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ORIGINAL ARTICLE

# Hypotransferrinemia and changes in plasma lipid and metabolic patterns in sepsis

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**Abstract** This study was performed to obtain a characterization of the changes in plasma transferrin (Tf, g/L) in sepsis. More than four hundred determinations of Tf, and of a large series of simultaneously collected blood and hemodynamic variables, were obtained in 17 patients with post-traumatic sepsis. Tf during sepsis was consistently low (mean ± SD = 1.46 ± 0.46) however fluctuated markedly according to changes in metabolic and hemodynamic patterns. Regression analysis showed that decreases in Tf were simultaneously correlated with the plasma lipid pattern (in particular with decreasing cholesterol and increasing triglycerides), with decreases in albumin and peripheral $O_2$ extraction, and with increasing cardiac index ($p < 0.001$ for all). Decreases in Tf were moderated by increasing the parenteral amino acid dose ($p < 0.001$). Combinations of these variables in multiple regressions explained nearly 80% of the variability of Tf. There were no similar correlations for other acute phase proteins except ceruloplasmin, which showed opposite changes compared to those of Tf. These results show that within the hypotransferrinemia which characterizes sepsis, Tf may oscillate remaining strongly correlated with changes in metabolic and hemodynamic patterns, which may account for nearly 80% of the variability of Tf.

**Keywords** Sepsis · Plasma transferrin · Hypocholesterolemia · Hypertriglyceridemia · Peripheral oxygen extraction · Ceruloplasmin · Hyperdynamic cardiovascular state

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## Introduction

Transferrin is a protein involved in iron transport and metabolism. It is already known that the plasma concentration of transferrin (Tf, g/L) commonly decreases in acute phase response, for instance in trauma and sepsis. However the changes in Tf have not been characterized according to the simultaneous modifications in other metabolic and cardiorespiratory variables, and in severity of illness. We have carried out such an assessment over a large number of measurements performed in post-traumatic sepsis.

## Materials and methods

The assessment was based on the analysis of 486 determinations of Tf and of a large series of complementary variables, performed prospectively on 17 patients who developed sepsis after trauma. The patients were 14 men and 3 women; age was 31 ± 15 years (mean ± SD), weight was 76.8 ± 15.9 kg, and height 174 ± 7 cm. They had a combination of abdominal, chest and head injuries, 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 (Stevens 1983; Skau 1985) upon diagnosis of sepsis was 24 (range 12–66). One patient died from multiple organ dysfunction syndrome and one patient from sudden cardiac arrest; the other patients survived. Serial determinations were performed every 8–12 h while criteria for the diagnosis of sepsis persisted or death occurred, for a total of 402 determinations. Eighty-four additional determinations were performed before the development of sepsis (time till diagnosis of sepsis 3.0 ± 1.8 days). All patients were undergoing total parenteral nutrition at 35 ± 13 kcal/kg/d (3/4 glucose and 1/4 fat) and 1.5 ± 0.5 g/kg/d amino acids. Each determination included the measurement of plasma Tf (normal value 2.00–4.00 g/L), albumin, alpha-1-antitrypsin, fibrinogen, C-reactive protein, alpha-2-macroglobulin, ceruloplasmin, glucose, blood urea nitrogen, creatinine, sodium, potassium, calcium, chloride, cholesterol, triglycerides, total bilirubin, alkaline phosphatase, lactate, blood cell count, the full amino-acidogram with estimation of amino acid clearances (Clowes et al. 1980a, b), daily urinary 3-methylhistidine and urea excretion, arterial blood gas analyses, mean blood pressure, heart rate, sepsis severity score and sepsis-related organ failure assessment score (Stevens 1983; Skau 1985; Vincent et al. 1996). In 242 determinations performed during sepsis, central venous blood gas analyses with peripheral $O_2$ extraction, thermodilution cardiac index, peripheral vascular resistance and pulmonary blood pressures were simultaneously measured. These determinations provided a continuous distribution of observations, from moderate to extremely severe septic illness, which was well suited to assessing the correlates of changes in Tf over a wide range of pathophysiological abnormalities. The statistical analysis and validation of the results were mostly 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 assess the hierarchy of correlations and to select the simplest possible regressions yielding the best control of variability of Tf (Seber 1977).

The protocol complied with the Helsinki declaration of 1975 as revised in 1996, and was approved by the Institutional Review Board.

## Results

Plasma Tf, at initial determination after trauma, was 1.81 ± 0.38 (mean ± SD; median 1.77, range 1.19–2.54). In the subsequent determinations, before the development of sepsis it was 1.63 ± 0.29 (median 1.65, range 1.08–2.33), and upon the diagnosis of sepsis it was 1.53 ± 0.36 (median 1.53, range 0.98–2.22). Thereafter Tf tended to remain low (1.46 ± 0.46, median 1.45, range 0.25–2.61) however fluctuating markedly according to changes in plasma lipid patterns and in metabolic and cardiorespiratory patterns. In particular, in all the determinations performed during sepsis, regression analysis showed that the best correlates of decreasing Tf were decreasing cholesterol and increasing triglycerides. The degree of hypocholesterolemia in several measurements was moderated by the presence of cholestasis, and when this effect was taken into account by including also the level of alkaline phosphatase in a multiple regression, this explained 47% of the variability of Tf (regression 1, Table 1). The additional inclusion in the same multiple regression of albumin and of amino acid infusion rate brought the total of the explained variability of Tf to 57% (regression 2, Table 1). With regard to nutritional substrates, it is important to specify that Tf was unrelated to the dose of the other substrates, and also that triglyceride levels were unrelated to the dose of fat.

Tf additionally decreased with increasing plasma bilirubin and decreasing phenylalanine clearance (reflecting a

**Table 1** Regressions

| | |
|---|---|
| 1. Tf = 0.006(Chol) −0.001(ALP) −0.002(Trig) + 1.476 | $r^2 = 0.47$ |
| $n = 402$ | |
| 2. Tf = 0.005(Chol) −0.001(ALP) −0.002(Trig) + 0.336(Alb) + 0.168(AAIR) + 0.476 | $r^2 = 0.57$ |
| $n = 402$ | |
| 3. Tf = 0.007(Chol) −0.002(ALP) −0.001(Trig) + 0.333(Alb) + 1.929($O_2$Ex) + 0.263(AAIR) −0.224 | $r^2 = 0.73$ |
| $n = 242$ | |
| $p < 0.001$ for each regression and each coefficient in the regressions | |

Mean ± SD of relevant variables: Tf 1.46 ± 0.46; Chol 77 ± 15 (measurements without cholestasis) and 107 ± 41 (with cholestasis); Trig 254 ± 120; Alb 2.4 ± 0.4; AAIR 1.5 ± 0.5; $O_2$Ex 0.21 ± 0.06

*Tf* plasma transferrin (g/L), *Chol* cholesterol (mg/dL), *ALP* alkaline phosphatase (U/L, n.v. < 100), *Trig* triglycerides (mg/dL), *Alb* albumin (g/dL), *AAIR* parenteral amino acid infusion rate (g/kg/day), *$O_2$Ex* peripheral $O_2$ extraction (fraction)

parallelism with degree of liver dysfunction) and with increasing sepsis severity and sepsis-related organ failure scores ($r^2$ between 0.29 and 0.09, $p < 0.001$ for all). Conversely, it increased with increasing plasma glutamate (this was associated with higher amino acid dose, and was likely related to expansion of the glutamate pool) ($r^2 = 0.25$, $p < 0.001$). Other variables were more weakly related ($r^2 < 0.08$) or unrelated to Tf. However the inclusion of any additional variable in regression 2, Table 1, did not further improve the explained variability of Tf.

In the set of determinations in which peripheral $O_2$ extraction and hemodynamic measurements were also performed, the previously assessed correlations were reconfirmed. However Tf was found to be additionally related to abnormally decreasing peripheral $O_2$ extraction, mean blood pressure and peripheral resistance, and to abnormally increasing cardiac index and increasing vasopressor doses to maintain blood pressure ($p < 0.001$ for all). Although several combinations of these variables in multiple regressions explained nearly 80% of the variability of Tf, the simplest most significant fit was obtained by including only $O_2$ extraction (regression 3, Table 1 and Fig. 1). Decreasing $O_2$ extraction and increasing cardiac index had a similar role in explaining the decreases in Tf related to abnormal hemodynamic patterns.

It is noteworthy that none of the other acute phase proteins was so evidently correlated with cardiac index, except for a direct and weak relationship with ceruloplasmin ($r^2 = 0.12$, $p < 0.001$). Furthermore, although ceruloplasmin was unrelated to Tf, there was an evident opposite balance between the changes in concentration of these two proteins. As a consequence the strength of some previously listed correlations was amplified when using the Tf/ceruloplasmin ratio, as occurred for instance for the correlations with triglycerides, $O_2$ extraction and cardiac index (Fig. 2).

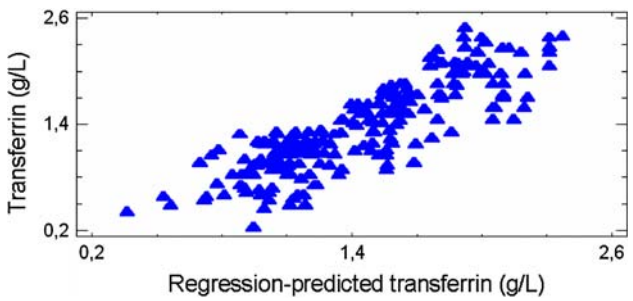


**Fig. 1** Actual transferrin measurements vs regression-predicted transferrin (predicted from regression 3 in Table 1, on the basis of the simultaneously occurring values of metabolic variables). The figure reflects the strong relationship between increasingly severe multiple metabolic abnormalities and decreasing transferrin

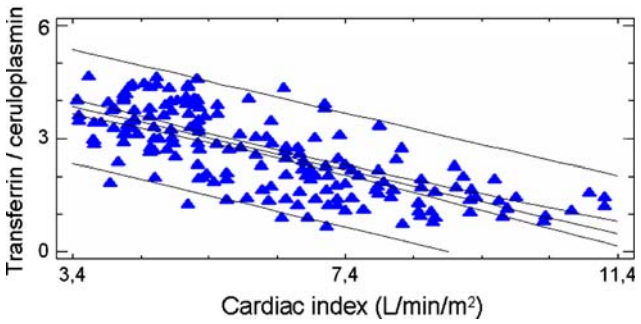


**Fig. 2** Relationship between cardiac index and the transferrin/ceruloplasmin ratio, with regression line and 95% confidence limits. Basic regressions: transferrin = −0.16 (cardiac index) + 2.42; $r^2 = 0.40$, $p < 0.001$; transferrin/ceruloplasmin = −0.42 (cardiac index) + 5.27; $r^2 = 0.51$, $p < 0.001$. Mean ± SD of variables (units): cardiac index 6.2 ± 1.9 (L/min/m$^2$); transferrin 1.45 ± 0.50 (g/L); ceruloplasmin 0.50 ± 0.11 (g/L)

Finally, the correlations with Tf assessed by regression analysis, were all reconfirmed in evaluating sequential determinations and evolutive patterns in individual patients.

## Discussion

These data characterize the correlations of Tf with plasma lipid and metabolic patterns in sepsis, and help to explain the fluctuations of Tf which are often observed in this condition. Indeed, the variables considered in the study accounted for nearly 80% of the variability of Tf, and for extreme decreases in Tf down to 0.25 g/L, which is about one-tenth of the normal level.

The data show the tendency of hypotransferrinemia to parallel the degree of hypocholesterolemia and hypoalbuminemia, which are landmarks of severity of acute phase response, and to decrease further with the increase in triglycerides (which reflects a further worsening of septic metabolic deterioration) (Siegel et al. 1979; Giovannini et al. 2005), with the fall in peripheral $O_2$ extraction and the development of progressively more unbalanced hemodynamic patterns. Hypotransferrinemia was partly counteracted by increasing the exogenous amino acid load (regressions 2 and 3, Table 1).

The evident correlation of Tf with cholesterol and triglycerides was confounded in some cases by superimposed cholestasis. Cholestasis is known to cause an upward shift in cholesterol above the level related to the underlying pattern, therefore moderating the degree of hypocholesterolemia in critical illness, and causing an upward shift in the correlations between cholesterol and plasma proteins (Cooper 1990; Giovannini et al. 1999, 2005, 2006). This was reconfirmed in our study, where the correlation

between cholesterol and Tf was influenced by cholestasis, and multiple regression analysis could account for this effect. It is also possible that cholestasis directly contributed to a decrease in Tf by enhancing the severity of acute phase response. At any rate, although the interplay between Tf, cholesterol and triglyceride levels in acute phase response still remains unclear (Khovidhunkit et al. 2004; Esteve et al. 2005) our data provide a well defined picture in clinical sepsis.

Another remarkable finding was the relationship between decreasing Tf and abnormally increasing cardiac index, because cardiac index is considered a dynamically changing variable and its correlation with a plasma protein is unexpected. Nevertheless this was independently confirmed by the measurements of thermodilution cardiac index and of peripheral $O_2$ extraction (which reflects the ratio between oxygen consumption and cardiac index). Furthermore the correlation with decreasing Tf became stronger when taking into simultaneous account the decreasing blood pressure and peripheral resistance, and the increased requirement for vasopressor medications. These findings reconfirm that in sepsis the hyperdynamic cardiovascular pattern, in the presence of reduced vascular tone and $O_2$ extraction, is strongly determined by the underlying metabolic patterns, and is a steady adaptive component of severe septic illness (obviously, unless profound shock or acute cardiovascular complications occur).

The pattern of Tf was unique, because similar hemodynamic correlations were not observed for other plasma proteins, except for weaker correlations of opposite sign with ceruloplasmin. Indeed, although ceruloplasmin was unrelated to Tf, there was the tendency for opposite changes in their concentrations. As a consequence several variables were better related to the Tf/ceruloplasmin ratio than to Tf, as occurred for instance for triglycerides, $O_2$ extraction and cardiac index (Fig. 2). Although it is difficult to speculate whether the increasing ceruloplasmin is part of an enhanced antioxidant and protective response (elicited by progressively worsening septic metabolic and inflammatory deterioration), or has a different explanation, our data clearly outline the tendency for an opposite balance between decreasing Tf and increasing ceruloplasmin. A similar pattern has been observed already in ARDS and myocardial infarction, and in chronic inflammatory conditions such as chronic hemodialysis and diabetes (Gutteridge et al. 1994; Kirschbaum 2000, Baykan et al. 2001; Memisogullari and Bakan 2004).

Before concluding, it should be mentioned for completeness that in a patient who died very rapidly from septic shock, with partial measurements and excluded from the study, we found Tf consistently higher than 3.2 g/L and displaced from the general distribution. This remained unsatisfactorily explained, although it was probably related to the use of contraceptive medications (Casabellata et al. 2007).

In conclusion, this study shows that the degree of hypotransferrinemia in sepsis is very strongly related to the changes in plasma lipid, metabolic and hemodynamic patterns. The data support the concept that these patterns drive the balance between synthesis and release of Tf (probably downregulated by the priority use of amino acids in host defence and tissue repair), removal of Tf from the intravascular space, and Tf catabolism (Piagnerelli et al. 2005). The exact implications within the patterns of endogenous iron economy (Ashrafian 2003) in extreme septic illness remain the object of future studies.

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