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Patterns of Correlation of Plasma Ceruloplasmin in Sepsis

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Background. **In sepsis, plasma ceruloplasmin (Cp, mg/L) is known to increase as part of the acute phase response. However, there is poor knowledge of the patterns of increase and correlation with changes in other biochemical variables, and our study has been performed to investigate this aspect.**

Materials and methods. **A total of 213 simultaneous measurements of Cp and other acute phase proteins, biochemical variables, and amino acids were performed on nine patients with severe sepsis, and processed by regression analysis.**

Results. **Mean Cp was 478 ± 119 mg/L (median 488, range 242–784). Significant direct correlations between Cp and C-reactive protein, α-1-antitrypsin and α-2-macroglobulin ($P < 0.001$ for all) were all simultaneously influenced by the level of alkaline phosphatase, which was an independent determinant of increased Cp ($P < 0.001$). Cp increased further with decreasing plasma pH and increasing triglyceride, taurine levels, and distance from the onset of sepsis ($P < 0.001$ for all). The maximum increases in Cp were associated with the presence of cholestasis, increasing triglyceride levels, and metabolic acidosis. With regard to septic liver dysfunction, while signs of cholestasis were mostly reflected in greater increases in Cp, increasing bilirubin in the presence of normal alkaline phosphatase was mostly correlated with abnormal increases in cyst(e)ine, cystathionine, and tyrosine levels.**

Conclusions. **These data characterize the patterns of correlation of Cp within the biochemical abnormalities of sepsis, and may provide new insights into the pathophysiology of septic hepatobiliary dysfunction.**

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Key Words: **plasma ceruloplasmin; acute phase proteins; sepsis; plasma triglycerides; acidosis; liver dysfunction; amino acids; taurine; cyst(e)ine; cystathionine.**

INTRODUCTION

Ceruloplasmin (Cp, mg/L) is a copper-containing α-2 glycoprotein of a mean molecular mass of 132 kDa that is mostly found in human plasma at a concentration of 200 to 400 mg/L. Its biological roles are not entirely clear and include ferroxidase activity, copper transport and detoxification, maintenance of copper homeostasis in tissues, antioxidant and antiinflammatory protection and, under peculiar circumstances, an impact on vascular tone through an interaction with nitric oxide and proinflammatory activity [1–14]. Abnormalities of plasma Cp levels include its decrease in Wilson and Menkes disease, several instances of liver disease, malabsorption, nutritional copper deficiency, excessive therapeutic zinc, and conditions associated with protein loss, or the total absence of Cp in aceruloplasminemia; conversely, Cp may increase in a variety of neoplastic and inflammatory states, copper intoxication, pregnancy, estrogen therapy, use of oral contraceptives, cholestasis, insulin-dependent diabetes mellitus, and alcoholic cirrhosis [8, 12, 15–32]. In sepsis, the increase in Cp is generically considered to be part of the acute-phase response; however understanding of function and patterns of changes of Cp is incomplete, and knowledge of the correlations with simultaneous changes in other variables is poor. This study was performed to assess the relationship between changes in Cp and in other metabolic variables and plasma amino acids (AA), in a group of patients with systemic sepsis.

MATERIALS AND METHODS

The study was based on the analysis of 213 measurements of Cp, of a large series of complementary variables, and of plasma amino

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acids, performed prospectively on nine patients who developed severe sepsis after trauma. The patients were three women and six men; median age was 25 years (range 17–38 y), weight was 70 kg (40–85), and height 170 cm (163–188). They had a combination of abdominal, chest, and head injuries (median injury severity score 31.5, range 13–43) [33], and the subsequent cause of sepsis was intra-abdominal, pulmonary, or extensive soft tissue infection. The diagnosis of sepsis was based on the simultaneous occurrence of a temperature >38.3°C, white blood cell count $>12 \times 10^9$/L or $<3 \times 10^9$/L, and clear evidence of infection confirmed by positive cultures from blood, surgical drainage of infected areas, or sputum in the case of pulmonary sepsis. Median sepsis severity score (SSS) [34, 35] upon diagnosis of sepsis was 25.5 (range 11–75). Four patients developed signs of multiple organ dysfunction syndrome, in moderate form in three, and in lethal form in one who did not survive. Serial measurements were performed every 8 to 12 h while criteria for the diagnosis of sepsis persisted or death, for a total of 213 measurements taken over a period of up to 17 days from the diagnosis of sepsis (mean distance after diagnosis of sepsis 6.1 ± 4.4 day). Thirty-three additional measurements were performed before the development of sepsis (distance before diagnosis of sepsis 2.3 ± 1.4 day). All patients were undergoing total parenteral nutrition (37 ± 14 kcal/kg/d, 77% glucose and 23% fat, and 1.6 ± 0.6 g/kg/d mixed amino acids). Each measurement included plasma Cp (normal laboratory range 200 to 400 mg/L), C-reactive protein (CRP, mg/L), α-2-macroglobulin (α-2-Macro, g/L), α-1-antitrypsin (α-1-Atrip, g/L), fibrinogen, transferrin, albumin, glucose, blood urea nitrogen, creatinine, sodium, potassium, calcium, chloride, triglycerides (Trig, mg/dL), total bilirubin, alkaline phosphatase (AP, U/L, n.v. < 100), lactate, blood cell count, the full amino-acidogram, arterial and central venous blood gas analyses with the calculation of peripheral O_2 extraction, blood base excess and daily 3-methylhistidine and urea excretion, heart rate, thermodilution cardiac index, systemic, and pulmonary blood pressures. The data provided a continuous distribution of observations, from moderate to extremely severe or preterminal septic illness, which was perfectly suited to assessing the correlates of changes in plasma Cp over a wide range of pathophysiological abnormalities. The statistical analysis and validation of the results were performed by least-square regression and covariance analysis, with skewness and kurtosis control, analysis of residuals (Statgraphics Plus; Manugistics, Rockville, MD), confidence intervals of regression coefficients (Scheffé) and Mallows' Cp criteria, to select the simplest possible regressions yielding the best control of variability of Cp [36]. The protocol complied with the Helsinki declaration of 1975 as revised in 1996, and was approved by the Institutional Review Board.

RESULTS

Mean Cp was 478 ± 119 mg/L (median 488, 10%–90% percentiles 292–626, range 242–784), and was significantly higher than the mean value of 406 ± 86 mg/L observed before the development of sepsis ($P < 0.01$). Along the septic course, Cp was found to be above 400 mg/L in 166 measurements performed in eight patients, and below this limit in 47 measurements performed in four of these same patients and in another patient in whom it remained consistently lower than 400 mg/L.

The correlations between Cp and all other variables were carefully investigated. Cp was related directly to Trig, AP, CRP, α-1-Atrip, α-2-Macro, cyst(e)ine, taurine, and pulmonary arterial pressure (r from 0.56 to 0.37), and inversely to arterial, venous pH, blood base excess and peripheral O_2 extraction (r from 0.45 to 0.37) ($P < 0.001$ for all). The relationship with Trig was independent of the dose of infused fat, and the relationship with pH was equally affected by the metabolic or respiratory component of altered pH. The correlations with all other variables were weaker ($r < 0.32$) or not significant. These variables included also the initial injury severity score, the type of injury, the source of sepsis, and the SSS and sepsis-related organ failure assessment (SOFA) scores [34, 35, 37] reassessed along the clinical course. A trend for a direct relationship between Cp and SSS or SOFA score could be observed, however with borderline significance. A direct correlation was found between the SSS and the SOFA scores ($r = 0.87$, $P < 0.001$). The correlations between Cp and the acute phase proteins CRP, α-1-Atrip, and α-2-Macro were simultaneously mediated by the level of AP: that is, for any given increase in CRP, α-1-Atrip, and α-2-Macro, the increase in Cp was stronger in the presence of a simultaneously increased AP (Table 1, regressions 1 to 3). A similar pattern was observed also for the direct correlation found between Cp and Trig (Table 1, regression 4). Multiple regression analysis selected AP, Trig, and arterial pH as the best simultaneous correlates of Cp, and these together explained 72% of the variability of Cp (Table 1, regression 5; $r = 0.85$, $r^2 = 0.72$). Covariation of Cp with AP and Trig was also reflected by the distribution of Cp values associated with low or high levels of these variables (Table 2). Regression 5 in Table 1 was not improved by including any other variable considered in the study, except the distance from the onset of sepsis, which brought the total explained variability of Cp to 78%, with a mean estimated increase in Cp of 10 mg/L/day ($r = 0.88$, $r^2 = 0.78$, $P < 0.001$). Cross-correlations among the best correlates of Cp, other acute-phase proteins and AA showed that also α-1-Atrip, α-2-Macro and

TABLE 1

Upper Case: Plasma Levels of Main Biochemical and Acute-Phase Correlates of Cp. Lower Case: Regressions with Coefficients of Correlation (r); $P < 0.001$ for Each Regression and for Each Coefficient in the Regressions. Symbols and Units as in the Text

	Median	10%–90% percentiles	Range
Trig	208	105–367	24–780
Arterial pH	7.4	7.34–7.47	7.25–7.54
AP	114	63–209	47–617
α-2-Macro	2.0	1.4–3.2	1.1–3.8
α-1-Atrip	8.1	5.6–10.2	4.6–14.3
CRP	204	91–330	18–406

1. Cp = 0.6(CRP) + 131.2(log AP) − 272.6	r = 0.68
2. Cp = 29.2(α-1-Atrip) + 110.0(log AP) − 297.2	r = 0.68
3. Cp = 47.6(α-2-Macro) + 101.4(log AP) − 111.9	r = 0.57
4. Cp = 0.7(Trig) + 136.2(log AP) − 323.5	r = 0.76
5. Cp = 0.7(Trig) − 480.0(arterial pH) + 160.4(log AP) + 3076.7	r = 0.85