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SEED Haematology

Recognising special patterns and spurious results in automated blood counting

Interferences caused by unstable haemoglobin variants

The focus of this SEED article series is to present the potential root causes of some well-characterised special patterns that lead to spurious results on the Sysmex 5-part differential haematology analysers, explain which reportable parameters might be affected and highlight aspects of the scattergrams and the flagging that can support the prompt identification of these patterns. This article focuses on the effect that unstable haemoglobin variants have on the WDF scattergram, which has been extensively described in the literature.

Unstable haemoglobin variants

There have been more than 1,200 haemoglobin variants identified [1, 2], many of which result in a less soluble protein that tends to precipitate in the red blood cells (RBC) (e.g. in the form of Heinz bodies). These rare variants seem to have diverse clinical effects, ranging from asymptomatic to severe haemolytic anaemia [1–4]. Although all the described variants are related to distinct genetic mutations of the haemoglobin β-globin gene (*HBB* gene), they all present the same pattern when samples of patients carrying a haemoglobin variant are measured using the WDF channel.

The detection of unstable haemoglobin variants on Sysmex analysers

Although case reports and studies with samples containing unstable haemoglobin variants exist on various analyser models [1–7], the common denominator is the incorrect classification of white blood cells (WBC) in the WDF channel due to very low side fluorescence signals (SFL).

It is hypothesised that the unstable haemoglobin variants that are released in the sample upon the lysis of the RBC by the lysing reagent interfere with the binding of the fluorescence reagent to the nucleic acids of the WBC. This may be linked to a higher affinity of the fluorescence marker to the unstable haemoglobin, which would prevent efficient interaction with the nucleic acids of the WBC. Another hypothesis is reduced permeability of the WBC cell membrane, due to the presence of the haemoglobin variants, which would result in lower availability of the fluorescence reagent in the cells and subsequently a lower SFL signal [1, 2, 5–7].

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List of haemoglobin variants that have been described in the literature as presenting interference on Sysmex haematology analysers

- Hb Indianapolis [1]
- Hb Himeji [1]
- Hb Köln [1, 4]
- Hb Hazebrouck [2]
- Mozhaisk haemoglobin [3]
- Hb Brussels [4]
- Hb Omlteld [4]
- Hb Baille [4]
- Hb Geneva [4]
- Hb Hana [4]
- Hb Leiden [5, 7]
- Hb M Dothan [6]

Typical scattergram patterns and flag messages

The presence of unstable haemoglobin variants in a blood sample can be easily noticed in the WDF scattergram, as the WBC exhibit a very low SFL signal and are displayed at the bottom of the scattergram (Fig. 1). In all case reports of unstable haemoglobin variants described on Sysmex analysers, it has been reported that relevant flag messages, such as the 'WBC Abn Scattergram' flag, were triggered to alert the user of the abnormal pattern observed [2–4, 7].

Although the WNR channel is also based on the principle of fluorescence flow cytometry, no distinct scattergram patterns related to unstable haemoglobin variants have been reported, probably due to the different reagents utilised in this channel. As shown in Fig. 1, some examples exhibit a bigger debris cloud in the WNR scattergram, which could be related to the precipitated haemoglobin, but this does not impact on the differentiation and on the count of WBC, NRBC or basophils.

Workflow improvement

As numerous studies describe a distinct scattergram pattern that can easily point towards the presence of a haemoglobin variant [1–7], this triggered the researchers' interest in conducting further investigations that would support the prompt identification of unstable haemoglobin variants and improve the laboratory's workflow. In one such study [4], the authors investigated several patients presenting different unstable haemoglobin variants and reported that the parameters lymphocyte side fluorescence (LY-SFL; equivalent to LY-Y) and neutrophil side fluorescence (NE-SFL) demonstrated excellent sensitivity and specificity, which allowed them to appropriately classify 100 % of the unstable haemoglobin variant patients in their study cohort. Bosma *et al.* tested the proposed algorithm and enriched it by additional criteria (NEUT#, LYMPH#, along with a minimum patient age requirement of over 30 days) to increase the specificity for their patient cohort [8].

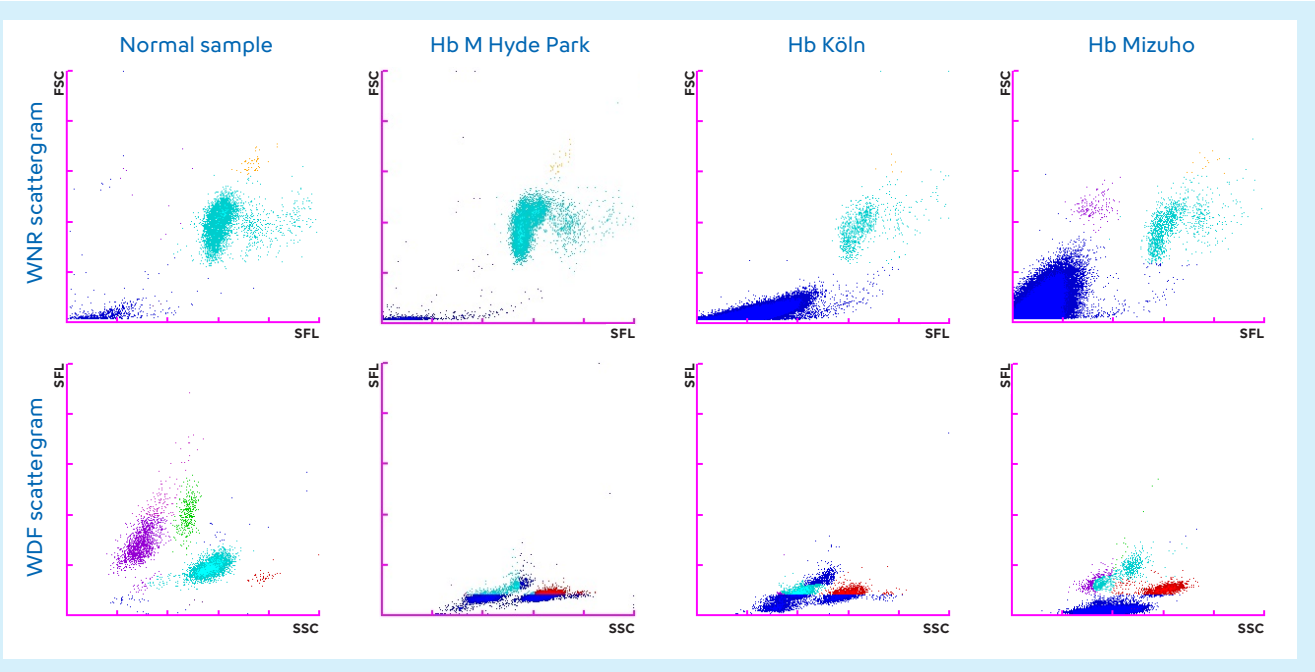

Fig. 1 Unstable haemoglobin variants create a distinct pattern in the WDF scattergram, due to interference with the reagent reaction of the WDF channel. As a result, the WBC subpopulations exhibit a low side fluorescent light (SFL) and all clusters appear at the bottom of the scattergram. The WNR channel is less affected and shows an extended debris area. In all the cases shown, the 'WBC Abn Scattergram' flag was triggered.

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Conclusion

- Cases of unstable haemoglobin variants show a distinct pattern in the WDF scattergram.
- Relevant flag messages associated with this distinct pattern can effectively alert the user of the need for further investigation.
- As shown in one study, investigation of the parameters LY-SFL (LY-Y) and NE-SFL could further support the identification of unstable haemoglobin variants and improve the laboratory's workflow.

References

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