ($p < 0.0001$), respectively, and in each ethnic group, about 20% of patients had values above 10 pg/ml (Fig. 2). There was no correlation between hsCRP and IL-6 levels. Also, levels of IL-6 were not statistically different in patients sampled <2 h, 2 to 4 h, or 4 to 6 h from onset of symptoms, but levels of IL-6 were slightly elevated in patients who had troponin I >0.2 pg/ml: median 5.7 (IQR: 3.3 to 10.8) pg/ml versus median 4.4 (IQR: 2.5 to 8) pg/ml ($p = 0.0025$).

Both Scottish patients and their control subjects had slightly higher admission values of hsCRP than the Italians and Chinese did ($p = 0.001$), but the absolute difference between patients and control subjects was similar in all 3 ethnic groups. The levels of IL-6 were not different across the 3 groups.

Overall, there was a significant association between hsCRP levels $\geq$2 mg/l and first myocardial infarction for the entire population and for all 3 ethnic groups. In the overall population adjusted for age, sex, smoking, dyslipidemia, diabetes, hypertension, and body mass index, the odds ratio was 2.24 (95% confidence interval [CI]: 1.42 to 3.28) for hsCRP (Fig. 3), which is comparable to that reported in previous primary prevention studies (14) and an odds ratio (OR) of 5.89 (95% CI: 4.37 to 7.96) for IL-6. The high predictive value of IL-6 levels suggests that, in spite of the early time of sampling after the onset of

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**Figure 2** hs-CRP and IL-6 Levels

Scatter plot distribution of patients and control subjects of high-sensitivity C-reactive protein (hsCRP) admission levels for the total population (A) and for each country (B). Scatter plot of patients and control subjects for interleukin (IL)-6 admission levels for the total population (C) and for each country (D). Note the scale change on the y axis for IL-6. For both markers in all graphs, patients versus control subjects: $p < 0.0001$. IQR = interquartile range.
symptoms, IL-6, but not hsCRP levels, reflected an acute myocardial infarction–related component, as indicated by the higher IL-6 values observed in patients with elevated troponin I.

The overall prevalence of the 4 major modifiable cardiovascular RF (smoking habit, dyslipidemia, hypertension, and diabetes) was significantly higher in patients than in control subjects (p < 0.0001). Each of them was found to have a significant predictive value for STEMI in the whole study group after adjustment for age, sex, and for the remaining RF (Fig. 3). There were no significant interactions with the ethnic groups in the multivariate analysis. The risk of STEMI associated with multiple RF was: 1 RF: OR: 1.21 (95% CI: 0.79 to 1.87); 2 RFs: OR: 2.64 (95% CI: 1.68 to 4.15); 3 RFs: OR: 6.41 (95% CI: 2.64 to 15.55); 4 RFs: OR: 10.12 (95% CI: 2.88 to 32.66). However, the majority of patients presenting with their first acute myocardial infarction had only 1 or 2 of the major RF, whereas the number of patients with 3 or 4 RF was rather small, which is consistent with previous reports (15). A similar trend was observed in all 3 ethnic groups.

The presence of hsCRP ≥2 mg/l had an incremental predictive value for STEMI in patients with 1 or more RF with an OR of 2.12 (95% CI: 1.63 to 2.75) (p < 0.0001) (Fig. 4). The incremental predictive value of hsCRP did not differ when only patients with troponin I <0.2 ng/ml were considered: OR: 2.09 (95% CI: 1.42- to 2.92). There were no significant differences in the number of cardiovascular RF among patients with hsCRP <2 mg/l and those with hsCRP ≥2 mg/l (Table 3).

Discussion

Our findings indicate that in over 40% of 887 patients admitted within 6 h from symptoms onset for an unequivocally documented STEMI, before emergency reperfusion strategies were performed in 89.6% of the cases, hsCRP serum levels were below 2 mg/l. The percentage of low baseline levels might have been even larger if, in some patients, the baseline hsCRP value became elevated above baseline just before the sampling time because of the acute infarction process. This possibility appears unlikely for myocardial necrosis because of the early sampling, but it cannot be excluded for potential inflammatory triggers of infarction.

At any rate, a substantial percentage of patients appears to develop acute STEMI in spite of baseline levels of hsCRP <2 mg/l and, hence, would not be eligible for preventive strategies now under consideration (16).

The median values and interquartile ranges of hsCRP in our patients and control subjects were similar to those reported in previous preventive studies (14), which however
were only focused on median values and did not give appropriate consideration to the wide dispersion of values, largely overlapping with those of control subjects, and to its practical implications.

Indeed a very wide dispersion of values of hsCRP was reported in the control subjects of PROVE IT–TIMI 22 (Pravastatin or Atorvastatin Evaluation and Infection Therapy–Thrombolysis In Myocardial Infarction 22) study (14) and found to be unrelated to the low-density lipoprotein cholesterol levels. Our findings also demonstrated that patients with unequivocally documented very acute STEMI exhibit a very wide dispersion of values and a broad overlap with control subjects (Fig. 2). The wide dispersion of hsCRP values was observed in spite of the selection of a phenotypically homogeneous group by restricting the inclusion criteria to patients with STEMI within 6 h from onset of symptoms occurring as their very first manifestation of coronary disease, very few of whom were on cardiovascular drugs. Previous reports from our group indicated that hsCRP was higher in patients in whom infarction was preceded by “severe unstable angina” (17), but the findings were not confirmed in the present report based on a much larger number of patients, in any of the 3 ethnic groups, possibly because of less stringent definition of “unstable angina.”

The intriguing observation of markedly elevated levels of IL-6 above 10 pg/ml in about 20% of patients in each ethnic group (Fig. 2) and the lack of correlation with hsCRP levels suggested the investigation of the inflammatory components of STEMI at the 2 extreme ends of the spectrum in matched patients with either markedly elevated or very low IL-6 levels. The biological and genetic characterization of these 2 extreme groups are currently in progress using some of the multiple samples stored in the central biological bank, but conclusive results are not yet available. Such differences are not related to commonly considered variables, because these 2 extreme groups had similar incidence of pre-infarction angina, similar estimated infarct size, similar levels of hsCRP, and similar RF profile. Among those patients with hsCRP <2 mg/l, more than 80% had ≥2 traditional RF, a proportion not significantly different from that observed in patients with hsCRP ≥2 mg/l (Table 3). Current work includes the study of a set of 48 single nucleotide polymorphisms potentially involved in multiple biological pathways.

### Table 3 Distribution of RF Among Control Subjects and Patients Divided on the Basis of hsCRP <2 or ≥2 mg/l

<table>
<thead>
<tr>
<th></th>
<th>0 RF n. (%)</th>
<th>1 RF n. (%)</th>
<th>2 RF n. (%)</th>
<th>3 RF n. (%)</th>
<th>4 RF n. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>254 (28.5)</td>
<td>381 (43.0)</td>
<td>204 (23.0)</td>
<td>45 (5.1)</td>
<td>3 (0.4)</td>
</tr>
<tr>
<td>Patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients with hsCRP &lt;2 mg/l</td>
<td>363</td>
<td>45 (12.2)</td>
<td>135 (37.3)</td>
<td>139 (38.3)</td>
<td>40 (11.1)</td>
</tr>
<tr>
<td>Patients with hsCRP ≥2 mg/l</td>
<td>524</td>
<td>48 (9.2)</td>
<td>176 (33.5)</td>
<td>215 (41.1)</td>
<td>76 (14.5)</td>
</tr>
</tbody>
</table>

Values are n (%).
hsCRP = high-sensitivity C-reactive protein; RF = risk factor(s).
At present, we have no plausible explanation for the finding of hsCRP levels <2 mg/l in 41% of the cases in all 3 ethnic groups considered.

Conclusions

We found that 41% of patients in 3 different ethnic groups develop an acute STEMI as the very first manifestation of coronary disease despite having hsCRP levels below 2 mg/l. This observation indicates that the current reductionist (“one size fits all”) approach based on statistically significant differences in median values might have limitations when the dispersion of values about the median is very wide and overlaps with control subjects (18,19). In such a large fraction of patients, it appears mandatory to search for new pathophysiological mechanisms in order to identify specific markers of risk and novel therapeutic targets for effective preventive strategies.

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Key Words: high-sensitivity C-reactive protein  myocardial infarction  ST-segment elevation myocardial infarction.

APPENDIX

For a list of centers participating in the FAMI study, as well as the FAMI Investigators’ acknowledgments, please see the online version of this paper.