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HAEMATOLOGY WHITE PAPER | May 2021*

Anaemia management

Advanced RBC parameters in the differential diagnosis and management of anaemia

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* Revision of the original article published in August 2020

Anaemia – basic facts and classification

Anaemia is a global public health issue affecting an estimated third of the world's population with women and young children being predominantly concerned. According to the World Health Organization (WHO), anaemia is a condition in which the number of red blood cells (RBC) or the RBC's capability of carrying oxygen is reduced, resulting in an insufficient oxygen supply to meet the individuals' physiological demand. In order to diagnose anaemia, the haemoglobin (Hb) concentration is a good first indicator. Appropriate Hb cut-offs were first published in 1968 by a WHO expert group and have since been set to < 13 g/dL (8 mmol/L) for healthy males, < 12 g/dL (7.4 mmol/L) for healthy females, and < 11 g/dL (6.8 mmol/L) for pregnant women [1]. Accordingly, fertile women are a vulnerable group for the potential development of anaemia, along with children, elderly people and patients with chronic diseases.

Typical anaemia symptoms include fatigue, shortness of breath, tachycardia and headache. These may occur mild but lead to a serious reduction of the individual's quality of life. In the long term, permanently insufficient oxygen supply caused by untreated anaemia may seriously impair organ function. Therefore, detecting pre-anaemic conditions and diagnosing anaemia in an early phase can facilitate a timely intervention to prevent irreversible damage. The most common type of anaemia is iron deficiency anaemia (IDA). Other anaemia types result from vitamin deficiencies, blood loss events and infectious/chronic diseases. IDA can often be treated by nutritional iron supplementation, while patients with haemolytic, aplastic or myelodysplastic anaemia subtypes or anaemia of chronic disease (ACD) require precise differential diagnosis for adequate medical treatment to prevent severe state progression.

Differential diagnosis usually requires a morphological classification of RBC via the measurement of the mean corpuscular volume (MCV), which allows to distinguish between microcytic, normocytic and macrocytic anaemia (Fig. 1). Normal MCV values range between 80 and 100 fL. Impaired haemoglobin production, which is typical of IDA and β-thalassaemia, results in microcytic RBC with MCV values < 80 fL. In contrast, MCV values > 100 fL are mainly associated with abnormal erythropoiesis. For example, vitamin B12 and folate deficiencies lead to macrocytic anaemia with megaloblastic RBC. A second subtype of macrocytic anaemia is the non-megaloblastic macrocytic anaemia. Normocytic anaemia, on the other hand, is characterised by normal MCV values and a reduced number of RBC due to acute bleeding, haemolysis and/or chronic diseases (Fig. 1).

Haemoglobin concentration and morphological RBC classification provide valuable clinical insights into anaemia status, especially if iron or vitamin deficiencies are involved. Hence, efficient medical intervention depends on precise differential diagnosis. Modern automated haematology analysers offer a variety of advanced RBC parameters which serve this need. Advanced parameters

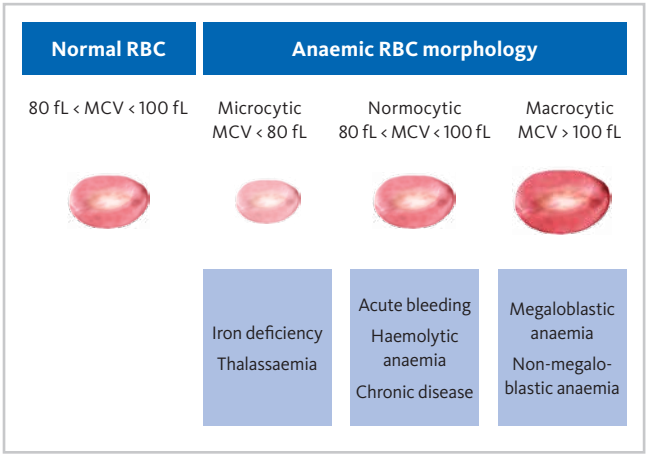


Fig. 1 Morphological classification of anaemia using the MCV.
Typically, low MCV values indicate the presence of a microcytic anaemia, which may be due to iron deficiency or thalassaemia. Normocytic types of anaemia can be caused by acute bleeding, increased haemolysis or chronic diseases, none of which alters the MCV. Macrocytic anaemia is characterised by an increased MCV and is further divided into non-megaloblastic anaemia caused by impaired erythropoiesis, and megaloblastic anaemia resulting from vitamin B12 or folate deficiency.

such as the percentage of microcytic and hypochromic RBC, reticulocyte maturation stages, immature reticulocytes and reticulocyte haemoglobin content (RET-H_e) deliver detailed data on specific cell populations. How these parameters can be used to facilitate and improve clinical decisions in anaemia management has been shown in numerous studies, which are summarised in the following paragraphs.

Detection of latent iron deficiency

Iron supply is driven by dietary intake. A lack of iron often starts latently, symptom-free, with normal MCV and Hb values, but is likely to manifest as IDA over a certain time. Especially pregnant women are at risk of complications due to latent or severe iron deficiency (ID). The same applies for toddlers and children since their bodies demand high levels of iron for rapid growth and development. Therefore, diagnosing latent ID in a subclinical stage is crucial for timely interventions with dietary changes or iron supplements. A powerful advanced clinical parameter for predicting iron deficiency is the reticulocyte haemoglobin content or equivalent (RET-H_e/ CHr, see box). For this approach, the reticulocyte haemoglobin content was recently investigated in high-risk population groups. A study conducted by Ulrich *et al.* demonstrated a better overall performance of CHr for predicting iron stores in a paediatric population of 9–12-month-old infants [2]. The authors determined the ideal threshold for CHr at 27.5 pg (1.707 fmol), with a sensitivity of 83 % and a specificity of 72 %, to include infants with a high likelihood to subsequently develop anaemia. In an adult population, Toki *et al.* compared RET-H_e to classical biochemical parameters for accurate ID screening [3]. The cut-off was set to 28.4 pg (1.762 fmol) with a corresponding sensitivity of 68 % and a specificity of 91 % –

results that match the classical biochemical values very well and indicate comparable accuracy of ID diagnosis. A similar cut-off was applied in a study assessing RET-H_e as a routine screening tool for latent ID of blood donors (Hb > 12.5 g/dL, > 7.8 mmol/L) [4]. The RET-H_e cut-off was set to 28 pg (1.73 fmol) with a sensitivity and specificity of 91.2% and 97.2%, respectively, when compared to the soluble transferrin receptor (sTfR) value. Postmenopausal, apparently healthy women were examined by Urrechaga *et al.* and it was also concluded that RET-H_e shows significant differences between latent ID and non-ID individuals [5].

RET-H*e* and CHr – two equivalent parameters

The reticulocyte haemoglobin content is reported by two different haematology analyser systems with different terminology: the reticulocyte haemoglobin content (CHr) provided by Siemens Healthineers' Advia® haematology series and the reticulocyte haemoglobin equivalent (RET-H_e) from Sysmex.

Several studies showed that these two parameters have the same clinical meaning and strongly agree with each other in paediatric patients (RET-H_e and CHr; $y = 1.04x - 1.06$; $r^2 = 0.88$) and adults (RET-H_e and CHr; $y = 1.06x - 0.43$; $r^2 = 0.83$) [31].

Also, Jarc *et al.* found a linear correlation between RET-H_e and CHr ($r = 0.895$) and provided cut-off values for the identification of iron deficiency in iron deficiency anaemia. Altogether these studies show that CHr and RET-H_e are equivalent parameters and directly comparable [26, 31, 32].

All mentioned studies pointed out the advantages of measuring reticulocyte haemoglobin content/equivalent for assessing iron store status at an early stage. The analysis is quick, cost-efficient and comparable with or better than conventional biochemical markers, which proves the application a suitable screening parameter for latent ID.

The use of advanced RBC parameters in the management of microcytic anaemia

Detection of iron deficiency anaemia

Iron deficiency is the root cause of an estimated 50 % of all anaemia diagnoses [1]. The reference method for assessing bone marrow iron stores and detecting iron deficiency is Perls' Prussian blue staining [6, 7]. This method requires bone marrow aspiration, which is an invasive and painful procedure. As an alternative, serum ferritin levels, transferrin saturation percent (TSAT), total iron binding capacity (TIBC) and soluble transferrin receptors (sTfR) are common biochemical indicators of iron deficiency [6]. However, these parameters have limitations. Since TSAT is influenced by the daily fluctuations of serum iron, and serum ferritin is an acute-phase reactant, both parameters may not be reliable under inflammatory conditions [8, 9]. In contrast to this, RET-H_e reflects the haemoglobin content of red blood cell progenitors, which have a lifetime of one or two days in peripheral blood, and therefore gives real-time information about iron availability in the bone marrow and its incorporation into haemoglobin. RET-H_e is not affected by the acute-phase reaction [10, 11], and shows a much lower degree of biological variation than TSAT and ferritin (Table 1) [12].

Table 1 *Analytical and biological variation in parameters assessing anaemia and iron status, adapted from [12].*

	Coefficient of variation (%)				
Source of variation	**Hb**	**Hct**	**RET-H_e/CHr**	**TSAT**	**Ferritin**
Analytical	2.0	2.2	2.4	2.7	6.9
Biological	4.0	4.0	4.8	38.0	15.1
Total	6.0	6.2	7.2	40.7	22.0

Hb: haemoglobin; Hct: haematocrit; RET-H_e/CHr: reticulocyte haemoglobin; TSAT: transferrin saturation

Mehta *et al.* investigated the ability of RET-H_e and serum ferritin to assess bone marrow iron in IDA side-by-side in an adult patient cohort. Their results showed a high correlation between RET-H_e and serum ferritin ($r = 0.786$; $P < 0.0001$) and that RET-H_e is slightly better in predicting bone marrow iron stores with an area under the curve (AUC) of 0.894 compared to 0.891 for serum ferritin [7].

Buttarello *et al.* compared the efficiency of HYPO-H_e, which gives the percentage of RBC with a haemoglobin content < 17 pg (1.055 fmol), and RET-H_e to diagnose iron-deficient conditions, which were defined by serum ferritin levels < 15 µg/L (12 µg/L in women) and TSAT < 16 %. With a sensitivity of 93.1 % and a specificity of 95.1 %, RET-H_e (cut-off 30.6 pg, 1.899 fmol) showed a remarkable ability to identify IDA and performed slightly better than HYPO-H_e (cut-off 0.9 %), which achieved 84.5 % and 95.7 % for sensitivity and specificity, respectively [6].

Together, these studies showed that the advanced RBC parameters – especially RET-H_e – are a promising tool for IDA detection and assessing bone marrow iron stores. However, these parameters do not allow differentiating iron deficiency conditions from β-thalassaemia. Therefore, they should be used with caution in populations with a high prevalence of β-thalassaemia.

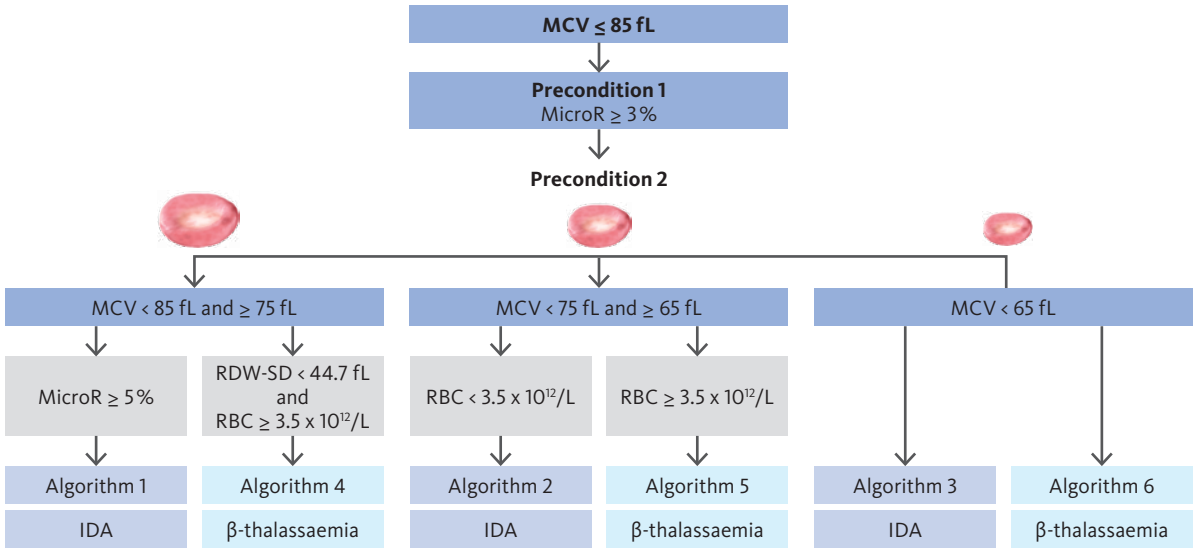
Differential diagnosis of microcytic anaemia and β-thalassaemia

Although IDA and β-thalassaemia arise from different aetiologies, their common decrease in MCV makes it difficult to differentiate them with classic haematological parameters. The most reliable

method for diagnosing β-thalassaemia is the measurement of the HbA_2 concentration using high-performance liquid chromatography. In search of a fast and easy-to-use screening tool, several authors used classic RBC parameters – MCV, mean corpuscular haemoglobin (MCH), Hb and the red cell distribution width (RDW) – to develop algorithms with the aim of differentiating IDA from β-thalassaemia patients. These algorithms turned out to be inappropriate to discriminate IDA subjects from β-thalassaemia carriers. With the availability of advanced RBC parameters, interest in such algorithms was renewed [13]. For example, Urrechaga *et al.* developed an index that combines MicroR – the percentage of microcytic RBC with a volume < 60 fL – and HYPO-H_e, which reflects the percentage of hypochromic RBC with a Hb content < 17 pg (1.055 fmol), together with the red cell distribution width (RDW). For β-thalassaemia screening, the so-called 'M-H-RDW index' provided a sensitivity of 100 % and a specificity of 92.6 %, at a cut-off value of -7.6 [13].

Schoorl *et al.* developed six algorithms – three for IDA and three for β-thalassaemia – which can be used to differentiate IDA and β-thalassaemia depending on defined preconditions (Fig. 2) [14]. For example, after the identification of a microcytic patient (MCV ≤ 85 fL), microcytic erythropoiesis should be confirmed by MicroR ≥ 3 %, which is the first precondition. Using more specific MCV ranges as a second precondition, in combination with MicroR, RDW and/or RBC cut-off values, guides the decision which algorithm should be used to identify IDA (algorithms 1–3) or β-thalassaemia (algorithms 4–6). Direct comparison of the diagnostic efficiency in a study population consisting of 142 IDA, 34 β-thalassaemia and 309 healthy subjects revealed that the algorithms developed by Schoorl *et al.* performed better than previously published indices. Algorithms 1–3 (IDA) showed a sensitivity of 79 % and a specificity of 97 %, while algorithms 4–6 (β-thalassaemia) demonstrated a sensitivity of 74 % and a specificity of 98 % [14].

The combination of advanced RBC parameters with conventional indices represents a valuable tool, which improves β-thalassaemia screening and helps in the differential diagnosis of IDA and β-thalassaemia.



No.	Algorithm	Condition
1	MicroR / HYPO-H_e < 4.0 and [(MCV^2 x RDW-CV) / (HGB x 100)] ≥ 75 and RET < 0.08	IDA
2	MicroR / HYPO-H_e < 3.4 and [(MCV^2 x RDW-CV) / (HGB x 100)] ≥ 77 and RET < 0.08	IDA
3	MicroR – HYPO-H_e – RDW-CV < -5.2	IDA
4	[(MCV^2 x RDW-CV) / (HGB x 100)] < 75 and [MCV – RBC – 3.4 – (5 x HGB)] < 4.0 and RET < 0.08 and Delta-H_e ≥ 0	β-thal
5	[(MCV^2 x RDW-CV) / (HGB x 100)] < 77 and MicroR / HYPO-H_e ≥ 2.0	β-thal
6	MicroR – HYPO-H_e – RDW-CV ≥ -5.2	β-thal

Fig. 2 Differentiation of IDA and β-thalassaemia using six novel algorithms developed by Schoorl et al.
After the identification of microcytic RBC (MCV ≤ 85 fL), precondition 1 (MicroR ≥ 3 %) is used to confirm microcytosis. Three different MCV ranges are used as precondition 2 to categorise this microcytosis. After precondition 2, further decision criteria guide to either an appropriate algorithm to identify IDA or an algorithm for β-thalassaemia identification. Formulas of respective algorithms are shown in the table below the flow chart. Adapted and modified from [14]. Units used for the parameters of the alorithm: RBC (× 10^{12}/L), Delta-H_e (pg), haemoglobin HGB (g/dL), HYPO-H_e (%), MCV (fL), MicroR (%), RDW-SD (fL), RDW-CV (%), RET (× 10^{12}/L).

Utility of advanced RBC parameters in the management of anaemia of chronic disease

Differentiation of iron deficiency anaemia and anaemia of chronic disease

ACD is the second most prevalent type of anaemia worldwide. It can evolve from many different aetiologies, for example kidney disorders and malignancies. In most cases, ACD develops as a normocytic anaemia, which means MCV values are unaffected. Biochemical markers such as sTfR and the ratio sTfR/log ferritin have been shown to be reliable in clinical use but have limitations.

Nevertheless, classical IDA, ACD and a combination of ACD/IDA are difficult to distinguish. Since RET-H_e is a useful parameter for the detection of ID and IDA in certain patient cohorts, it was also considered useful in the differentiation between IDA and ACD patients. The study of Canals *et al.* reported significant differences in the values obtained for RET-H_e between IDA and ACD patients [15]. The ability of RET-H_e to discriminate IDA from ACD was further confirmed by Urrechaga *et al.* Interestingly, not only RET-H_e but also HYPO-H_e showed significant differences between patients with iron deficiency, chronic kidney disease (CKD) and haemodialysis patients [11, 16].

A powerful tool for discriminating classic IDA from ACD and the combined condition of functional iron deficiency with ACD is the so-called 'Thomas plot', developed by Thomas *et al.* The four-bay diagnostic plot combines RET-H_e with the sTfR/log ferritin ratio to guide the treatment decision whether iron supplementation (IDA and ACD with IDA) or erythropoietin application (ACD and ACD with IDA) is applicable (Fig. 3) [10].

The Thomas plot was shown to be attractive in preoperative anaemia management. Enko *et al.* used the Thomas plot to guide the decision whether a patient with preoperative anaemia (Hb < 13 g/dL, < 8.1 mmol/L) should be supplemented with 200 mg iron intravenously and 40,000 international units (I.U.) of erythropoiesis-stimulating agents (ESA) in case the plot indicated ACD, or with 1,000 mg iron intravenously and 10,000 I.U. ESA if ID or combined ACD/ID was indicated. When using these criteria, patients with elective hip or knee surgery showed higher pre- and post-operative Hb levels compared to untreated anaemic patients and received 44 % less RBC transfusion units [17].

In addition to RET-H_e, also Delta-H_e has come into focus as a useful parameter in the management of anaemia and the differential diagnosis of ACD/IDA [18]. Delta-H_e represents the difference between reticulocyte and red blood cell haemoglobin content, therefore reflecting the trend of iron incorporation into erythroid precursor cells.

By combining Delta-H_e and RET-H_e, Weimann *et al.* developed a novel diagnostic plot. The 'Haema plot' (Fig. 4) provides rapid information about changes in erythropoiesis, which could help to decipher the root cause of disease-related types of anaemia under inflammatory conditions [18]. Interestingly, both parameters, Delta-H_e and RET-H_e, were able to distinguish ACD and sepsis patients receiving or not receiving therapy, which makes them interesting parameters for therapy monitoring.

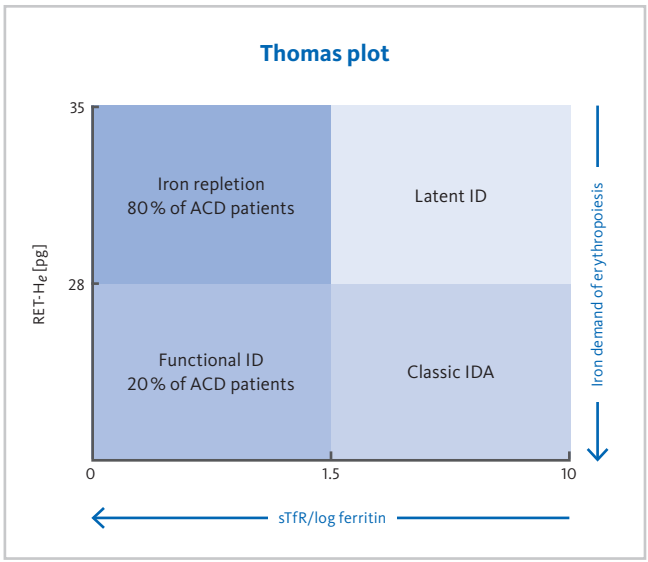


Fig. 3 Diagnostic plot for the assessment of iron status in iron deficiency (ID) according to Thomas et al. *The Thomas plot is a four-bay plot which combines sTfR/log ferritin and RET-He. It gives information about the iron-deficient state and helps to differentiate IDA from ACD. Adapted and modified from [10].*

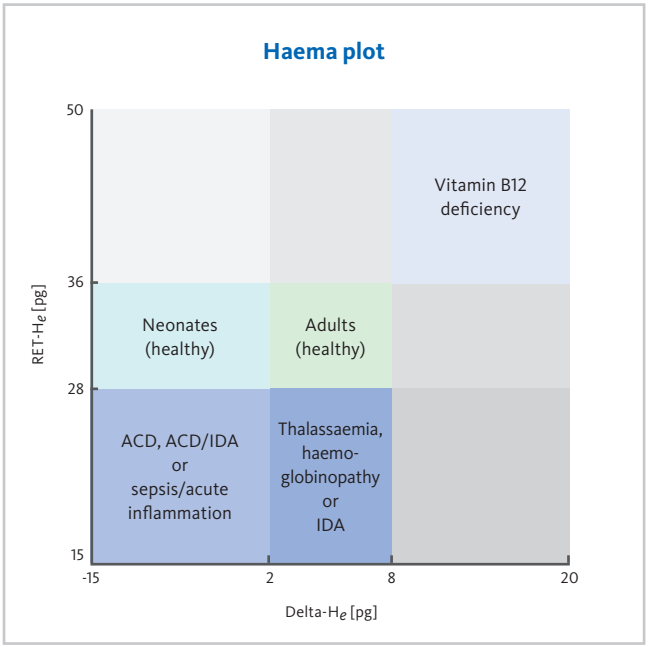


Fig. 4 Haema plot for the management of various disease-specific types of anaemia [18]. *The nine-bay Haema plot merges RET-He and Delta-He values. The bays are consecutively numbered from one to nine and patients can be assigned according to the respective values for the two biomarkers. Adapted and modified from [18].*

Iron deficiency and management of anaemia in renal disorders

In chronic kidney disease (CKD), anaemia develops from impaired erythropoiesis, which is often due to a lack of erythropoietin (EPO) production. Administration of ESA, such as recombinant human erythropoietin (rHuEPO), is an efficient treatment and has the potential to rectify impaired RBC production fully. However, insufficient iron availability, caused by an absolute or functional iron deficiency, significantly limits the therapeutic efficiency of rHuEPO [19]. Therefore, the identification of patients who need additional iron supplementation is necessary. Since biochemical parameters such as serum ferritin and transferrin saturation were shown to be less accurate in assessing functional iron deficiency under inflammatory conditions [6], several clinical practice guidelines propose the percentage of hypochromic red cells and the reticulocyte haemoglobin content as parameters for the evaluation of iron deficiency and assessment of iron therapy targets in CKD patients (Fig. 5) [20, 21].

Initial evaluation of iron deficiency in CKD

Cellular assessment

- Hb < 11 g/dL
- RBC indices (MCH, MCHC, MCV)
- White blood cell and differential count
- Platelet and reticulocyte count

Iron assessment

- Hypochromic cells % (if sample < 6 h old)
- Reticulocyte Hb (RET-H_e)
- Serum ferritin
- C-reactive protein

Iron therapy targets

- Hypochromic cells < 6 %
- Reticulocyte Hb (RET-H_e) > 29 pg
- Ferritin > 100 µg/L
- TSAT > 20 %

Fig. 5 Renal association's clinical practice guideline on anaemia of chronic kidney disease [21]. *Hb < 11 g/dL is equal to Hb < 6.8 mmol/L in SI units. Accordingly, RET-H_e > 29 pg is equal to RET-H_e > 1.8 fmol.*

By now, several studies showed that RET-H_e can be used to assess the target of iron supplementation in CKD patients undergoing haemodialysis [22] and is a useful parameter for evaluating the need of iron supplementation during rHuEPO treatment [20]. Especially in children on chronic dialysis, where anaemia evolves from iron deficiency rather than insufficient EPO production, RET-H_e was shown to be a much better biomarker for iron deficiency than TSAT and ferritin [23]. In end-stage renal disease (ESRD) patients undergoing peritoneal dialysis, Danielson *et al.* observed a correlation of Delta-H_e with inflammation markers IL-6 and hs-CRP. Additionally, their study revealed an association of Delta-H_e with response to ESA treatment and all-cause mortality risk, suggesting it to be a useful marker for risk assessment and prediction of ESA response in ESRD patients undergoing peritoneal dialysis [24].

In summary, RET-H_e is a valuable parameter for assessing the iron status in CKD patients and more reliable than other biochemical parameters. Especially in patients undergoing haemodialysis, the use of RET-H_e is advantageous and allows adjusting iron supplementation and EPO therapy according to a patient's needs. Besides RET-H_e, also Delta-H_e is of great interest as an alternative inflammation marker for the prediction of ESA response and risk assessment in renal disorders.

Reference ranges for advanced RBC parameters

For the advanced RBC parameters, the reference ranges on a large healthy cohort of Caucasians has not been published so far. However, in a recent study, van Pelt *et al.* analysed 12,782 blood samples from Dutch healthy individuals and established reference ranges for these parameters (Table 2) [33]. Despite this comprehensive data and respective reference ranges, the suitability of reference ranges in a given patient population should always be tested according to the recommendations of the International Federation of Clinical Chemistry and Laboratory Medicine [30].

Table 2 *Reference ranges for advanced RBC parameters on Sysmex XN-Series haematology analysers [33].*

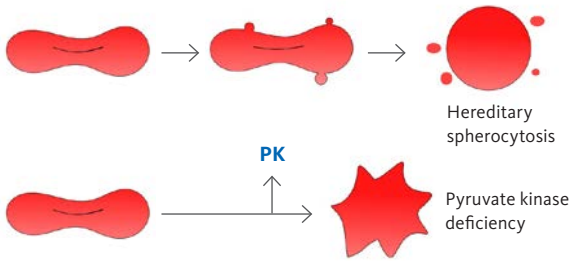
XN parameter	Reference range
RET-H_e	29.7 – 35.4 pg (1.731 – 2.197 fmol)
IRF	2.7 – 14.9 %
Delta-H_e	1.4 – 3.7 pg (0.087 – 0.23 fmol)
MicroR	0.3 – 3.9 %
MacroR	2.9 – 4.8 %
HYPO-H_e	0.0 – 0.2 % (male) 0.0 – 0.4 % (female)
HYPER-H_e	0.5 – 0.9 % (male) 0.4 – 0.8 % (female)

Differential diagnosis of rare haemolytic anaemia

Haemolytic anaemias such as hereditary spherocytosis (HS) and pyruvate-kinase deficiency (PKD) are classified as normocytic types of anaemia and diffential diagnosis is particularly challenging due to the lack of sensitivity and specificity of commonly used methods [25]. HS evolves from different molecular defects leading to membrane skeleton abnormalities and the formation of spherically shaped RBC.

In this context, the normal CBC with a slightly elevated MCHC (mean cellular haemoglobin concentration) value was shown to be relevant to identifying certain patients with RBC membrane diseases including HS. The RBC score in Sysmex's CBC-O concept has been found to allow highly sensitive detection of RBC disease [26]. The search for an easy-to-use HS screening tool based on routine haematology parameters revealed that an increased reticulocyte (RET) count and an elevated ratio of total reticulocytes to the immature reticulocyte fraction (RET/IRF) are associated with HS. This discovery led to their implementation in the International Council for Standardization in Haematology (ICSH) guidelines as criteria for the diagnosis of HS [27, 28]. These findings were based on a study conducted by Mullier *et al.*, who used a reticulocyte count ≥ 80,000/µL and RET/IRF > 7.7 to identify HS patients. Their study also showed that RET/IRF > 16 could be used to identify trait or mild HS and further defined HS severity by MicroR ≥ 3.6 % and MicroR/HYPO-H_e ≥ 2.5 (moderate) and ≥ 2.0 (severe). This tool was further adapted by Persijn *et al.*, who found a better performance by increasing the reticulocyte count threshold to ≥ 100,000/µL and decreasing the cut-off for MicroR to ≥ 2.6 % [29].

Based on these findings, Bobeé *et al.* developed a specific and easy-to-use screening tool for HS, which achieved a sensitivity of 100 % and a specificity of 92.1 % by combining the RET/IRF ratio with haemoglobin level, reticulocyte count, MicroR and HYPO-H_e. Using the same parameters, Bobeé *et al.* also developed criteria for diagnosing PKD and established the first automated blood count-based screening tool for the identification of PKD patients with a sensitivity of 100 % and a specificity of 96.5 % (Fig. 6). Both the HS and PKD screening criteria proved to be effective in HS patients with anaemia and in children under the age of three years [25].



Screening criteria Parameter	**HS** Cut-off	**PKD** Cut-off
1. RET	> 80 x 10^9/L	> 150 x 10^9/L
2. RET/IRF	> 9.1	> 9.5
3. MicroR (Hb < 12 g/dL*)	< 2.2 %	< 5.5 %
4. MicroR / HYPO-H_e (Hb > 12 g/dL*)	≥ 3.5	< 6.0

Fig. 6 Scheme of abnormal RBC membrane formation due to HS and PKD with the respective screening criteria based on advanced RBC parameters for the haematological identification of HS and PKD patients.

By using extended RBC parameters, Bobee et al. developed a screening tool for HS, which allows to identify HS and PK patients with the given cut-off values for reticulocyte count (RET), the ratio of reticulocytes to the immature reticulocyte fraction (RET/IRF), and, depending on the Hb level, the percentage of microcytic RBC (MicroR) or the ratio of microcytic to hypochromic RBC (MicroR/HYPO-H_e). Hb < 12 g/dL is equal to Hb < 7.4 mmol/L. Adapted and modified from [25].

**Hb concentrations only considered for HS patients.*

Conclusion

Modern haematology analysers easily measure a multitude of different parameters, which allow obtaining a fast and clear picture of the anaemic status of a patient. Several studies showed that the advanced red blood cell parameters are a valuable tool for anaemia management and can guide clinicians in their decision on the best and most efficient therapy for an anaemic patient. Moreover, combining advanced RBC parameters with each other or with classic parameters opens a variety of new opportunities for the early diagnosis of different types of anaemia, and the monitoring of disease progression and response to therapy.

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