Soluble Transferrin Receptors and Iron Deficiency, a Step beyond Ferritin. A Systematic Review

Anastasios Koulaouzidis¹, Elmhuhtady Said², Russell Cottier³, Athar A Saeed⁴

1) Centre for Liver and Digestive Disorders, Royal Infirmary of Edinburgh, Medical Day Case & Endoscopy Unit, Edinburgh; 2) Barnsley General Hospital, Gawber Road, Barnsley; 3) Warrington General Hospital, Lovely Lane, Warrington, Cheshire; 4) Queen Elizabeth Hospital, Gateshead, Tyne & Wear, UK

Abstract
Iron deficiency is a common disorder; it can also be the first indicator of significant gastrointestinal pathology. Total iron stores are evaluated by ferritin measurement, but this is often difficult, as coexistent disease can obscure ferritin results. Bone marrow (BM) examination was previously felt to be superior to all known serological markers of iron status, but it has a number of disadvantages. The validity of measurement of soluble transferrin receptors (sTfR) as a surrogate marker of BM iron stores has been the subject of various studies so far. Aim: To critically review the use of sTfR as a marker for the evaluation of iron stores. Methods: A systematic computerised literature search, in order to identify studies that compared sTfR measurement against BM iron stain. Results: Twenty prospective studies were identified, of which nine fulfilled the inclusion criteria (sTfR measurement in anaemic adults alongside BM iron staining). For a total of 979 patients, a different sTfR reference range was used, but levels of 2.5mg/L (29.5nmol/L) were consistently used as the threshold for iron-deficiency anaemia resulting in good specificity and sensitivity. Conclusion: The use of sTfR improves the clinical diagnosis of iron deficiency anaemia, especially in the presence of coexisting chronic disease or gastrointestinal malignancies. The safety and cost-effectiveness of a ferritin/sTfR-based approach to exclude gastrointestinal cancer in the presence of iron deficiency has to be proven with a prospective, well standardised, multicentre trial or a meta-analysis.

Keywords

Introduction
Iron deficiency (ID) is a common disorder, affecting almost 1.2 billion people worldwide [1]. In 1985, the World Health Organization reported that 15% to 20% of the world’s population had iron deficiency anaemia (IDA) [2]. In developed countries more than 500 million people are iron deficient [3-5]. Iron deficiency anaemia is a particularly important problem for men and postmenopausal women, as it can be the first presenting feature of an occult gastrointestinal malignancy [6-8].

It is important to remember that for most clinicians ID is associated with anaemia; the truth is that it is a continuous process evolving in three stages. The first phase is the depletion of storage iron (stage I), where total body iron is decreased but haemoglobin (Hb) synthesis and red cell indices remain unaffected. Both these indexes change when the supply of iron to bone marrow (BM) becomes problematic (iron deficient erythropoiesis – IDE, or stage II). In stage III the iron supply is insufficient to maintain a normal Hb concentration, and eventually IDA develops [9].

Evaluation of iron deficiency
Evaluation of iron deficiency is performed by various methods. Red cell indices [mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCHC)] may confirm a microcytic, hypochromic picture, with rerum ferritin often low. Unfortunately both these tests are less reliable with coexistent illness. Anaemia of chronic disease (ACD) is a syndrome associated with chronic inflammatory and autoimmune conditions, malignancies, or infections (acute or chronic), and causes a state of ‘functional’ ID. Recently, hepcidin (a liver-produced peptide) was found to be a link between the immune system and the movement of iron to and from the storage depots. Hepcidin binds to ferroportin, a transmembrane iron exporter present on macrophages, enterocytes and hepatocytes. It has been demonstrated that in vitro hepcidin induces the internalization and degradation of ferroportin. By regulating the number of iron exporters, hepcidin is involved in the control of iron uptake and release from enterocytes or macrophages. It is conceivable that
The two entities (IDA & ACD) can coexist and so the detection of ID in the presence of chronic disease can be an important diagnostic challenge. Efficient management of IDA requires, on most occasions, not only administration of iron therapy to replenish iron stores, but also invasive investigations (i.e. endoscopies) to find and treat an underlying cause [11-12]. Endoscopies are known to have potential life-threatening complications [13], whereas inappropriate administration of iron in ACD may aggravate the underlying disease.

The British Society of Gastroenterology guidelines recommend confirmation of ID before embarking on endoscopic tests [12]. Measurements of peripheral blood indices (i.e. MCV, MCH and ferritin) can prove inadequate in certain patient groups because of known confounders (infection, inflammation or cancer). Haemoglobin (Hb) determination, the most widespread screening method for ID, has serious limitations when used as the only laboratory marker for iron deficiency because of its low specificity and sensitivity. This is because any individual must loose a large portion of total body iron for iron stores to decrease and the Hb to fall below normal levels [14].

Despite that, when hereditary anaemias are excluded, in the majority of cases the presence of hypochromia and microcytosis in the face of low ferritin makes the diagnosis of ID fairly straightforward [15]. The difficulty is in interpreting low markers in isolation, particularly when the Hb is normal [16]. The work of Jacobs, Walters and Bentley established serum ferritin as an accurate indicator of storage iron [17-21]. Numerous studies since have demonstrated the superiority of ferritin over other measurements in identifying ID, and thus ferritin has been the most useful laboratory marker over the past 30 years [15].

In their review of 55 studies, Guyatt et al. reported that the mean area of the receiver-operator characteristic (ROC) curves for ferritin was 0.95 ± 0.1, while that of MCV was only 0.76 [22]. A ferritin level of ≤12μg/L is considered a highly specific indicator of ID (98% specificity). The sensitivity for this is only 25% [23, 24], and can be improved to 92% if a cut-off ferritin level of 30μg/L is used for ID diagnosis, thus resulting in a positive predictive value (PPV) of 92% [24, 25]. However it is known that ferritin is an acute phase reactant and can be elevated irrespective of iron status in patients with concurrent chronic inflammatory or infectious diseases, recurrent acute infections and malignancies [26].

The ‘gold standard’

The examination of bone marrow (BM) aspirate, stained with Prussian blue for iron, is still considered as the best method for evaluating iron status in patients with indeterminate laboratory findings. Its drawbacks are that it is invasive, expensive, and that results are operator dependant. Barron et al. studied a cohort of 108 consecutive unselected patients in whom the BM examinations were reported as iron deficient, and found that reports of absent BM haemosiderin may be inaccurate in more than 30% of cases – even when accurate, this may not necessarily signify the presence of ID [27]. Measurement of serum ferritin levels is still required to confirm a clinical diagnosis. Furthermore, up to 35% of BM aspirate may be inadequate for interpretation [20]. In a small retrospective study, Ganti et al. concluded that the PPV of absent BM iron stores for the diagnosis of ID was only 50% [28]. Another recent study showed that patients who had stainable iron in their BM had in fact ‘functional’ ID [29].

The invasive nature of BM aspiration and biopsy has made it tempting to identify a peripheral blood test which could act as surrogate marker of BM iron stores.

Witte et al. examined a cohort of 97 consecutive anaemic patients and devised a graph relating serum ferritin with erythrocyte sedimentation rate (ESR); they suggested that it could allow accurate confirmation or exclusion of ID [30]. Two years later, they reported that a two-dimensional linear graphic relationship between ferritin and ESR provided a PPV of 97%, thus aiding the confirmation or exclusion of ID in community hospital practice [31]. Others have proposed that a ferritin level of 70μg/L was the necessary safety limit for exclusion of ID [32]. Guyatt et al. showed that 40% of patients with ferritin levels between 18μg/L and 100μg/L had ID on BM examination [33].

There are two main categories of laboratory tests for identifying ID. Screening measurements (Hb, transferrin saturation, MCV, MCH, zinc protoporphyrin and reticulocytes Hb) that detect iron deficient erythropoiesis (IDE) by demonstrating either a reduced supply of serum iron or poor haemoglobination of red cells and definitive measurements that evaluate tissue iron status by measuring proteins derived from either the iron store compartment in macrophages (ferritin), or the iron utilization compartment in red cell precursors [34]. Of note, red cell distribution width (RDW) usually precedes the other markers of IDE [35]. Soluble transferrin receptors (sTfR) can detect stages II and III of ID, and are considered as a ‘dual’ index.

**Soluble transferrin receptors**

The first description of the presence of transferrin receptors (TfR) on the surface of reticulocytes was made in 1963 [36]; they were further characterised as cell surface glycoproteins in 1981 [37]. The overall dynamics of these receptors was studied using the mouse erythroleukemia K562 cell line [38]. Ward et al. found that in these cells, TfR mRNA synthesis was increased when iron-deficient serum, or iron-chelating agents were added to the culture medium [39, 40]. It was later proposed that, the up-regulation of receptors must be mediated (at least in part) by the transferrin levels.

The transferrin receptor mediates cellular uptake of iron by binding the iron carrier-protein transferrin (Tf). Following internalisation of the iron-transferrin-TfR complex, iron is released from its binding sites in the acidic milieu of...
the endosomes (acidosomes), and the Tf–TfR complex is returned back to the cell surface, where apo-transferrin is released again [41].

The transferrin receptor is comprised of two identical subunits, each composed of 760 amino-acids with a molecular mass of 95 kDaltons. Each polypeptide subunit contains a trans-membrane segment with 21 amino acid residues, an N-terminal cytoplasmic domain with 61 residues, and a large C-terminal extracellular domain with 671 amino acids. The soluble form of TfR in serum was recognised since 1986 [42]. Kohgo et al. described its use as a surrogate marker of BM erythropoiesis and red cell production [43].

Humans use 80% of their body iron for erythropoiesis, and almost the same proportion of TfR in the body is found in erythroid progenitor cells. Reticulocytes entering the peripheral bloodstream carry a high surface concentration of the receptor; as the cells mature the receptors are shed into the circulation [44]. Release of TfR has been shown to be mediated by an integral membrane proteinase, and inhibited by a matrix metalloproteinase and the TNF-N protease inhibitor-2 [41].

As the idea behind Witte’s work remained attractive, serum or soluble TfR (sTfR) was the next to be scrutinised with regard to the validity of its use as a marker of BM iron. We critically reviewed the existing evidence in order to evaluate if sTfR measurement or any calculated values (such as the sTfR/Log 50 ferritin ratio or sTfR–F index) could stand as a surrogate marker of BM iron.

### Review criteria

A comprehensive computerized literature search of English-language studies was performed using the [Mesh] terms “receptors, transferrin” and “bone marrow” in the MEDLINE database with limits in humans, in the English Language, and over the age of 19 years. We checked Medline 1966 – May 2007 and we tracked appropriate references. A total of 31 references were found. Twelve more references were tracked from a manual review of related articles. From the total 43 articles, twenty relevant references (sTfR measurement and BM iron staining) were identified; nine of which fulfilled the inclusion criteria and were included in our analysis.

**Inclusion Criteria:** Studies were included only if they satisfied the following criteria:

1. Examined anaemic adult patients who had sTfR measurements documented.
2. Bone marrow aspiration and iron stain was the reference standard for detection of iron status.

In some studies, only a subgroup of patients fulfilled the inclusion criteria. Therefore, included studies ought to have at least 50% of patients subjected to BM examination.

**Exclusion criteria:** letters, comments, and articles without original data and conference abstracts were excluded. We also excluded 2 studies because recruited patients had malignant lymphomas and myelodysplastic syndromes [45, 46]. Haematological malignancies are known to interfere with the level of sTfR and qualify as exclusion criteria for most of the other studies. Two more studies were not included in our final analysis as they contained preliminary results reported later in a larger study by Punnonen et al. [47-49].

**Outcome measures**

- **A. Population**
  - ideal - prospective study, consecutive anaemic patients (with explicit definition of anaemia), exclusion criteria; marked as 3
  - best - prospective study, not consecutive but with exclusion criteria; marked as 2
  - acceptable - any other prospective study; marked as 1

- **B. Intervention**
  - ideal - specified method of testing (i.e. how laboratory tests were done); marked as 3
  - acceptable - anything else; marked as 1

- **C. Outcome – Bone marrow**
  - ideal - BM examined by 2 or more blinded (to each other and the lab tests) readers; marked as 3
  - acceptable - anything else; marked as 1

**Quality assessment**

- ideal - prospective study, consecutive anaemic patients (with explicit definition of anaemia), exclusion criteria; marked as 3
- best - prospective study, not consecutive but with exclusion criteria; marked as 2
- acceptable - any other prospective study; marked as 1

- data extraction: Data was extracted independently from each report (by AK and ES) using a predefined review form, and disagreement was resolved by consensus. The

<table>
<thead>
<tr>
<th>Study (year)</th>
<th>Population</th>
<th>Intervention outcomes</th>
<th>Total score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Punnonen et al. 1997</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Rimon et al. 2002</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Means et al. 1999</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Hanif et al. 2005</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Joosten et al. 2002</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Junca et al. 1998</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Lee et al. 2002</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Suominen et al. 2000</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Fitzsimons et al. 2002</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Quality assessment</th>
<th>No. studies (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population</td>
<td></td>
</tr>
<tr>
<td>ideal</td>
<td>2 (22.2)</td>
</tr>
<tr>
<td>best</td>
<td>4 (44.5)</td>
</tr>
<tr>
<td>acceptable</td>
<td>3 (33.3)</td>
</tr>
<tr>
<td>Intervention</td>
<td></td>
</tr>
<tr>
<td>ideal</td>
<td>9 (100)</td>
</tr>
<tr>
<td>acceptable</td>
<td>- (0)</td>
</tr>
<tr>
<td>Outcome measures</td>
<td></td>
</tr>
<tr>
<td>ideal</td>
<td>- (0)</td>
</tr>
<tr>
<td>best</td>
<td>2 (22.2)</td>
</tr>
<tr>
<td>acceptable</td>
<td>7 (77.8)</td>
</tr>
</tbody>
</table>

**Table I. Quality assessment of the studies evaluated**
extractors were aware of the site of origin of publication, journal and year of publication. The following data was recorded from each article: author and year of publication, sample size, number of anaemic patients, number of bone marrow examinations performed, classification of patients according to bone marrow iron status and sensitivity, and specificity of sTfR and/or sTfR–F index if that had been calculated (Table II).

**Studies breakdown and discussion**

Punnonen et al (1997) studied 129 consecutive anaemic patients. All underwent BM aspiration to define the type of anaemia and determine iron stores [49]. All blood samples were obtained before any blood transfusions, and patients on iron therapy were excluded. Haematological malignancies, haemolytic anaemias, B12 or folic acid deficiencies were also considered as exclusion criteria [50–52].

Bone marrow aspirations were performed from sternum or iliac crest sites. Patients were assigned to three groups according to their BM iron. Forty-eight belonged to the group of ID (no stainable iron), 64 were classified as having ACD (stainable iron present), whilst 17 with both no stainable iron in the BM plus a concurrent inflammatory process or non-haematological malignancy, were placed in the combined (COMBI) anaemia group. Measurement of sTfR was performed using a polyclonal antibody in a sandwich Enzyme ImmunoAssay (EIA). The reference distribution levels were 0.85 to 3.5 mg/L. The calculated areas under the curve (AUC) for sTfR were 0.85 to 3.5 mg/L. The calculated areas under the receiver operator characteristics (AUCROC) for sTfR were 0.98 for the whole patient population. The same value was obtained for the subpopulation of uncomplicated IDA and ACD patients, as well as the one containing the ACD and COMBI anaemias. The logarithmic transformation of the ferritin values, and calculation of the sTfR–F Index provided an outstanding indicator of iron depletions (AUCROC: 1.00).

The efficiency curves for distinction between IDA and ACD suggest cut-off limits for sTfR and for sTfR–F index of 2.7 and 1.5 mg/L respectively. Such a cut-off level for sTfR (2.7 mg/L) had sensitivity, specificity, PPV, negative predictive value (NPV) and efficiency of 0.94 each. The equivalent values for the sTfR–F index were 0.98, 1.00, 1.00, 0.98, 0.99.

A year later (1998), Juncà et al. reported on their study of 37 anaemic patients (10 of them had hypoferritinaemia due to chronic blood losses and were used as controls of the sTfR technique) with concurrent infectious or inflammatory pathology [53]. Those from the group (n=27) with hyperferritinaemia underwent BM aspiration. Twelve were found to have low BM iron and fifteen (n=15) normal or increased BM iron content. The BM smears were evaluated by two pathologists and marrow iron grade was recorded as 0 (absent), + (reduced), ++ (normal) or +++ (increased).

They simplified the results into absent or low vs. normal or high BM iron. sTfR was measured with an immunoenzymometric assay and the reference range was 3.1–4.5 mg/L. Applying multivariate logistic regression analysis they found that sTfR was the first variable to be excluded from the model as non-significant, as it did not improve its predictive value at any cut-off level. At a level of 4.5 mg/L the sensitivity was only 50% and the specificity

### Table II. Review results

<table>
<thead>
<tr>
<th>Name of study</th>
<th>IDA on BM</th>
<th>sTfR mean</th>
<th>ACD- total</th>
<th>no sTfR mean</th>
<th>sTfR cut-off level 2.7</th>
<th>sens% 94</th>
<th>spec% 94</th>
<th>PPV% 94</th>
<th>NPV% 94</th>
<th>Index level 1.5</th>
<th>sens% 98</th>
<th>spec% 100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Punnonen 1997</td>
<td>48</td>
<td>6.2 ± 3.5</td>
<td>64</td>
<td>1.8 ± 0.6</td>
<td>5.1 ± 2.0</td>
<td>2.7</td>
<td>94</td>
<td>94</td>
<td>94</td>
<td>1.5</td>
<td>98</td>
<td>100</td>
</tr>
<tr>
<td>Junca 1998</td>
<td>n/a</td>
<td>n/a</td>
<td>15</td>
<td>3.39 ± 1.9</td>
<td>5.63 ± 3.3</td>
<td>2.8</td>
<td>84</td>
<td>47</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Means 1999</td>
<td>24</td>
<td>52.7 ± 39.9</td>
<td>121</td>
<td>23.7 ± 12.3*</td>
<td>n/a</td>
<td>n/a</td>
<td>71</td>
<td>74</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Suominen 2000</td>
<td>19</td>
<td>2.92 ± 0.76</td>
<td>11</td>
<td>1.81 ± 0.48</td>
<td>n/a</td>
<td>n/a</td>
<td>2.3</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>13.5</td>
<td>n/a</td>
</tr>
<tr>
<td>Joosten 2002</td>
<td>27</td>
<td>34±11</td>
<td>38</td>
<td>25.5 ± 10</td>
<td>n/a</td>
<td>7</td>
<td>28</td>
<td>68</td>
<td>61</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Rimon 2002</td>
<td>49</td>
<td>n/a</td>
<td>14</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>1.5</td>
<td>88</td>
<td>93</td>
</tr>
<tr>
<td>Lee 2002</td>
<td>31</td>
<td>n/a</td>
<td>48</td>
<td>n/a</td>
<td>n/a</td>
<td>1.8</td>
<td>97</td>
<td>88</td>
<td>n/a</td>
<td>n/a</td>
<td>1.36</td>
<td>100</td>
</tr>
<tr>
<td>Fitzsimons 2002</td>
<td>6</td>
<td>n/a</td>
<td>12</td>
<td>23.9*</td>
<td>40.2*</td>
<td>28.1*</td>
<td>93</td>
<td>92</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Hanif 2005</td>
<td>86</td>
<td>9.68 ± 2.48</td>
<td>90</td>
<td>2.96 ± 1.28</td>
<td>n/a</td>
<td>83.3</td>
<td>100</td>
<td>100</td>
<td>87</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
</tbody>
</table>

| **Note:** IDA: iron deficiency anaemia, sTfR: soluble Transferrin receptor, ACD: anaemia of chronic disease, Combi anaemia: ACD+IDA, no: number, sens: sensitivity, spec: specificity, PPV: positive predictive value, NPV: negative predictive value, *sTfR levels in nmo/L (sTfR levels for the rest expressed in mg/L).
74%. This study involved a small number of patients and the sTfR–F index was not estimated.

A year later (1999), Means et al. studied the predictive value of sTfR for the BM iron results in a heterogeneous group of patients [54]. This was a large, three-centre study that included 145 anaemic patients undergoing BM examination for diagnostic purposes (no BM aspiration was performed solely for study purposes). The investigators examined the stained marrow smears in a blinded manner, i.e. without any information about the patients and reported the BM iron content in a semi-quantitative scale. Subjects receiving iron or erythropoietin therapy were excluded. Of the 216 individuals recruited, 71 were excluded for various reasons [15 were excluded later due to concomitant treatment with iron, some had BM biopsy for cancer staging or haematological malignancies (leukaemia/myeloma), 11 had B12 deficiency or other haemolytic states, while 45 patients had unsatisfactory or uninterruptible BM samples]. The remaining 145 entered the final analysis, including some with malignancies and liver disease (n=87). Soluble TIR was measured by enzyme-linked immunosorbent assay (ELISA) and the reference range was 8.8 – 28.1 nmol/L for white and 10.6 – 29.9 nmol/L for coloured subjects. Mean sTIR concentration for patients with stainable iron on the BM aspirate was 23.7 ± 12.3 nmol/L (mean ± SD), while the value for those with absent BM iron were 52.7 ± 39.9 nmol/L. Means et al. tested a sequential algorithm (predictor of BM iron) for both ferritin alone and ferritin/sTfR in combination. The ferritin algorithm correctly identified 42% of the patients with absent BM iron (sensitivity) and 70% of those with BM stainable for iron (specificity).

The authors found that sTfR was the only laboratory test used (amongst serum iron, Tf, Tf saturation, ferritin, MCV) which showed mean values in the normal range for patients with detectable BM iron and mean values outside the normal range in those without detectable BM iron. Despite this, the sTfR assay in this study failed to identify 29% of the patients with absent BM iron. The authors explained this to be inherent to the study design as only 7% of the recruited subjects underwent BM examination primarily for the evaluation of anaemia.

Suominen et al. (2000) studied a group of 30 anaemic patients with rheumatoid arthritis (RA) and performed BM examination in all of them [55]. The investigators determined both sTIR and sTIR–F index and according to their protocol they supplemented patients with diminished or exhausted BM stores with iron. In one more study from Turku, Finland, it was reported that an sTIR level of 2.3 mg/L and an sTIR–F index of 1.35 was the most efficient in discriminating between absent and replete BM iron stores.

In 2002, four more studies on that subject were published. Joosten et al. prospectively studied elderly hospitalised patients (mean age 83 years) in order to check the validity of sTfR in discriminating IDA from ACD [56]. After excluding cases of haematological malignancies, haemolytic anaemia, B12 or folate deficiency, moderate renal failure, recent surgery or therapy with iron/transfusion, they recruited 83 anaemic patients who all underwent BM aspiration. Thirty-four had no stainable BM iron and were classified as IDA; 38 had iron replete iron stores from either acute infections or chronic inflammatory conditions, and were classified as ACD; 11 were excluded from further analysis as they had normal BM iron and no evidence of chronic/acute infection, inflammation or malignancy. Their results showed that at a level of 2.8 mg/L for the detection of ID, the sTIR measurement had a sensitivity of 68% and specificity of 61%. The authors concluded that sTIR measurement provided results similar to that of ferritin in their study population, partly because of the broader spectrum of coexisting underlying diseases.

Rimon et al. also focused on elderly inpatients and studied a population of 49 anaemic and 14 non-anaemic individuals (n=63) [57], all of whom had BM examinations. As well as measuring the usual haematological parameters and sTIR values, they also calculated the sTIR–F index. This was a well-conducted, prospective study with stringent inclusion and exclusion criteria and a good study size. The mean age of the cohort was 83 years. Using a sTIR–F index level of 1.5 they came up with a specificity of 92.9% and sensitivity of 87.8% for the detection of IDA (PPV of 97.7% and NPV of 68.4%).

Fitzsimons et al. (2002) examined BM from 29 anaemic patients with rheumatoid arthritis (RA), 6 patients with simple IDA, and healthy volunteers. They employed 125I-Tf and 59Fe-Tf in order to assess the in vitro erythroblast surface TfR expression, and the uptake of high purity erythroblast fractions prepared from the BM samples. Serum TfR values were measured only for the RA anaemia group, which was subdivided as either RA-IDA (marrow iron present) or RA-ACD (marrow iron absent), on the basis of visible reticuloendothelial BM iron stores. They concluded that sTfR levels in RA-ACD remain within the normal range, RA erythroblasts, however are still able to respond to any additional worsening of the iron supply caused by absent reticuloendothelial iron stores. This additional response causes the highly significant increase in serum sTfR values seen between RA-IDA and RA-ACD [58].

Later that year, Lee et al. reported their study of the use of sTfR and sTfR–F index in anaemic patients with non-haematological malignancies and chronic inflammation [59]. The authors recruited 120 anaemic adults (mean age 54 years) who underwent BM examination for anaemia and 81 non-anaemic controls. The group was divided into 4 subgroups according to BM results: IDA (n=31), ACD (n=48), COMBI with chronic inflammatory disease (n=15) and COMBI with malignancy (n=26). Exclusion criteria were similar to those of previous studies and sTfR was measured with the use of an immunoturbidimetric method. At an sTfR–F Index level of 1.36, the sensitivity for detection of IDA was 100% and the specificity 98%, while the same parameters for a sTIR level of 1.8 mg/L were 97% and 88%, respectively.

Hanif et al. (2005) conducted a study wherein 176 patients were subjected into BM examination [60]. The authors reported excellent PPV for the test (sTfR) in both
IDA and ACD, but the NPV for the latter state was only 74.1%. This study had no clear sTfR level used for defining IDA, and the reported PPV and NPV in different subgroups of IDA and ACD appear confusing.

The studies we examined are heterogeneous as to the population of recruited patients (Table III) and the exclusion criteria used (Table IV). Most of them, with four notable exceptions [49, 54, 59, 60], were limited by the number of BM examinations performed, and were restricted to a maximum of 3 centres [54]. Furthermore reference ranges for the measurement of sTfR are blighted as there are several commercial assays available, and the technique is not standardized [61, 62]. This leads to confusion in the transferability of results from one study to another, both nationally and internationally; however this problem is not unique within the remit of diagnostic testing. There must be increased pressure to establish international guidelines for the calibration of sTfR, as first highlighted by Skikne in 1998 [62].

Currently, there is no National Quality Assessment Scheme so it still remains impractical to compare across different laboratory analyses, which is necessary if sTfR is to be utilized on a wider basis. The sTfR assay is also claimed to be cost-prohibitive in comparison to established ‘traditional’ laboratory indices of iron assessment, but with the continued proliferation of high-throughput analyzers these indices are blighted as there are maximum of 3 centres [54]. Furthermore reference ranges of BM examinations performed, and were restricted to a maximum of 3 centres [54].

The safety and cost-effectiveness of a ferritin/sTfR-based approach to exclude gastrointestinal cancer in the presence of coexisting chronic disease, but further research is required [64, 65].

**Conclusion**

The use of sTfR improves the clinical diagnosis of iron deficiency anaemia, especially in the presence of coexisting chronic disease or gastrointestinal malignancies. The safety and cost-effectiveness of a ferritin/sTfR-based approach to exclude gastrointestinal cancer in the presence of coexisting chronic disease or gastrointestinal malignancies.
of iron deficiency has to be proven with a prospective, well standardised, multicentre trial or a meta-analysis.

Acknowledgements

We thank Mageed Abdalla, MSc for his invaluable help with Figure 1, A Karagiannidis, MD for his help with the tables, and Shivaram Bhat, MBBCh & Sarah Douglas, BSc for their help with proof reading.

References