Iron and vitamin D deficiency in children living in Western-Europe
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Red blood cell distribution width and the platelet count in iron-deficient children aged 0.5-3 years


ABSTRACT

Early detection of iron deficiency (ID) and iron deficiency anaemia (IDA) in young children is important to prevent impaired neurodevelopment. Unfortunately, many biomarkers of ID are influenced by infection thus limiting their usefulness. The aim of this study was to investigate the value of red blood cell distribution width (RDW) and the platelet count for detecting ID(A) among otherwise healthy children.

A multi-center prospective observational study was conducted in the Netherlands to investigate the prevalence of ID(A) in 400 healthy children aged 0.5-3 years. ID was defined as serum ferritin (SF) <12 µg/l in the absence of infection (CRP <5mg/l) and IDA as hemoglobin <110 g/l combined with ID. RDW (%) and the platelet count were determined in the complete blood cell count.

RDW was inversely correlated with SF and not associated with CRP. Calculated cut-off values for RDW to detect ID and IDA gave a relatively low sensitivity (53.1% and 57.1%, respectively) and specificity (64.7% and 69.9%, respectively). Anaemic children with a RDW >14.3% had a 2.7 higher odds (95% CI 1.2-6.3) to be iron deficient, compared to anaemic children with a RDW <14.3%. The platelet count showed a large range in both ID and non-ID children.

In conclusion, RDW can be helpful for identifying ID as the cause of anaemia in 0.5-3 year old children, but not as primary biomarker of ID(A). RDW values are not influenced by the presence of infection. There appears no role for the platelet count in diagnosing ID(A) in this group of children.
CHAPTER 2

Red blood cell distribution width and the platelet count in iron-deficient children aged 0.5-3 years

INTRODUCTION

Early detection of iron deficiency (ID) and iron deficiency anaemia (IDA) in young children is important to prevent impaired neurodevelopment.1-4 Studies report prevalence rates of ID and IDA of 0-85% and 0-53% in young children living in Europe, respectively.5 The great variation could be explained by the investigated population, nutritional habits and definition of ID(A) that is used. Ideally, iron status should be measured with as little biomarkers as possible, that provide as much as possible information, without being influenced by infection or inflammation, in order to minimize the required amount of blood.6 Many studies have used a combination of several biomarkers to detect ID in children, but these are not always easily available. Comparison between studies is also hampered by the lack of consensus about which combination of biomarkers is best. Moreover, the presence of infection or inflammation should be taken into account since it increases the concentration of biomarkers such as serum ferritin (SF). Red cell distribution width (RDW) and the platelet count may be promising biomarkers of ID and IDA since they are both influenced by iron status and easily available.

Due to inadequate iron supply, erythrocytes become smaller and show a larger variation in size. RDW is an index of this variation in red blood cell size (anisocytosis). It is part of routine red blood cell measurements in laboratories using automated hematology analyzers and therefore could be easily used for screening for ID(A). Studies have shown that an increased RDW is the earliest hematological manifestation of ID7-10 and that it can be used to differentiate between β-thalassemia and ID in anaemic patients11,12. Few studies have investigated the role of RDW in diagnosing ID in children. These studies are difficult to compare since ID was defined using different criteria, the presence of a possible infection was not taken into account and different cut-off values for RDW were used.13-18

It is known that iron is an important element for platelets and that ID can affect the platelet count.19-21 Both thrombocytosis22 and thrombocytopenia21,23,24 have been described in patients with ID(A). The exact mechanism of these reactions is not completely understood.

The present study aimed to evaluate whether RDW and the platelet count are useful to detect ID(A) among otherwise healthy children aged 0.5 – 3 years in a developed country, while using the World Health Organization (WHO) criteria for ID(A) and taking into account the presence of infection.
MATERIALS AND METHODS

A multi-center prospective observational study was conducted in the Netherlands in 2011-2012 to investigate the prevalence of ID and IDA in 400 healthy children aged 0.5 to 3 years (IROSTAT study). In this study, included children were undergoing general anaesthesia because of simple elective surgery or a diagnostic procedure. The study protocol was approved by the Medical Ethics Committee of South-West Holland. Informed consent was obtained from all the parents of the participating children. Further methodological details, including inclusion and exclusion criteria, and the results of this study have been published previously.6,25-27

Biochemical analysis and definitions

During insertion of a peripheral venous catheter necessary for administering anesthetics, venous blood was collected and then analyzed for a complete blood cell count, SF and C-reactive protein (CRP). RDW and the platelet count were analyzed using Sysmex XE-2100 or XE-5000 (Sysmex Corporation, Kobe, Japan) automated hematology analyzers. RDW is quantified by means of a equation, in which the standard deviation of red blood cell volume is divided by the mean corpuscular volume (MCV) of the erythrocytes and then further multiplied by 100 to express data as a percentage.28

ID was defined as SF <12 µg/l in the absence of infection (CRP <5mg/l) and IDA as Hb <110 g/l in combination with ID, according to criteria of the WHO.29

Statistical analysis

SPSS (version 21.0; SPSS Inc, Chicago, IL, USA) was used for all statistical analysis. Before analysis, data were checked on normality using histograms. Univariate analysis were performed, using a Student’s t-test for normally distributed continuous variables and a Chi-square test for dichotomous variables, for comparison of groups. In case of a non-normal distribution we used Mann-Whitney tests to compare groups. Thereafter, binary logistic regression models were used to analyze associations of laboratory parameters with ID(A) and to correct for certain confounders like age. Correlations between laboratory parameters were calculated using Pearson’s coefficients and partial coefficients. Finally, to investigate the value of RDW for discriminating (1) iron deficient children from non-iron deficient children and (2) anaemic children with ID from anaemic children without ID, we constructed receiver operating characteristic (ROC) curves. Statistical significance was defined as p<0.05.
RESULTS

Study population
The study initially included 400 children: 248 children from the Juliana Children’s Hospital in the Hague and 152 children from the Sophia Children’s Hospital in Rotterdam. Five children with underlying causes for anaemia, 43 with elevated CRP levels (10.8%) and 1 with the combination of an underlying cause and an elevated CRP were excluded. The remaining 351 children had a mean age of 18.2 months (± 8.8 months) of which 71.5% were males. Other characteristics of the study population, for example ethnicity, socio economical status and dietary factors, have been published previously. ID and IDA were found in 66 (18.8%) and 29 (8.3%) children, respectively. Laboratory parameters are presented in Table I.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g/l)</td>
<td>110.2 ± 9.5</td>
</tr>
<tr>
<td>Mean Corpuscular Volume (fl)</td>
<td>77 ± 4</td>
</tr>
<tr>
<td>Trombocytes (10⁹/l)</td>
<td>312 ± 86</td>
</tr>
<tr>
<td>Red blood cell distribution width (%)</td>
<td>14.0 ± 1.1</td>
</tr>
<tr>
<td>Serum ferritin* (µg/l)</td>
<td>19.0 (14.1 - 26.7)</td>
</tr>
</tbody>
</table>

Data are expressed as means (with standard deviation), unless otherwise noted. *No normal distribution and therefore we report the median with IQ1 and IQ3. Abbreviation: SD = standard deviation.

Iron deficiency
Univariate analysis showed that the children with ID (or IDA) were significantly older, had significant higher RDW levels and slightly lower platelet counts, compared to the non-iron deficient children (Table II). Binary logistic regression analysis showed that a higher age (p=0.001), a higher RDW level (p=0.001) and also a lower platelet count (p=0.033) were all independently associated with ID(A).

When comparing the children with ID (but without anaemia) to the children with an adequate iron status, there was no statistical difference in mean RDW levels and the platelet count (Table II). Binary logistic regression analysis showed that only a higher age (p=0.000) was independently associated with ID (without anaemia). RDW (p=0.059) and the platelet count (p=0.264) were not associated with primarily ID.
Table II Characteristics of children with and without iron deficiency (anaemia)

<table>
<thead>
<tr>
<th></th>
<th>No ID (n=285)</th>
<th>ID(A) (n=66)</th>
<th>p1</th>
<th>ID (n=37)</th>
<th>p2</th>
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<tbody>
<tr>
<td><strong>Demographic data</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male sex</td>
<td>200 (70.2%)</td>
<td>51 (77.3%)</td>
<td>0.250</td>
<td>27 (73.0%)</td>
<td>0.726</td>
</tr>
<tr>
<td>Age (months)</td>
<td>17.4 ± 8.8</td>
<td>21.4 ± 8.0</td>
<td>0.001*</td>
<td>23.2 ± 7.9</td>
<td>0.000*</td>
</tr>
<tr>
<td><strong>Laboratory data</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hb (g/l)</td>
<td>110.2 ± 9.3</td>
<td>110.5 ± 10.5</td>
<td>0.776</td>
<td>118.4 ± 5.9</td>
<td>0.000*</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>77 ± 3</td>
<td>76 ± 5</td>
<td>0.083</td>
<td>77 ± 4</td>
<td>0.424</td>
</tr>
<tr>
<td>Trombocytes (10^9/l)</td>
<td>316 ± 90</td>
<td>292 ± 66</td>
<td>0.045*</td>
<td>303 ± 67</td>
<td>0.384</td>
</tr>
<tr>
<td>RDW (%)</td>
<td>13.9 ± 0.9</td>
<td>14.4 ± 1.6</td>
<td>0.000*</td>
<td>14.2 ± 1.4</td>
<td>0.068</td>
</tr>
</tbody>
</table>

Data are expressed as numbers (with percentage) or means (with standard deviation). p1 No ID versus ID(A). p2 No ID versus ID. *Statistically significant with p<0.05. Abbreviations: ID = iron deficiency, IDA = iron deficiency anaemia, Hb = hemoglobin, MCV = mean corpuscular volume, RDW = red blood cell distribution width.

Iron deficiency anaemia

The children with IDA had significant higher RDW levels and lower platelet counts compared to the children without IDA (but possibly ID) (Table III). Binary logistic regression analysis showed that a higher RDW level (p<0.001) and a lower platelet count (p=0.038) were both independently associated with IDA. Age was not associated with IDA (data not shown).

When comparing the children with IDA to the children with an adequate iron status, similar associations were found (Table III).

Table III Characteristics of children with and without iron deficiency anaemia

<table>
<thead>
<tr>
<th></th>
<th>No ID (n=322)</th>
<th>IDA (n=29)</th>
<th>p1</th>
<th>No ID (n=285)</th>
<th>p2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographic data</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male sex</td>
<td>227 (70.5%)</td>
<td>24 (82.8%)</td>
<td>0.161</td>
<td>200 (70.2%)</td>
<td>0.153</td>
</tr>
<tr>
<td>Age (months)</td>
<td>18.1 ± 8.9</td>
<td>19.1 ± 7.6</td>
<td>0.557</td>
<td>17.4 ± 8.8</td>
<td>0.325</td>
</tr>
<tr>
<td><strong>Laboratory data</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>77 ± 4</td>
<td>76 ± 5</td>
<td>0.069</td>
<td>77 ± 3</td>
<td>0.054</td>
</tr>
<tr>
<td>Trombocytes (10^9/l)</td>
<td>314 ± 87</td>
<td>279 ± 63</td>
<td>0.034*</td>
<td>316 ± 90</td>
<td>0.032*</td>
</tr>
<tr>
<td>RDW (%)</td>
<td>13.9 ± 1.0</td>
<td>14.7 ± 1.8</td>
<td>&lt;0.001*</td>
<td>13.9 ± 0.9</td>
<td>0.000*</td>
</tr>
</tbody>
</table>

Data are expressed as numbers (with percentage) or means (with standard deviation). p1 No IDA versus IDA. p2 IDA versus no ID. *Statistically significant with p<0.05. Abbreviations: ID = iron deficiency, IDA = iron deficiency anaemia, MCV = mean corpuscular volume, RDW = red blood cell distribution width.
Of the 66 children with ID in our study population, 29 (43.9%) were also anaemic and therefore diagnosed with IDA. This latter group of children with IDA had similar RDW levels compared to the non-anaemic but still iron deficient children (14.7% vs. 14.2%, p=0.217). So, RDW values did not significantly further increase in children with IDA when compared to children with ID but without anaemia.

**RDW cut-off for diagnosing ID(A)**

Two ROC curves were constructed for RDW in children with and without ID or IDA. The AUC-ROC represents the diagnostic efficacy of RDW to predict the development of ID or IDA. The AUC-ROC for ID was 0.599 (95% CI 0.519-0.679, p=0.013). The best cut-off value for RDW to detect ID was 14.1% (sensitivity 53.1%; specificity 64.7%). This cut-off of RDW for ID gave a positive predictive value of 25.2% and a negative predictive value of 85.1% in our study population. The AUC-ROC for IDA was 0.661 (95% CI 0.559-0.763, p=0.005). The best cut-off value for RDW to detect IDA was 14.3% (sensitivity 57.1%; specificity 69.9%). This cut-off of RDW for IDA gave a positive predictive value of 14.3% and a negative predictive value of 66.6% in our study population.

**RDW cut-off for discriminating between ID and other causes of anaemia**

Based on the WHO criteria for anaemia 152 children (42.7%) (after exclusion of children with an elevated CRP) were anaemic. Of these 152 anemic children, 29 (19.1%) had IDA. These children with IDA had significantly higher RDW levels compared to anaemic children without ID (14.7% vs. 14.0%, p=0.004).

We constructed a ROC curve to investigate whether RDW is valuable in the work-up of anaemic children to discriminate between ID and other causes of anaemia. Therefore we also included the 5 children with other causes of anaemia.

The AUC-ROC for ID in anaemic children was 0.638 (95% confidence interval 0.524-0.751, p=0.023) (Figure 1). The best cut-off value for RDW to detect ID in anaemic children was 14.3% (sensitivity 57.1%; specificity 67.2%). Anaemic children in our study with a RDW >14.3% had a 2.7 higher odds (95% CI 1.2 – 6.3, p=0.019) to be diagnosed with ID as the cause of their anaemia, compared to anaemic children with a RDW <14.3%.

We did not construct a ROC curve to investigate the value of the platelet count in diagnosing ID in anaemic children since the children with IDA and the anaemic but not-iron deficient children had similar platelet counts (279 ± 63 vs. 312 ± 97, p=0.085).
Figure 1 ROC curve

Receiver operating characteristic curve for RDW to predict ID in anaemic children. Area under the curve is 0.683 (95% CI 0.524 – 0.751).

RDW: correlation with other laboratory parameters and the influence of infection

RDW was inversely correlated with SF (correlation coefficient -0.181, p<0.001), Hb (-0.205, p<0.001) and MCV (-0.435, p<0.001), after adjusting for age. After also including the 43 children with a CRP ≥5mg/l, RDW was not associated with serum CRP (p=0.320). The mean RDW levels of children with and without elevated CRP level (≥5mg/l vs. <5mg/l) were also similar (14.1% vs. 13.9%, p=0.258).
DISCUSSION

This is the first observational study that has investigated the usefulness of RDW and the platelet count as indicators of ID(A) in a population of healthy Dutch children aged 0.5-3 years in which an infection was excluded. We found that, after adjusting for age, ID and IDA were both associated with higher RDW levels and lower platelet counts. We showed that RDW can be used in anaemic children to discriminate between ID and other causes of anaemia, but is less useful as predictor of ID(A) in otherwise healthy children. Furthermore, we showed that RDW values were not influenced by the presence of infection. Finally, there appears no role for the platelet count in diagnosing ID(A) in our study population.

RDW

In accordance with other studies, we found higher RDW levels in children with ID(A) compared to children with an adequate iron status. However, the discriminative power of RDW to detect ID(A) seems limited. Our calculated cut-off values for RDW to detect ID and IDA are suboptimal because of a relatively low sensitivity and specificity. Hinchcliffe reported a better diagnostic capacity of RDW for ID in 13-month old children. In this study an adult-derived RDW reference limit of 13.9% was used showing a sensitivity of 100% (specificity not reported). Sazawal reported an excellent diagnostic capacity of the combination of a low Hb (≤10g/dl) and a RDW >15% to detect IDA in Indian toddlers (sensitivity 99%; specificity 90%). The difference between our study and the two aforementioned studies can be explained by different definitions of ID that have been used. We used the WHO criteria for defining ID instead of the presence of >2.5% hypochromic red cells or a SF <11µg or a zinkprotoprofyrin >80µmol/mole of heme. Another explanation could be the different machines that have been used for analysis. One should realize that RDW varies between analysers depending the technology used for red cell analysis. Reference limits for RDW are therefore instrument-specific and not interchangeable between different laboratories.

We showed that RDW has the potential to discriminate between ID and other causes of anaemia in anaemic children aged 0.5-3 years, while using ferritin as the ‘gold standard’ for ID. However, RDW does not discriminate between infants with an adequate iron status and those with ID but without anaemia. We determined that anaemic children in our study with a RDW >14.3% had an almost three times higher odds to be diagnosed with ID as the cause of their anaemia. To our knowledge this is the first study that investigated the diagnostic
capacity of RDW for ID in anaemic children (defined as a low Hb). Other studies investigated the value of RDW for diagnosing ID in case of microcytosis (defined as a low MCV) and/or microcytic anaemia (defined as a low MCV in combination with a low Hb) and they report a higher diagnostic capacity.\textsuperscript{15,16,31} In these studies the value of RDW for diagnosing ID is investigated in patients with depleted iron stores that have already led to suppression of the erythropoiesis as indicated by a low MCV. Severe and long-standing ID(A), which would give the strongest association between ID(A) and RDW, is uncommon in the Netherlands and this could explain the lower diagnostic capacity in our study.

We found that RDW levels were not associated with infection. To our knowledge, only two other studies investigated the influence of an infection on RDW levels in children. In the first prospective study among 113 children, haematological parameters, including RDW, did not change significantly between the 1st, 3rd and 15th day of an infection.\textsuperscript{32} In the second study RDW did not correlate with serum CRP in children living in Chile.\textsuperscript{33} A study in morbidly obese adult patients also showed that RDW did not correlate with CRP.\textsuperscript{34} Associations between elevated RDW levels and bad prognosis in patients with congestive heart failure, acute myocardial infarction and sepsis have been described in adults.\textsuperscript{28,35} It is thought that an increased RDW mirrors a profound deregulation of erythrocyte homeostasis involving both impaired erythropoiesis and abnormal red blood cell survival, which may be attributed to a variety of underlying metabolic abnormalities.\textsuperscript{28} We speculate that simple short-term infections, which are common in children, do not affect RDW levels, but longer-lasting infections or inflammatory processes do. So, in case of an infection and hereby possibly an incorrect normal or elevated SF, RDW might be helpful in the diagnostic work-up of anaemic children. Further studies are necessary to confirm our results and to investigate the value of RDW in children with infections or inflammation.

**Thrombocytes**

Children with ID(A) in our study had slightly lower platelet counts compared to children without ID(A). It has been shown that moderate IDA is usually associated with thrombocytosis whereas severe IDA is likely to be accompanied by thrombocytopenia.\textsuperscript{20,21,36} Most paediatric patients with ID have normal or elevated platelet counts at diagnosis (in the range of 500 to 700 $\times 10^9$/l);\textsuperscript{21} thrombocytopenia in combination with ID is less common.\textsuperscript{21,23,24} Thrombocytosis may be due to stimulation of platelet production by increased erythropoietin concentrations, that occur in patients with IDA.\textsuperscript{21} The mechanism(s) for the thrombocytopenia associated with ID is not known although theories exist
about stem cell competition and bone marrow crowding. It is thought that megakaryocytic and erythroid cell lineages share a common progenitor cell. Chronic erythropoietin stimulation can lead to increased red cell production at expense of platelet production. Since the platelet count in both iron deficient and non-iron deficient children showed a large range (data not shown), we believe that the observed difference is of no clinical relevance.

The strength of this study is that we investigated the value of RDW as an indicator of iron status in a well-defined population of healthy children aged 0.5 – 3 years in a developed country. In this study, we used the definition of the WHO for ID and we took into account the presence of an infection. A limitation of this study is that a limited number of children (8.3%) had the most severe form of ID, namely IDA. We suggest that the diagnostic capacity of RDW might be higher in a population with more severe ID and/or IDA.

**CONCLUSION**

RDW can be helpful for identifying ID as the cause of anaemia in 0.5-3 year old children because it is inversely correlated with serum ferritin and not affected by the presence of infection. It is not useful as a primary biomarker of ID or IDA. Further studies are necessary to produce instrument-specific cut-off values and to investigate its diagnostic capacity in children with infection and/or inflammation. There appears no role for the platelet count in diagnosing ID(A) in these children.
REFERENCE LIST

Red blood cell distribution width and the platelet count in iron-deficient children aged 0.5-3 years