Invited critical review

Soluble transferrin receptor in complicated anemia

Federica Braga, Ilenia Infusino, Alberto Dolci, Mauro Panteghini

Centre for Metrological Traceability in Laboratory Medicine (CIRME), University of Milan, Milan, Italy
Clinical Biochemistry Laboratory, ‘Luigi Sacco’ University Hospital, Milan, Italy

Abstract

Determination of serum soluble transferrin receptor (sTfR) has been proposed to identify iron-deficiency anemia (IDA) in patients affected by concurrent inflammatory disease that may spuriously increase ferritin concentration. The aim of this study was to critically review the available literature to assess the diagnostic efficacy of sTfR in complicated anemia. The criteria for study selection were: enrolment of patients with complicated anemia; bone marrow examination used as diagnostic gold standard for IDA; evaluation of sTfR vs. ferritin and binary data presentation. Six published studies met the criteria. However, the small size and wide heterogeneity of the studies did not allow us to conduct a meta-analysis. sTfR was overall more sensitive, even though it was evident that the ferritin sensitivity was influenced by selected cut-offs. Well-designed studies are still needed to define the added value, if any, of sTfR to ferritin for IDA detection in complicated anemia.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

Iron deficiency is one of the most frequent clinical disorders in humans, caused by dietary deficiency, chronic blood loss or pregnancy [1]. Iron-deficiency anemia (IDA) occurs when this condition is severe enough to reduce erythropoiesis. The diagnosis of IDA can be very difficult in patients with coexistent pathologies, such as inflammatory and autoimmune diseases, malignancies, or infections (acute or chronic), because anemia of chronic disease (ACD), frequently occurring in these conditions, can be a confounding factor [2]. Differentiating between IDA and ACD and identifying the coexistence of mixed IDA+ACD is, therefore, a serious diagnostic challenge, mainly because these conditions of anemia need different treatments and in ACD patients an inappropriate supplementation of iron could aggravate the underlying disease [3].

The gold standard for diagnosing iron deficiency is the bone marrow examination (BME), which establishes the absence of stainable iron [4,5]. BME is, however, invasive, expensive and operator-dependent,

Abbreviations: IDA, iron-deficiency anemia; ACD, anemia of chronic disease; BME, bone marrow examination; sTfR, soluble transferrin receptor; LR+, positive likelihood ratio; LR−, negative likelihood ratio; ISs, International Standards; ROC, receiver operating characteristic.
* Corresponding author at: Laboratorio Analisi Chimico-Cliniche, Azienda Ospedaliera ‘Luigi Sacco’, Via GB Grassi 74, 20157 Milan, Italy. Fax: +39 02 503 19835.
E-mail address: federica.braga@unimi.it (F. Braga).
so that it cannot be routinely performed in clinical practice. Consequently, the measurement of serum ferritin concentration is the first-level test for diagnosing IDA [6]. Blood ferritin concentrations are reported to reflect the amount and the changes of intracellular ferritin, the main iron storage protein, as confirmed by robust data obtained in experimental studies [6,7]. By aggregating data across different studies Guyatt et al. [5] showed that an effective rule-in for IDA can be obtained at a ferritin decision level of 15 μg/L and an effective rule-out by adopting thresholds of 40 μg/L for the general population and 70 μg/L for patients with infection or malignancy, respectively. This is because serum ferritin may be markedly increased by inflammation, working as a positive acute phase reactant. Recently, Ferraro et al. [6] argued on the current validity of these absolute numbers, based on studies published from 1970 to the 1980s using non-harmonized assays. Independent of the assay calibration, the concept that the optimal interpretation of ferritin values should rely on the use of different decision limits depending on the possible presence of co-morbidities is widely accepted [8–10].

The availability of further biomarkers, not affected by concurrent chronic disease and inflammation and therefore able to identify mixed IDA+ACD, could have a great clinical value. The soluble transferrin receptor (sTfR) may play this role, as its serum concentration is expected to rise in IDA, but not in ACD [11]. This is a single polypeptide chain of 85 kDa, produced by the proteolytic cleavage of the 190 kDa transmembrane transferrin receptor, a glycoprotein primarily expressed in cells requiring iron. Plasma sTfR concentrations reflect the receptor density on cells and the number of cells expressing the receptor, therefore it is closely related to cellular iron demands and erythroid proliferation rate [12]. Some authors also reported the use of the sTfR/log ferritin ratio, i.e. the sTfR index, theoretically taking advantage of the reciprocal relationship between the two variables influenced by iron deficiency (increase of sTfR and decrease of ferritin concentrations) [13,14]. However, a recent meta-analysis demonstrated the greater clinical value of sTfR rather than the sTfR index [15]. In the same paper, we concluded that further studies are needed to define the overall diagnostic accuracy of sTfR and its possible position in the diagnostic flowchart of IDA. In particular, its added value to serum ferritin determination for detection of IDA across an exhaustive population of anemic patients with inflammatory disease should have been demonstrated. Accordingly, the aim of the present study was to critically review the available literature comparing the diagnostic accuracy of sTfR vs. ferritin as biomarkers of IDA in patients with clinical suspicion of mixed IDA+ACD to check the scientific evidence of these data in supporting the efficacy of sTfR as an additional diagnostic tool in complicated conditions.

2. Materials and methods

2.1. Literature search

We searched the published peer-reviewed literature from 1986 up to December 2012 in the Medline and Embase databases, with MeSH terms [soluble transferrin receptor] or [ferritin] and [iron deficiency anemia], and with limits “Title/Abstract, Human Subjects, English”. In addition, the reference lists of retrieved articles were screened to identify further studies for possible inclusion. The final aim of the search was to identify original articles in which ferritin and sTfR were head-to-head investigated as diagnostic tools for IDA identification in complicated clinical cases.

2.2. Selection criteria

First, two reviewers (FB and II) evaluated the title and abstract of the identified records to determine whether the studies were relevant to the aim of the review. Observational studies, case reports, narrative reviews, letters to the editor and other similar contributions were excluded. Then, by evaluating the complete manuscript, it was determined whether the preliminary selected papers met the following inclusion criteria:

1. Enrolment of potentially anemic patients with complicated conditions (e.g., malignancy, infection, etc.);
2. Use of BME as the diagnostic gold standard for IDA;
3. Evaluation of sTfR vs. ferritin as diagnostic tools for detecting IDA;
4. Binary data presentation allowing the calculation of diagnostic sensitivity and specificity.

In addition, the employed analytical methods and way of cut-off selection were carefully considered.

2.3. Statistical analysis

As the binary data presentation was one of the inclusion criteria, we derived for each selected study diagnostic sensitivity and specificity. In addition, positive (LR+) and negative (LR−) likelihood ratios, corresponding to sensitivity / (1 – specificity) and (1 – sensitivity) / specificity, were estimated. In particular, the strength of the indication for the presence of the disease provided by a positive test result is relevant when LR+ ≥ 10, modest when 5 ≤ LR+ < 10 and poor when 2 ≤ LR+ < 5, and the strength of the indication for the absence of the disease provided by a negative test result is relevant when LR− ≤ 0.10, modest when 0.10 < LR− ≤ 0.20 and poor when 0.20 < LR− ≤ 0.50 [16]. We also considered the meta-analysis of data as a highly informative statistical tool, but the small size and the huge heterogeneity among study characteristics did not permit any data pooling and further statistical elaboration.

3. Results

3.1. Retrieved studies

The search strategy retrieved a total of 2283 potential records, after removing duplicates. After evaluation of titles and abstracts, 2253 studies were excluded because they were not relevant to the aim of the study, and a total of 30 original papers were selected for full-text examination. Among those, 24 papers were further excluded for the following reasons:

1. BME was not used as the diagnostic gold standard for IDA (n=19);
2. enrolment of uncomplicated patients (n=2);
3. binary data not available (n=2);
4. ferritin test not carried out (n=1).

Finally, only six articles met all the established inclusion criteria [17–22]. Table 1 shows the main characteristics of the selected studies. A total of 357 subjects were enrolled. Five out of 6 studies displayed higher sensitivity for sTfR (from 0.71 to 1.00). On the other hand, the specificity of ferritin measurement (from 0.67 to 1.00) appeared to be superior in the majority of studies. Accordingly, sTfR displayed relatively low LR−, indicating an adequate (from modest to relevant, i.e. LR− ≤ 0.20) ability in four studies to exclude the presence of IDA when the test results are negative. As expected, this was not the case for ferritin for which the ability to rule out IDA in the studied patients was rather poor.

3.2. Type of enrolled subjects

Four out of the 6 retrieved studies declared to have enrolled only anemic patients [17,18,20,21]. The anemia condition was clearly defined in two papers [17,18] as a hemoglobin concentration <128 g/L in men and <117 g/L in women [17], and <140 g/L in men and <120 g/L in women [18], respectively. The other two studies did not declare the applied criteria for defining anemia for patient selection [20,21]. With only one exception [17], studies did not ensure a consecutive enrolment, thus introducing a selection bias.
Table 1

<table>
<thead>
<tr>
<th>Author/Ref.</th>
<th>No. of subjects</th>
<th>Patient enrollment</th>
<th>Setting</th>
<th>IDA prevalence</th>
<th>ferritin Assay Cut-off, μg/L</th>
<th>sensitivity</th>
<th>specificity</th>
<th>LR+</th>
<th>LR−</th>
</tr>
</thead>
<tbody>
<tr>
<td>Punnonen et al.[17]</td>
<td>81</td>
<td>Consecutive</td>
<td>Undergone BME</td>
<td>21%</td>
<td>Orion Diagnostica RIA</td>
<td>0.71</td>
<td>0.98</td>
<td>3.55</td>
<td>0.30</td>
</tr>
<tr>
<td>Means et al.[18]</td>
<td>145</td>
<td>NA</td>
<td>Undergone BME</td>
<td>17%</td>
<td>NA</td>
<td>0.26</td>
<td>0.99</td>
<td>2.50</td>
<td>0.76</td>
</tr>
<tr>
<td>Nagral et al.[19]</td>
<td>63</td>
<td>Selected patients with chronic liver disease</td>
<td>NA</td>
<td>63%</td>
<td>NA</td>
<td>0.12</td>
<td>0.92</td>
<td>1.16</td>
<td>0.08</td>
</tr>
<tr>
<td>Fitzsimons et al.[20]</td>
<td>12</td>
<td>Selected RA patients</td>
<td>NA</td>
<td>12%</td>
<td>NA</td>
<td>0.00</td>
<td>1.00</td>
<td>25.0</td>
<td>0.76</td>
</tr>
<tr>
<td>Baillie et al.[21]</td>
<td>76</td>
<td>Retrospective</td>
<td>Undergone BME</td>
<td>18%</td>
<td>Siemens Advia turbidimetry</td>
<td>0.86</td>
<td>0.79</td>
<td>4.1</td>
<td>0.18</td>
</tr>
<tr>
<td>Chang et al.[22]</td>
<td>76</td>
<td>NA</td>
<td>Undergone BME</td>
<td>18%</td>
<td>NA</td>
<td>0.86</td>
<td>0.79</td>
<td>4.1</td>
<td>0.18</td>
</tr>
</tbody>
</table>

Sens, sensitivity; Spec, specificity; LR+, positive likelihood ratio; LR−, negative likelihood ratio; BME, bone marrow examination; RIA, radioimmunoassay; IEMA, immunoenzymometric assay; RA, rheumatoid arthritis; ACD, anemia of chronic disease.

a Original cut-off values in nmol/L converted to mg/L by using a multiplication factor of 0.0738.

As per inclusion criteria, all enrolled patients were complicated cases. The case-mix was, however, highly heterogeneous and often very much selected in order to a priori exclude the presence of different disturbing factors (particular diseases or conditions) known to interfere with sTfR concentration [17,18,22]. In this regard, it has been demonstrated that hematological malignancies, hemolytic anemia and a deficiency of vitamin B12 or folic acid may be associated with elevated sTfR concentrations regardless of the iron status of the patient [23–25]. Two studies did not describe in detail the criteria for recruiting patients and for the reason for submitting only a relatively small subgroup of them to BME [18,21]. In two studies, only patients with seropositive rheumatoid arthritis or chronic liver disease were enrolled [19,20]. Confounding factors for iron metabolism studies and bone marrow iron stores, e.g. blood transfusion, erythropoietin therapy, cytotoxic medication, the iron chelator penicillamine and iron therapies, were additionally considered in the four studies [17,18,20,22].

3.3. Gold standard for IDA diagnosis

One of the inclusion criteria applied in this systematic review was the use of BME as the gold standard for IDA identification. Therefore, by definition all the retrieved papers classified the patients and estimated the diagnostic accuracy of ferritin and sTfR on the basis of the BME report. However, there was some disagreement over the definition of iron status groups among papers that could have affected the diagnostic efficacy of the evaluated biomarkers. If Chang et al. [22] included patients with both reduced and absent bone marrow iron stores in the same IDA group, other studies differently categorized patients on the basis of presence/absence of stainable iron in bone marrow [17,18,20,21]. In these papers, patients with reduced, but not absent, bone marrow iron were therefore mostly included in the non-IDA control group. For instance, Baillie et al. [21] specified that in this study iron stores were graded as 0 to 6+ and grades from 1+ on were still considered as not iron deficient. In another study evaluating eight patients [19], two independent hematologists similarly graded iron stores as absent to 6+. However, there was discrepant reporting on iron stores in two of them (25% of studied patients). Since in both cases one hematologist reported the absence of and the other reported 1+ marrow iron, the authors decided to take absent/1+ iron as indicative of IDA.

3.4. Analytical methods and diagnostic thresholds

As it is well known, the results of most immunoassays, including ferritin and sTfR, are method-dependent [6,15]. Consequently, information that cannot absolutely be omitted in a clinical study evaluating these markers are the assay name and type, the manufacturer of the reagents and the platform used. Surprisingly, in half of the retrieved papers [18–20] the employed method for ferritin determination was not specified. In one of them [19], the authors paradoxically declared that serum ferritin was measured by a “standard technique”, which clearly is quite general and scientifically not acceptable. With regard to this marker, current requirements for assay traceability, as outlined in the European Union Directive on In Vitro Diagnostic Medical Devices [26], ask manufacturers to align their analytical systems to available higher-order WHO International Standards (ISs) [6,27]. The introduction of these preparations as reference materials at the beginning of the new century required a change in the assay calibration that in turn produced different results (and, possibly, different clinical thresholds), with a bias of approximately 5–10% between the calibrations [28]. Because of this realignment, data from clinical studies performed before the implementation of traceability of ferritin assays to ISs cannot directly be compared with those obtained in more recent years using traceable assays [6]. The lack of standardization of sTfR measurements also limits the robustness of studies performed to define its diagnostic power, making the comparison of sTfR results difficult among the various studies using different assays [15].
How diagnostic cut-off values were established in different studies is another important issue to take into account. As serum ferritin is a positive acute phase protein, the use of clinical thresholds derived from uncomplicated populations can cause false negative results in IDA detection when this condition is associated with an inflammatory state, leading to a decrease of test sensitivity. As shown in Table 1, half of the retrieved studies used rather low cut-off values for ferritin, just hazarding this pitfall [18,19,21]. In some papers [18,19], the employed cut-off was simply the lower limit of the value distribution in an apparently healthy population used as reference in the corresponding laboratory, whereas another study [21] did not specify how the cut-off was obtained. In the remaining three studies, a probably more correct, higher ferritin threshold adjusted for co-morbidity presence was used [17,20,22]. Particularly, Punnonen et al. [17] correctly selected the optimal decision threshold (41 μg/L) using the receiver operating characteristic (ROC) curve obtained in their studied population, which was considerably above their conventional gender-specific lower reference limits (5 μg/L for women and 15 μg/L for men, respectively). The 75 μg/L cut-off used in the study on the rheumatoid arthritis patients [20] was derived by another published paper investigating the use of serum ferritin for diagnosing IDA in the same type of population [29]. This approach could be correct if the method for ferritin used in the two papers were the same. Unfortunately, since in the retrieved paper the employed ferritin method was not reported, we were unable to confirm the correctness of the approach.

With regard to the sTfR cut-offs, values were similar when the R&D Systems ELISA was used [17,18,20]. However, while Punnonen et al. [17] correctly relied on the ROC curve approach, the other two studies [18,20] uncritically endorsed a previously published reference interval established in 105 healthy adults by Allen et al. [30]. Other studies using Orion Diagnostica reagents [19,21,22] applied quite different thresholds (from 2.00 to 8.50 mg/L), but without clearly specifying how these values were obtained.

The overall evaluation of the retrieved studies is summarized in Table 2.

### Table 2

<table>
<thead>
<tr>
<th>Author [Ref.]</th>
<th>Recruitment of anemic patients</th>
<th>Exclusion of patients with hematological malignancies, hemolytic anemia and deficiency of vitamin B12, or folic acid</th>
<th>Traceable ferritin assay</th>
<th>Ferritin co-morbidity-adjusted cut-off</th>
<th>Way of sTfR cut-off selection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Punnonen et al. [17]</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes (ROC)</td>
<td>ROC</td>
</tr>
<tr>
<td>Means et al. [18]</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No (LRL)</td>
<td>Published URL</td>
</tr>
<tr>
<td>Nagral et al. [19]</td>
<td>No</td>
<td>Only patients with chronic liver disease enrolled</td>
<td>No</td>
<td>No (LRL)</td>
<td>?</td>
</tr>
<tr>
<td>Fitzsimons et al. [20]</td>
<td>Yes</td>
<td>Only patients with rheumatoid arthritis enrolled</td>
<td>?</td>
<td>Yes</td>
<td>Published URL</td>
</tr>
<tr>
<td>Ballie et al. [21]</td>
<td>Yes</td>
<td>No (but very selected subgroup)</td>
<td>Yes</td>
<td>No</td>
<td>Manufacturer's URL</td>
</tr>
<tr>
<td>Chang et al. [22]</td>
<td>No</td>
<td>Yes (only hemolytic anemia)</td>
<td>Yes</td>
<td>Yes</td>
<td>URL (source unspecified)</td>
</tr>
</tbody>
</table>

ROC, receiver operating characteristic curve-derived; LRL, lower reference limit; URL, upper reference limit.

In conclusion, a clear message from this systematic review of the literature by defining the appropriate criteria for judging the eligibility of studies to be included in the analysis. In particular, BME was retained as the diagnostic gold standard method for IDA [4,5], although it shows some technical limitations [31]. Accordingly, the majority of the recruited papers (19 out of 30) which did not use BME as a diagnostic reference method for classifying patients were excluded from further analysis.

One of the greatest concerns in the general applicability of obtained results was related to patient selection. Except for two papers [17,18], the studies retrospectively enrolled patients, strictly selecting them on the basis of the availability of BME findings, and did not consider the wider clinical framework of the mixed IDA + ACD condition. In addition, also the two studies [17,18] excluded patients that had hematologic conditions other than IDA, that have been reported to spurious increase sTfR values [23–25]. Thus, there is a possibility that the true diagnostic performance of this marker might be overestimated.

From the sensitivity and specificity data shown in Table 1 it is worthy to note that ferritin was overall more specific than sTfR, while sTfR was shown to be more sensitive. As already mentioned, the low sensitivity of ferritin may, however, be due, at least partly, to the low cut-offs used by some authors. In spite of the inclusion of patients with inflammatory co-morbidities, these authors employed the lower reference limit of ferritin, usually partitioned by gender, as a cut-off resulting in low sensitivity of ferritin to predict BME iron findings. On the other hand, the total or partial recruitment of patients with clinical conditions associated with elevated sTfR concentrations, irrespective of iron status (e.g. hemolysis or megaloblastosis), could have worsened sTfR specificity. The relatively high rate of false positive sTfR results has specifically been discussed by some authors. For instance, Lee et al. [32] concluded that sTfR was not superior to ferritin for detecting IDA and that, in patients with nonhematologic malignancy, sTfR may not reflect the iron status because of a still unknown mechanism. Means et al. [18] pointed out that in chronic inflammatory conditions, elevated sTfR concentrations may indicate a functional iron deficiency due to the inflammatory suppression of iron mobilization, even though iron stores assessed by BME are not completely depleted. Other authors have more recently supported this hypothesis, suggesting sTfR measurement as an index of iron-deficient erythropoiesis more than a marker of the amount of stored iron [33].

In conclusion, a clear message from this systematic review of available literature is the obvious lack of robust data supporting the diagnostic accuracy of sTfR for identifying the IDA condition in patients with complicated clinical presentation. With regard to its possible introduction in the clinical setting, better designed studies are needed to reliably define the additional role, if any, of sTfR for diagnosing mixed IDA + ACD in complicated conditions when combined with (or alternatively to) serum ferritin.