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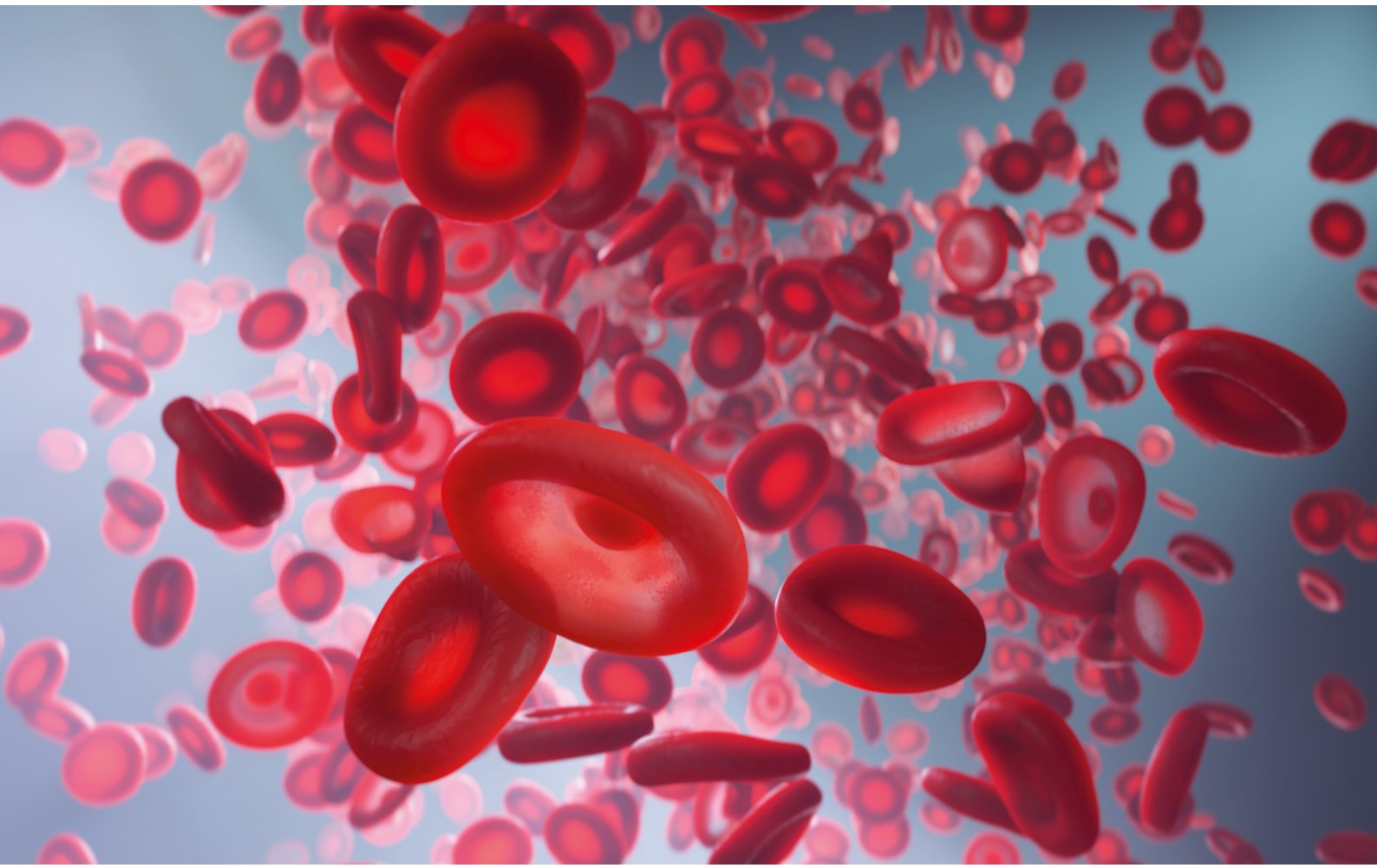
HAEMATOLOGY CASE REPORT | June 2024



Haemoglobinopathies

Presence of unstable haemoglobin variants

More than 1200 haemoglobin variants (Hb variants) have been identified so far [1, 2], which are related to distinct genetic mutations of the haemoglobin β-globin gene (*HBB* gene) and are leading to different structures and biochemical properties (e.g. oxygen affinity) of the haemoglobin molecule [3]. While the largest portion of haemoglobin variants have no clinical effect, approximately 150 unstable Hb variants are known to cause haemolytic anaemia of varying severities [1, 2, 4-6].

Fig 1 Haematology analysis results displayed by the XN IPU.

Clinical case information and laboratory results

In this patient case, a blood test was requested for a male patient during a routine check-up. The complete blood count (CBC) appeared mostly normal, except for a slightly decreased haematocrit and increased RDW-CV, triggering the flag message 'Anisocytosis'. A low MCHC triggered the flag message 'Hypochromia'. A subsequently ordered DIFF measurement presented all WBC subpopulations with very low fluorescence signals appearing in the bottom end of the scattergram. Consequently, the XN-Series analyser triggered the 'WBC Abn Scattergram' and pointed out the abnormal positioning of the cell clusters. The patient's blood smear showed some irregularly shaped RBC together with a moderate amount of target cells. A follow-up HPLC analysis revealed the presence of the mildly unstable haemoglobin variant 'Hb G-Ferrara', which was initially described in an Italian family [7]. This variant shows replacement of an asparagine residue by a lysyl residue at position β57 (β57 Asn › Lys).

Typical scattergram patterns and WBC flags in case of unstable haemoglobin variants

The presence of unstable haemoglobin variants in a blood sample can be easily noticed in the WDF channel of Sysmex haematology analysers, as the WBC exhibits a very low SFL signal, and all clusters are displayed at the bottom of the scattergram (Fig. 2). While the cause of this WDF channel alteration is not entirely explained, there are two hypotheses for this observation:

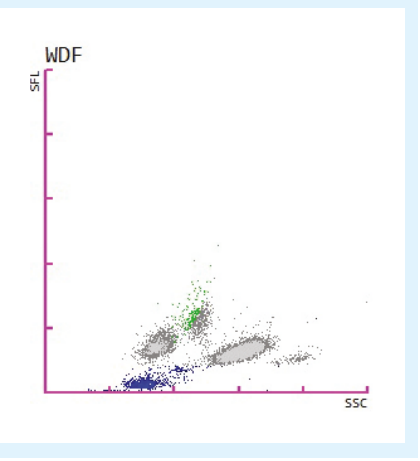


Fig. 2 WDF scattergram with confirmed unstable haemoglobin variant 'Hb G-Ferrara'.

1) The polymethine fluorescent marker, which is used in Sysmex's haematology analysers for WBC differentiation, might bind to the variant haemoglobin molecule with a greater affinity than to the nucleic acids of WBC. Thus, WBC staining is reduced showing abnormally low fluorescence levels in the WDF channel [5].

2) The presence of haemoglobin variants reduces the permeability of the WBC cell membrane, resulting in lower availability of the fluorescent reagent in the cells and subsequently a lower SFL signal.

Relevant flag messages, such as the 'WBC Abn Scattergram' flag, associated with this distinct pattern can effectively alert the user of the need for further investigation. The WNR channel is less affected and showed an extended debris area. As described in a study by Moioli *et al.* (2019), investigation of the parameters LY-SFL (LY-Y) and NE-SFL showed excellent sensitivity and specificity and could further support the identification of unstable haemoglobin variants to improve the laboratory's workflow [4].

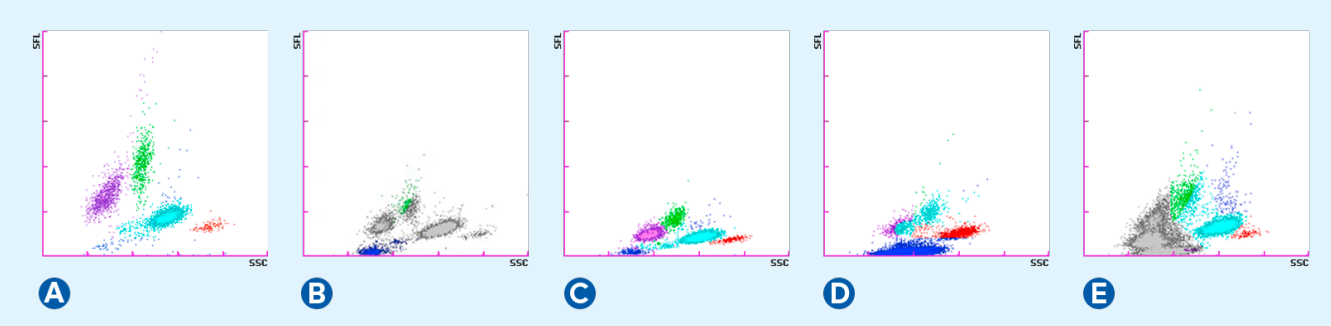


Fig. 3 Comparison of WDF scattergrams of patients with different confirmed unstable Hb variants. A: WDF scattergram of a healthy person. B: Hb G Ferrara, C: Hb Koeln, D: Hb Mihuzo, E: Hb Volga

References

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[4] 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-e327.

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[7] Giardina B *et al.* (1978): Properties of hemoglobin G. Ferrara (β57(E1) Asn›Lys). Biochim Biophys Acta. 534(1): 1-6.