Thrombosis Research (2009) **123**, 617–621

THROMBOSIS RESEARCH

www.elsevier.com/locate/thromres

REGULAR ARTICLE

Diurnal variation of soluble CD40 ligand in patients with acute coronary syndrome. Soluble CD40 ligand and diurnal variation

Alberto Dominguez-Rodriguez [a,*], Pedro Abreu-Gonzalez [b], Martín J. Garcia-Gonzalez [a], Juan Carlos Kaski [c]

[a] *Department of Cardiology (Coronary Care Unit), University Hospital of Canarias, Tenerife, Spain*
[b] *Department of Physiology, University of La Laguna, School of Medicine, Tenerife, Spain*
[c] *Department of Cardiological Sciences, St. George's Hospital Medical School, London, United Kingdom*

Received 13 February 2008; received in revised form 5 May 2008; accepted 8 May 2008
Available online 24 June 2008

KEYWORDS
Soluble CD40 ligand;
Diurnal variation;
Acute coronary syndrome;
ST-segment elevation myocardial infarction;
Preanalytical conditions

Abstract

Introduction: We sought to investigate whether day-night variations occur in the concentration of circulating soluble CD40 ligand in patients with acute coronary syndrome, as this may have practical implications.
Materials and Methods: We assessed 70 consecutive ST-segment elevation myocardial infarction patients admitted into the Coronary Care Unit and 50 control subjects. Each subject was studied under strictly controlled light/dark conditions. Blood samples were drawn at 09:00 h (light phase) and 02:00 h (dark phase). Nocturnal blood samples were drawn by a trained nurse, with the help of a minute torch with a dim red light in order to avoid any direct lighting on the patient during sleep. The soluble CD40 ligand was measured using a commercially available ELISA.
Results: Soluble CD40 ligand levels showed no diurnal variations in control subjects. In the ST-segment elevation myocardial infarction group, however, soluble CD40 ligand concentration (pg/mL) in the light phase was significantly higher than that in the dark phase (167.3 ± 63.2 *vs* 118.9 ± 48.3 pg/mL, $p<0.001$).
Conclusions: The study shows for the first time the existence of diurnal variations in soluble CD40 ligand levels in ST-segment elevation myocardial infarction patients,

Abbreviations: sCD40L, Soluble CD40 ligand; CD40L, CD40 ligand; STEMI, ST-segment elevation myocardial infarction; CCU, Coronary Care Unit.

* Corresponding author. Coronary Care Unit, University Hospital of Canarias, Ofra s/n La Cuesta E-38320, Tenerife, Spain. Tel.: +34 922 679030; fax: +34 922 362716.
E-mail address: adrvdg@hotmail.com (A. Dominguez-Rodriguez).

0049-3848/$ - see front matter
doi:10.1016/j.thromres.2008.05.006

which indicates the need for standardizing the time of blood sampling for the assessment of this molecule, at least in studies involving ST-segment elevation myocardial infarction patients.

Introduction

CD40 ligand (CD40L) is a 50 KDa type I transmembrane protein. Its C-terminus contains a tumor necrosis factor homology domain required for binding to its receptor CD40. Intracellular CD40L is expressed on platelet membranes in response to platelet activation and endothelial cells [1]. Subsequent cleavage by metalloproteases produces soluble CD40L (sCD40L), an 18-kDA soluble fragment [2]. Many reports have suggested that sCD40L is a promising clinical biomarker of atherothrombotic risk. Increased concentrations of sCD40L were reported mostly in disorders associated with platelet activation such as acute and stable coronary artery disease [3].

Several cardiovascular disease states show a daily cycle of activity, i.e. a peak incidence of cerebrovascular and cardiovascular events has been documented in the early morning hours [4]. Previously, we have shown diurnal variations in the production of another inflammatory systemic markers, interleukin-6, C-reactive protein, matrix metalloproteinase-9 and soluble vascular cell adhesion molecule-1 in patients with acute myocardial infarction [5–8].

In spite of the great interest on this biomarker, sCD40L still awaits appropriate clinical validation [9]. Although preanalytical conditions may influence sCD40L concentrations significantly, they have not been standardized for the measurement of this marker [10–14]. The present study was designed to investigate whether day-night variations occur in the concentration of sCD40L in patients with ST-segment elevation myocardial infarction (STEMI). Such variations may have important implications for the timing of blood sampling when measuring sCD40 for the assessment of cardiovascular risk stratification.

Patients and methods

Patients

Seventy consecutive patients who were admitted into the Coronary Care Unit (CCU) of the University Hospital of Canarias with a diagnosis of STEMI were assessed [15]. Interventional cardiologists blinded to all study assay results performed diagnostic coronary angiography and primary percutaneous coronary intervention of the culprit coronary artery within 6 h of symptoms onset, according to our standard hospital protocols. We also recruited 50 control subjects with cardiovascular risk factors but those in whom relevant coronary artery disease had been ruled out by angiography or stress test. We considered hypertension, hyperlipidaemia, smoking and positive family history as cardiovascular risk factors. The study was approved by the local research ethics committee and all subjects gave written informed consent before study entry. Exclusion criteria for both groups were as follows: presence of any active infection, autoimmune diseases, collagen tissue diseases, malignancies, illicit drug consumption, ongoing radiotherapy, presence of acute or chronic renal or liver diseases, immunosuppressive treatment, sleeping disorders, and diabetes mellitus. We did not include shift workers or subjects with jet-lag syndrome.

Study Protocol

In the STEMI group we obtained venous blood samples at two time points: 09:00 h (light period) and 02:00 h (dark period). In every case these samples were obtained after the angiographic confirmation that TIMI III flow had been achieved after primary angioplasty.

After angiography, the samples were taken between 1 and 12 hours. Each subject was studied under strictly controlled light/dark conditions. The CCU of the University Hospital of Canarias has 12 independent bedrooms. Each room is isolated from external light and noise, the environmental conditions mimicking the normal light/dark cycle. The light period in the CCU lasted 14 hours (1745 ± 33 lux) and the dark period 10 hours (1.33 ± 0.3 lux). Lights were turned on at 07:00 h. Light intensity was measured in the subject's faces, as per our standardized protocol. Nocturnal blood samples were drawn by a trained nurse, with the help of a minute torch with a dim red light (intensity <30 lux) thus avoiding directing any light to the patient's face during sleeping.

All subjects in the control group were assessed for detection of depression, or other psychiatric illnesses, epilepsy and thyroid disorders. We did not include subjects with a history of drug use, active infections or any inflammatory condition, all of which were assessed specifically. Medical history and examinations in these control subjects were established by two independent cardiologists who were not involved in this study and they were blinded to sCD40L values. In 18 subjects of the control group with a moderate or high risk of a fatal or non-fatal cardiovascular event, blood was sampled after relevant coronary artery disease and it was ruled out by angiography. Thirty two subjects of the control group with a low cardiovascular risk profile the coronary artery disease have been ruled out by stress test. All subjects in the control group were assessed in the CCU and followed exactly the same protocol as the STEMI patient in relation to light environment and blood sampling.

Analytical Methods

Blood samples were drawn from a cubital median vein for measurement into BD vacutainer tubes (BD vacutainer SST II, BD, USA), without any anticoagulant. The blood sample was allowed the formation of clot at room temperature during 15 min. The blood was centrifuged at 3000 rpm 10 min and immediately the serum was separated into aliquots and frozen at $-70\ ^{\circ}C$ until assay. Serum sCD40L concentrations were measured using a high–