Homocysteine and Laminin Are Not Prognostic Markers in Patients with Septic Inflammatory Response Syndrome

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The aim of this study was to measure plasma homocysteine and laminin concentrations in patients with nonbacteraemic systemic inflammatory response syndrome (SIRS) and to compare them with those of a healthy control group. Concerning laminin, significant increased concentrations could be observed in the SIRS group compared to the control group, but for homocysteine, no significance could be observed. In summary, homocysteine and laminin levels are not useful in the prediction of a patient’s outcome.

The cause of endothelial cell damage in patients with septic shock is not fully understood (3). The presence of elevated levels of the biological thiol homocysteine is a well known independent risk factor for endothelial damage in atherosclerosis (1, 2). Up to now, the influence of homocysteine on endothelial cell damage has not been investigated in patients with systemic inflammatory response syndrome (SIRS).

Furthermore, the presence of increased concentrations of laminin, a high-molecular-weight, noncollagenous glycoprotein, which is a component of basement membranes (14) at the boundary between epithelial or endothelial cells and the tissue stroma, has been shown in several diseases (7–9, 11, 16). It has been argued that increased values of laminin might be an indicator of endothelial cell damage (4). It was the aim of the present study to determine the time course of production of homocysteine and laminin in 14 patients with SIRS and to compare it with that of 14 patients suffering from local inflammatory disease (LID) and with that of an age- and sex-matched healthy control group (n = 14 volunteers). The study was performed at a medical intensive care unit between October 1994 and March 1995. Fourteen nonbacteraemic patients (9 male, 5 female, median age of 63 years, range of 27 to 72 years) fulfilling four of Bone’s criteria (3) for SIRS (e.g., body temperature, >38°C or <36°C; heart rate, >90 beats/min; tachypnoe, >20 breaths/min; leukocyte count, >12 0000/μl or <4,000/μl) were included in the study. The local inflammatory group consisted of 14 patients (7 male, 7 female, median age of 63 years, range of 27 to 75 years), and the healthy control group consisted of 14 age- and sex-matched volunteers. Plasma samples, obtained by using endotoxin-free tubes (Kabi-tubes; Chromogenix) containing 120 IU of heparin, were drawn daily. The tubes were centrifuged at 1,500 × g and stored at −70°C until analysis. For statistical analysis, only samples of patients fulfilling Bone’s criteria for SIRS were used and analyzed at day 1 (the day that patients fulfilled SIRS criteria), day 4, and day 7 (or 1 day before the patients died).

Blood cultures, urine and stool cultures, wound swabs, and bronchoalveolar lavages were performed whenever clinically indicated.

Patient survival was defined as being alive 30 days after fulfilling sepsis criteria. Levels of homocysteine in plasma were measured by high-performance liquid chromatography as recommended by the manufacturer (Bio-Rad Laboratories GmbH, Munich, Germany). Values are expressed as micromoles per liter; the minimal detectable concentration was 0.1 μmol/liter.

Plasma nitric oxide (NO) concentrations were determined by measuring nitrate concentrations by high-performance liquid chromatography as described by El-Menyawí et al. (6). Values are expressed as micromoles per liter; the minimal detectable concentration was 0.1 μmol/liter.

Concentrations of laminin in serum were measured with a sandwich enzyme immunoassay (laminin EIA kit; Takara Bio-medica, Otsu Shiga, Japan) as described by the manufacturer. Values are expressed in nanograms per milliliter.

For comparison between patients and controls, analysis of variance and Tukey’s test were used. Comparisons between survivors and nonsurvivors were calculated by Student’s t-test. For correlation, Pearson’s correlation matrix was used. All analyses were two sided, and P values of less than 0.05 were considered significant.

For patient characteristics, see Table 1. Seven out of 14 patients died. Twelve patients were mechanically ventilated and received either parenteral (Kabi Mix 1800; Pharmacia, Upjohn) or enteral nutrition via a nasogastric tube. Thirteen patients required at least one catecholamine treatment because of hemodynamic instability. All blood cultures showed negative results; no underlying infection could be detected.

The patients with LID presented with osteomyelitis (four patients), wound infection (two patients), abscess (three patients), pacemaker infection (two patients), erysipelas (two patients), and endocarditis.

The mean ± standard deviation concentrations of homocysteine in plasma were significantly lower in the SIRS group than those in the LID group (42.1 ± 31.04 μmol/liter; P < 0.05), but only numerically lower than those in the control group (21.4 ± 5.4 μmol/liter). This value was 6.88 ± 6.7 μmol/liter at day 1, had decreased to 5.82 ± 5.14 μmol/liter at day 4, and had increased to 9.36 ± 11.15 μmol/liter at day 7. There was no significant difference between survivors and nonsurvivors at any time of observation; however, the values tended to be numerically lower in the nonsurvivor group (Table 2).

The nitrate concentrations were significantly higher (P < 0.05) in the SIRS group than those in the LID and control...
groups (20.5 ± 6.92 μmol/liter at day 1, 24.01 ± 6.62 μmol/liter at day 4, and 19.25 ± 7.11 μmol/liter at day 7 in the SIRS group; 4.48 ± 1.19 μmol/liter in the LID group; and 3.26 ± 1.29 μmol/liter) in the control group. As for homocysteine, no significant difference was calculated between survivors and nonsurvivors (Table 2). Only at day 4, could a significant correlation be calculated between plasma homocysteine and nitrite concentrations and homocysteine and fibrinogen concentrations (P < 0.01). No correlation could be calculated between plasma homocysteine concentrations and the following parameters at any time of observation: creatinine, alanine aminotransferase, C-reactive protein (with an exception at day 1, 24.01 ± 6.62 μmol/liter at day 4, and was 2,283 ± 1,479 mg/ml on day 7. In nonsurvivors, plasma laminin concentration was 889 ± 1,291 ng/ml; in the control group, plasma nitrate concentration was 1,395 ± 1,045 mg/ml, respectively. The plasma lamini concentrations in the control and LID groups were 433 ± 3.17 and 941 ± 6.92 μmol/liter at day 1, decreased to 2,283 ± 1,479 mg/ml at day 4, and was 313 ± 1,164 μmol/liter at day 7 in the SIRS group than those in the control group (P < 0.05).

Concerning laminin, concentrations were significantly higher in the SIRS group than those in the control group (P < 0.05). The mean ± standard deviation serum laminin concentration was 1,438 ± 3.8 mg/ml on day 4, and was 2,283 ± 1,479 mg/ml on day 7. In nonsurvivors, plasma laminin concentrations tended to be numerically higher than those in survivors; however, no significance was achieved (Table 2). The factors determining plasma laminin levels are not known at present. In healthy people, laminin levels increase with age, ethanol intake, and pregnancy (10). Laminin concentrations 1.8 times higher than those in a control group were found in patients with mesangial proliferative and membranoproliferative glomerulonephritis (10). The increased laminin concentrations in our patients could partly be explained by an induction through cytokines, such as IL-1β, which has been demonstrated to increase laminin production in patients with glomerulonephritis (13). Additionally, increased laminin concentrations might be due to the damage of the microcirculation caused by SIRS.

Homocysteine is a sulfur-containing amino acid that is formed during methionine metabolism. It is well established that mild to moderate elevations in serum homocysteine are associated with an increased risk of vascular disease (1). It is postulated that homocysteine may act as an endogenous NO antagonist in diverse processes including infection (5). It is known that NO inhibits methionine synthase and consequently homocysteine production (11). On the other side, NO and homocysteine form nitrosothiols stabilizing NO (15). In our septic patients, we found significantly higher plasma nitrate concentrations than those in both the control group and the LID group. Overall the particular mechanism causing decreased levels of homocysteine in patients with SIRS

### TABLE 1. Characteristics of patients in this study

| Sex   | Age (yr) | Underlying disease                          | Clinical evaluation scorea | MOF | Survivora, b |
|-------|----------|---------------------------------------------|----------------------------|-----|-------------|
| Male  | 47       | Intracerebral bleeding                      | 43                         | 5   | S           |
| Female| 45       | Systemic lupus erythematosus, osteomyelitis | 77                         | 9   | Ns          |
| Female| 75       | Cardiopulmonary resuscitation               | 31                         | 7   | S           |
| Male  | 40       | Renal failure, coma                         | 70                         | 6   | S           |
| Male  | 69       | Ketoacidotic coma                           | 88                         | 2   | S           |
| Female| 68       | Hepatoma, cardiopulmonary resuscitation     | 48                         | 7   | S           |
| Male  | 62       | N. bronchi, lung cancer                     | 39                         | 6   | Ns          |
| Male  | 27       | Cirrhotic hepatitis, gastrointestinal bleeding | 51                       | 12  | Ns          |
| Female| 72       | Leukemia                                    | 50                         | 5   | S           |
| Male  | 63       | Aortocoronary bypass, aortal valve replacement | 26                       | 10  | S           |
| Male  | 65       | Acute pancreatitis                          | 103                        | 8   | Ns          |
| Female| 70       | Chronic lymphocytic leukemia                | 57                         | 8   | Ns          |
| Male  | 63       | Insulin-dependent diabetes mellitus         | 25                         | 5   | S           |
| Male  | 46       | Gastrointestinal bleeding                   | 37                         | 7   | Ns          |

a APACHE III, Acute Physiology and Chronic Health Evaluation III; MOF, multiorgan failure.
b S, survivor; Ns, nonsurvivor. See reference 17.

### TABLE 2. Time course of plasma homocysteine, laminin, and nitrate concentrations in patients with SIRS

| Parametera | Survivors | Nonsurvivors | Survivors | Nonsurvivors | Survivors | Nonsurvivors |
|-------------|-----------|--------------|-----------|--------------|-----------|--------------|
| Homocysteine (μmol/liter) | 10.44 ± 8.0 | 4.1 ± 4.66 | 6.58 ± 1.2 | 5.46 ± 6.56 | 14.1 ± 14.0 | 4.61 ± 4.35 |
| Nitrate (μmol/liter) | 20.5 ± 4.5 | 21.2 ± 9.5 | 22.0 ± 3.83 | 26.0 ± 8.8 | 19.4 ± 6.3 | 19.0 ± 9.3 |
| Laminin (ng/ml) | 1,395 ± 1,171 | 1,639 ± 1,252 | 941 ± 732 | 1,751 ± 1,164 | 2,097 ± 1,641 | 2,447 ± 1,397 |
| C-reactive protein (mg/100 ml) | 15.3 ± 13.5 | 21.7 ± 6.63 | 18.2 ± 14.5 | 11.36 ± 8.0 | 15.3 ± 13.4 | 21.7 ± 6.63 |
| Creatinine (mg/100 ml) | 2.59 ± 3.17 | 1.27 ± 0.41 | 2.06 ± 2.49 | 1.56 ± 0.64 | 1.95 ± 2.23 | 2.06 ± 0.64 |
| Leukocytes (10⁹/liter) | 26.7 ± 29.2 | 7.2 ± 3.2 | 17.5 ± 8.9 | 13.1 ± 11.6 | 15.7 ± 10.7 | 12.33 ± 5.28 |
| IL-6 (pg/ml) | 661 ± 1,104 | 857 ± 1,108 | 80.4 ± 75.1 | 710 ± 729 | 243 ± 481 | 1,557 ± 1,162 |

a The plasma homocysteine concentrations in the control and LID groups were 21.1 ± 5.4 and 42.1 ± 8.3 μmol/liter, respectively. The plasma nitrate concentrations in the control and LID groups were 4.32 ± 4.04 and 4.49 ± 3.6 μmol/liter, respectively. The plasma laminin concentrations in the control and LID groups were 433 ± 25 and 889 ± 1,291 μmol/liter, respectively. Normal values for leukocytes, C-reactive protein, and creatinine are 4 × 10⁹/liter, <1 mg/100 ml, and 0.7 to 1.3 mg/100 ml, respectively.
b Values are expressed as means ± standard deviations.
depressed homocysteine synthesis or increased formation of nitrosothiols) remains unclear.

The present study also showed that there was no correlation between homocysteine and parameters of endothelial damage, such as sVCAM and laminin in patients with SIRS. Although significantly higher concentrations of laminin were observed in the SIRS group, no significant difference could be demonstrated in a comparison of survivors and nonsurvivors. Thus, laminin cannot be used as a prognostic parameter in patients with SIRS. We speculate that increased laminin levels in our patients might be partly due to the damage to the microcirculation caused by SIRS.

In summary we found decreased concentrations of homocysteine in plasma and increased concentrations of laminin in plasma in patients with SIRS compared to those of the control group. Whether the decrease in homocysteine is caused by inhibition of methionine synthase caused by increased NO formation or increased nitrosothiols remains unclear. None of the evaluated parameters could be used as an early prognostic marker.

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