**Abstract**

**Introduction:** For critically patients, enteral immunonutrition results in notable reductions in infections and in length of stay in hospital, but not on mortality, raising the question as to whether this relate to the heterogeneous nature of critically ill patients or to the absence of the altered absorption of specific nutrients within the immunonutrient mix (e.g. iron). Immune-associated functional iron deficiency (FID) is not only one of the many causes or anaemia in the critically ill, but also a cause of inappropriate immune response, leading to a longer duration of episodes of systemic inflammatory response syndrome and poor outcome.

**Objective:** This prospective cross-sectional study was undertaken to assess the prevalence of FID in critically ill patients during their stay in intensive care (ICU) in order to find the more appropriate population of patients that can benefit from iron therapy.

**Method:** Full blood cell counts, including reticulocytes (RETIC), serum iron (SI), transferring levels (TRF) and saturation (satTRF), serum TFR receptor (sTfR), ferritin (FRT) and C-reactive protein (CRP) were measured in venous blood samples from 131 random patients admitted to the ICU for at least 24 h (Length of ICU stay, LIS; min: 1 day; max: 38 days).

**Results:** Anaemia (Hb < 12 g/dL) was present in 76% of the patients (Hb < 10 g/dL in 33%), hypoferremia (SI < 45 µg/dl) in 69%; satTRF < 20% in 53%; FRT < 100 ng/mL in 23%; sTfR > 2.3 mg/dL in 13%; and CRP > 0.5 mg/dL in 88%. Statistically significant correlations (r of Pearson; *p < 0.05, **p < 0.01) were obtained for serum CRP levels and WBC**, Hb*, TRF**, satTRF*, and FRT**. There was also a strong correla-
tion between TRF and FRT (-0.650**), but not between FRT and satTRF or SL. LIS correlated with Hb*, CRP**, TRF*, satTRF* and FRT**.

**Conclusion:** A large proportion of critically ill patients admitted to the ICU presented the typical functional iron deficiency (FID) of acute inflammation-related anaemia (AIRA). This FID correlates with the inflammatory status and the length of stay at the ICU. However, 21% of the ICU patients with AIRA had an associated real iron deficiency (satTRF < 20; FRT < 100 and sTfR > 2.3). Since oral supplementation of iron seems to be ineffective, all these patients might benefit of IV iron therapy for correction of real or functional iron deficiency, which in turn help to ameliorate their inflammatory status.

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Key words: Enteral route. Anemia. Iron. Inflammatory status.

**Introduction**

The potential to modulate the activity of the immune system by interventions with specific nutrients is termed immunonutrition, which has three potential targets, namely the mucosal barrier function, the cellular defence, and the local or systemic inflammation. This concept may be applied to any situation in which an altered supply of nutrients is used to modify inflammatory or immune responses. However, immunonutrition has become associated most closely with attempts to improve the clinical course of critically ill and surgical patients, who will often require an exogenous supply of nutrients through the parenteral or enteral routes.

Four meta-analyses give a fairly consistent view of the clinical efficacy of enteral immunonutrition. All four considered only randomised controlled trials in either surgical or critically ill patients and found that immunonutrition results in notable reductions in infections and in length of stay in hospital, but not on mortality. In general, the reduced infection rate and length of hospital stay are more pronounced in surgical than critically ill patients, and the reasons for these differences need to be investigated further in order to address whether these relate to the heterogeneous nature of critically ill patients or to the presence or absence of specific nutrients within the immunonutrient mix.

Critically ill patients are at greater risk of adverse outcomes than surgical patients. A biphasic response with an early hyperinflammatory response followed by an excessive compensatory response associated with immunosuppression is seen in many such patients. Therefore, early treatment needs to focus on decreasing the inflammatory response and prevent the compensatory immunosuppression.

Functional iron deficiency (FID) is a condition in which there is a decrease in iron available for metabolic processes and critically ill patients may develop FID in response to immune activation. At laboratory level, FID is characterized by serum transferring saturation < 20%, serum ferritin < 100 ng/l, hypochronic red cells > 5%, or reticulocytes with low Hb content (CHr < 28 pg), in the presence of increased concentration of an inflammatory marker (e.g., C-reactive protein > 0.5 mg/dl). Research in animals and human beings has suggested that adequate iron scores are important not only for erythropoiesis but also for immune function and a deficiency may, therefore, predict patients with inappropriate immune responses.

To this regard, Bellamy et al. found that duration of episodes of systemic inflammatory response syndrome and duration of stays at the intensive care unit were longer in critically ill patients with FID compared with patients without FID. This FID status can not be corrected by oral iron, since intestinal iron absorption is decreased in the presence of normal iron stores. However, in a recent study, the administration of iron sucrose, alone or in combination with EPO, effectively reduced the requirements for allogeneic blood transfusion, and resulted in an amelioration of systemic inflammatory response and a reduction in mortality rate.

Hence, functional iron status may be a marker of nutritional status or general health. Accordingly, this stud was initiated to determine the prevalence of FID in critically ill patients during their stay in intensive care in order to find the more appropriate population of patients that can benefit from iron therapy.

**Material and methods**

**Study design**

In this prospective cross-sectional observational study, we included adult medical or surgical patients ad-
mitted to the general intensive care unit (ICU) of a tertiary referral teaching hospital over 3 months. Patient’s enrolment was performed every Monday, and only those patients with a ICU stay longer than 24 h were included (Length of ICU stay, LIS; min: 1 day; max: 38 days).

Blood samples

Venous blood samples for determination of haematometric parameters were drawn in K2-EDTA (3 mL; VenoJet II, Terumo, Belgium), whereas those for determination of biochemical parameters were drawn in serum separator (4 mL; Sepacell, VenoJet II, Terumo, Belgium). All samples were collected at the laboratory, after daily routine analyses had been performed, and no extra blood samples were taken from any patient.

Laboratory parameters

Full blood cell counts, including reticulocytes (RETIC) (Pentra 120 Retic, ABX, France). Serum iron (SI, µg/dL), transferrin levels (TRF, mg/dL) and saturation (satTRF, %) (Cobas Integra, Roche, Germany), ferritin (FRT, ng/mL), serum transferring receptor (sTfR, mg/dL) and C-reactive protein (CRP, mg/dL) (Image, Beckman-Coulter, USA) were measured in venous blood samples. Corrected RETIC counts for the degree of anaemia (reticulocyte index, RI) was calculated according to the expression: RI = % RETIC \times (observed Htc/normal Htc) \times 0.5^6.

Statistical analysis

All data are shown as the mean ± standard deviation (n) and an unpaired Student t test was used for comparison of means. We used the test r of Pearson to correlate values. All statistics were performed using Microsoft Excel 2000 and SPSS 11.0 packages (Licensed to the University of Málaga, Spain). A P value < 0.05 was considered statistically significant.

Results

Demographic and baseline characteristics of patients

A total of 131 patients were included in this observational cross-sectional study. Haematological and biochemical parameters were measured once by rescuing blood samples drawn for routine analyses. LIS at the moment of blood sample procurement varied between 1 and 38 days, with the following distribution: 57 (43.5%) out 131 were in the ICU for up to 3 days (Group A), 35 (26.7%) between 4 and 7 days (Group B), and 39 (29.9%) 8 days or more (Group C). Since patient enrolment were performed every Monday, we paid especial attention to avoid duplicate patient inclusion.

Mean age for the sample was 56 ± 17 years (range, 35-91 years). The majority of the patients were men (76%). Among surgical patients (50/131, 38%), the more frequently performed procedure was coronary artery bypass grafting, whereas sepsis and chronic obstructive pulmonary disease were the more frequent diagnostics among medical patients (81/131, 62%).

Haematologic parameters

Mean values, ranges, and 95% CI of full blood count are summarised in table I. One hundred (76.3%) out of 131 patients were anaemic (Hb < 12 g/dL), with 43 (32.8%) presenting Hb levels below 10 g/dL. However, most of them presented normal MCV. RETIC counts were between 0.52 and 6.72%, with inadequate RETIC corrected counts in many cases. WBC counts were elevated in 75 (57%) with neutrophilia in 65 (50%), whereas 16 (12%) presented lymphocyte count below 800/µL (lymphopenia). Mean values of haematologic data after patient stratification according to LIS are summarised in table III.

Iron metabolism and inflammatory status

Iron metabolism status was assessed by measuring SI, TRF, satTRT, sTfR, and FTR (table II). Hypoferraemia (SI < 45 µg/dL) was observed in 90 (68.7%), satTRF < 20% in 69 (52.6%), sTfR > 1.9 mg/dL in 23 (17.6%) and > 2.3 in 17 (13%), FTR < 100 ng/mL in 30 (22.9%). The patient’s inflammatory status was assessed by measuring serum levels of CRP (table II), which was found to be above the upper limit of normality (0.5 mg/dL) in 116 (88.1%) patients. Additional information was provided by WBC counts (table I) and TFR and FRT levels (table II). Mean values of iron metabolism parameters and CRP levels after patients stratification according to LIS are summarised in table III.
Correlations between inflammatory status and haematologic and iron parameters

Statistically significant positive and negative correlations (r of Pearson) were obtained for serum CRP levels and WBC, RBC, Hb, TRF, satTRF, and FRT, but not for CRP and sTfR (table IV, fig. 1). After stratification of patients according to LIS, most of these correlations remain significant for Groups A and B, whereas in Group C this was true only for SI (table III).

There was also a strong correlation between TRF and FRT (-0.369; p < 0.01), but not between FRT and satTRF or SI. In the other hand, LIS correlated with Hb*, CRP**, TRF*, satTRF* and FRT**.

Discussion

Although there is no clear relationship between dietary iron intake and iron status, isotope studies have identified multiple dietary factors that influence iron absorption, such as ascorbic acid, animal tissue, phytates and polyphenols. The modern diet contains less red meat and is lower in iron than that consumed 30 years ago; however, there is no evidence to suggest that current dietary changes will have a major impact on iron status in the general population12.

In elderly populations, mean prevalence of folate (< 7 nmol/l) and vitamin B12 (< 148 pmol/l) deficiencies is high13, 14, whereas iron deficiency used to be infrequent, although is more prevalent in women13, 15. In these populations, prevalence of anaemia based on the WHO criteria (Hb < 13 g/dL in men; < 12 g/dL in women) has been reported to be 3-6%13, 14, 16. On the contrary, subjects with inflammatory process had a higher prevalence of anaemia (22.2% men, 31.6% women)13.

However, ICU patients show a completely different picture. Henche-Morilla et al.17 evaluated oligoelement and trace element levels when patients are admitted into the ICU and were included into a total parenteral nutrition (TPN) program. They found that, on admission to the unit, patients showed low serum levels iron, transferrin, zinc and calcium, and normal magnesium, phosphorus and copper figures. It is well known that major surgery, trauma and sepsis is followed by a systemic inflammatory response, whose humoral mediators (e.g., interleukin-1, interferon-γ, and tumor necrosis factor-α) inhibit erythropoiesis both directly by suppressing erythroid colony growth and indirectly by suppressing erythropoietin production18. Most studies in critically ill adults focus on the longer stay patients and report an impaired EPO response to anaemia, based on comparison either with normal reference ranges or with a control group of patients with non-renal anaemia19-21. In contrast, one study of 10 patients with sepsis or septic shock in the first 4 days following admission demonstrated marked increases in EPO levels in those patients who subsequently died, paralleling changes in the acute phase response22. Similarly, Elliot et al.23 found that EPO levels are high in the first 48 hours of critical illness in patients with ARF.
suggesting an acute renal response to injury. These patients also had the highest APACHE II scores and mortality rates. EPO levels then decline over time, together with a reduction in haemoglobin concentrations, and by day 3 in the ICU the EPO levels for almost all patients are in the low normal range. This indicates failure of EPO effect rather than failure of production as the one of the mechanisms for the anaemia of critical illness.

In addition, these cytokines induce a functional iron deficiency (FID) status24, 25; a clinical situation where the iron stores in the bone marrow macrophages is normal, but this iron is not available for erythropoiesis, due to an alteration of it release from the macrophages, of it incorporation to transferrin, or both6. Hepcidin, an hepatic acute phase protein recently discovered, seems to play a crucial role in these alterations26.

Most of our patients presented with a low serum iron, low transferrin level and saturation, and high ferritin: a pattern which is typical of the anaemia of chronic disease. These changes occur rapidly in acute as well as chronic illness as part of the systemic inflammatory response27. In fact, correlation between CRP and iron metabolism parameters were stronger in Group B (LIS: 4-7 days), and it is well known that effects of major surgery on iron metabolism are maximum during the first postoperative week25,26.

As expected, there was a strong negative correlation between TRF (a negative acute phase protein) and FRT (a positive acute phase protein) (-0.650; p < 0.01), but not between FRT and satTRF or SI, indicating that stored iron is not available. Thus, the high C-reactive protein combined with the low transferring saturation (fig. 1) and high ferritin support the view that, at least in part, the anaemia was a consequence of FID27, whereas only a small proportion of patients (13%) showed a real iron deficiency combined with inflammation, as suggested by high sTfR levels28. On average, the incidence of anaemia and the levels of Hb in our patients are similar to those recently reported for several European ICUs29.

However, FID leads not only to a blunted erythropoiesis but to an inappropriate immune response as well8. This might explain why systemic inflammatory response episodes last longer in critically ill patients with FID, with prolonged stay at the ICU and increased morbidity8. This FID status could not be corrected by oral iron, since intestinal iron absorption is inhibited, due to an hepcidin-induced reduction of its release through the enterocyte basolateral membrane26. On the contrary, once injected in vivo, the iron-carbohydrate complexes are metabolized, the iron is released where it then binds transferrin in the plasma, and the redundant carbohydrate moiety is then cleared via the liver26. Hence, the intravenous iron emerged as a therapeutic option for the treatment of FID in these patients, since the increased erythropoietic effect (4.5-5.5 times that of basal) of IV iron last 7 to 10 days, after which the iron is se-
questered by the reticuloendothelial system\textsuperscript{31}. In a recently published study the effectiveness of the administration of iron sucrose alone or in combination with EPO, was assessed in a population of anemic critically ill patients\textsuperscript{10}. Compared to those in the control group who only received folic acid, both treatments effectively reduced the requirements for ABT. In addition, patients treated with iron sucrose experienced an amelioration of systemic inflammatory response and a reduction in mortality rate. These beneficial effects were not as evident in patients receiving iron sucrose plus EPO, probably due to persistence of FID caused by the EPO-enhanced erythropoietic activity. Hence, it is possible that the correction of FID by administering iron sucrose has not only contributed to reduce the requirements for ABT by improving the erythropoietic response, but also to reduce postoperative morbi-mortality rate by restoring an adequate immune response.

Based on their findings, Henche-Morilla et al.\textsuperscript{17} arrived to the conclusion that the daily parenteral supplements of oligoelements should be higher than those recommended. We believe that this also applies to enteral nutrition, especially for those micronutrients, such as iron, for which intestinal absorption is limited by the systemic inflammation. From a theoretical point of view, our data support the idea that a significant amount of ICU patients might benefit from intravenous iron supplements, including those with enteral and parenteral nutrition. However, further research, preferably as a large randomised clinical trial, is needed to ascertain whether intravenous iron therapy might improved clinical outcomes in these patients.

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References