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HAEMATOLOGY WHITE PAPER | September 2023*

RBC diseases

Management of RBC indices abnormalities in the context of RBC diseases

Introduction

Diseases of the red blood cells affect both children and adults and require immediate attention in order to alleviate the symptoms. These diseases can be either hereditary, where one or both parents pass a defective gene to their offspring, or temporary, where the symptoms are acquired during lifetime but can be reversed with the appropriate treatment. Hereditary RBC diseases arise from defects in haemoglobin or the cell membrane, which are the two main structural components of a red blood cell, or from enzymatic defects in the RBC metabolism. Advancements in haematology analysers have made it possible to suspect such diseases through the interpretation of advanced complete blood count (CBC or FBC) parameters and intelligent algorithms.

Haemoglobinopathies

An introduction to haemoglobin

A red blood cell is mainly comprised of haemoglobin and water, making the haemoglobin molecules the centre of interest in an RBC. A haemoglobin molecule is always comprised of four globin chains, each of which includes the haem group, a cyclic porphyrin with coordinated Fe^{2+} that binds and transports oxygen (see Figure 1). A variety of different globin chains exists and different types of haemoglobin molecules are built with various globin chain compositions. The most common haemoglobin type in healthy adults is HbA_0 (accounting for 97.5 % of the total molecules) made of two α-globin and two β-globin chains ($\alpha_2\beta_2$). The remaining 2.5 % consists of HbA_2 ($\alpha_2\delta_2$), while HbF, the fetal haemoglobin ($\alpha_2\gamma_2$), is the main molecule during gestation [1].

What characterises haemoglobinopathies?

Haemoglobinopathies are a large group of genetic disorders, describing quantitative or qualitative disturbances in the production of the haemoglobin globin chains (see Figure 1). Qualitative disturbances in haemoglobin production can result in haemoglobin variants. To date, more than 1,000 haemoglobin variants caused by mutations in specific genes have been identified [1]. The majority of variants are of no clinical consequence for the carrier, but some are unstable and can lead to haemolytic anaemia. Unstable haemoglobin carriers are rare and often remain undiagnosed.

Quantitative disturbances in haemoglobin synthesis often lead to thalassaemias. This large group of blood disorders affects the synthesis of the α- or β-chain on a genetic level. Beta-thalassaemia is the most frequent form exclusively affecting the β-chain of the haemoglobin which can be reduced or absent. The three main forms of beta-thalassaemias are thalassaemia major, thalassaemia intermedia and thalassaemia minor [2].

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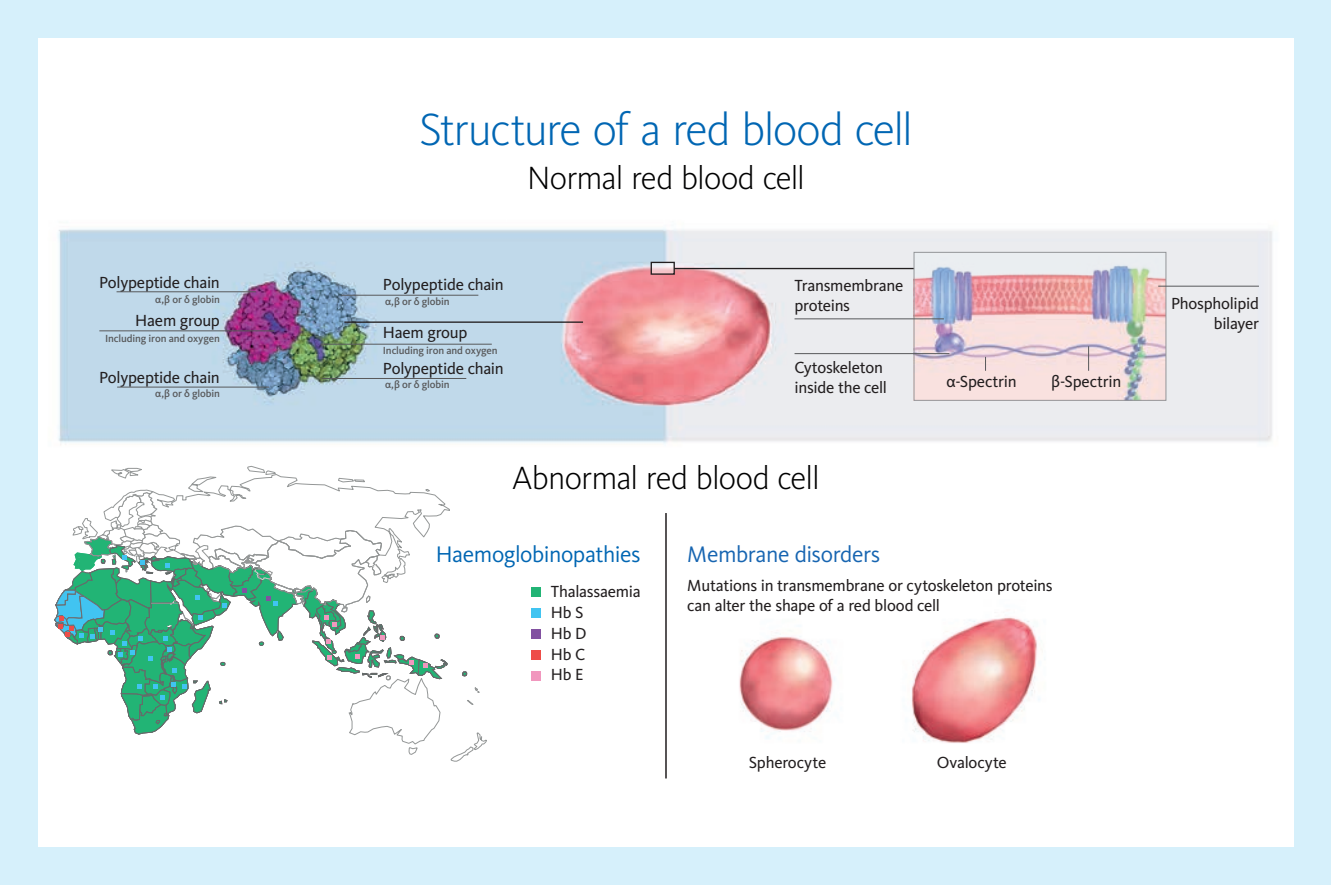


Fig. 1 Structure of a red blood cell. *A healthy red blood cell acquires its distinctive shape (top middle) from the unique composition of its membrane (top right) and can carry oxygen with the help of haemoglobin molecules (top left). RBC diseases can derive from abnormalities in the haemoglobin (bottom left) or in the structure of the cell membrane (bottom right).*

Suspicion of unstable haemoglobin variants

Several case studies have described the occurrence and consequent diagnosis of unstable haemoglobin variants from peripheral blood using Sysmex' automated haematology analysers [3–7]. According to these case studies, unstable haemoglobin variants do not act like their stable counterparts during the lysis of red blood cells, an important step during measurement of white blood cells.

Samples with unstable haemoglobin variants showed a decreased fluorescence signal in the differential channel, triggering an alarm, indicating that the differentiation of the individual white blood cell populations had not been performed properly. The authors hypothesised that the released unstable haemoglobin variants had interfered with the fluorescence marker used for white blood cell differentiation and hence interfered with the separation of the subpopulations [4, 5]. The triggered flag, in conjunction with the described abnormal scatterplots and a clinical haemolytic anaemia, should lead to further tests, such as a high-performance liquid chromatography (HPLC) analysis of the haemoglobin composition and/or genetic sequencing.

Indicators of thalassaemia

An indication for thalassaemia can be derived from several diagnostic tests, using different technologies. The results from a CBC are the first point of contact with a patient who is unaware of an underlying thalassaemia, with several blood parameters having been shown to effectively identify those patients. In such cases, it is crucial to distinguish between different root causes for the anaemic condition in order to establish the appropriate therapy strategy.

Danise *et al.* utilised the nucleated red blood cell (NRBC) count to effectively distinguish thalassaemias from other hereditary RBC diseases. In a study cohort of thalassaemia major, thalassaemia intermedia and hereditary spherocytosis patients, the presence of NRBC in the peripheral blood was shown to be a clear indicator for thalassaemia, correctly identifying 100 % of thalassaemia major and 87 % of thalassaemia intermedia cases, while hereditary spherocytosis patients had no NRBC [8].

A large research effort has focused on distinguishing beta-thalassaemia from iron deficiency anaemia (IDA), two conditions with different aetiologies, but which share the presence of microcytic

red blood cells, making the differentiation between the two difficult. IDA is an acquired condition where there is little iron for haemoglobin synthesis. Urrechaga *et al.* compared the blood profiles of beta-thalassaemia carriers and IDA patients, and identified the differences between the two groups. Beta-thalassaemia carriers had increased red blood cell counts, decreased mean corpuscular volume (MCV), high percentage of microcytic red blood cells (MicroR) and moderately increased immature reticulocyte fraction (IRF). Patients with IDA, on the other hand, had increased presence of hypochromic red blood cells (Hypo-He) and an increased red blood cell distribution width (RDW-CV), a parameter that indicates anisocytosis [9].

Several groups also developed algorithms based on the blood profiles in order to differentiate between beta-thalassaemia and IDA, all starting with the shared precondition of the presence of microcytic red blood cells (MCV < 85 fL or MCV < 80 fL). Schoorl *et al.*, for instance, developed six algorithms to distinguish the two conditions, achieving 79 % sensitivity for beta-thalassaemia and 74 % sensitivity for IDA [10]. More details on the last case can be found in the white paper *'Advanced RBC parameters in the differential diagnosis and management of anaemia'* [11]. Moreover, using a cohort of 2,664 patients with microcytic anaemia, Urrechaga *et al.* validated the performance of over 20 different discriminating algorithms. Based on the diagnostic needs, there were algorithms that performed better for identifying the conditions or excluding them [12]. Further classification of beta-thalassaemias among other cases with abnormal RBC indices, not exclusively with IDA, is further discussed in the RBC Defect Workflow Optimisation chapter.

Modern automated HPLC analysers can be integrated into the laboratory workflow and provide valuable information for the identification of thalassaemias, such as the Tosoh HLC-723G11 analyser that is equipped with a dedicated beta-thalassaemia (beta-thal) mode. In the beta-thal mode the analyser separates and quantifies HbA_2 and HbF, both significantly elevated in beta-thalassaemia patients [13]. Of special interest are also beta-thalassaemia screening programmes in the Mediterranean and Middle East where inherited thalassaemias are a public health concern. The aim of these programmes is to identify the carrier status of haemoglobinopathies in engaged couples in order to consult and prevent the spread of thalassaemias. A study from the Kurdistan region of Iraq described more than 50,000 couples being consulted, resulting in 223 identified high-risk couples. The screening programme managed to reduce the rate of major haemoglobinopathies by 21.1 % [14].

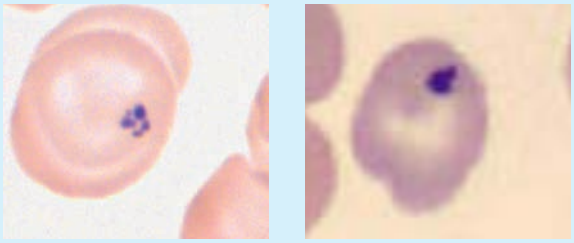
Apart from reducing smear rates in a laboratory, the careful investigation of flags from haematology analysers can lead to unexpected indications for conditions such as haemoglobinopathies or unstable haemoglobin variants and can be of great benefit to the affected carrier.

RBC inclusions

Although they do not constitute an RBC disease themselves, RBC inclusions have been described in association with anaemias or thalassaemias and could serve as an additional indication for those diseases. Inclusions are a common phenomenon and can result from many different sources. Amongst them, Pappenheimer bodies and Howell-Jolly bodies are more prominent. Pappenheimer bodies are named after the discovering physician – Alwin M. Pappenheimer – and describe RBC inclusion bodies consisting of iron in form of ferritin in the cytosol [15]. Increased erythropoiesis (e.g. caused by haemolytic anaemia) or impaired haemoglobin production (e.g. thalassaemia) can increase the presence of red blood cells with Pappenheimer bodies [16]. Howell-Jolly bodies on the other hand are DNA inclusions. These RBC inclusions can derive from a pathologic fragmentation of the erythroblast nucleus or of remaining chromosomal material after mitosis during erythropoiesis. The remaining DNA content in the cell can interfere with the reticulocyte count on a haematology analyser and lead to a pseudo-reticulocytosis [17].

Moreover, these types of inclusions are present in patients having undergone a splenectomy, because the spleen usually filters affected red blood cells and removes the inclusions without destroying the cells – a process called pitting [15]. Other inclusions such as Heinz bodies (denatured haemoglobin aggregates) or basophilic stippling (aggregates of ribosomes or fragments of ribosomal RNA) can also be found. These might influence the reticulocyte count like other red cell membrane disorders.

Stainable inclusions such as Howell-Jolly bodies interfere in the fluorescence reticulocyte (RET) channel of the analysers where reticulocytes are measured after DNA/RNA labelling. Resulting abnormalities in flags, scatterplots and count data alert the user to perform further investigations, such as a microscopic or automatic digital imaging examination of a blood smear [17].



Pappenheimer bodies left. Source: Copyright Universitätsklinikum Aachen AÖR, Klinik für Hämatologie, Onkologie, Hämostaseologie und Stammzelltransplantationen, Medizinische Klinik IV, Uniklinikum RWTH Aachen. Howell-Jolly bodies right. Source: Sheikh Khalifa Medical City, Hematology Section, Abu Dhabi, UAE

Red cell membrane disorders

Physiology of the red cell membrane

Mature red blood cells circulating in the peripheral blood have the distinctive shape of a biconcave disc that looks compressed in the middle, with a greater thickness on the periphery (see Figure 1). This shape, along with the absence of a nucleus, allows the cells to carry more oxygen molecules compared to a spherically shaped cell of the same volume (increased surface area-to-volume ratio) and to alter their shape when they navigate through capillaries that are smaller than their diameter (deformability) [18].

The membrane of the red blood cells, as with virtually all human cells, is comprised of a lipid bilayer embedded with transmembrane proteins that facilitate the exchange of ions and the free movement of water molecules. Specific to red blood cells, a network of cytoskeleton proteins, mainly spectrins and actins, is anchored in protein complexes that include the transmembrane proteins and form a skeleton mesh that laminates the cytoplasmic side of the membrane bilayer. This double structure comprised of the lipid bilayer with its proteins and the skeleton mesh gives the shape, integrity, and deformability properties in the red blood cells to enable them to circulate and survive for 120 days in the peripheral blood (see Figure 1) [19].

What characterises membrane disorders?

Any abnormality in the transmembrane or cytoskeleton proteins will result in a deviation from the biconcave disc shape, altering the physiological properties of the cells, causing reduced motility and half-life. Several such red cell membrane disorders have been the subject of extensive research in the past decades, due to the need for faster and more easy-to-use detection tests.

Hereditary spherocytosis is an inherited red cell membrane disorder characterised by the presence of spherical red blood cells that have lost their central concavity (see Figure 1). The disease arises from defects in membrane proteins, that lead to the uncoupling of the skeleton mesh from the lipid bilayer, completely altering the physiology of the cells. Spherocytes lose their deformability and become trapped as the blood is being filtered in the spleen. The increased function of the spleen to remove the spherocytes causes the organ to enlarge (splenomegaly), one of the typical clinical conditions of the disease, along with jaundice and anaemia at varying levels. Some spherocytes though escape and re-enter the circulation, and can be identified with laboratory tests [20].

The destruction of red blood cells in the spleen is compensated with a higher production and release of reticulocytes from the bone marrow. The reticulocytes in hereditary spherocytosis patients have almost abolished their genetic material, and only a few cells with higher genetic content can be found. This differentiates hereditary spherocytosis from other conditions, such as haemolytic disorders and iron deficiency, where a higher number of immature reticulocytes is found.

Similar to hereditary spherocytosis, Southeast Asian ovalocytosis is a hereditary red cell membrane disorder, arising from a mutation in the band 3 transmembrane protein that causes a dysregulation of the ionic exchange, together with structural abnormalities. The red blood cells acquire a distinctive oval shape which affects the deformability (see Figure 1), but individuals with the disease do not usually present with severe clinical symptoms, apart from signs of mild haemolysis and jaundice [21].

Another important condition that has attracted the need for a faster diagnostic test is sickle cell disease. Although it is caused by a mutation in the β-globin chain gene of haemoglobin A, which results in the generation of haemoglobin HbS, sickle cell disease has been associated with other red cell membrane disorders due to the characteristic sickle shape that the red blood cells acquire under low oxygen level conditions (see Figure 1). This sickle shape can cause ischaemic episodes as the red blood cells block the flow of blood in the capillaries. Another important factor in the pathophysiology of the disease is the very short lifespan of a sickled red blood cell of up to 20 days, which contributes to a rate of haemolysis for which the bone marrow cannot compensate [22].

Algorithm for hereditary spherocytosis

With the advancement in the technology of haematology analysers and the ability to measure reticulocytes and their different fractions, reticulocytosis has been introduced as an additional clinical and diagnostic feature of the disease. The first researchers to utilise these features were Mullier *et al.* in their diagnostic tool for the screening of hereditary spherocytosis based on blood count results. In their cohort, all 45 patients with confirmed disease had a reticulocyte count (RET) ≥ 80,000/µL and a reticulocyte to immature reticulocyte fraction ratio (RET/IRF) greater than 7.7. These values served as a precondition to screen hereditary spherocytosis cases with a sensitivity of 100 % in differentiating them from haemolytic disorders, iron deficiencies, healthy controls and other routine samples. Trait or mild cases were characterised by haemoglobin levels (HGB) greater than 12.0 g/dL (7.4 mmol/L) and RET/IRF ≥ 19.

Further severity of the disease was assessed with the use of advanced clinical parameters derived from the blood count. More severe cases are characterised by the release of microparticles, parts of the membrane that have detached from the red blood cell due to the uncoupling from the skeleton mesh, which is reflected in an increase in the MicroR parameter. Moreover, red blood cell hypochromia can be assessed with the Hypo-H*e* parameter. Those two parameters combined allowed the identification of moderate or severe cases: patients with MicroR ≥ 3.5 %, and MicroR/Hypo-H*e* ≥ 2.5 (for HGB between 8.0 and 12.0 g/dL, 5.0 and 7.4 mmol/L) or MicroR/Hypo-H*e* ≥ 2.0 (for HGB < 8.0 g/dL, 5.0 mmol/L) [23].

This algorithm for the identification of hereditary spherocytosis, using only the CBC results from a Sysmex haematology analyser, was integrated in the guidelines for the laboratory diagnosis of red cell membrane disorders issued by the International Council for Standardization in Haematology (ICSH) in 2015. The guidelines acknowledge the good performance of the algorithm and state that a positive result from the algorithm should be used in conjunction with a confirmation test in cases where there is no family history of hereditary spherocytosis documented [24].

After the publication from Mullier *et al.*, several other groups validated the algorithm and adjusted the cut-offs to better reflect the individual patient cohorts. Persijn *et al.*, working in a university hospital, dealt with more severe pathologies and splenectomised patients. Splenectomy, a common surgical procedure in hereditary spherocytosis patients, has been found to lower the reticulocyte levels in the peripheral blood. Compensation from the bone marrow is no longer needed, as the red blood cell and haemoglobin levels return to physiological levels. Since the goal was to identify only unknown patients, the group was able to increase the initial threshold of RET to 100,000/µL and decrease the MicroR threshold for severe cases to 2.6 %. These thresholds achieved 100 % sensitivity [25].

Similarly, Sottiaux *et al.*, confirmed that the algorithm is not well suited for splenectomised patients, although they did retain the initial RET threshold proposed by Mullier *et al.* and lowered the RET/IRF cut-off for trait and mild cases to 14, achieving 94 % sensitivity [26]. Finally, using the same blood parameters, Bobeé *et al.* were able to identify all hereditary spherocytosis cases, as well as all patients with pyruvate kinase deficiency, an enzymatic deficiency of the red blood cells. The following criteria were introduced for hereditary spherocytosis: RET ≥ 80,000/µL, RET/IRF > 9.1 and MicroR > 2.2 % for HGB < 12.0 g/dL (7.4 mmol/L) or MicroR/Hypo-H*e* ≥ 3.5 for HGB > 12.0 g/dL (7.4 mmol/L). Similarly, for pyruvate kinase deficiency: RET > 150,000/µL, RET/IRF > 9.5, MicroR < 5.5 % and MicroR/Hypo-H*e* < 6.0 [27]. The cut-offs for the parameters included in the algorithm are summarised in table 1.

Table 1 *Overview of cut-off values for the screening and identification of hereditary spherocytosis.*

	Mullier *et al.* [23]	Persijn *et al.* [25]	Sottiaux *et al.* [26]	Bobeé *et al.* [27]
HS patients				
■ number	n = 45	n = 20	n = 27	n = 47
Precondition				
■ RET#	≥ 80,000/µL	≥ 100,000/µL	≥ 80,000/µL	≥ 80,000/µL
■ RET/IRF	> 7.7	> 7.7	> 7.7	> 9.1
Severity				
Trait and mild RET/IRF				
■ RET/IRF	≥ 19	≥ 19	≥ 14	> 9.1
Moderate and severe				
■ MicroR	≥ 3.5 %	≥ 2.6 %	≥ 3.5 %	> 2.2 % for HGB < 12.0 g/dL for HGB < 7.4 mmol/L
■ MicroR/Hypo-H*e* (8.0 g/dL < HGB < 12.0 g/dL) (5.0 mmol/L < HGB < 7.4 mmol/L)	≥ 2.5	≥ 2.5	≥ 2.5	≥ 3.5 for HGB > 12.0 g/dL for HGB > 7.4 mmol/L
■ MicroR/Hypo-H*e* (HGB < 8.0 g/dL) (HGB < 5.0 mmol/L)	≥ 2.0	≥ 2.0	≥ 2.0	
Performance				
■ Sensitivity	100 %	100 %	94.1 %	100 %
■ Specificity	99.3 %	99 % (without splenectomy)	96.7 %	92.1 %

Decision tree for spurious red blood cell indices

Although the targeted identification of hereditary spherocytosis from other haemolytic conditions improves the diagnostic and workflow efficiency, it does not fully reflect the needs of physicians and the everyday workload of laboratories. Berda-Haddad *et al.* identified that cases of RBC diseases, mainly sickle cell disease and hereditary spherocytosis, could be initially suspected from an elevated mean corpuscular haemoglobin concentration(MCHC) (> 36.5 g/dL, 22.7 mmol/L) and developed a decision tree to differentiate those diseases from other aetiologies of spurious RBC indices.

MCHC is considered a stable red blood cell index with a very narrow range. Whenever an elevated count occurs, it is important for the treating physician to understand if this phenomenon is an artefact or if it actually reflects a pathological condition (see box for more information). To solve this issue, Berda-Haddad *et al.* compared the measurements of RBC and HGB between the standard measuring technologies and the fluorescence flow cytometry analysis offered by the Sysmex automated haematology analysers. Based on the biology of the condition, along with a visual inspection of the blood sample, they created a decision tree that could identify cases of RBC cold agglutination, optical interference and RBC diseases.

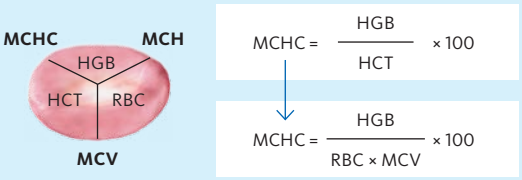
RBC cold agglutination could be detected from a difference between the optical measurement and the hydrodynamically focussed direct current (DC) detection method of red blood cell count, and since the optical method did not have any interference, it is the one reported.

Cases of optical interference and RBC disease showed the same differences in the haemoglobin between the two technologies. A distinction between the two was made possible with the RBC score, calculated from the reticulocyte count and the fragmented red cell count (FRC), the two parameters that increased in the samples with known RBC diseases.

Cases of sickle cell disease and hereditary spherocytosis had a positive RBC score and reflected cases of true MCHC elevation. The described decision tree is provided in the Sysmex *Extended* Information Processing Unit (IPU) as the CBC-O application (see box for more information) [28].

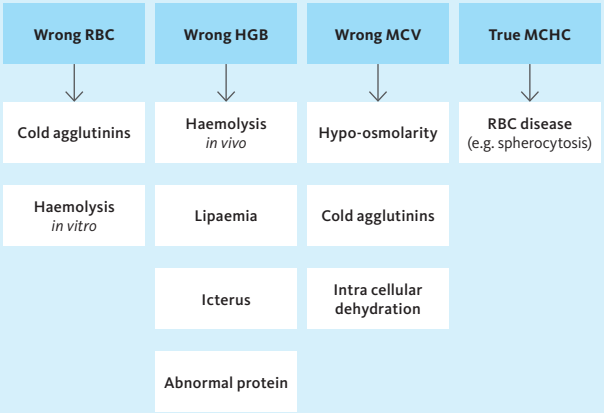
Elevated MCHC and CBC-O application

MCHC is calculated from the ratio of HGB to HCT, which in turn is calculated from the RBC count and MCV.



The CBC-O application compares the RBC indices generated by the hydrodynamically focussed DC detection and sodium lauryl sulphate (SLS)-haemoglobin methods with those from the optical channel, which along with a visual inspection of the sample suggests the most probable cause of the elevated MCHC, and proposes which values should be reported.

Pre-analytical and analytical errors with the samples can lead to incorrect RBC, HGB or MCV values which generate falsely elevated MCHC values. A typical example are cold agglutinins. A true MCHC elevation is characteristic for red blood cell diseases, such as hereditary spherocytosis.



RBC Defect Workflow Optimisation

The studies described in the previous chapters show the high interest and need for reliable diagnosis of haemoglobinopathies and red cell membrane disorders from other causes of RBC indices abnormalities. In a series of two studies, Nivaggioni *et al.* were able to provide a holistic approach and described a reliable algorithm that distinguished several hereditary red blood cell diseases (including heterozygous haemoglobinopathies, sickle cell disease, hereditary spherocytosis and Southeast Asian ovalocytosis) from patients with IDA (an acquired condition) or other conditions. The indication for each disease was accompanied by the recommended follow-up tests that can confirm the diagnosis, thus avoiding unnecessary tests that could generate spurious results and ultimately reducing the diagnostic laboratory workload.

The algorithm evaluated the presence of several advanced red blood cell and reticulocyte parameters, namely MicroR, NRBC, RDW-SD, MCHC and Hypo-H*e* to MicroR ratio. Samples with normal MCHC values (≤ 36.5 g/dL, 22.7 mmol/L) entered the algorithm from the beginning, while samples with truly elevated MCHC (> 36.5 g/dL, 22.7 mmol/L), as identified by the CBC-O application described in the previous chapter, entered the algorithm directly in the lower part (see Figure 2).

Heterozygous haemoglobinopathies and IDA presented as cases with normal values of MCHC (< 36.5 g/dL, 22.7 mmol/L) along with the presence of microcytic cells (MicroR > 12.9 %). A distinction between the two was made possible based on the fact that patients with haemoglobinopathies had a low RDW-SD (< 38.5 fL). Cases of sickle cell disease presented with both normal and elevated MCHC. In the former instance, the cases had varying levels of microcytic cells, but were distinguishable from other conditions or IDA by the higher NRBC frequency (> 0.9 %) with MCHC > 33.8 g/dL (21.0 mmol/L) or the higher RDW-SD (> 38.5 fL) with MCHC > 33.0 g/dL (20.5 mmol/L). These cases were then forwarded to the lower part of the algorithm, where they were evaluated along with the samples derived from the CBC-O application.

Hereditary spherocytosis, Southeast Asian ovalocytosis and a portion of sickle cell disease cases were all identified by the CBC-O application as true cases of elevated MCHC. Along with the remaining sickle cell disease cases from the upper part of the algorithm, they were initially evaluated by the RBC score. Sickle cell disease and hereditary spherocytosis cases had a positive RBC score, due to elevated reticulocyte counts. Southeast Asian ovalocytosis on the contrary did not manifest with increased reticulocyte release in the peripheral blood, so the RBC score remained very low (< 0.15). A differentiation between the two diseases with a positive RBC score was conducted based on the fact that cases of sickle cell disease had an IRF higher than 20 %. The cases of Southeast Asian ovalocytosis were confirmed by a Hypo-H*e*/MicroR ratio higher than 1.5 (see Figure 2 and Table 2) [29, 30].

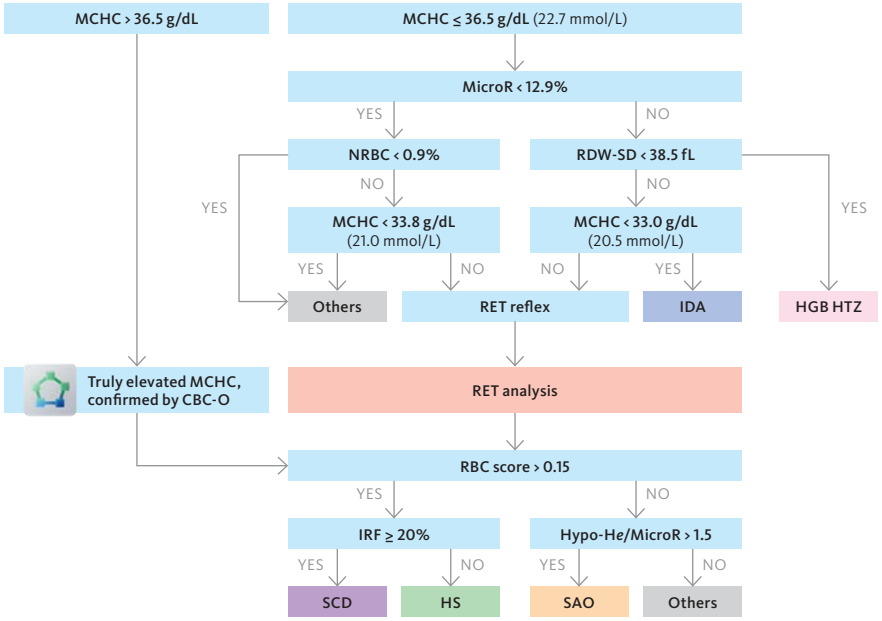


Fig. 2 RBC Defect Workflow Optimisation. *An algorithm that supports identifying cases of heterozygous haemoglobinopathies (HGB HTZ), iron deficiency anaemia (IDA), sickle cell disease (SCD), hereditary spherocytosis (HS), and Southeast Asian ovalocytosis (SAO) based on results from the CBC-O application, the RBC score, as well as advanced red blood cell and reticulocyte parameters. Adapted from Nivaggioni V et al. 2022 [30]*

Table 2 *RBC indices and RBC score from RBC diseases samples. Mean with 95 % confidence intervals are shown. Highlighted values indicate the data used in the algorithm [30].*

	Sickle cell disease	Sphero-cytosis	Ovalo-cytosis	Others
Patients	n = 24	n = 14	n = 18	n = 68
MCHC (g/dL)	36.9 [36.6 – 37.6]	37.4 [36.6 – 38.3]	37.4 [36.7 – 38.2]	37.0 [36.6 – 38.7]
RBC score	0.918 [0.15 – 1.00]	0.560 [0.01 – 1.00]	0.004 [0.00 – 0.06]	0.013 [0.00 – 1.00]
IRF (%)	30.0 [10.0 – 42.8]	9.7 [6.1 – 16.0]	14.4 [6.0 – 36.8]	10.8 [0.0 – 49.6]
Hypo-He/ MicroR	0.46 [0.11 – 1.33]	0.07 [0.00 – 0.32]	6.72 [2.78 – 11.87]	0.10 [0.00 – 0.86]
Hypo-He (%)	1.7 [0.2 – 10.5]	0.3 [0.1 – 2.5]	20.5 [9.5 – 38.7]	0.2 [0.0 – 4.6]
MicroR (%)	5.5 [0.3 – 19.9]	3.3 [1.7 – 17.2]	2.8 [1.5 – 10.1]	2.5 [0.5 – 23.0]

Conclusion

Although haematology analysers are not able to directly determine morphologic abnormalities, the biology of RBC diseases is reflected in the red blood cell indices and in advanced red blood cell parameters. Intelligent interpretation of blood counts and algorithms taking advantage of disease features can represent a novel way of supporting the identification of certain RBC diseases, with the ability to distinguish them from other aetiologies that result in abnormal red blood cell indices. The workflow in the laboratory can be optimised due to the elimination of unnecessary blood smears and cytological examination.

References

[1] ***Thom CS et al. (2013):*** *Hemoglobin variants: biochemical properties and clinical correlates. Cold Spring Harb Perspect Med 3 (3): a011858.*

[2] ***Kohne E. (2011):*** *Hemoglobinopathies: clinical manifestations, diagnosis, and treatment. Dtsch Arztebl Int 108 (31-32): 532–40.*

[3] ***Rosetti M et al. (2015):*** *A rare case of Hemoglobin Leiden interfering with the DIFF channel of Sysmex XE-2100. Scand J Clin Lab Invest 75 (5): 436–7.*

[4] ***Jongbloed W et al. (2018):*** *Unstable haemoglobin variant Hb Leiden is detected on Sysmex XN-Series analysers. Clin Chem Lab Med 56(9): e249-e50.*

[5] ***Moioli V et al. (2019):*** *Mozhaisk haemoglobin variant effects on leukocyte differential channel using the Sysmex XN-Series. Clin Chem Lab Med 57 (12): e324–e7.*

[6] ***Mongelli F et al. (2016):*** *A rare and unstable hemoglobin variant, Hb M Dothan beta 25/26 (-GTG), detected by the anomalous cytogram on Sysmex XE-2100. Clin Chem Lab Med 54 (2): e31–3.*

[7] ***Teixeira C et al. (2017):*** *Automated detection of unstable hemoglobin variants by Sysmex XE-Series analyzers. Clin Chem Lab Med 55 (11): e243–e6.*

[8] ***Danise P et al. (2009):*** *Nucleated red blood cells and soluble transferrin receptor in thalassemia syndromes: relationship with global and ineffective erythropoiesis. Clin Chem Lab Med 47 (12): 1539–42.*

[9] ***Urrechaga E et al. (2011):*** *Erythrocyte and reticulocyte parameters in iron deficiency and thalassemia. J Clin Lab Anal 25 (3): 223–8.*

[10] ***Schoorl M et al. (2012):*** *Efficacy of advanced discriminating algorithms for screening on iron-deficiency anemia and beta-thalassemia trait: a multicenter evaluation. Am J Clin Pathol 138 (2): 300–4.*

[11] ***White Paper:*** *Advanced RBC parameters in the differential diagnosis and management of anaemia.*

[12] ***Urrechaga E et al. (2017):*** *Critical appraisal of discriminant formulas for distinguishing thalassemia from iron deficiency in patients with microcytic anemia. Clin Chem Lab Med 55 (10): 1582–91.*

[13] ***Merono F et al. (2011):*** *Analytical evaluation of the Tosoh HLC-723 G8 automated HPLC analyzer for hemoglobin analysis in beta-thalassemia mode. Clin Biochem 44 (5-6): 441–3.*

[14] ***Al-Allawi NA et al. (2015):*** *Premarital screening for hemoglobinopathies: experience of a single center in Kurdistan, Iraq. Public Health Genomics 18 (2): 97–103.*

[15] ***Sears DA et al. (2004):*** *Pappenheimer bodies: a brief historical review. Am J Hematol 75 (4): 249–50.*

[16] ***Thomas L. (2020):*** *Clinical Laboratory Diagnostics 2020, 10th edition. TH-Books.*

[17] ***Jiang H et al. (2019):*** *Interferents of Automated Reticulocyte Analysis Integrated with Relevant Clinical Cases. Clin Lab 65 (7).*

[18] ***Tomaiuolo G. (2014):*** *Biomechanical properties of red blood cells in health and disease towards microfluidics. Biomicrofluidics 8 (5): 051501.*

[19] ***Lux SEt. (2016):*** *Anatomy of the red cell membrane skeleton: unanswered questions. Blood 127(2): 187-99.*

[20] ***Perrotta S et al. (2008):*** *Hereditary spherocytosis. Lancet 372 (9647): 1411–26.*

[21] ***Flatt JF et al. (2020):*** *Expression of South East Asian Ovalocytic Band 3 Disrupts Erythroblast Cytokinesis and Reticulocyte Maturation. Front Physiol 11: 357.*

[22] ***Kato GJ et al. (2018):*** *Sickle cell disease. Nat Rev Dis Primers 4: 18010.*

[23] ***Mullier F et al. (2011):*** *Additional erythrocytic and reticulocytic parameters helpful for diagnosis of hereditary spherocytosis: results of a multicentre study. Ann Hematol 90 (7): 759–68.*

[24] ***King MJ et al. (2015):*** *ICSH guidelines for the laboratory diagnosis of nonimmune hereditary red cell membrane disorders. Int J Lab Hematol 37 (3): 304–25.*

[25] ***Persijn L et al. (2012):*** *Screening for hereditary spherocytosis in routine practice: evaluation of a diagnostic algorithm with focus on non-splenectomised patients. Ann Hematol 91 (2): 301–2.*

[26] ***Sottiaux JY et al. (2020):*** *Evaluation of a hereditary spherocytosis screening algorithm by automated blood count using reticulocytes and erythrocytic parameters on the Sysmex XN-Series. Int J Lab Hematol 42 (2): e88–e91.*

[27] ***Bobee V et al. (2018):*** *Screening of hereditary spherocytosis and pyruvate kinase deficiency by automated blood count using erythrocytic and reticulocytic parameters. Int J Lab Hematol 40 (6): 697–703.*

[28] ***Berda-Haddad Y et al. (2017):*** *Increased mean corpuscular haemoglobin concentration: artefact or pathological condition? Int J Lab Hematol 39 (1): 32–41.*

[29] ***Nivaggioni V et al. (2020):*** *Use of Sysmex XN-10 red blood cell parameters for screening of hereditary red blood cell diseases and iron deficiency anaemia. Int J Lab Hematol 42 (6): 697–704.*

[30] ***Nivaggioni V et al. (2022):*** *Detection of Southern Asian Ovalocytosis with Sysmex XN-10: A complement to the decision tree previously described. Int J Lab Hematol. 44 (2): 84–86.*

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