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The use of soluble transferin receptor to assess iron deficiency in adults with cystic fibrosis

Sabiha Khalid [a], Donovan McGrowder [b,*], Mike Kemp [a], Pauline Johnson [c]

[a] Department of Clinical Biochemistry, Royal Brompton Hospital, Chelsea, London, UK
[b] Department of Pathology, University of the West Indies, Kingston 7, Jamaica
[c] Caribbean Food and Nutrition Institute, University of the West Indies, Kingston 7, Jamaica

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Abstract

Background: Iron deficiency (ID) is common in cystic fibrosis (CF) and the soluble transferrin receptor (sTfR) is a sensitive, quantitative measurement of tissue iron deficiency. The study investigated the use of sTfR together with serum iron, transferrin saturation (TS) and serum ferritin, in assessing iron status in adult CF patients.
Methods: The patient population consisted of 127 CF patients which consisted of 51 inpatients with infected exacerbation (IE) and 76 outpatients at the time of their annual review (AR). Serum sTfR was measured using a particle-enhanced immunoturbidimetric assay on the Beckman Coulter LX20.
Results: Sixty five percent (65%) of CF patients in the IE group and 28% in the AR group had ID as determined TS, but only 18% (IE group) and 20% (AR group) as determined by ferritin. Serum sTfR detected 20% in the IE group and 12% in the AR group. We found significant correlation between C-reactive protein and TS ($r=-0.56$; $P<0.01$) but not with ferritin ($r=0.22$; $P=0.380$) in the IE group.
Conclusion: Iron status of patients with CF can be accurately assessed by sTfR which is unaffected by the acute phase response and can be used in conjunction with serum ferritin.

Keywords: Soluble transferrin receptor; Iron deficiency; Serum ferritin; Transferrin saturation; Serum iron

1. Introduction

Iron deficiency (ID) is a common clinical problem. The correct diagnosis of ID is essential for successful patient management because it may be the presenting sign of a serious illness such as a gastrointestinal malignancy [1]. In many cases, ID is relatively simple to diagnose and treat. However, in some patients with medical problems such as infection, inflammation and malignancy, the diagnosis can be difficult [2]. Measurement of serum ferritin is currently the accepted laboratory test for diagnosing ID, and a serum ferritin value < 12 μg/l is a highly specific indicator of ID [3]. Other commonly used laboratory tests such as serum iron, total iron-binding capacity (TIBC), mean corpuscular volume (MCV), and transferrin saturation (TS) provide little additional diagnostic value [4]. These common laboratory tests for measuring ID in patients with an inflammatory condition such as cystic fibrosis (CF) are considerably influenced by inflammatory acute phase reactions, which may complicate the clinical interpretation of the test results [5].

Cystic fibrosis is a very common with about 1 in 2000 live births in the U.K. It is a defect in the cystic fibrosis transmembrane receptor gene, which controls ion transport in mucus formation [6]. The build-up of the mucus leads to recurrent infections in the lungs and blockage of pancreatic enzymes from the gut. This cause iron deficiency anemia (IDA) by preventing the absorption of iron required for erythropoiesis from the gut. Other possible causes of iron deficiency in cystic fibrosis include dietary deficiency, gastrointestinal blood loss, and chronic inflammation where iron is taken up by macrophages and becomes unavailable for use in erythropoiesis [7].

The soluble transferrin receptor (sTfR), a truncated form of the membrane-associated transferrin receptor is a sensitive

* Corresponding author. Tel.: +1876 927 1410; fax: +1876 977 1811.
E-mail address: donovan.mcgrowder@uwimona.edu.jm (D. McGrowder).

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indicator of ID [8]. The amount of sTfR in the serum reflects both the receptor density on cells (tissue iron deficiency) and the number of cells with receptor (erythropoietic activity). Consequently, sTfR correlates inversely with the amount of iron available for tissues, and directly with the rate of erythropoiesis [2]. The quantification of sTfR has been shown to differentiate effectively between iron-deplete and iron-replete anemic states, irrespective of the presence of acute or chronic inflammatory reactions [9,10]. This finding emphasizes the clinical usefulness of the quantification of the circulating receptor in assessing iron status in patients, as ferritin is a known acute-phase reactant [11] and concentrations of serum iron and transferrin or total binding capacity are known to decrease in the presence of an acute-phase reaction [12].

Confirmation of the absence of iron stores in a bone marrow aspirate is still considered the gold standard for the diagnosis of ID, but its routine use, particular in a large number of patients is impractical because of the invasive nature of the test [13]. In recent years sTfR is measured by fully automated methods such as immunoturbidimetric and immunoenzymometric methods. However, the method of choice is the enhanced particle immunoturbidimetric assay rather than the immunoenzymometric assay. Even though both methods of measurements are reliable and sensitive, the immunoenzymometric method takes up to 2 h to measure sTfR while the immunoturbidimetric method takes 20 min [14].

The assessment of iron status in cystic fibrosis patients is an unexplored area in clinical research and to date only 2 studies have been done using sTfR to detect ID in this group of patients [15,16]. In this study, we investigated the utility of sTfR with other traditional iron indices such as TS and serum ferritin in the assessment of iron status in adult CF patients who are stable (annual review group) and infective (infective exacerbation group).

2. Materials and methods

The patient population consisted of 127 CF patients at the Royal Brompton Hospital who were recruited in the study in a three-month period. The diagnosis of CF was based on accepted clinical criteria including a typical clinical history, altered pulmonary function, and elevated levels of sodium and chloride in repeated sweat tests. These patients were in 2 separate groups; 51 inpatients with infected exacerbation, and 76 outpatients coming in for their annual review who are generally well. The infective exacerbation group consisted of 26 men and 25 women with mean age 26.4±1.54 y while the annual review group consisted of 38 men and 38 females with mean age 28.4±2.20 y. A total of 50 normal individuals were recruited for the study having a mean age of 29.4±2.20 y. The study was approved by the Royal Brompton Hospital local ethics committee and informed consent was obtained in writing from all patients.

The annual review group was assessed in the clinic while the infective exacerbation group on the ward. A full history of anemia, bleeding, menorrhagia, liver disease, gastrointestinal disease (including varices and ulcers), blood transfusions, symptoms in the previous week of cough, breathlessness, increase in the volume or a change in the colour of the sputum, absenteeism from work, decreased exercise tolerance and fever. All medication, including pancreatic supplements, iron, vitamin K, antacids and proton pump inhibitors were recorded.

An infective exacerbation is defined as the presence of at least three new findings or changes in clinical status compared to the recent baseline visit (Consensus conferences). This includes: increased sputum production and/or a change in the appearance of sputum, fever (>38 °C for at least 4 h in a 24-h period) on more than one occasion in the previous week, weight loss >1 kg or 5% of body weight, work absenteeism due to illness in the previous week, new findings on chest examination, decreased exercise tolerance and decrease in oxygen saturations from baseline within past 3 months of >10%. Therefore, patients were defined as having an infective exacerbation if they had 2 out of the following 4: (i) a raised erythrocyte sedimentation rate (ESR)>20 mm/h, (ii) an elevated neutrophil count ($>10\times10^9$/l), (iii) increased C-reactive protein (CRP)>20 mg/l, or (iv) an infective exacerbation as defined above.

Blood samples for routine measurements were drawn from each patient by venipuncture. The whole blood was sent to the Hematology Department for a complete blood count and determination of hematological parameters. Hemoglobin (Hb), mean cell volume (MCV), mean corpuscular hemoglobin (MCH), neutrophils and platelets were measured on an Advia™cell counter (Bayer, Newbury, UK). ESR was measured using the Westergren Method. Iron deficiency anemia was defined using multiple laboratory criteria. These included: Hb<13.0 g/dl in men and <11.5 g/dl in women [17], MCF<80 fl or MCHC<27 pg [18]. Laboratory values for ID included: serum ferritin<12 μg/l in women and <20 μg/l in men which represents the point of total depletion of iron stores [19], serum iron<12 μmol/l, TS<15% and TIBC>70 μmol/l [15]. CF patients with C-reactive protein values >10 mg/l were considered as individuals with possible inflammation [20].

A Beckman LX20 analyser was used to measure: serum iron, TIBC using Ferrozine, CRP and transferrin by immunoturbidimetry, and albumin by bromocresol purple. Serum ferritin was measured on the Beckman Access by a paramagnetic chemiluminescent immunoassay. The remaining serum was dispensed in 500 μl aliquots in eppendorf tubes and stored at −20 °C for batch analysis of the sTfR concentration.

In this study the IDeA sTfR particle enhanced immunoturbidimetric (IDeA sTfR-IT) application on the Beckman Coulter LX20 was evaluated. The application of the assay on the Hitachi 911 analyzer (Roche) was previously described [21]. The method requires 20 μl of sample, 250 μl of IDeA sTfR-IT buffer, and 20 μl of IDeA sTfR-IT reagent. The reagent consists of polyclonal anti-human TfR $F(ab)_2$ antibodies bound to SVBC-latex particles. In the presence of sTfR, the latex particles are agglutinated in a dose-dependent manner, causing increased turbidimetry. The increase in turbidimetry is detected at 660 nm. The amount of sTfR in the sample is determined by means of a calibration curve based on five ready-to-use calibrators, which may be also prepared by automatic dilution of the highest calibrators, which contains 8–9 mg/l sTfR. Intra and inter assay batch precisions were performed. The mean, standard deviation, and percentage CV were calculated and compared with the manufacturer's value.

The data was entered into the computer and managed by using SPSS for windows (11.5; SPSS Inc, Chicago, IL). Results were expressed as mean±SE.

Table 1
Comparison of hematological and biochemical parameters of iron status of CF patients in the annual review and infective exacerbation groups

	Mean±SE		*P*
	Annual review group	Infective exacerbation group	
n	76	51	
Hb (g/l)	13.98±0.18	13.91±0.21	NS
MCV (fL)	87.67±0.58	84.76±0.72	NS
MCH (pg)	33.77±0.17	33.02±0.26	NS
WBC ($\times10^9$/l)	9.90±0.42	13.17±0.64	NS
ESR (mm/h)	11.63±0.97	27.90±2.47	<0.0001
CRP (mg/l)	6.55±0.22	31.98±3.18	<0.0001
Iron (μmol/l)	14.37±0.80	7.64±0.65	0.035
TIBC (μmol/l)	67.03±1.22	66.14±1.57	NS
Ferritin (μmg/l)	50.86±13.43	36.98±4.34	NS
TS (%)	12.04±1.02	12.53±1.74	NS
sTfR (mg/l)	1.40±0.10	1.52±0.10	NS

Values shown are mean±SE. Hb = hemoglobin; MCV = mean corpuscular volume; TIBC = total iron-binding capacity; CRP = C-reactive protein; sTfR = soluble transferrin receptor.