Inflammation Markers and Risk Stratification in Patients With Acute Coronary Syndromes. Design of the SIESTA Study (Systemic Inflammation. Evaluation in Patients With Non-ST Segment Elevation Acute Coronary Syndromes)

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**Background and objective.** Evidence is growing regarding the prognostic value of markers of inflammation in unstable angina/non-ST segment elevation myocardial infarction (UA/NSTEMI). However, the independent value of these variables has not been systematically investigated in prospective studies. The main objective of the SIESTA study is to assess the relative prognostic roles of C-reactive protein, fibrinogen, neopterin, interleukins 6, 8, 10 and 18, tumor necrosis factor, e-selectin, endothelin 1, tissue factor, VCAM-1, ICAM-1, pregnancy-associated plasma protein-A, B-type natriuretic peptide, leukocytes, troponin I or T and serum creatine kinase-MB (CKMB) in UA/NSTEMI patients.

**Patients and method.** SIESTA is a prospective, multicenter trial involving patients with chest pain suggestive of acute coronary syndrome (ACS) within 48 hours of enrollment and at least one of the following: abnormal troponin levels, electrocardiographic signs of ischemia or previously documented vascular disease. Clinical outcome data and serial biochemical determinations will be assessed during hospital admission and at 30, 180 and 365 days of follow-up. The TIMI (Thrombolysis In Myocardial Infarction) and PEPA (Proyecto de Estudio del Pronóstico de la Angina) risk scores will be also validated. Study variables will include death due to any cause, cardiac death, non-fatal myocardial infarction, unstable angina requiring re-admission, emergency revascularization and a composite of death, myocardial infarction and need for emergency hospitalization or myocardial revascularization. Each of these conditions will be treated as secondary end-points when assessed individually.

**Key words:** Acute coronary syndromes. Unstable angina. Inflammation markers. Risk stratification.

This study will provide valuable prospective information about the prognostic value of inflammatory markers in «real life» ACS patients of Mediterranean origin.

**Marcadores de inflamación y estratificación de riesgo en pacientes con síndrome coronario agudo: diseño del estudio SIESTA (Systemic Inflammation Evaluation in patients with non-ST Segment elevation Acute coronary syndromes)**

**Introducción y objetivos.** A pesar de que se conoce el valor pronóstico de varios marcadores de inflamación en el síndrome coronario agudo sin elevación del segmento ST (SCASEST), aún se ignora qué subconjunto de éstos proporciona mejor información y qué grado de asociación existe entre ellos.

El objetivo del estudio SIESTA es establecer el valor pronóstico de la proteína C reactiva, fibrinógeno, neopterina, interleucinas 6, 8, 10 y 18, factor de necrosis tumoral, e-selectina, endotelina 1, factor tisular, molécula de adhesión celular vascular-1 (VCAM-1) e intercelular-1 (ICAM-1), proteína plasmática-A asociada al embarazo (PAPP-A), péptido natriurético ventricular (tipo B), troponina I o T, leucocitos e isoforra MB de la creatinfosfocinasa (CK-MB), en pacientes con SCASEST.

**Patients y método.** SIESTA es un estudio prospectivo, multicéntrico, que incluirá a pacientes que hayan presentado dolor torácico sugestivo de síndrome coronario agudo en las últimas 48 h y alguna de las siguientes condiciones: signos electrocardiográficos de isquemia miocárdica, enfermedad vascular documentada o elevación de la concentración de troponinas. Se realizará un seguimiento clínico durante un año, con determinaciones hematoxínicas y bioquímicas en el momento del ingreso, del alta, y a los 30, 180 y 365 días. Se validarán las escalas TIMI (Thrombolysis In Myocardial Infarction) y PEPA (Proyecto de Estudio del Pronóstico de la Angina).

La variable principal estará compuesta de muerte por cualquier causa, muerte de origen cardíaco, infarto de...
Abbreviations

NSTEMI: non-ST segment elevation acute coronary syndrome.
CRP: C-reactive protein.
PAPP-A: pregnancy-associated plasma protein-A.
BNP: brain natriuretic peptide.
NFKB: nuclear factor kappa-B.
PCTA: percutaneous coronary transluminal angioplasty.

Introduction

Acute coronary syndrome is one of the most severe forms of heart disease, and is the most frequent cause of morbidity and mortality in the western world. Patients with ACS are at serious risk of developing cardiovascular events within the first year of acute coronary syndrome.

Recently, Marrugat et al estimated that in the year 2002 more than 40,000 patients will have been admitted to Spanish hospitals with a diagnosis of acute myocardial infarction (60% of the total estimated admissions). The mortality rate of these patients in the first 28 days (excluding the pre-hospital phase) will be close to 25%. Nearly 33,000 individuals will be hospitalized with the diagnosis of non-ST segment elevation acute coronary syndrome (NSTEMI), of which 4.5% will die within the first 3 months.

Adequate risk classification would allow for more precise therapeutic management of these patients.

The problem, however, is the heterogeneity of this syndrome, as it includes patients with varying clinical pictures. In addition, prognosis depends on various clinical, electrocardiographic, biochemical, and angiographic variables which, to a degree, reveal the presence of ischemia or myocardial necrosis, frequently associated with heart disease. In patients with ACS, distinct complex physiopathological mechanisms, including erosion and rupture of atherosclerotic plaque, cause acute obstruction of coronary flow. The principal events associated with clinical instability in patients with NSTEMI are the degree of inflammatory activity, increase in vasomotor tone, and plaque activation; similarly, systemic inflammation is found to be associated with the development of atherosclerosis, changes in hemostasis, and acute coronary thrombosis. The presence of inflammatory cells within the atheromatous plaque plays an important role in the process that leads to fissure, coronary thrombosis, and vascular occlusion characteristic of ACS. The activation of macrophages, T cells, and nuclear factor kappa-B (NFkB), as well as the production and freeing of pro-inflammatory cytokines and neurohumoral substances, significantly contribute to the presence of clinical symptoms in these patients.

Currently, risk classification of patients with NSTEMI is mainly based on clinical, electrocardiographic, and angiographic data, along with markers for cardiac damage, inflammatory activity, increase in vasomotor tone, and plaque activation; similarly, systemic inflammation is found to be associated with the development of atherosclerosis, changes in hemostasis, and acute coronary thrombosis. The presence of inflammatory cells within the atheromatous plaque plays an important role in the process that leads to fissure, coronary thrombosis, and vascular occlusion characteristic of ACS. The activation of macrophages, T cells, and nuclear factor kappa-B (NFkB), as well as the production and freeing of pro-inflammatory cytokines and neurohumoral substances, significantly contribute to the presence of clinical symptoms in these patients.

In spite of these findings, reports are contradictory and the controversy continues regarding the usefulness of CRP in everyday clinical practice. CRP values are good predictors of risk since it has been shown that elevated CRP value in a hospital setting was shown to be a powerful predictor of death, myocardial infarct, and the need for urgent myocardial revascularization.
ous variations that can occur in the same patient; it is also unknown what cut point is appropriate for CRP to be considered elevated. Recently, other biochemical markers for risk have been proposed, such as brain natriuretic peptide (BNP) and pregnancy-associated plasma protein-A (PAPP-A)\(^\text{27,38}\); these markers must be validated in the context of markers already studied, as is the case with various markers of inflammation whose possible independent prognostic value is unknown.

### Cardiac risk in Mediterranean patients and the role of markers for inflammation in risk stratification

The Mediterranean population has a lower incidence of myocardial infarction and mortality than other populations\(^\text{39,40}\) despite the fact that the prevalence of conventional risk factors in this population is similar to that in other areas of Europe and in the United States. In Spain, the mortality rate due to cardiovascular disease is lower than in other regions of the world\(^\text{41}\) and it is believed that genetic factors and dietary habits (Mediterranean diet) may play an important role. Whether markers for inflammation are of independent prognostic value in patients with ACS in the Mediterranean population has not been systematically studied.

The PEPA registry provides useful data on risk classification in patients with NSTEACS through the use of conventional variables, but does not provide data on the role of markers for inflammation\(^\text{5}\).

SIESTA (Systemic Inflammation Evaluation in Patients with Non-ST Segment Elevation Acute Coronary Syndromes) is a multicenter, observational, prospective Spanish study of patients admitted with a diagnosis of NSTEACS; the goal of the study is to determine the prognostic value of various markers of inflammation as well as markers of endothelial activation in patients with NSTEACS.

### Objectives

The principle objectives of the SIESTA study are to:

1. Compare the prognostic value of CRP with other markers for inflammation, including pro- and anti-inflammatory cytokines, chemokines, adhesion molecules, neopterin, fibrinogen, serum amyloid A protein, endothelin 1, and the recently described PAPP-A and BNP.
2. Compare the prognostic value of markers for inflammation with other established risk indicators (clinical, electrocardiographic, and biochemical [troponin]).
3. Establish the predictive value of a single test result vs repeated measurement of various markers of inflammation.

A secondary objective is to establish the prognostic usefulness of the TIMI21 and PEPA5 risk scores.

### Hypotheses

The SIESTA study will attempt to confirm the following hypotheses:

- The presence of elevated values of circulating markers for inflammation, chemokines, and markers for endothelial activation are valuable for establishing prognosis for patients with NSTEACS.
- BNP and PAPP-A are independent risk markers in the population studied.
- The presence of persistently elevated values of markers for inflammation is associated with prognosis in patients with NSTEACS.
- The TIMI or PEPA risk scores, or both, are clinically useful for classification of risk in patients of Mediterranean origin with NSTEACS.

### Patients and methods

Patients of both sexes will be included in the study, not limited by age, who have chest pain suggestive of ACS during the previous 48 hours and at least 1 of the following conditions:

1. Electrocardiographic signs of myocardial ischemia (decline of the ST segment or T-wave inversion, or both).
2. Documented heart, cerebrovascular, or peripheral vascular disease.
3. Percutaneous coronary transluminal angioplasty (PCTA) or myocardial revascularization surgery, or both, performed no less than 12 weeks prior to the current episode.
4. Elevated cardiac troponin values.

Patients will not be included in the study who present with: a) ST segment elevation; b) complete left branch block; c) moderate or severe aortic stenosis; d) hypertrophic or dilated myocardial pathology; e) myocardial infarct during the last 12 weeks; f) PCTA or revascularization surgery, or both, during the prior 12 weeks; g) a history of heart failure; h) cerebrovascular or peripheral accident during the prior 12 weeks, and i) uncontrolled arterial hypertension, anemia, evidence of infection, tyrotoxosis, local or systemic inflammatory disease, terminal renal insufficiency, neoplasia, or any other disease that seriously compromises the prognosis for survival or generates a systemic inflammatory response, or both.

### Sample size

In patients with NSTEACS, the incidence of new cardiovascular events during the first year ranges...
from 16% to 30%.\(^\text{42,43}\) To calculate the sample size for this study, we assumed that the probability of patients presenting with new cardiovascular events was 20%, and we chose CRP as the variable to calculate in the sample size, as CRP is the value that presents with the greatest variability (in different studies its standard deviation ranged from 5 mg/dL to 10 mg/dL).\(^\text{44}\) We assumed a loss rate of 5%, a bilateral alpha risk of 0.05, and a beta error of less than 0.10, we would require 1044 of patients without events and 313 of patients with events in order to detect a difference equal to 1.5 mg/dL in the concentration of CRP between the 2 groups.

**Therapeutic management and clinical followup**

Patients will be treated in accordance with the recommendations of the Spanish Cardiology Society,\(^\text{45}\) and will receive aspirin, beta blockers, heparin, clopidogrel, nitrates, hypolipemiantis, and angiotensin-converting enzyme inhibitors or angiotensin II receptor antagonists (ARA II). Glycoprotein inhibitors IIb/IIIa will be used in high-risk patients, those with elevated troponin, or those about to undergo angiography and possible PCTA.

Data will be collected on any other medications—cardiologic or not—used during the study. The decisions regarding the treatment of patients will be left to the judgment of the attending physician, and the results of markers of inflammation in this study will not be available for patient management. It is expected that all patients with refractory or incapacitating angina that persists despite optimal medical treatment, and patients with signs of serious ischemia will undergo coronary angiography in order to provide PCTA or revascularization surgery. In patients who undergo cardiac catheterization, the ejection fraction, the severity of coronary lesions, and the number of occluded vessels will be noted.

The ethics committee of each participating hospital has approved the study protocol, and the patients will sign informed consent forms before their admission to the study, signifying their agreement to participate in the SIESTA study. Patient recruitment began in June, 2002, and the last patients are expected to be admitted at the end of 2003. The patients will be followed for 1 year.

Demographic and clinical variables that will be analyzed are shown in Table 1.

**Blood analysis**

Peripheral venous blood will be obtained and immediately centrifuged. Hemogram and biochemistry will be determined, to include: MP isoenzyme of creatin kinase (MB-CK), troponin, lipid profile, creatinine concentrations, urea, etc., at each participating hospital. The samples collected will be quickly frozen and maintained at –70 °C until they are sent to the central laboratories, which will produce a complete lipid profile, high sensitivity CRP, fibrinogen, serum amyloid A protein, interleukins 6, 8, 10 and 18, tumor necrosis factor alpha (TNF-alpha), intracellular cellular adhesion molecule 1 (ICAM-1); vascular cellular adhesion molecule 1 (VCAM-1); e-selectin; von Willebrand factor; metalloproteinase 1, 2, and 9; tissue factor; neopterin; PAPPA; BNP; neopterin; and endothelin 1. Blood samples will be obtained at the time of admission and hospital discharge, and at 6 months and 1 year followup.

Two laboratories, the Instituto Carlos III de Madrid and Saint George’s of London, have been selected to analyze the samples. The Madrid lab will perform lipid studies and will keep the frozen samples until they are transferred to London, where the markers of inflammation will be determined. The blood samples will be codified in a manner that masks the identity and clinical characteristics of the patients to the laboratories.

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**TABLE 1. Demographic and Clinical Variables**

<table>
<thead>
<tr>
<th>Risk factors</th>
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<tbody>
<tr>
<td>Smoking (habitual, ex-smoker, never smoked; years smoking), hypertension, hyperlipidemia, diabetes mellitus, history of myocardial infarction, NSTEMI, revascularization surgery, cerebrovascular accident or peripheral vascular disease, family history of cardiovascular disease</td>
</tr>
<tr>
<td>Medication at the time of admission</td>
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<tr>
<td>Aspirin, beta blockers, heparin, IIb/IIIa inhibitors, clopidogrel, nitrates, calcium antagonists, hypolipemiantis, angiotensin converting enzyme inhibitors, ARA II, hormone replacement therapy, diuretics, digoxin, others</td>
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<tr>
<td>Coronary angiography</td>
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<tr>
<td>Left ventricular ejection fraction, severity of coronary stenosis and number of diseased vessels</td>
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<tr>
<td>Blood samples (on admission and hospital discharge, at 1, 6 and 12 month followup)</td>
</tr>
<tr>
<td>CK-MB, cardiac troponins, lipid profile, high sensitivity</td>
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<tr>
<td>CRP, neutrophils, fibrinogen, interleukins 6, 8, 10 and 18, serum amyloid A protein, TNF-alpha, tissue factor, ICAM-1, VCAM-1, von Willebrand factor, e-selectin, metalloproteinase 1, 2, and 9, neopterin, endothelin 1, PAPP-A, and BNP</td>
</tr>
</tbody>
</table>

**Variables**

The principle variable will consist of death due to any cause, death of cardiac origin, non-lethal myocardial infarction and unstable angina that requires urgent hospitalization or revascularization. Individual evaluation of each variable is considered to be a secondary objective.
Study variables

The principal variable will be death due to any cause, death of cardiac origin, non-lethal myocardial infarction, and angina that requires hospitalization, PCTA, or urgent revascularization surgery. Each component of the principal variable will be a secondary variable when evaluated individually.

Patients who are discharged will be seen in clinic by the research physicians at 1 month, 6 months, and 1 year after discharge. During this follow-up period, an independent committee will analyze the events, defining death according to the criteria of the International Classification of Diseases (ICD).

Definitions

The definitions used in this study are based on the recent guidelines of the American College of Cardiology/American Heart Association (ACC/AHA)47: a «new» episode of angina is defined as an episode of pain that occurs at rest and last no less than 5 minutes, with ST segment elevation or decline greater than 1 mm or inversion of the T-wave, or both, in 2 contiguous leads, with the exception of the aVR lead. Myocardial infarction is defined as an increase in CK-MB or of troponin values with the exception of the aVR lead. Myocardial infarction is classified as «death» (including when the patient survives the event) for the purpose of statistical analysis.

The TIMI risk score21 is a new risk scale that includes variables easily obtained from patients with NSTEACS: age greater than 65 years, the presence of 3 or more risk factors (smoking, diabetes, hypercholesterolemia, arterial hypertension, family history), heart disease with lesions >50%, the use of aspirin during the last 7 days, acute symptoms of angina (more than 2 episodes of angina during the last 24 hours), elevated cardiac enzymes, and changes in the ST segment greater than 0.5 mm. A point was assigned to each of these variables, with 0 being the lowest score and 7 the maximum possible score.

The PEPA risk scale5 includes clinical, electrocardiographic, and biochemical variables: age ≥65 years, diabetes, peripheral vascular disease, more than 2 episodes of angina during the 24 hours prior to admission, post-infarct angina within the first 30 days, Killip class ≥2, ST segment decline, and elevation of markers of cardiac necrosis.

Discussion

This study will answer a number of important clinical questions about the prognostic role of markers of inflammation in patient of Mediterranean origin. The prospective studies in these populations are eagerly anticipated due to the specific characteristics of the population, which are different from Anglo-Saxon and Scandinavian populations who comprise the majority of epidemiological studies in the field of cardiovascular disease.

The patients who comprise this study will be a representative sample of the general population with NSTEACS who are treated in Spanish hospitals. The SIESTA study will include patients from everyday medical practice who present with NSTEACS and, therefore, the results could be extrapolated to everyday medical practice.

For the first time a comparative study will be performed that analyzes the prognostic role of various markers of inflammation, of variables that indicate endothelial activation of conventional risk factors, in the context of NSTEACS. The SIESTA study will, in addition, allow us to answer, questions about the prognostic value of persistently elevated values vs values that are only transiently elevated.

The prognostic clinical and electrographic markers of risk have also not been compared previously or systematically with markers of inflammation or with markers most recently proposed (BNP and PAPP-A) in Mediterranean patients with NSTEACS.

The SIESTA study will provide the possibility of validating scoring indices such as TIMI in Mediterranean patients. When an increased number of biochemical markers are noted, our findings may help to establish new scoring indexes that are more in accordance with the lifestyle and diet of the Spanish population. Patient recruitment has already started, and we hope that at the beginning of 2004 the first results will be analyzed.

PARTICIPANT IN THE SIESTA STUDY

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