Utility of Access Soluble Transferrin Receptor (sTfR) and sTfR/log Ferritin Index in Diagnosing Iron Deficiency Anemia

Dong Hoon Shin¹, Hyun Soo Kim¹, Min Jeong Park¹, In Bum Suh², and Kyu Sung Shin¹

¹Department of Laboratory Medicine, Hallym University College of Medicine, Chuncheon, and ²Department of Laboratory Medicine, Kangwon National University School of Medicine, Chuncheon, South Korea

Abstract. The Access® soluble transferrin receptor (sTfR) is considered the world’s first automated chemiluminescence immunoassay. In this study, the diagnostic utility of this and other tests for serum iron were evaluated by studying their interrelationships with inflammation. A total of 367 patients with anemia (iron deficiency anemia [IDA], 157; anemia of chronic disease [ACD], 210) and 80 normal controls were subjected to a battery of diagnostic tests, including complete blood cell count, serum iron, total iron-binding capacity (TIBC), C-reactive protein (CRP), ferritin, sTfR, and hepcidin. The accuracy of test parameters was determined by the area under the receiver operating characteristic curve (AUC). Patients falling within the ferritin greyzone (10-100 ng/ml) were evaluated separately, given that such individuals are typically difficult to detect and manage in actual clinical practice. CRP was used to assess the correlation between the aforementioned markers of iron and inflammation. The single most accurate diagnostic test used to differentiate IDA from ACD was serum ferritin (AUC 0.989). However, sTfR assay outperformed other tests in the ferritin greyzone (AUC 0.931), and the sTfR/log ferritin index was the most reliable parameter in both scenarios (AUC 0.994 and 0.962, respectively). Ferritin, TIBC, and hepcidin showed the highest correlation with CRP, whereas sTfR displayed the lowest. The Access sTfR and sTfR/log ferritin index enabled highly accurate diagnosis of IDA in the ferritin grey zone. This is an easy-to-use automated chemiluminescence immunoassay, amenable to routine use in hospitals.

Keywords: anemia, iron deficiency, soluble transferrin receptor, ferritin, hepcidin.

Introduction

Iron deficiency anemia (IDA) is the most common form of anemia, followed by anemia of chronic disease (ACD, or anemia of inflammation) [1]. Causes of IDA vary by age and gender. Nutritional deficiency, gastrointestinal bleeding, and menstrual bleeding/myoma are considered the primary etiologies of anemia in children, adult males, and adult females, respectively [2]. On the other hand, ACD is indicative of underlying disease, where diverse mechanisms are involved, including inadequate erythropoietin production, diminished bone marrow response to erythropoietin, cytokine-induced inhibition of erythropoiesis, insufficient available iron, and shortened erythrocyte survival [1]. ACD is the most common cause of anemia in hospitalized patients. According to one source, about 55% of hospitalized patients studied suffered from anemia [3]. Treatment of IDA usually entails iron supplementation, whereas ACD is corrected by resolving underlying conditions. Thus, differentiating the nature of anemia is critical. If infection or inflammation co-exists, it may be quite difficult to distinguish IDA from ACD [4]. The prevalence of anemia is higher among the elderly, and its treatment improves their survival [5]. In our aging societies, differentiating IDA and ACD will therefore become increasingly important.

Tests generally used by hospitals to detect iron deficiency are serum iron, total iron-binding capacity (TIBC), and ferritin. Soluble transferrin receptor (sTfR) and hepcidin are still used exclusively for research purposes. Although ferritin best reflects body iron store, it is an acute phase reactant that readily fluctuates with active infection or inflammation [6, 7]. The specificity of ferritin is high at its optimal cut-off point (10-20 ng/mL), so it is commonly used in diagnosing IDA. However, the sensitivity of ferritin is very low [8]. Staining bone marrow for iron is widely touted as the diagnostic gold standard in IDA, but this invasive procedure has a relatively low accuracy and is rarely done for diagnosis.
of IDA [9]. Plasma levels of sTfR reflect the body’s iron demand for erythropoiesis, and an elevated sTfR concentration indicates iron deficiency [10]. Hepcidin is the master hormone that regulates iron homeostasis [11]. Hepcidin is an acute phase reactant and shows strong correlation with C-reactive protein (CRP) [12].

The present study was designed to compare the new Access® sTfR immunoassay system with traditional iron determinants and hepcidin levels in terms of differentiating IDA and ACD. Access sTfR chemiluminescence immunoassay is the only method of its kind that is designed for automated testing and is ready for immediate implementation by hospitals. However, sTfR test is rarely used in the clinical field despite the many studies revealing its usefulness. We aimed to clarify the utility of sTfR in the clinical setting.

Materials and Methods

Subjects. The study protocol was approved by our hospital’s Institutional Review Board. We recruited adults ≥20 years of age who underwent iron determinations at our Department of Laboratory Medicine between July 2012 and March 2013. In accordance with WHO guidelines, eligible males were those with hemoglobin (Hb) concentrations <13 g/dL, and qualifying females had hemoglobin levels <12 g/dL [13]. Patients were excluded from study on the following grounds: 1) hematologic diseases other than IDA and ACD; or 2) active bleeding; or 3) blood transfusion within the last 3 months; or 4) use of any iron supplements. Patients with IDA (with or without ACD) were defined as follows: 1) serum ferritin levels <15 ng/mL in males and <10 ng/mL in females; or 2) serum ferritin <100 ng/mL and transferrin saturation (TSAT) <15%, with elevation of sTfR or reduction in hepcidin; or 3) serum ferritin <200 ng/mL (with increased CRP) and microcytic hypochromic anemia responsive to the therapeutic trial of iron (i.e., more than

![Figure 1. Box and whisker plots delineating median and interquartile range (box) values of ferritin (A), hepcidin (B), sTfR (C), and sTfR/log ferritin index (D) distributions in normal control, IDA, and ACD groups. Y-axis is logarithmic in scale. ACD: anemia of chronic disease; IDA: iron deficiency anemia; sTfR: soluble transferrin receptor.](image-url)
10% increase in mean cell volume [MCV] and mean cell Hb concentration [MCHC] within 3 weeks of iron supplementation [14,15]. Patients with ACD (also called anemia of inflammation) were defined as anemias due to infections, inflammations, tumors and other chronic diseases such as chronic liver or kidney diseases [1]. Overall, 175 patients qualified as IDA, and another 210 were assigned to the ACD group. Eighty non-anemic adults who visited our hospital for regular health checkups with no underlying disorders served as controls.

**Methods.** The automated ADVIA 2120 analyzer (Siemens Diagnostics, Deerfield, IL, USA) was utilized for all hematologic tests. Blood chemistries (serum iron, TIBC, and CRP) were quantified on the Hitachi 7600 analyzer (Hitachi, Tokyo, Japan). Serum ferritin and sTfR were determined via UniCel DxI 800 (Beckman Coulter Inc, Fullerton, CA, USA) automated immunoassay system. In the calculation of the sTfR/log ferritin index, log refers to base-10 log and not to natural log [8]. The Hepcidin-25 ELISA kit (DRG Instruments, Marburg, Germany) was used to quantify serum hepcidin. In order to avoid circadian variation, only samples drawn in a fasting state before 8:00 AM were acceptable. Serum samples were frozen for subsequent ferritin, sTfR, and hepcidin assays. The diagnostic accuracy of each test in the ferritin greyzone (10-100 ng/mL), where IDA and ACD often coexist as a true clinical
dilemma, was analyzed separately [15]. In addition, all test parameters were checked for correlation with CRP to gauge the impact of ongoing infection or inflammatory disease.

Statistical analysis. The Kolmogorov-Smirnov test was routinely applied to assess normality, with normal distributions expressed as mean ± SD and non-normal distributions as median and interquartile range (25th percentile, 75th percentile). Inter-group differences of categorical variables, such as gender, were analyzed by Pearson’s chi-square test. Continuous variables, with normal distribution, were analyzed by ANOVA or by non-parametric Kruskal-Wallis test. Independent t-test (with normal distribution) and Mann-Whitney U test (with non-normal distribution) were used to explore differences between IDA and ACD groups. Optimal cut-off points of ROC curves were based on the optimal Youden index. The DeLong test served to compare the diagnostic performance shown by ROC curves. A p value <0.05 was considered statistically significant. Correlation of CRP and iron determinants was analyzed by the Spearman rank correlation test. All statistical computations relied on standard software (MedCalc v13; MedCalc Software, Mariakerke, Belgium).

Results

Demographic and laboratory findings of each group are summarized in Table 1. In the IDA group, the median age was 56 years (interquartile range [IQR]: 43-75 years) and the ratio of males to females was 1:1.7. The median age of the ACD group (72 years; IQR: 58-80 years) was significantly higher by comparison, though the ratio of males to females was lower, at 1:1.2. With the exception of gender, all variables (i.e., age and laboratory findings) differed significantly among the three study groups (p <0.001) and between both groups with anemia (IDA vs ACD; p <0.001). Other than MCV, all parameters tested showed non-normal distributions, as illustrated by box and whisker plots of ferritin, hepcidin, sTfR, and sTfR/log ferritin index (Figure 1). Log transformation was used for highly skewed distributions.

The capacities of individual tests to differentiate IDA and ACD were compared based on the area under the receiver operating characteristic curves (AUC), as shown in Table 2. Diagnostic accuracy was highest for the sTfR/log ferritin index (AUC 0.994; optimal cut-off point 1.80; sensitivity 95.5%; specificity 98.6%). As a solitary serum test, ferritin (AUC 0.989; optimal cut off point 32.7 ng/mL; sensitivity 96.8%; specificity 93.3%) proved to be the most accurate, followed by TIBC, MCHC, sTfR, MCV, TSAT, hepcidin, and serum iron. Ferritin and sTfR/log ferritin index each displayed superior and similar (p =0.1927) discriminatory capacity, performing significantly better than all other tests. Although TIBC and MCHC performed comparably (p =0.1937), they fared significantly better than sTfR (p =0.0232), which was on par with hepcidin (p =0.2169). Serum iron showed a significantly lower discriminatory capacity compared with all other tests. The discriminatory capacity of sTfR was lower than that of ferritin and TIBC in all patients, underscoring its limited role in differentiating IDA and ACD.

The diagnostic performance of each test in the ferritin greyzone (10-100 ng/mL) is recorded in Table 3. Values within the ferritin greyzone (IDA 46; ACD 61) accounted for 29.2% of patients overall. Here as well, the highest diagnostic accuracy was shown by sTfR/log ferritin index (AUC 0.962), and sTfR displayed the highest accuracy (AUC 0.931) of any single test in differentiating IDA and ACD, followed by TSAT, TIBC, MCHC, MCV, ferritin, iron, and hepcidin. The sTfR/log ferritin proved significantly more accurate than ferritin (p =0.0086). Hepcidin showed the lowest accuracy (AUC 0.738). In the ferritin grey zone, sTfR (or

Figure 2. ROC curves of sTfR/log ferritin index, sTfR, TSAT, ferritin, and hepcidin for differentiating IDA from ACD in ferritin grey zone. ACD: anemia of chronic disease; IDA: iron deficiency anemia; ROC: receiver operating characteristic; sTfR: soluble transferrin receptor; TSAT: transferrin saturation.
sTfR/log ferritin index played a very important role. ROC curves of sTfR/log ferritin index, sTfR, TSAT, ferritin and hepcidin are shown in Figure 2.

Correlations of iron markers and CRP were compared based on Spearman’s Rho (correlation coefficient) (Table 4). In all groups, TIBC and CRP showed the strongest correlation, followed by ferritin, hepcidin, iron, and sTfR. No correlation between CRP and TSAT was evident. In normal control subjects, only ferritin correlated with CRP. In the IDA group, ferritin showed the strongest correlation with CRP, followed by TIBC and iron, whereas sTfR, hepcidin, and TSAT showed no correlation. In the ACD group, serum iron showed the strongest correlation with CRP, followed by hepcidin, TIBC, ferritin, TSAT and sTfR. On the whole, classic iron markers (i.e., serum iron, TIBC, and ferritin) correlated with CRP strongly in anemia patients, demonstrating the vulnerability of these markers to inflammatory influence. As with the previous studies, ferritin was acting as positive acute phase reactant (APR), whereas serum iron and TIBC were acting as negative APR. The new marker sTfR was least influenced by inflammation.

Discussion

Soluble transferrin receptor (sTfR) is a marker of iron status that was first demonstrated in humans by Kohgo et al. (1986) [16]. Elevated sTfR is proportionate to deficits of iron in tissue, reflecting the body’s iron demand for erythropoiesis. As sTfR is
Diagnostic Utility of Access soluble transferrin receptor (sTfR)

not affected by inflammation, it has emerged as a primary parameter for the evaluation of iron status in cases where serum ferritin was unreliable [17,18]. An upsurge in sTfR level usually coincides with storage iron depletion and serum ferritin decline, but elevations of sTfR may occur for other reasons, such as erythroid hyperplasia (due to hemolytic or megaloblastic anemia and thalassemia), hypoxia, malignancies, and physiologic stress (as in pregnancy) [10,19]. If sTfR remains low in the face of iron deficiency, chronic renal failure or a hypoproliferative state (e.g., aplastic anemia) is probable. An 18-study meta-analysis aimed at sTfR supports its high diagnostic accuracy (AUC, 0.912) in IDA [20]. sTfR has also been analyzed by Kari et al. in conjunction with ferritin as sTfR/log ferritin index. Values <1 are indicative of ACD, whereas values >2 are classifiable as IDA [8]. Although studies on cut-off points are numerous, the Thomas plot has been widely applied in this setting [21]. For CRP<5 mg/L, the cut off point is 1.5, and for CRP>5 mg/L, the cut-off point is 0.8.

Hepcidin likewise has frequently been targeted for study since its initial discovery by Park et al. (2001) as a peptide hormone that regulates iron homeostasis in humans [11,22]. However, because hepcidin is also an acute phase reactant, its diagnostic accuracy is diminished by infection or inflammation [12]. Despite the cumulative data that substantiates the diagnostic accuracy of sTfR in iron deficiency, in-hospital testing was not easily implemented. The sTfR ELISA kits are labor-intensive and may therefore result in data sensitive to the particular laboratory staff and the environment, resulting in low precision. This first and only automated sTfR immunoturbidmetric assay, developed by Roche, saw little use in clinical studies [23,24]. We expected the Access sTfR immunoassay, an automated chemiluminescent system recently launched by Beckman Coulter, to improve sensitivity and precision of sTfR testing, but again, clinical trial activity has not been substantial. The first study of Access sTfR, conducted by Skikne et al., lacked statistical power, and the diagnostic criteria for defining types of anemia were ambiguous [25].

A sizable number of patients were tested in our study, which showed a significantly lower diagnostic accuracy of sTfR (AUC 0.944) compared with ferritin (AUC 0.989; \( p=0.0001 \)) in differentiating IDA (with or without ACD) and ACD in patients overall. Furthermore, the sTfR/log ferritin index, combining ferritin and sTfR values, exhibited only a marginally higher accuracy (AUC 0.994) than that of ferritin alone (\( p=0.1927 \)). The latter was likely due to the many typical IDA and ACD patients included, with either very low or very high ferritin levels. However, in the ferritin grey zone (10-100 ng/mL), where disease distinctions are difficult, sTfR showed the highest accuracy (AUC 0.931) of any single test used to differentiate IDA and ACD, followed by TSAT (AUC 0.915), MCHC (AUC 0.906), MCV (AUC 0.895), and ferritin (AUC 0.875). Besides sTfR, traditional markers (i.e., TSAT, MCHC, MCV) also exhibited a higher accuracy than ferritin. As with patients overall, the sTfR/log ferritin index showed the highest diagnostic accuracy (AUC 0.962) in patients of the ferritin grey zone. Ferritin and sTfR complement each other. For instance, ferritin reflects storage iron, whereas sTfR corresponds with tissue iron supply. While only 29.2% of our study population fell within the ferritin grey zone, this figure might be different according to the nature of the study subjects.

Our cut-off point point of sTfR/log ferritin index for diagnosis of IDA was 1.8, which is in accordance with prior research [21,25]. However, our values are not directly applicable to in-hospital use, given that the cut-off points and reference intervals of any test will vary according to the diagnostic method and reagents used, as well as demographic factors, like the race, age, and gender of patients. For routine clinical use, hospitals must generate their own reference limits. In particular, sTfR has yet to be standardized. We used the sTfR values of the control group in the calculation of the reference range, which may vary according to the subjects of study or test method. Recent efforts to develop recombinant sTfR as a reference material may facilitate sTfR standardization, reducing the variability introduced by test method [26].

In correlation analysis, ferritin, TIBC, and hepcidin showed the strongest correlation with CRP, whereas sTfR showed the least. Because sTfR is not influenced by inflammation, it may be used to complement other, more vulnerable tests. It is very likely that this property explains the high diagnostic accuracy of sTfR in the ferritin grey zone, which is where many patients with IDA and inflammation seemed to gravitate. The fact that diagnostic markers for IDA such as iron, TIBC, and ferritin...
fluctuate with inflammatory activity must always be considered when interpreting results. Previous studies have largely explored the relationship between CRP and single markers, failing to incorporate several parameters at once in their comparisons. Thus, the outcomes of this study may provide valuable insight for interpreting various markers of iron in different clinical situations.

In conclusion, the sTfR/log ferritin index, combining ferritin and sTfR values, proved to be the most effective means of identifying IDA or differentiating IDA and ACD, especially in the ferritin grey zone. The role of sTfR is particularly important if infection or inflammation co-exist in iron-deficient patients. In our view, sTfR testing is highly recommended for hospitalized patients, many of whom experience ACD and IDA concurrently. Access sTfR is an easy-to-use automated chemiluminescence immunoassay, amenable to routine use in hospitals. In our study, pure IDA and IDA with ACD were grouped as one because both conditions necessitate iron supplementation. However, if anemic patients were divided into three groups (ie, pure IDA, IDA with ACD, ACD), results may differ.

Acknowledgment

This research was financially supported by the Ministry of Trade, Industry & Energy (MOTIE), the Korea Institute for Advancement of Technology (KIAT), and the Gangwon Institute for Regional Program Evaluation (GWIRPE) through the Leading Industry Development for Economic Region.

References