

# Clinical utility of calculated haematological parameters in the diagnosis of iron deficiency anaemia in pregnant women

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**Abstract.** Iron deficiency anaemia (IDA) is a common condition during pregnancy. The aim of the present study was to determine the diagnostic accuracy measures and define the optimal parameters for diagnosing IDA. Simple parameters, such as erythrocyte count (E), iron (Fe), haematocrit (HCT), mean corpuscular volume of erythrocytes (MCV), and red blood cell distribution width (RDW), were included and several ratios were calculated (Fe/E, MCV/RDW, RDW/E, RDW/Fe, and RDW/HCT). Additionally, a scoring model was proposed. A total of 623 pregnant women were included in the present study, and the blood was obtained a day before or on the day of delivery. Simple parameters and calculated ratios were determined. The clinical criterion for IDA diagnosis was defined based on the World Health Organization threshold of haemoglobin <110 g/l, and pregnant women were classified as anaemic or non-anaemic based on this metric. The values of all assessed parameters were significantly different ( $P < 0.001$ ) between the two groups. A weak correlation was identified for MCV, Fe/E, and MCV/RDW; a moderate correlation for E, Fe, RDW/E, and RDW/Fe; and a strong correlation for HCT and RDW/HCT. No correlation was identified for RDW. Markedly high diagnostic accuracy for IDA diagnosis was obtained with an area under the curve (AUC) of 0.921 for the calculated parameter  $\text{RDW/HCT} > 43.64 \text{ l/l} \times 10^{-2}$ , and an AUC of 0.973 for the simple parameter  $\text{HCT} \leq 0.32 \text{ l/l}$ . RDW/HCT and HCT demonstrated the highest diagnostic accuracy, and may be useful parameters in routine practice for the diagnosis of IDA.

## Introduction

Anaemia is defined as a low blood haemoglobin (Hb) concentration and is a public health problem (1). It can occur in all stages of life, particularly during pregnancy and childhood (2). Iron deficiency anaemia (IDA) is the predominant cause of anaemia during pregnancy and is one of the primary consequences of iron deficiency (ID) (3).

It is hypothesised that the occurrence of IDA in pregnancy is >40%. IDA occurs due to an increased need for the development of the fetoplacental unit, an increase in the mass of erythrocytes (E), and an increase in the volume of plasma to compensate for iron loss during childbirth. In developed countries, the iron intake is often below nutritional needs (4).

Undiagnosed and untreated IDA can have a significant impact on the health of both the mother and the child. In mothers, IDA is associated with a generally poor condition, decreased working capacity, fatigue, pallor, breathlessness, palpitations and headaches. The risk of postpartum depression is significantly increased compared with pregnant women without IDA. Fatigue and depression often affect the relationship between mother and child. During pregnancy, IDA is also associated with problems with the placenta, intrauterine death, infections, retardation of intrauterine growth, low birth weight, preterm delivery and perinatal mortality. In infants, IDA is associated with poor postnatal growth, reduced cognitive ability, and early ID (5). Iron is also necessary for the activity of enzymes related to the development of the central nervous system (4).

The World Health Organization (WHO) defines IDA as anaemia accompanied by depleted iron stores and a compromised supply of iron to the tissues (1). IDA develops when the supply of iron is insufficient to sustain erythropoiesis, leading to a decreased concentration of Hb (6).

To prevent the development of IDA, the WHO has developed a system for classifying IDA in pregnant women (1).

According to the WHO, IDA in pregnancy is present if Hb is <110 g/l, serum ferritin <15  $\mu\text{g/l}$ , and haematocrit (HCT) <0.33 l/l (7). To determine the presence of anaemia in pregnant women, the WHO recommends the determination of Hb and ferritin concentrations (8).

Laboratory determination plays a crucial role in diagnosing IDA. For the differential diagnosis of IDA, it is necessary to determine the complete blood count (CBC),

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which includes Hb concentration and HCT, as diagnostic criteria for IDA. In addition, ferritin concentration in the serum sample should be determined. Ferritin is considered the gold standard for diagnosing IDA, but it has its limitations. As an acute-phase reactant, ferritin levels can be influenced by inflammation; therefore, it is essential to determine C-reactive protein (CRP) levels for an accurate diagnosis. It is recommended to determine ferritin levels once CRP levels have returned to normal. Conversely, serum iron (Fe) exhibits significant daily variation; therefore, determining transferrin levels is also recommended (4). Hb concentration is not a reliable parameter during pregnancy due to physiological changes in plasma volume and E mass in pregnant women. Therefore, Hb is not a sensitive or specific indicator of IDA (3). Regarding erythrocyte constants, the mean corpuscular volume (MCV) initially rises slightly and then falls, which can lead to drawing an incorrect conclusion. Mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) are reduced only when anaemia is severe. Red blood cell distribution width (RDW) represents a quantitative measure of the variation in the size of E. This is a routine parameter and part of a CBC, easily measurable, independent indicator, and increases at least 4 weeks before MCV. RDW is an early indicator of the change in E, which is essential for diagnosing IDA (9). Hb and HCT effectively provide the same data. They are roughly described by the following formula:  $Hb \times 3/1,000 = HCT$ . Hb is measured directly using spectrophotometry, while HCT is calculated as the product of the E count and MCV (10,11).

During a normal pregnancy, Fe concentration, the percentage of Fe saturation, and total iron binding capacity (TIBC) have less diagnostic importance, as Fe and the percentage of Fe saturation decrease as TIBC increases (9). It is also important to emphasise the pre-analytical requirements for Fe determination. A pregnant woman should not drink vitamin drinks up to 48 h before drawing blood. Since Fe shows significant daily variation (up to 70%), blood should be drawn before 10 AM. If a pregnant woman is taking Fe preparations, the determination should be postponed for at least 10 days after the last oral Fe dose, 3 days after intravenous Fe preparations, and 1 month after intramuscular Fe preparations. It is also important not to carry out Fe determination during an acute infection, as this can cause Fe to be trapped within the cells of the reticuloendothelial system. Pregnant women often undergo Fe therapy before pregnancy or begin taking Fe supplements early in pregnancy, and this information should be considered when interpreting laboratory results (12).

It should also be noted that reference intervals differ between pregnant and non-pregnant women of the same age, primarily due to the previously mentioned increase in plasma volume during pregnancy (4).

It is essential to diagnose IDA as early as possible to prevent complications for both the mother and the child. Therefore, it is also important to use diagnostic parameters that are simple, safe and cost-effective (13).

Inspired by the notion of calculated parameters with strong diagnostic accuracy, in the present study, new parameters, as ratios of routinely available parameters (E, Fe, HCT, MCV, and RDW), are proposed for their diagnostic value.

The aim of this study was to evaluate the diagnostic accuracy of various parameters and define the best possible parameter for diagnosing IDA. Simple and widely applicable calculated ratios were assessed, including Fe/E, MCV/RDW, RDW/E, RDW/Fe and RDW/HCT, and a scoring model was proposed. In view of the importance of early diagnosis and the limitations of current diagnostic parameters, the scoring model may represent a meaningful and easy-to-use diagnostic tool, allowing for diagnosis of IDA earlier, while making it more accessible and cost-effective, thereby facilitating timely therapeutic intervention (14).

## Materials and methods

**Study plan.** The present study was performed at the Clinical Hospital Center Rijeka (Rijeka, Croatia) between August 2019 and January 2020. A total of 623 pregnant women who had low-risk, full-term pregnancies, including women who had received Fe supplements, were enrolled in the study. The median age of the pregnant women was 32 years (age range, 16–47). Blood samples were collected either on the day of delivery or the day before when they were admitted to the hospital.

The study was performed in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the Clinical Hospital Center Rijeka, Rijeka (approval no. 2170-29-02/1-19-2; class, 003-05/19-1/110; approval date, 15 July 2019; Rijeka, Croatia). Written informed consent was obtained from all participants in the study.

The inclusion criteria were women who delivered between 38 and 41 weeks of gestation, those with premature rupture of membranes lasting no longer than 12 h, women giving birth for the first time, and those who had multiple births (up to three births).

The exclusion criteria were twin pregnancies, those with gestational diabetes requiring medical treatment, hypertension, preeclampsia, uterine anomalies, bleeding during pregnancy, women who had had multiple births with  $\geq 4$  deliveries, smokers and women with chronic bowel disease.

**Sample collection and analysis.** For blood collection, two blood tubes were used; one tube ( $K_3$ EDTA tube 3 ml, 13x75 mm; cat. no. 454217; VACUETTE®, Greiner Bio-One Ltd.) to determine CBC and the other tube (serum clot activator tubes with gel separator 8 ml, 16x100 mm; cat. no. 455071; VACUETTE®, Greiner Bio-One Ltd.) for determining Fe concentration. The blood samples were sent to the Medical Biochemical Laboratory of the Clinical Hospital Center Rijeka (Rijeka, Croatia), and all test measurements were performed within 3 h of collection.

The determination of CBC parameters (E, Hb, HCT, MCV and RDW) were performed using the ADVIA 2120 Hematology Analyzer (Siemens Healthineers) based on colourimetric, peroxidase-based optical and laser optical methods, while the serum test (Fe) was performed on an Olympus AU 640 Automatic Biochemical Analyzer (Olympus Corporation), based on the spectrophotometric principle with manufacturer-provided reagents. In addition to internal quality control procedures, external quality control CROQALM HDMBLM (croqalm.hdmblm.hr) and LABQUALITY EQAS

Table I. Assessed parameters in the anaemic and non-anaemic pregnant women.

| Parameter (unit)                                | Anaemic, n=133   | Non-anaemic, n=490 | P-value             |
|-------------------------------------------------|------------------|--------------------|---------------------|
| Hb (g/l <sup>b</sup> )                          | 102 (97-106)     | 123 (117-130)      | <0.001 <sup>a</sup> |
| E (x10 <sup>12</sup> /l <sup>c</sup> )          | 3.77±0.38        | 4.20±0.33          | <0.001 <sup>a</sup> |
| Fe (μmol/l <sup>b</sup> )                       | 8 (6-10)         | 14 (10-18)         | <0.001 <sup>a</sup> |
| HCT (l/l <sup>b</sup> )                         | 0.31 (0.29-0.32) | 0.36 (0.34-0.38)   | <0.001 <sup>a</sup> |
| MCV (fl <sup>b</sup> )                          | 82.5 (75.4-87.3) | 87.7 (84.6-90.8)   | <0.001 <sup>a</sup> |
| RDW (% <sup>b</sup> )                           | 15.0 (14.1-15.9) | 14.3 (13.7-15.0)   | <0.001 <sup>a</sup> |
| Fe/E (μmol x10 <sup>-12b</sup> )                | 2.0 (1.5-2.8)    | 3.2 (2.5-4.4)      | <0.001 <sup>a</sup> |
| MCV/RDW (fl x10 <sup>-2b</sup> )                | 5.5 (4.8-6.1)    | 6.1 (5.7-6.6)      | <0.001 <sup>a</sup> |
| RDW/E (%lx10 <sup>-12b</sup> )                  | 4.0 (3.8-4.3)    | 3.4 (3.2-3.6)      | <0.001 <sup>a</sup> |
| RDW/Fe (lx10 <sup>-2</sup> /μmol <sup>b</sup> ) | 2.0 (1.4-2.7)    | 1.1 (0.8-1.4)      | <0.001 <sup>a</sup> |
| RDW/HCT (l/lx10 <sup>-2b</sup> )                | 49.3 (46.3-54.5) | 39.4 (36.8-43.0)   | <0.001 <sup>a</sup> |

<sup>a</sup>P<0.001. <sup>b</sup>Median and interquartile range (IQR). <sup>c</sup>Mean ± SD. Hb, haemoglobin; E, erythrocyte count; Fe, iron; HCT, haematocrit; MCV, mean corpuscular volume; RDW, red blood cell distribution width; Fe/E, iron/erythrocyte ratio; MCV/RDW, mean corpuscular volume/red blood cell distribution width ratio, RDW/E, red blood cell distribution width/erythrocyte ratio; RDW/Fe, red blood cell distribution width/iron ratio; RDW/HCT, red blood cell distribution width/haematocrit ratio.

Table II. Correlations between Hb and all tested parameters.

| Parameter (unit)                  | Correlation coefficient (95% CI) | P-value             |
|-----------------------------------|----------------------------------|---------------------|
| E (x10 <sup>12</sup> /l)          | r=0.650 (0.602 to 0.693)         | <0.001 <sup>a</sup> |
| Fe (μmol/l)                       | ρ=0.545 (0.487 to 0.598)         | <0.001 <sup>a</sup> |
| HCT (l/l)                         | ρ=0.941 (0.932 to 0.950)         | <0.001 <sup>a</sup> |
| MCV (fl)                          | ρ=0.423 (0.356 to 0.485)         | <0.001 <sup>a</sup> |
| RDW (%)                           | ρ=-0.243 (-0.316 to -0.167)      | <0.001 <sup>a</sup> |
| Fe/E (μmol x10 <sup>-12</sup> )   | ρ=0.426 (0.360 to 0.489)         | <0.001 <sup>a</sup> |
| MCV/RDW (fl x10 <sup>-2</sup> )   | ρ=0.360 (0.289 to 0.426)         | <0.001 <sup>a</sup> |
| RDW/E (%lx10 <sup>-12</sup> )     | ρ=-0.743 (-0.777 to -0.706)      | <0.001 <sup>a</sup> |
| RDW/Fe (lx10 <sup>-2</sup> /μmol) | ρ=-0.552 (-0.604 to -0.495)      | <0.001 <sup>a</sup> |
| RDW/HCT (l/lx10 <sup>-2</sup> )   | ρ=-0.804 (-0.830 to -0.774)      | <0.001 <sup>a</sup> |

<sup>a</sup>P<0.001. Hb, haemoglobin; E, erythrocyte count; Fe, iron; HCT, haematocrit; MCV, mean corpuscular volume; RDW, red blood cell distribution width; Fe/E, iron/erythrocyte ratio; MCV/RDW, mean corpuscular volume/red blood cell distribution width ratio, RDW/E, red blood cell distribution width/erythrocyte ratio; RDW/Fe, red blood cell distribution width/iron ratio; RDW/HCT, red blood cell distribution width/haematocrit ratio; r, Pearson's correlation coefficient; ρ, Spearman's rank correlation coefficient; CI, confidence interval.

(my.labscala.fi) were also performed at the Clinical Hospital Center Rijeka laboratory.

The clinical criterion for IDA diagnosis in the present study was defined based on the WHO threshold of Hb <110 g/l, and pregnant women were classified as anaemic and non-anaemic based on this threshold (6).

**Statistical analysis.** Statistical analysis was performed using MedCalc software version 20.104 (MedCalc Software Ltd.). The Shapiro-Wilk test was used to determine the normality of the distribution of variables. E followed a normal distribution, thus data are presented as the mean ± standard deviation (SD), while other parameters did not follow a normal distribution, and are presented as the median and interquartile range (IQR). Pearson's correlation coefficient (r) was used for correlation

analysis of normally distributed data and Spearman's rank correlation coefficient (ρ) was used for correlation analysis of non-normally distributed data with a corresponding 95% confidence interval (95% CI).

The difference between anaemic and non-anaemic groups was determined by the unpaired t-test for normal data distribution and the Mann-Whitney U test for non-normal data distribution. P<0.05 was considered to indicate a statistically significant difference.

The diagnostic sensitivity and specificity, with the respective 95% CI of the evaluated parameters in anaemic and non-anaemic pregnant women in diagnosing IDA, were determined. Receiver operating characteristics (ROC) analysis and the area under the curve (AUC) were used for this purpose. The optimal cut-off value was selected according to the Youden index.

Table III. Diagnostic accuracy of tested parameters in distinguishing anaemic (n=133) and non-anaemic (n=490) pregnant women.

| Parameter (unit)                           | Area under the curve (95% CI) | P-value             | Cut-off     | Sensitivity (95% CI) | Specificity (95% CI) |
|--------------------------------------------|-------------------------------|---------------------|-------------|----------------------|----------------------|
| E ( $\times 10^{12}/l$ )                   | 0.802 (0.768-0.833)           | <0.001 <sup>a</sup> | $\leq 3.98$ | 73.7 (65.3-80.9)     | 75.9 (71.8-79.6)     |
| Fe ( $\mu\text{mol}/l$ )                   | 0.824 (0.791-0.853)           | <0.001 <sup>a</sup> | $\leq 10$   | 75.9 (67.8-82.9)     | 74.9 (70.8-78.6)     |
| HCT (l/l)                                  | 0.973 (0.957-0.984)           | <0.001 <sup>a</sup> | $\leq 0.32$ | 92.5 (86.6-96.3)     | 94.7 (92.3-96.5)     |
| MCV (fl)                                   | 0.740 (0.704-0.774)           | <0.001 <sup>a</sup> | $\leq 82.9$ | 54.1 (45.3-62.8)     | 85.9 (82.5-88.9)     |
| RDW (%)                                    | 0.661 (0.622-0.698)           | <0.001 <sup>a</sup> | $> 14.7$    | 60.2 (51.1-68.7)     | 66.9 (62.6-71.1)     |
| Fe/E ( $\mu\text{mol} \times 10^{-12}$ )   | 0.771 (0.735-0.803)           | <0.001 <sup>a</sup> | $\leq 2.53$ | 70.5 (61.9-78.1)     | 73.4 (69.3-77.3)     |
| MCV/RDW (fl $\times 10^{-2}$ )             | 0.722 (0.685-0.756)           | <0.001 <sup>a</sup> | $\leq 5.8$  | 65.4 (56.7-73.4)     | 69.9 (65.7-74.0)     |
| RDW/E ( $\% \times 10^{-12}$ )             | 0.884 (0.856-0.908)           | <0.001 <sup>a</sup> | $> 3.63$    | 87.8 (80.9-92.9)     | 73.9 (69.8-77.8)     |
| RDW/Fe (l $\times 10^{-2}/\mu\text{mol}$ ) | 0.835 (0.804-0.864)           | <0.001 <sup>a</sup> | $> 1.27$    | 84.9 (77.6-90.5)     | 68.1 (63.8-72.2)     |
| RDW/HCT (l/l $\times 10^{-2}$ )            | 0.921 (0.897-0.941)           | <0.001 <sup>a</sup> | $> 43.64$   | 88.6 (81.8-93.4)     | 79.5 (75.7-83.0)     |

<sup>a</sup>P<0.001. Hb, haemoglobin; E, erythrocyte count; Fe, iron; HCT, haematocrit; MCV, mean corpuscular volume; RDW, red blood cell distribution width; Fe/E, iron/erythrocyte ratio; MCV/RDW, mean corpuscular volume/red blood cell distribution width ratio; RDW/E, red blood cell distribution width/erythrocyte ratio; RDW/Fe, red blood cell distribution width/iron ratio; RDW/HCT, red blood cell distribution width/haematocrit ratio.

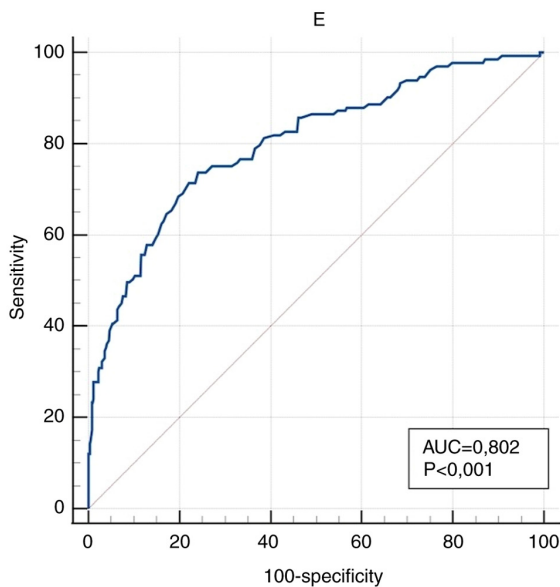


Figure 1. Receiver operating characteristic curve for E. E, erythrocyte count; AUC, area under the curve.

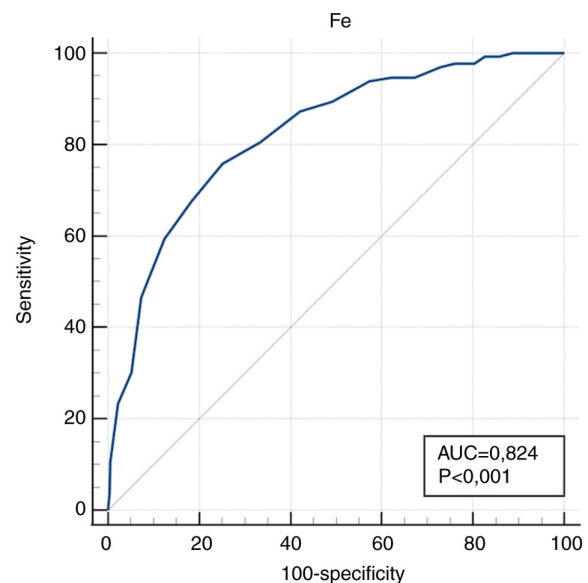


Figure 2. Receiver operating characteristic curve for Fe. Fe, iron; AUC, area under the curve.

**Scoring model.** For the purpose of earlier and more accurate IDA recognition, a scoring model based on parameters routinely determined in a medical biochemical laboratory was developed. The model consisted of five components, each representing a ratio of simple haematological and biochemical parameters: Fe/E, MCV/RDW, RDW/E, RDW/Fe and RDW/HCT. Whether the measured values were higher or lower than the limit values was considered. The limit values of the ratios were obtained using ROC curve analysis based on data from all 623 pregnant women (both anaemic and non-anaemic). Concentrations higher than the limit values where the criterion was met were scored 1, and those lower than the limit values where the criterion was not met were scored 0. The final score was calculated as the

sum of all five components (ranging from 0-5) and reflects the severity of anaemia, allowing for earlier diagnosis and timely therapeutic intervention.

## Results

Of the 623 pregnant women included, 51% were primigravida, 32% had one previous birth and 17% had  $\geq 2$  previous births (Table SI). Based on the Hb concentration, 133 women (21%) were classified as anaemic, 209 (34%) were anaemic according to RDW/HCT and 149 (24%) according to HCT (Table SII). These findings suggest that RDW/HCT and HCT are more sensitive indicators of IDA than Hb concentration.

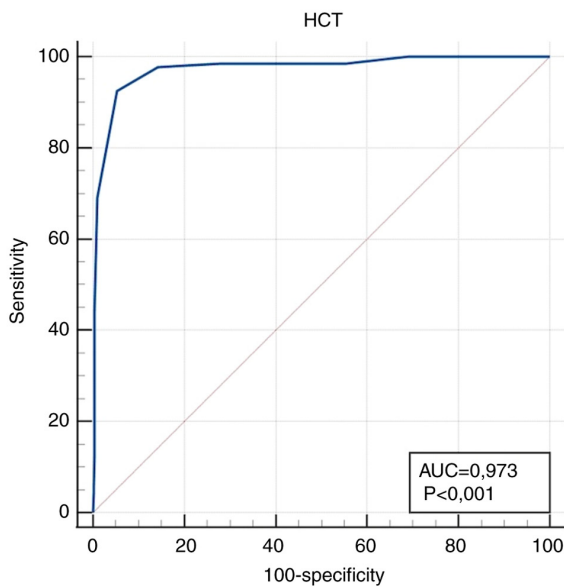


Figure 3. Receiver operating characteristic curve for HCT. HCT, haematocrit; AUC, area under the curve.

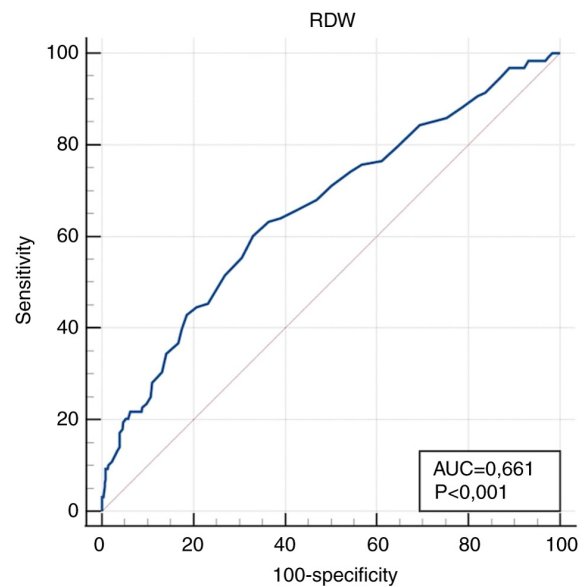


Figure 5. Receiver operating characteristic curve for RDW. RDW, red blood cell distribution width; AUC, area under the curve.

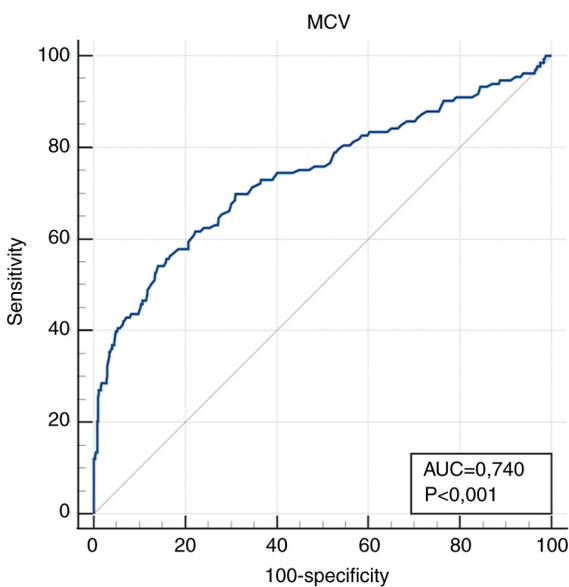


Figure 4. Receiver operating characteristic curve for MCV. MCV, mean corpuscular volume; AUC, area under the curve.

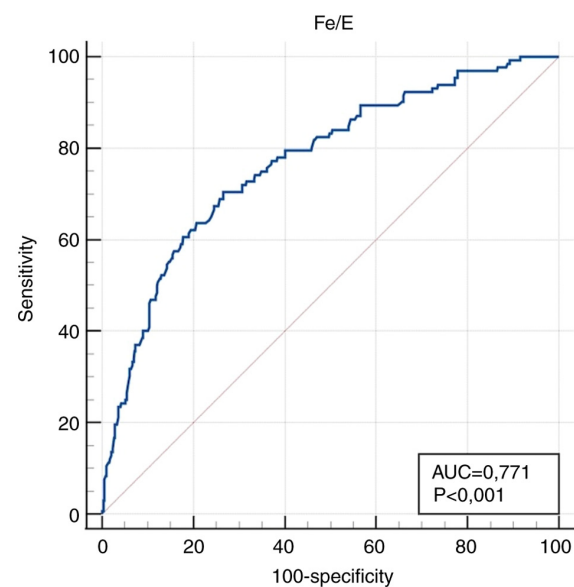


Figure 6. Receiver operating characteristic curve for Fe/E. Fe/E, iron/erythrocyte ratio; AUC, area under the curve.

All tested parameters in anaemic and non-anaemic pregnant women are presented in Table I. The values of all tested parameters Fe/E, MCV/RDW, RDW/E, RDW/Fe, RDW/HCT, E, Fe, Hb, HCT, MCV and RDW were significantly different between the anaemic and non-anaemic pregnant women ( $P<0.001$ ). In the anaemic group, E, Fe, HCT, MCV, Fe/E and MCV/RDW values were statistically lower, and RDW, RDW/E, RDW/Fe and RDW/HCT were statistically higher compared with non-anaemic pregnant women.

The correlations between Hb and all parameters are shown in Table II. A weak correlation (correlation coefficients from 0.250-0.500) was found for MCV, Fe/E and MCV/RDW, likely due to their previously mentioned limitations in diagnosing IDA. A moderate correlation (correlation

coefficients from 0.500-0.750) was found for E, Fe, RDW/E and RDW/Fe. A strong correlation (correlation coefficients  $>0.750$ ) was found for HCT and RDW/HCT. No significant correlation (correlation coefficient  $<0.250$ ) was found for RDW.

The diagnostic accuracy of the measured values obtained from the ROC curves for the tested parameters at their optimal diagnostic cut-off values is presented in Table III.

Markedly high diagnostic accuracy (AUC value  $>0.9$ ) for IDA diagnosis was obtained for  $\text{RDW/HCT} > 43.64 \text{ l/l} \times 10^{-2}$  as a calculated parameter, and  $\text{HCT} \leq 0.32 \text{ l/l}$  as a simple parameter. High diagnostic accuracy ( $0.9 > \text{AUC} > 0.8$ ) was obtained for RDW/E, RDW/Fe, E and Fe.

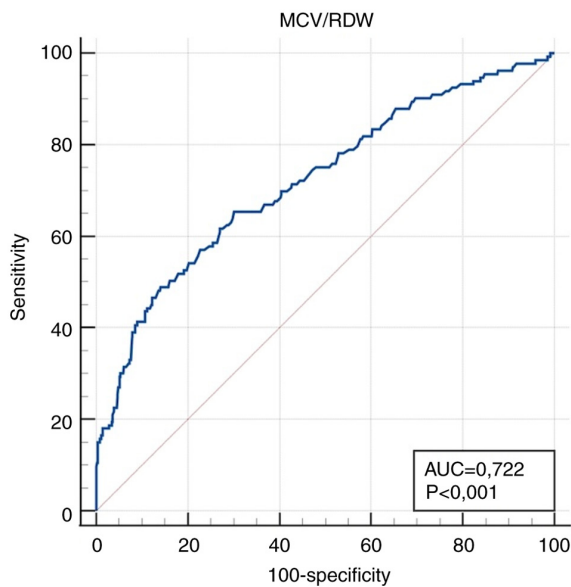


Figure 7. Receiver operating characteristic curve for MCV/RDW. MCV/RDW, mean corpuscular volume/red blood cell distribution width ratio; AUC, area under the curve.

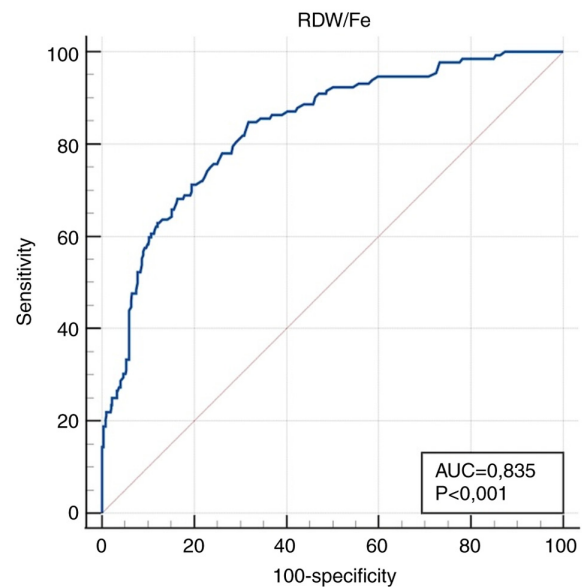


Figure 9. Receiver operating characteristic curve for RDW/Fe. RDW/Fe, red blood cell distribution width/iron ratio; AUC, area under the curve.

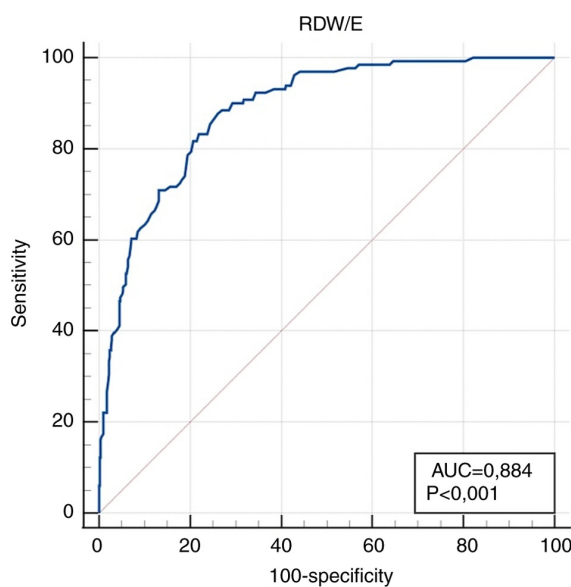


Figure 8. Receiver operating characteristic curve for RDW/E. RDW/E, red blood cell distribution width/erythrocyte ratio; AUC, area under the curve.

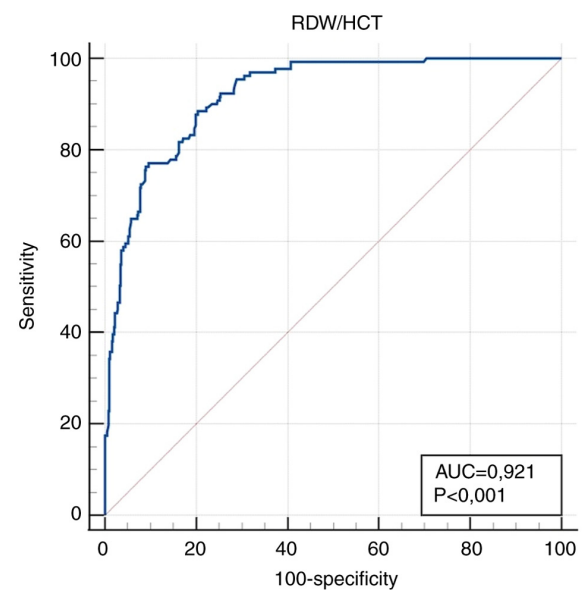


Figure 10. Receiver operating characteristic curve for RDW/HCT. RDW/HCT, red blood cell distribution width/haematocrit ratio; AUC, area under the curve.

The ROC curves for all parameters, both simple and calculated are shown in Figs. 1-10, and the comparison of all the ROC curves of all calculated parameters is presented in Fig. 11.

According to the cut-off values of other calculated and simple parameters, the following numbers and percentages of anaemic pregnant women were obtained: 223 (36%) for Fe/E, 234 (38%) for MCV/RDW, 242 (39%) for RDW/E, 267 (43%) for RDW/Fe, 216 (35%) for E, 224 (36%) for Fe, 141 (23%) for MCV, and 237 (38%) for RDW (Table SIII).

The components and criteria of our scoring model are shown in Table IV. According to the of the scoring model, the

following numbers and percentages of 133 anaemic pregnant women were obtained: 0, 0/133 (0%); 1, 3/133 (2%); 2, 14/133 (11%); 3, 20/133 (15%); 4, 34/133 (26%), and 5, 62/133 (47%). For the 490 non-anaemic pregnant women, the numbers and percentages were as follows: 0, 217/490 (44%); 1, 80/490 (16%); 2, 76/490 (16%); 3, 60/490 (12%); 4, 33/490 (7%), and 5, 24/490 (5%) (Table SIV).

## Discussion

During pregnancy, women are prone to developing IDA. Routine laboratory parameters are only effective in detecting already visible and manifested anaemia (3). Despite the availability of

Table IV. Components and criteria of the scoring model.

| Component                                     | Criterion   | No/Yes |
|-----------------------------------------------|-------------|--------|
| Fe/E ( $\mu\text{mol} \times 10^{-12}$ )      | $\leq 2.53$ | 0/1    |
| MCV/RDW ( $\text{fl} \times 10^{-2}$ )        | $\leq 5.8$  | 0/1    |
| RDW/E ( $\% \times 10^{-12}$ )                | $> 3.63$    | 0/1    |
| RDW/Fe ( $1 \times 10^{-2} / \mu\text{mol}$ ) | $> 1.27$    | 0/1    |
| RDW/HCT ( $1 / 1 \times 10^{-2}$ )            | $> 43.64$   | 0/1    |
| All                                           |             | 0-5    |

The table presents the scoring model criteria and components: Fe/E, iron/erythrocyte ratio; MCV/RDW, mean corpuscular volume/red blood cell distribution width ratio; RDW/E, red blood cell distribution width/erythrocyte ratio; RDW/Fe, red blood cell distribution width/iron ratio; RDW/HCT, red blood cell distribution width/haematocrit ratio; Criteria: Cut-off values obtained from the ROC curve of all 623 pregnant women; No/Yes indicates a score of 0 or 1, depending on whether the criterion is not met or met.

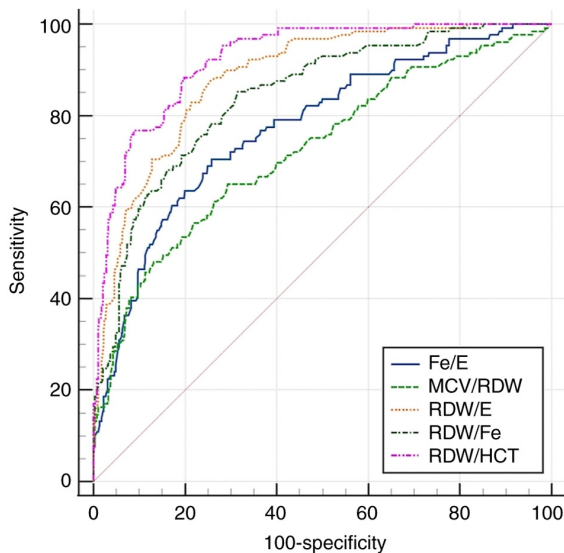


Figure 11. Comparison of the receiver operating characteristic curves for all calculated parameters. Fe/E, iron/erythrocyte ratio; MCV/RDW, mean corpuscular volume/red blood cell distribution width ratio; RDW/E, red blood cell distribution width/erythrocyte ratio; RDW/Fe, red blood cell distribution width/iron ratio; RDW/HCT, red blood cell distribution width/haematocrit ratio.

clinical and laboratory indicators, adverse events may sometimes go unrecognised in a timely manner, increasing the risk of complications in both mother and child. The introduction of a novel, simple and accessible laboratory indicator, either as a standalone or as part of a summary model, allows timely recognition and treatment of IDA (14). In the present study, several calculated parameters were evaluated with the aim of finding the one with the highest diagnostic accuracy. In addition to introducing new combined parameters, a scoring model based on the sum of these parameters was developed, and this model may facilitate the timely recognition of IDA and help in making clinical decisions.

The goal of the present study was to develop novel combined parameters for IDA that demonstrate high diagnostic accuracy,

using simple, routine procedures performed in a standard medical-biochemical laboratory. Additionally, another goal was to establish a model that can be successfully applied at different stages of pregnancy, thereby enabling timely diagnosis and effective follow-up. The study subjects were pregnant women whose blood samples were collected either the day before or on the day of delivery, upon admission to the hospital. This is also a limitation, as early recognition of IDA is crucial for reducing complications and improving outcomes. The model that is proposed requires validation, as the values obtained from the present study would otherwise be applicable only to our specific sample of 623 pregnant women. When the original sample was divided into training and validation subsets, the two groups produced statistical results similar to those of the full sample. Although slight differences were observed in the validation group during statistical analysis, it is important to note that the same parameters demonstrated the highest diagnostic accuracy. It is suggested that the model be validated using a larger, entirely new cohort of pregnant women, assessed at various stages of gestational age. The absence of such validation is also acknowledged as a limitation of our study. The scoring model presented in Table IV should also be tested on an entirely independent cohort to confirm its performance across various stages of the gestational period, as this remains another limitation of the present study.

It is hypothesised that the novel indicators are superior for diagnostic purposes and may have potential for prediction when applied in early pregnancy or at the initial stages. The scoring model may be useful for predicting IDA in the early stages of pregnancy. This will serve as a topic for further research.

In the present study, RDW/HCT was revealed to be the most effective parameter with high diagnostic sensitivity and specificity. Among the simple parameters, HCT exhibited the best performance. Both parameters showed the strongest correlation with Hb. The RDW/HCT ratio was influenced by HCT, but compared with RDW alone, RDW/HCT demonstrated superior diagnostic performance in terms of greater sensitivity and specificity, as well as stronger correlation with Hb. The ratio adjusts RDW in relation to the overall red blood cell mass, highlighting subtle cases in which the HCT may remain at the lower end of the normal range, while RDW is disproportionately elevated. This increases sensitivity by capturing both early anisocytosis and relative reductions in red cell mass. In this way, a more independent indicator was obtained, superior to HCT in terms of independence and to RDW in terms of diagnostic accuracy. According to the available literature, there were no similar studies with which to compare the findings obtained in the present study.

While RDW/HCT and HCT were identified as the most effective parameters, several other combined and simple parameters also demonstrated good diagnostic performance. As aforementioned, parameters that exhibited slightly lower AUC with good sensitivity and specificity were Fe/E, RDW/E, RDW/Fe, E, and Fe. According to the available literature, there were no studies with which to compare these results. All combined and simple parameters exhibited high AUC values, with the exception of RDW.

The values of all tested calculated parameters were statistically significantly different between the anaemic

and non-anaemic pregnant women, indicating that these parameters were good indicators of IDA. However, caution should be exercised when interpreting the results, taking into account the pre-analytical requirements of certain parameters. It should also be emphasised that several factors must be considered when making a diagnosis; laboratory findings are only one part of the process. The patient's medical history and clinical manifestations, together with laboratory results, contribute to the overall diagnostic picture. In the present study, some patients with borderline anaemia were asymptomatic, exhibiting no signs typically associated with anaemia. By contrast, other patients presented with symptoms that correlated with the severity of their anaemia. The patients reported symptoms such as shortness of breath, rapid heartbeat, increased fatigue, weakness, headache, dizziness, and poor concentration (5). Although some of these complaints may also be attributed to typical pregnancy symptoms, particularly in the third trimester, laboratory analyses confirmed anaemia in these patients. Conversely, pregnant women in the control group who did not have anaemia did not report such complaints, apart from fatigue and headaches (5), which were sporadic and only mildly pronounced. It should be emphasised that exclusion criteria were carefully designed, ensuring that pregnant women with conditions or illnesses that could contribute to such symptoms were not included in the study.

According to the reviewed literature, no studies similar to the present one were identified. Most studies were conducted during the first, second or third trimesters of pregnancy (1,3,9,11,15,16,17). As noted in the introduction, parameters from basic blood count measurements were combined to determine IDA.

A low Hb concentration is an accepted and recommended indicator of IDA. Unlike ferritin, Hb reflects functional Fe but does not provide information regarding Fe stores. This could explain its reduced ability to distinguish ID, especially during the early stages. However, Hb, along with the number of red blood cells and Fe, remains a good late indicator of ID (18). Red blood cell counts and Hb concentrations in pregnant women were useful but not entirely reliable indicators of anaemia, due to physiological changes in plasma volume and red blood cell mass as well as serum Fe concentration, which is subject to diurnal variations (12).

The first laboratory indicator of ID is an increase in RDW, which reflects the variation in the size of E and is a good marker of anisocytosis. This occurs before anaemia becomes visible. Often, RDW is elevated while MCV remains within the normal range, and only with disease progression does MCV decrease (19). In the present study, RDW did not prove to be a strong enough parameter, but calculated parameters that included RDW showed notably improved diagnostic accuracy.

Earlier studies have shown that MCV can be used as an indicator of ID and IDA (3,19). This aligns with the position of the WHO, that MCV and MCH are the most sensitive indicators of ID among standard CBC parameters (19). However, in the present study, MCV did not exhibit sufficient sensitivity.

As for HCT, it has been shown to have low distinguishing power for ID but high distinguishing power for IDA. A possible explanation is the similarity between Hb and HCT in representing the oxygen-carrying capacity of E. Additionally,

HCT is often included as a parameter in point-of-care testing devices, allowing for quick determination and reducing the time required for sample transport to the laboratory (19). In the present study, HCT proved to be an excellent indicator with high sensitivity and specificity.

In the present study, a novel scoring model was developed that was shown to offer an improved tool for identifying IDA in the cohort, with each component contributing individually. The criteria for a scoring model and the threshold values for each component were established. The calculated parameters included in the score demonstrated high sensitivity and specificity and are considered superior indicators than the individual parameters alone. These novel parameters make the score unique for detecting IDA and have not been previously reported. Furthermore, as the score increases, so does the probability of IDA. As aforementioned, each component in the scoring model contributes individually. While an individual calculated parameter may fall within a reference range, the sum of the scores may still reflect the presence and severity of IDA, which is the strength of the scoring model. The prediction score proposed in the present study showed potential for routine clinical use in the early detection of IDA.

In a published study by Sultana *et al* (3), haematological parameters (RDW, Hb, MCV, MCH and MCHC) were compared in pregnant women within the first 20 weeks of pregnancy. The study concluded that RDW (sensitivity 82.3%, specificity 97.4%) demonstrated the highest diagnostic value for detecting IDA and was a reliable and useful parameter. Conversely, in the present study, RDW showed a sensitivity of 60.2% and a specificity of 66.9%, which may be attributed to the different gestational ages of the pregnant women included in the study.

In another study by the same authors (9), RDW, Hb and MCV were compared in all pregnant women who visited their hospital during pregnancy. Once again, RDW proved to be the most reliable parameter for determining IDA. However, in the present study, RDW did not exhibit sufficient sensitivity and specificity (Table III).

Rabindrakumar *et al* (17) evaluated the role of red cell indices as a screening tool for the early detection of ID in pregnant women. They concluded that ID could be predicted in the early stages using Hb and red cell indices, which are much less expensive. The results of the present study support the conclusions of the study by Rabindrakumar *et al* (17).

Bencaiova and Breymann (20) investigated the relationship between Hb, Fe status and pregnancy outcome during the second trimester. They concluded that mild anaemia and depleted Fe stores, when detected early during pregnancy, were not associated with adverse maternal and perinatal outcomes in women receiving Fe supplementation. The present study also included pregnant women who received Fe supplements, and similarly, there was no significant association between Fe supplementation and adverse maternal outcomes (21).

In conclusion, it was found that RDW/HCT and HCT had the highest diagnostic accuracy and can be used in routine practice for diagnosing IDA. These parameters, alongside the scoring model, may be used in the clinic to predict maternal IDA based on parameters obtained from routine blood tests. This would enable clinicians to diagnose IDA more accurately

in pregnant women and initiate treatment promptly to ensure the best perinatal outcomes.

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## Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

## Authors' contributions

DŽ, AV and TŠ conceived and designed the study. AV, TŠ and DŽ collected the material and data. DŽ, AV, TB, GJ and LH performed the data analysis. DŽ wrote the first draft of the manuscript. All authors revised the manuscript. DŽ, AV and TŠ confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

## Ethics approval and consent to participate

The study was performed in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Clinical Hospital Center Rijeka (Rijeka, Croatia; approval no. 2170-29-02/1-19-2; class, 003-05/19-1/110; approval date, 15 July 2019). Written informed consent was obtained from all participants for participation in the study.

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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