Regression-based Reference Limits for Serum Transferrin Receptor in Children 6 Months to 16 Years of Age, Pauli Suominen,1,2 Arja Virtanen,1,3 Marjo Lehtonen-Veromaa,1,3 Olli J. Heimonen,1,6 Toivo T. Salmi,3 Markku Alonen,4 Timo Mättönen,5 Allan Rajamäki,5 and Kerttu Irjala1.

Serum soluble transferrin receptor (sTfR) has been established in recent years as a powerful tool for detecting iron deficiency (ID) in adults, especially in distinguishing between iron deficiency anemia (IDA) and anemia of chronic disease (ACD). Investigations regarding sTfR as a measure of iron status in infants and children have provided promising results, including evidence that, in infants, sTfR concentrations may be superior to ferritin measurements in diagnosing ID (9). However, to date, concrete reference values and other decision-supporting limits for the commercially available methods have been virtually absent, and the age-relatedness of sTfR concentrations, although introduced as a concept, has not been unequivocally modeled statistically (10–15).

In this study we measured the sTfR concentrations from a selection of 301 healthy children, 6 months to 18 years of age, using a commercially available automated immunoturbidimetric assay. We then used a regression-based method to construct age-dependent 2.5% and 97.5% reference limits for sTfR as well as 95% confidence intervals for these limits in our population (16). The purpose was to demonstrate consistent age-dependent changes in sTfR concentrations and to establish appropriate reference limits to enable the use of sTfR measurements in routine pediatric clinical practice.

A total of 301 children (130 boys and 171 girls; age range, 6 months to 18 years) were included in the healthy population of this study. The selection was made on the basis of detailed anamnesis and laboratory tests. Febrile episodes during the preceding 6 weeks, diet restrictions, chronic inflammatory or renal diseases, hematological diseases, recent iron supplementation, ongoing inflammation (C-reactive protein >10 mg/L; erythrocyte sedimentation rate >6 mm/h), anemia or abnormal red cell indices (17), and low ferritin concentrations (<10 μg/L) were considered criteria for exclusion from the study population. A population of 64 healthy boys and 36 girls (6 months to 8 years of age) were selected from patients undergoing elective short-term surgery or procedures (superficial hemangiomas, nevi, and other cosmetic surgeries; vesico-ureteral reflux, cystoscopies) in the Turku University Central Hospital. Additionally, 133 healthy girls and 66 boys (8–18 years of age) were recruited from local schools and athletic clubs.

We obtained bone marrow and serum samples from 24 children (13 boys and 11 girls; age range, 1.25–16.5 years), who were admitted to the hospital because of severe anemia, and analyzed them for iron and sTfR, respectively. The results were used to evaluate the validity of the reference limits for each age group by means of a “gold standard”.

The blood samples were obtained before any intravenous infusions. The normality of the hemoglobin and ferritin was evaluated according to Dallman and Siimes (17) and Lockitch et al. (18). Written informed consent was obtained from the parents of all subjects, and the study design was approved by the Joint Committee of Ethics of the Turku University Central Hospital and University of Turku.

Blood counts were measured using an automated analyzer (Technicon H*2; Bayer Diagnostics). sTfR assays were performed using an automated immunoturbidimetric assay (IDea® sTfR-TF; Orion Diagnostica) on a Hitachi 917 analyzer. The method has been described in detail elsewhere (19).

P values for between-gender difference and differences between age groups were derived by the Student t-test using the Windows for Workgroups™ software package (Microsoft Corporation). The reference limits and 95% confidence intervals were calculated using SAS® 6.10 software, as was the P value for the significance of age. Logarithmic transformation was applied to sTfR concentrations. The Shapiro–Wilk statistic and graphic analysis of residuals were used to assess the normality and constancy of residuals. Diagnostic measures (Cook’s statistic, high leverage points, dfits, and dbetas) were also calculated (16). Reference limits and confidence intervals were constructed as described by Virtanen et al. (16).

No significant between-gender difference in sTfR concentrations was observed (P = 0.37) in infants and children up to 10 years of age. A significant between-gender difference (P <0.001) was observed in adolescents (10–16 years), but this difference was evened out by excluding subjects with ferritin concentrations <10 μg/L (P = 0.28). A consistent age-related decrease in sTfR concentrations could be demonstrated (Fig. 1 and Table 1). Differences between children 0.5–4 and 4–10 years of age (P <0.001) and between children 4–10 and 10–16 years of age (P <0.01) were significant. The decrease was such that the 95% interval in the children 16 years of age paralleled the respective adult values obtained in a previous study (0.9–2.3 mg/L) (19). The effect of age was statistically significant (P <0.0001) with a coefficient of determination (R2) of 19% (P <0.0001). Of the 24 anemic patients whose iron status was verified by a bone marrow iron stain, 10 presented with exhausted bone marrow iron stores. The iron status was correctly determined in 23 of 24 patients by their sTfR concentrations (area under ROC curve, 0.9196; SE, 0.0843).

Measurement of sTfR has been brought up as a potentially useful tool for the pediatrician for several reasons. ID is known to be a common condition in infants, children, and adolescents, although the prevalence of frank
IDA has decreased significantly. Because of their relatively rapid growth, the amount of storage iron in children often is low, thus causing ferritin concentrations to frequently lie close to the iron-deficient range. Measurement of sTfR offers an advantage over ferritin in that it accurately detects iron-deficient erythropoiesis and can therefore be used to signal the point where subclinical ID progresses from storage iron depletion to depletion of the functional compartment (8). The importance of this property is underlined by the recent associations made between subclinical ID and impairments in psychomotor development (20–23). Furthermore, the results are not influenced by acute-phase reactions, and the sample volume required to perform the test in the commercially available assays is small (6, 19). It is therefore not surprising that sTfR has recently been shown to be superior to ferritin in distinguishing ID in infants in a study by Olivares et al. (9).

It has been reported that sTfR concentrations decrease with age (14, 15). In this study, our aim was to construct an accurate statistical model for the suggested age relatedness of sTfR concentrations and to derive decision-supporting cutoff values for ID on this particular assay. We investigated whether the method for regression-based 2.5% and 97.5% limits and confidence intervals recently introduced by Virtanen et al. (16) could be applied to calculating pediatric reference limits and confidence intervals of sTfR. A gold standard, namely bone marrow examination, was used to test the clinical validity of these reference limits.

The regression-based model showed that the age dependency of sTfR concentrations is quite substantial ($R^2 = 19\%; P < 0.0001$). The decrease was such that the reference limits merged with adult values at the age of 16 years, which was therefore established as the age limit between adults and adolescents. A relatively small ($n = 301$) population seems to have been statistically sufficient to establish the 2.5% and 97.5% reference limits as implied by the relatively narrow 95% confidence intervals. Conventional partitioning methods would have required a considerably larger population size to achieve a sufficient number of well-represented subgroups (16).

Because borderline iron status (subclinical stages of ID) is known to be quite prevalent in the pediatric population, using hemoglobin concentrations as the only criterion for normality could have unduly compromised the sensitivity of sTfR reference values for ID (9). We used a cutoff value for ferritin concentrations ($<10 \mu g/L$) to exclude subjects with obvious subclinical storage iron deficits (18). Using the upper (97.5%) reference limits of sTfR as cutoff values for ID in the separate age groups, we achieved a good distinction between IDA and anemia of other causes in the 24 patients who were subjected to bone marrow examination. The results indicated that although the sensitivity of sTfR was probably improved, the specificity remained uncompromised by the exclusion of patients with decreased ferritin concentrations. Although the population was small, the clinical accuracy (area under the ROC curve, 0.9196; SE, 0.0843) was similar to that reported for adults (6, 19), which indicates that the reference limits presented here may be useful in clinical work.

We conclude that these age-dependent reference intervals could be implemented for sTfR in children 6 months to 16 years of age on the IDEA sTfR-IT assay to enhance the differential diagnosis of IDA.

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References

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**Table 1. The 2.5% and 97.5% reference limits displayed separately for each age group as suggested by the regression-based method.**

<table>
<thead>
<tr>
<th>Age groups</th>
<th>2.5% and 97.5% reference limits, mg/L</th>
</tr>
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<tbody>
<tr>
<td>6 months–4 years</td>
<td>1.5 (1.4–1.5)–3.3 (3.1–3.4)</td>
</tr>
<tr>
<td>4–10 years</td>
<td>1.3 (1.3–1.4)–3.0 (2.9–3.2)</td>
</tr>
<tr>
<td>10–16 years</td>
<td>1.1 (1.1–1.2)–2.7 (2.7–2.8)</td>
</tr>
<tr>
<td>&gt;16 yearsb</td>
<td>0.9 (0.9–1.0)–2.3 (2.2–2.4)</td>
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| a 95% confidence interval for each limit given in parentheses.  
| b The values for subjects >16 years (adults) were established in a previous study (19). |

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**Fig. 1. sTfR concentrations as a function of age.**

The plot was produced using the regression-based method described previously by Virtanen et al. (16). The thick lines represent the upper and lower limits of the 95% reference interval, and the thin lines represent the 95% confidence intervals for these limits. The numerical values corresponding to the lines shown here are given in Table 1.
Fetal DNA Analyzed in Plasma from a Mother’s Three Consecutive Pregnancies to Detect Paternally Inherited Aneuploidy, Chih-Ping Chen,1,2* Schu-Rern Chen,2 and Wayseen Wang2 (Departments of 1 Obstetrics and Gynecology, and 2 Medical Research, Mackay Memorial Hospital, Taipei, Taiwan; * address correspondence to this author at: Department of Obstetrics and Gynecology, Mackay Memorial Hospital, 92, Section 2, Chung-Shan North Rd., Taipei, Taiwan; fax 886-2-25343642, e-mail cpc_mmh@yahoo.com)

The recent demonstration of fetal DNA in maternal plasma and serum at concentrations much higher than those present in the cellular fraction has introduced new possibilities for noninvasive prenatal diagnosis of paternally inherited dominant disorders (1–3). To date, prenatal detection of fetal aneuploidy in maternal blood has focused on searching intact cells using fluorescence in situ hybridization. The use of fetal DNA in maternal plasma to determine fetal aneuploidy has rarely been described. We previously reported prenatal detection of a paternally inherited fetal aneuploidy from fetal DNA in maternal plasma (4). Here we report the application of such a technique in an additional case involving a mother’s three consecutive pregnancies.

We studied fetal DNA in maternal plasma from a pregnant woman whose fetuses possibly had paternally inherited aneuploidy. Her husband had a balanced reciprocal translocation between the long arm of chromosome 10 and the short arm of chromosome 22, 46,XY,t(10;22)(q24.1;p11.2). The woman’s karyotype was normal. During her first pregnancy, genetic amniocentesis was performed at 19 weeks of gestation, and the maternal blood sample was collected at 22 gestational weeks before termination of the pregnancy. In contrast, during her second and third pregnancies, the maternal blood samples were collected at 14 and 18 gestational weeks, respectively, before amniocentesis. The amniocentesis of the first pregnancy revealed fetal distal 10q trisomy (10q24.1→qter), 46,XX,der(22)(t(10;22)(q24.1;p11.2), resulting from paternal t(10;22) reciprocal translocation. The amniocentesis of the second and third pregnancies showed a balanced translocation the same as the paternal karyotype, 46,XY,t(10;22)(q24.1;p11.2).

We collected 5 mL of both paternal and maternal peripheral blood into EDTA-containing tubes. Blood samples were centrifuged at 3000 g, and the plasma was carefully removed without disturbing theuffy coat. The maternal plasma sample was re enrificed, and the supernatant was collected for processing. DNA was extracted from buffy coat and 600-μL plasma samples using a DNA extraction reagent set (QIAamp® DNA Blood Mini Kit). We used fluorescent PCR assays and polymorphic small tandem repeats (STRs) to analyze DNA in maternal plasma. Five pairs of highly polymorphic primers were used separately to amplify the following loci: D10S574, D10S541, D10S534, D10S186, and D10S187. Each of the forward primers was labeled at the 5' end with fluorescein (HEX); or 4,7,2'9-triacetoxysuccinimidyl tetramethylrhodamine (TAMRA). We used fluorescent PCR assays and polymorphic small tandem repeats (STRs) to analyze DNA in maternal plasma. Five pairs of highly polymorphic primers were used separately to amplify the following loci: D10S574, D10S541, D10S534, D10S186, and D10S187. Each of the forward primers was labeled at the 5' end with fluorescein (HEX); or 4,7,2'9-triacetoxysuccinimidyl tetramethylrhodamine (TAMRA).