CARDIOVASCULAR MEDICINE

Increased serum concentrations of soluble CD95/Fas and caspase 1/ICE in patients with acute angina


Objective: To investigate the expression of death inducing receptors in the sera of patients with stable and unstable angina.

Design: 80 consecutive patients with stable (n = 40) or unstable (n = 40) angina pectoris were studied. Serum concentrations of soluble CD95 (sCD95), soluble CD95 ligand (sCD95L; CD178), tumour necrosis factor (TNF) α, soluble TNFα receptor type 1 (sTNFR1), and interleukin 1β converting enzyme (ICE; caspase 1) were measured by enzyme linked immunosorbent assay (ELISA).

Results: Significant increases in the concentrations of sCD95 and ICE (p < 0.001 and p < 0.023, respectively) were found in the serum from patients with unstable angina relative to those with stable angina. There were no significant differences in the concentrations of sCD95L, TNF α, and sTNFR1 between the groups.

Conclusions: These data provide the first evidence that sCD95 and ICE are important serological markers that may help to discriminate between stable and unstable angina. This observation may warrant further clinical study to elucidate the clinical impact of sCD95 and ICE in acute coronary syndromes.

Coronary artery disease is associated with systemic immune responses and signs of chronic inflammation. Apart from the well known traditional risk factors, seroepidemiological studies have suggested a link between atherosclerosis and microbial infections. The atherosclerotic lesions contain large numbers of immune cells, particularly monocytes, macrophages, and T cells. Most T cells are CD3+, CD4+, and T cell receptor 8αβ. They are largely T helper cell 1 subtype, which secrete interferon γ, interleukin 2, and tumour necrosis factor (TNF) α. These proinflammatory cytokines, as well as interleukin 1, interleukin 6, interleukin 8, and C reactive protein, cause activation of T cells, macrophages, and vascular endothelial cells. They also augment expression of adhesion molecules, chemoattractants, and other cell surface markers.

The maintenance of T cell homeostasis and peripheral tolerance to self antigens following repeated cell stimulations is regulated mainly by apoptosis. Apoptosis, or programmed cell death, is initiated by death-inducing receptors. These are transmembrane receptors that include the TNF receptors and a variety of immune cell receptors (CD95, CD40, CD30, and CD27) and their corresponding ligands. It has been previously shown that interaction between CD95 (Fas; apolipoprotein 1) and CD95 ligand (CD95L; Fas ligand, CD178) induces apoptosis in human plaque derived vascular smooth muscle cells. Activated cytotoxic CD4+/CD161+ T cells can lyse capillary endothelial cells in the presence of C reactive protein at concentrations frequently found in patients at risk for coronary heart disease. In addition, patients with unstable angina have increased concentrations of shed membrane microparticles, originating from activated CD3+ cells, endothelial cells, and macrophages, hence indicating increased intravascular peripheral apoptotic turnover.

It is not well known whether patients with acute coronary syndrome have increased concentrations of soluble CD95 (sCD95) and sCD95L, soluble TNF receptor type 1 (sTNFR1), and interleukin 1β converting enzyme (ICE; caspase 1). Therefore, we investigated comparatively the concentration of these molecules, which are involved in the apoptotic pathways, in the serum from patients with stable and unstable angina.

METHODS

Study group
Eighty consecutive patients admitted to our institution for the assessment of angina chest pain were studied and had undergone coronary angiography. Table 1 summarises demographic and some baseline clinical characteristics. Stable angina (n = 40) was defined by typical exertional chest pain angina relieved by rest, glyceryl trinitrate administration, or both with positive responses to exercise ECG stress testing and ≥ 50% diameter stenosis in ≥ 1 coronary arteries at catheterisation. Unstable angina (n = 40) was defined according to Braunwald. All patients with unstable angina class IIIIB had diagnostic ST segment changes, T wave inversion, or both, as well as negative cardiac enzymes. No patient included in the study had evidence of ongoing systemic or cardiac inflammatory processes as defined by clinical history. Blood samples were taken at the time of admission into hospital. The investigation conformed to the principles outlined in the Declaration of Helsinki.

Angiographic analysis
Coronary angiography was carried out according to the Judkins technique. Images of the coronary tree were obtained in routine, standardised projections with standard equipment (Siemens BICORTOP, Erlangen, Germany). To assess the extent of atherosclerotic disease the coronary artery tree was

Abbreviations: CD95L, CD95 ligand; ELISA, enzyme linked immunosorbent assay; FADD, Fas associated death domain; ICE, interleukin 1β converting enzyme; sCD95, soluble CD95; sCD95L, soluble CD95 ligand; sTNFR1, soluble tumour necrosis factor receptor type 1; TNF, tumour necrosis factor; TNFR1, tumour necrosis factor receptor type 1
assessed according to vessel score, stenosis score, and extension score.13

**Laboratory analysis**

Serum samples were obtained after clinical assessment grading the patient affected with stable or unstable angina and before cardiac catheterisation. Serum was then aliquoted and kept frozen. Circulating sCD95 and sCD95L were measured by ELISA with polyclonal antibodies against human TNF receptor type 1 (TNFR1) and TNFα with monoclonal antibody to ICE 1 antibody (Bender MedSystems Inc, McKinley, Minnesota, USA). ICE was measured in the serum by ELISA with monoclonal antibody to ICE 1 antibody (Bender MedSystems, Vienna, Austria). The sensitivity of the ELISA was 20 pg/ml for sCD95 and sCD95L, 3 pg/ml for sTNFR1, 80 pg/ml for TNFα, and 5 pg/ml for human ICE. The analytical unit of the biochemistry department following standard methods carried out all other biochemistry measurements.

<table>
<thead>
<tr>
<th>sCD95L (pg/ml)</th>
<th>Stable angina (n = 40)</th>
<th>Unstable angina (n = 40)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>78.29 (1.2)</td>
<td>62.34 (10.3)</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>ICE/caspase 1 (pg/ml)</td>
<td>46.94 (6.2)</td>
<td>78.21 (13.1)</td>
<td>0.023</td>
</tr>
<tr>
<td>sTNFR1 (pg/ml)</td>
<td>1.72 (0.2)</td>
<td>1.42 (0.3)</td>
<td>0.39</td>
</tr>
<tr>
<td>TNFα (pg/ml)</td>
<td>15.35 (6.4)</td>
<td>17.43 (5.4)</td>
<td>0.83</td>
</tr>
</tbody>
</table>

Data are mean (SEM).

### Statistical analysis

Results are presented as mean (SEM) for continuous, normally distributed variables and as percentages for categorical data. Continuous variables were analysed by two tailed t test for unpaired observations. A probability value of p < 0.05 was considered significant. The statistical software package SPSS (SPSS Inc, Chicago, Illinois, USA) was used for all calculations.

### RESULTS

#### Demographic and clinical features

Table 1 presents baseline characteristics of patients with stable and unstable angina. In both groups, a similar proportion of patients had a history of myocardial infarction or previous coronary interventions (coronary artery bypass graft or percutaneous transluminal coronary angioplasty). Established risk factors for coronary artery disease were similar in the two groups. Total cholesterol concentrations did not differ significantly between the groups. Angiographic findings were similar in both groups. The chronic stable angina group had slightly higher extension scores but this did not reach significance.

### Soluble death inducing receptors and ICE concentrations

The results listed in the table 2 show a significant increase in the concentration of ICE in serum from patients with unstable angina (p < 0.023). The concentrations of sCD95L, TNFα, and sTNFR1 were not significantly different (table 2). Interestingly, as shown in fig 1, the concentration of sCD95 was greatly increased in the unstable angina group compared with the stable group (mean (SEM) 3235.78 (380.2) and 668.23 (143.1) pg/ml, respectively; p < 0.001).

### DISCUSSION

Our study showed for the first time that serum sCD95 and ICE concentrations are significantly higher in patients with unstable angina than in patients with stable angina. This

![Figure 1](http://heart.bmj.com/) Concentration of soluble CD95 (sCD95) in the serum of patients with stable and unstable angina. Box plot shows the median, quartiles, and extreme concentrations of sCD95. Differences were significant (p < 0.001).
suggests that increased apoptosis specific markers are associated with clinical instability. We have also found that the serum concentrations of TNF-α, sTNFR1, and sCD95L were not significantly different in the study cohorts. These results indicate that patients with unstable angina may be in a paradoxical state of increased apoptotic turnover, as indicated by increased secretion of sCD95 and ICE/caspase 1.

Apoptosis is a highly organized, controlled, and energy dependent mechanism whereby a cell commits suicide. Distinct morphological features such as cell shrinkage, chromatin condensation, DNA fragmentation, membrane blebbing, and formation of apoptotic bodies are characteristic of this cell death mechanism. A family of cysteine proteases, termed caspases, homologous to the Caenorhabditis elegans cell death gene CED3, play a pivotal part in apoptosis, particularly caspase 8, which initiate the caspase activation cascade through caspase 3 and caspase 9. Caspases are present in the cell in an inactive form and are activated by other caspases or granzyme B. Activation of caspase requires transduction of a signal from cell membrane receptors, such as CD95 and TNFR1. Binding of ligand to death inducing receptors leads to trimerisation and cross linking of the aggregated cytoplasmic domain of the receptor complex and recruitment of the FLICE1 (MACH1; caspase 8), or FLICE2/caspase 10. This is one of several caspase activation pathways that may help to discriminate between stable and unstable angina. We conclude that our results showing increased concentrations of sCD95 and ICE in unstable angina may merely reflect inflammatory cell activation and the role in relation to apoptosis specific immune activation remains to be determined. However, to elucidate the role of these molecules in the pathogenesis, disease progression and stabilization, diagnosis, and prognosis of acute coronary syndromes further clinical studies as well as new experimental designs are needed.

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references


New combined treatments avoided transplantation in a child with severe pulmonary hypertension

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herapeutic options for the management of pulmonary hypertension have been limited for many years. However, recent progresses in the understanding of its pathology have led to the introduction of new treatments that have changed its prognosis, such as the continuous infusion of prostacyclin. Several other treatments (oral, inhaled, or subcutaneous prostacyclin, phosphodiesterase inhibitors, and endothelin receptor blockers) have shown beneficial effects in recent trials. An 8 year old boy with severe primary pulmonary hypertension showed a dramatic improvement following combined treatment with inhaled iloprost (a prostacyclin analogue) and oral bosentan (a dual endothelin receptor blocker). In the six minute walk test he increased the distance from 295 m before bosentan to 400 m after three months of combined treatment. Dramatic changes were observed at echocardiography. Echocardiographic short axis views were taken at the same time in the cardiac cycle before combined treatment (panel A) and three months after combined treatment of iloprost and bosentan (panel B). There was a significant reduction in right ventricular (RV) size and changes in interventricular septal curvature (IVS), which went from flat before treatment (panel A) to normal left to right on combined treatment (panel B). Echocardiography showed a decrease in right ventricular surface, from 39 cm² to 34 cm². The left ventricle (LV) is squeezed by the dilated right ventricle before (panel A) and is almost normal after three months (panel B). This allowed the lung transplantation to be postponed and improved the patient’s quality of life.

This case shows that combined therapies may offer further improvement in the therapeutic options for the treatment of pulmonary hypertension. Appropriate clinical trials should be performed to confirm this beneficial result.

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