Elevated serum transferrin receptor concentration in children with juvenile chronic arthritis as evidence of iron deficiency

S. M. Kivivuori, P. Pelkonen, H. Ylijoki¹, P. Verronen² and M. A. Siimes

Hospital for Children and Adolescents, University of Helsinki, Helsinki, ¹Rheumatism Foundation Hospital, Heinola and ²Department of Paediatrics, University of Tampere, Tampere, Finland

Abstract

Objectives. Active juvenile chronic arthritis (JCA) is accompanied by anaemia of chronic disease, which may be indistinguishable from anaemia due to iron deficiency. We speculate that elevation of the serum transferrin receptor (sTfR) concentration, which should not be influenced by inflammation, would be useful for detecting the role of iron status in anaemic children with JCA.

Methods. sTfR concentrations were measured in 30 children with JCA.

Results. The median sTfR was elevated, 6.1 (range 3.4–13.0) mg/l. In 13 patients (43%) the concentrations exceeded the upper limit for healthy subjects. Haemoglobin ($r = −0.48$, $P = 0.008$), mean corpuscular volume ($r = −0.47$, $P = 0.009$) and mean corpuscular haemoglobin ($r = −0.65$, $P = 0.0001$) correlated inversely with sTfR concentration.

Conclusions. In 13 of the 30 patients with JCA, the sTfR concentration, which is an indicator of iron status and erythropoiesis, was elevated. The results raise the possibility that sTfR is able to distinguish iron-deficiency anaemia from anaemia of chronic disease. It should be further explored as a candidate.

Key words: Transferrin receptor, JCA, Children, Iron deficiency, Erythropoiesis.

As originally introduced by Cartwright and Lee [1], juvenile chronic arthritis (JCA) is accompanied by anaemia of chronic disease, which may often be indistinguishable from anaemia due to iron deficiency. Although impaired flow of iron from reticuloendothelial cells to the bone marrow, a shortened red cell life-span, reduced erythropoietin responsiveness, an impaired marrow response to anaemia, and an accelerated plasma iron clearance rate have all been proposed as mechanisms responsible for the anaemia of chronic disease, the pathophysiology is not well understood [1–5]. An additional factor may be impaired iron uptake by erythroblasts, probably due to decreased binding of transferrin to erythroblasts [6]. The development of this anaemia may also be associated with local production of cytokines, such as tumour necrosis factor-$\alpha$ and interleukin-6 (IL-6), in the bone marrow [7, 8].

Because, in patients with inflammatory diseases, an increased rate of ferritin synthesis is associated with inflammation, a normal or even elevated serum ferritin level does not exclude iron deficiency [9, 10]. Serum transferrin receptor (sTfR) concentration is a parameter which reflects the iron requirements on the cell level. It becomes elevated soon after signs of iron deficiency appear, the rise reflecting the paucity of available tissue iron. We speculate that the elevated sTfR concentration, which should not be influenced by inflammation in adults [11–13], might be useful in detecting iron deficiency in anaemic children with JCA. The present study was undertaken to investigate sTfR levels in 30 children with JCA and to determine the usefulness of this parameter for detecting iron deficiency in these patients.

Patients and methods

Patients

Iron status, including sTfR concentrations, was measured in 30 consecutive children (22 girls and eight boys,
aged 1.8–15.2 yr) with JCA. The diagnosis of juvenile arthritis was defined according to the proposed American College of Rheumatology (ACR) criteria [14] except that the duration of a minimum of 3 months was required for diagnosis. The children were treated at the Hospital for Children and Adolescents, University of Helsinki, Helsinki, the Rheumatism Foundation Hospital, Heinola, and the Department of Paediatrics, University of Tampere, Tampere, Finland. The ages of the patients at onset were 1.3–12.6 yr. At the time of the study, arthritis was clinically active [14] in all but eight patients. Of the 30 patients with JCA, 16 had polyarthritis (seronegative in all 16), 13 had oligoarthritis (two of them with late onset) and one systemic JCA. During the preceding 6 months, 24 patients had received non-steroidal anti-inflammatory drugs, and 25 were being treated with slow-acting anti-rheumatic drugs. Corticosteroids had been given to 19 patients, 10 of whom received them orally and 15 intra-articularly. One patient had no medication during the time of the study. None of the patients received iron supplementation during the previous 6 months.

Methods

Haemoglobin concentration and red blood cell indices were measured with automatic counters. Erythrocyte sedimentation rate (ESR) was also measured. The serum ferritin concentration was measured by an immunoluminometric method. The serum transferrin concentration was measured by an immunoturbidimetric method. The sTfR concentration was measured using a two-step sandwich-type time-resolved immunofluorimetric assay [15]. In healthy pre-pubertal boys the 95% reference interval for sTfR is 2.2–6.3 mg/l. An elevated sTfR/ferritin ratio is thought to be an additional sensitive indicator of iron deficiency [16]. The ratios (mg/mg) were calculated. The mean ratio in iron-supplemented healthy boys has been reported to be 210 ± 19 (mg/mg) [mean ± standard error of the mean (s.e.m.)] [17]. However, the method used here for measuring sTfR gave 10–20% lower values [15, 17]. Accordingly, we estimate that, with the method used in this study, the upper limit in healthy children is about 200 µg/µg.

In each patient the joints with active arthritis at the time of the study [14] were counted, the total number of joints with active arthritis being taken as a measure of activity of the disease.

Permission

The study protocol was approved by the Ethics Committee at the Hospital for Children and Adolescents, University of Helsinki. Informed consent to the study was obtained from the parents of the patients.

Statistical analysis

Because of the skewness of the values, we decided to use Student’s unpaired, two-tailed t-test and simple regression analysis. A P value of <0.05 was considered significant. Medians and ranges are given.

Results

The median sTfR was elevated, 6.1 (range 3.4–13.0) mg/l, in the children with JCA (Fig. 1). In 13 patients (43%) the concentrations exceeded the upper 95% reference limit of normal, 6.3 mg/l. The sTfR levels associated with haemoglobin concentrations (r = −0.48, P = 0.008), mean corpuscular volume (MCV) (r = −0.47, P = 0.009) (Fig. 2), mean corpuscular haemoglobin (MCH) (r = −0.65, P = 0.0001), and ESR (r = 0.52, P = 0.004). However, there was no connection between the sTfR concentration and the serum ferritin or transferrin concentrations. Nor were age at onset, age at the time of the study, or the number of active joints associated with sTfR. The median ratio of sTfR/ferritin was 285 (range 75–1150) (µg/µg). In 20 patients the ratio exceeded 200 µg/µg.

Of these 30 patients with JCA, 15 had anaemia, which means haemoglobin values below the age-dependent −2 s.d. [18]. Anaemia was equally common among the
Table 1. The numbers, among the 30 children with JCA, who had anaemia, low ferritin and high sTfR concentrations and the ratio of sTfR/ferritin (based on estimation as described in the Methods section) divided according to the type of onset of the arthritis. Anaemia means haemoglobin values below the age-dependent –2 s.d.

<table>
<thead>
<tr>
<th>Type of onset</th>
<th>n</th>
<th>Anaemia</th>
<th>Ferritin &lt;10 µg/l</th>
<th>sTfR &gt;6.3 mg/l</th>
<th>Ratio &gt;200 µg/µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyarthritis</td>
<td>16</td>
<td>8</td>
<td>3</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>Oligoarthritis</td>
<td>13</td>
<td>6</td>
<td>1</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>Systemic disease</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

patients with polyarthritic and with oligoarthritic onset (Table 1). Only four of the 30 patients had microcytosis (MCV < 75 fl); all of these children were also hypochromic (MCH < 25 pg), and three had anaemia. The median haemoglobin concentrations of the anaemic and non-anaemic patients were 110 and 123 g/l, respectively. The sTfR concentration and ESR were elevated in the patients with anaemia, 7.5 vs 5.7 mg/l (P = 0.03), 27 vs
13 mm/h (P = 0.02), respectively. The median concentration of sTfR in the anaemic patients was 7.2 (range
4.1–13.0) mg/l and in the non-anaemic patients 5.6 (range 3.4–9.8) mg/l. There were no differences between these two groups in MCV, MCH, ferritin, transferrin, age at onset, age at the time of the study, the type of onset of the arthritis, or number of active joints.

Low serum ferritin values (<10 µg/l, n = 4) and high serum transferrin levels (>3.5 g/l, n = 4) were infrequent in this series. The serum ferritin concentration was inversely correlated with the transferrin concentration (r = −0.67, P = 0.0001). In contrast, it was unrelated to the haemoglobin level, the ESR or the number of active joints. Only one of the 30 patients with JCA had obvious iron-deficiency anaemia according to conventional criteria, i.e. anaemia, ferritin <10 µg/l, and transferrin >3.5 g/l. He also had a subnormal level of MCV (71 fl), and a high level of sTfR concentration (8.5 mg/l). With a ferritin cut-off value of 30 µg/l, there were two patients with iron-deficiency anaemia. Of the 13 patients with high sTfR, eight had both ferritin and transferrin levels within the reference limits.

The median ESR was 13 (range 3–77) mm/h. There were no associations between the number of joints with active arthritis and sTfR, haemoglobin, MCV, or ESR. The relationships between the type of onset and the three parameters of iron-deficiency anaemia are shown in Table 1.

Discussion

The exact mechanism of the anaemia of chronic disease is not known. In this study, 50% of the patients with JCA had anaemia. In adults with rheumatoid arthritis (RA), serum erythropoietin levels are elevated [19] but the normal relationship between erythropoietin and the degree of anaemia is impaired [5]. It has been proposed that recombinant human erythropoietin may be effective in improving anaemia of chronic disease in both adults and children with RA [20–22]. According to another study, the microcytic anaemia associated with arthritis is chiefly due to a defective iron supply to the erythron [7]. Indeed, in most adult patients with RA, several causes of anaemia are present simultaneously [3]. Iron deficiency has been reported in up to 70% of patients with anaemia of chronic disease and RA [23]. On the other hand, it is also assumed that iron-deficient patients without inflammatory disease have features of anaemia of chronic disease [3].

In patients with chronic inflammatory diseases, the determination of conventional haematological indices is of limited help in demonstrating iron status. MCV and MCH are often decreased in patients with anaemia of chronic disease without iron deficiency [3, 24, 25]. According to a Dutch study, the finding of hypochromic microcytic anaemia resulted in only slight overdiagnosis of iron deficiency [23]. In contrast, we think that microcytosis and hypochromia are apparent only when iron deficiency is advanced. Of the 30 children of this study, only four had both microcytosis and hypochromia. In patients with inflammation, serum iron and transferrin do not necessarily change because of iron deficiency [1, 3, 12, 23, 24]. Serum ferritin is an acute-phase reactant, and increases in inflammation [9, 26]. The combined use of these parameters might help in predicting iron deficiency. In the Dutch study, the combination of MCV, ferritin and transferrin resulted in 100% validity, and 79% sensitivity in adults [23], whereas in the present study we had only one patient with values indicating iron deficiency in all three of these parameters. Of course, this depends on the cut-off values used. We have used the normal reference values of our laboratory. With a higher ferritin cut-off value, 30 µg/l, we found two patients with iron-deficiency anaemia, instead of one. However, it has been proposed, for example, that in adult patients with RA the lower limit of ferritin should be 50–60 µg/l [23, 27]. All in all, there is very much to resolve in the pathogenesis of iron deficiency. Anttila and Siimes [28] gave oral iron to 60 pre-pubertal or early pubertal healthy boys in whom haemoglobin levels increased significantly in spite of the absence of iron-deficiency anaemia before treatment. So, even the reaction to iron treatment does not necessarily help in diagnosing iron deficiency.

We measured sTfR concentrations in 30 children with JCA. Our aim was to determine whether these measurements would make it easier to distinguish iron-deficiency anaemia from anaemia of chronic disease. The utility of sTfR in diagnosing iron-deficient erythropoiesis in adult patients with inflammation has already been shown [11]. However, Danish researchers found no difference in sTfR levels between adults with RA and depleted or replete iron stores [29]. Besides this discrepancy, it is difficult to compare sTfR levels in different studies,
because of the great variation between methods, and there are as yet no international standards or age-matched reference values. Flowers et al. [30] measured the sTfR in healthy adult volunteers using enzyme-linked immunosorbent assay (ELISA), the mean sTfR being 5.6 mg/l. They found no differences in sTfR concentrations between healthy men and women. This finding allowed us to ignore the gender influence. According to our previous study, the 95% reference interval for healthy pre-pubertal boys is 2.2–6.3 mg/l. We therefore used 6.3 mg/l as the upper limit of sTfR, since the method used was the same as in this study and, as far as we know, there are no other published reference values for sTfR in children. For the ratio of sTfR/ferritin, there are no international reference values either for adults or for children. However, we used an upper limit of 200 μg/μg, as described in the Methods section.

We wanted to calculate the value, because measurement of the ratio is thought to be a more sensitive method of evaluating iron status [16]. There are not many studies concerning sTfR in children with inflammation. Cazzola et al. reported that sTfR levels in children with systemic-onset JCA are about three times as high as in healthy age-matched controls [7]. In their study, sTfR values were associated with haemoglobin levels. These findings are in accord with ours. It has also been noticed that, when children with JCA and severe anaemia are given iron intravenously, their sTfR values return to normal [7, 31]. In our study, the median concentration of sTfR in the children with JCA was elevated, 6.1 mg/l, as was the median ratio of sTfR/ferritin, 285 μg/μg. We had eight children with high values of sTfR, and both ferritin and transferrin levels within reference limits. Here, the sTfR level may indicate iron deficiency in a situation where inflammation disturbs the interpretation of serum ferritin and transferrin levels.

The anaemia of chronic disease is correlated to some extent with the parameters of disease activity [1, 3, 19, 23], and anaemia is usually more common among patients with polyarthritis [32]. In adults, Ferguson et al. [11] have shown that sTfR is not influenced by inflammation, but they did not measure ESR or CRP values. According to Nielsen et al. [29], sTfR concentrations were not associated with levels of CRP. In an Italian study, however, sTfR levels were related both to the degree of anaemia and to the activity of the inflammatory process [33]. We observed a correlation between the sTfR and ESR and the type of onset of the arthritis, in rheumatoid arthritis. Am J Clin Pathol 1987;87:196–200. Indeed, Ravelli et al. [34] have recently reported that usually clinical and laboratory parameters of the activity of the disease do not correlate with each other. However, they stated that the use of different indices of activity of the disease would yield the same clinical information without loss of relevant data.

In summary, the sTfR concentration, which is an indicator of iron status and erythropoiesis, was elevated in 13 of the 30 children with JCA. sTfR was associated with haemoglobin, MCV, and ESR levels, but with neither serum ferritin nor transferrin. In situations where inflammation disturbs the interpretation of serum ferritin and transferrin levels, a new parameter to indicate iron deficiency would be necessary. The results raise the possibility that sTfR is able to distinguish iron-deficiency anaemia from anaemia of chronic disease. It should be further explored as a candidate.

**References**