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Detection of unstable haemoglobin variants with Sysmex XN-10

Sophie Combaluzier [1], Marie Loosveld [1 2], Vanessa Nivaggioni [1]

[1]Laboratoire d'Hématologie, Hôpital de la Timone, Assistance Publique–Hôpitaux de Marseille, Marseille, France
[2]CNRS, INSERM, CIML, Aix Marseille University, Marseille, France

Unstable haemoglobin variant (UHV) are a rare cause of haemolytic anaemia. UHV are less soluble and precipitate in the red blood cell (RBC) as Heinz bodies. There are several variants described worldwide, albeit rarely, and often as family disorders. These rare variants have variable clinical effects ranging from asymptomatic to severe haemolytic anaemia. Thereby these haemoglobin variants often remain undiagnosed. Moreover, the diagnosis by electrophoresis cannot highlight the abnormal fraction in such cases. Only genetics permits a certain diagnosis.

Recent studies described the detection of UHV with a Sysmex analyser.[1] The Sysmex XN-10 uses fluorescence flow cytometry with polymethine dye to differentiate the leukocyte subpopulations in the white blood cell differential (WDF) channel. The resulting staining is represented on the *Y*-axis of scattergrams as side fluorescence light (SFL). UHV seems to interfere in the WDF channel, showing a lower lymphocyte fluorescence signal than normal scattergrams (Figure 1). Nevertheless, after microscopy examination, a quantitative and qualitative analysis of the leukocytes appeared to be normal.

We present two family cases: a 33-year-old woman and her 12-year-old son, both carrying the UHV "Brussels." At their admission, the haemoglobin levels were respectively 99 and 112 g/L with mean corpuscular volume 101.3 and 81.2 fL. Reticulocytes were elevated (0.486 and 0.226 10^{12}/L) indicating highly regenerative anaemia. All other haematological parameters were within the reference ranges. Analysis performed on the Sysmex XN-10 caused an abnormal scattergram with the flag "WDF abnormal scattergram" for the mother, as shown in Figure 1. The fluorescence signal of lymphocytes and neutrophils were lower than a normal patient for both, as expected.

To establish a standard detection protocol for UHV, we completed our observation with the analysis of seven other UHV patients: four (1 Omlteld, 1 Baille, 1 Geneva, 1 Koeln) from Marseille University Hospital, Assistance Publique-Hopitaux de Marseille (AP-HM) France, two (1 Koeln, 1 Hana) from Robert Debré University Hospital, Assistance Publique-Hopitaux de Paris (AP-HP), France and one UHV Koeln from Besancon University Hospital (France). The study was approved by the local Ethics Committee of Assistance Publique des Hôpitaux de Marseille (Marseille, France). All blood samples were collected in EDTA K3 and complete blood counts were performed using Sysmex XN-10 analysers. A diagnosis of UHV was obtained using optical microscopy (blood smears with supravital staining cresyl blue and May-Grünwald-Giemsa stains), haemoglobin electrophoresis and genetics. Erythrocyte, reticulocyte and leukocyte parameters of interest were analysed as quantitative variables and summarised as median and range alongside a routine laboratory population ($n = 1585$) from our institute (Marseille). All analyses were performed using XLSTAT 2020 from Microsoft Corporation TM (Version

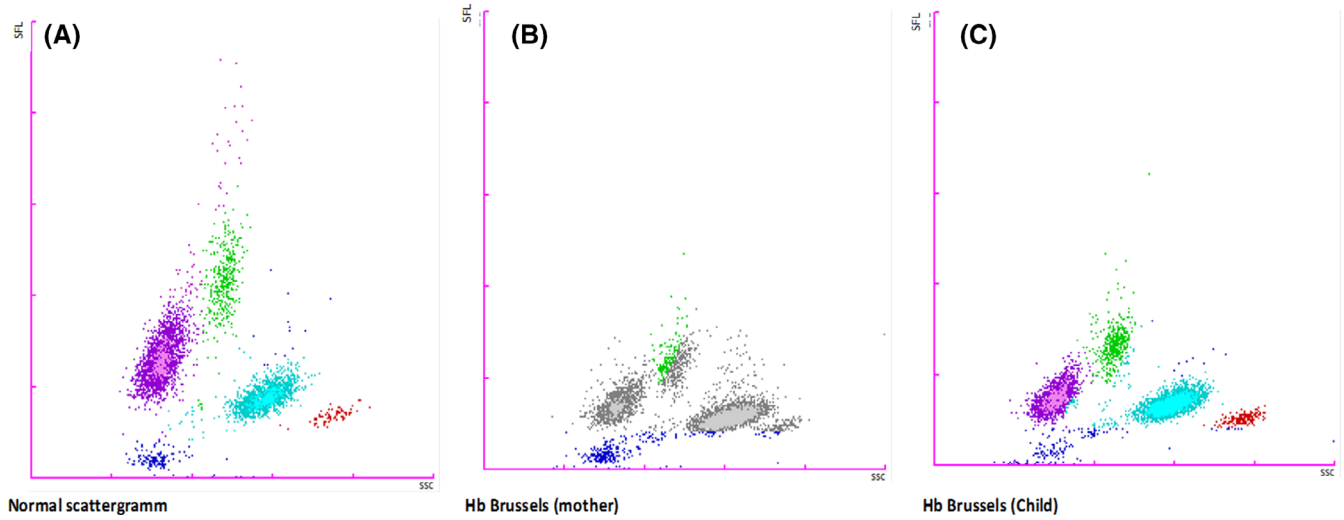


FIGURE 1 White blood cell differential (WDF) channel scattergrams on Sysmex XN-10 from a normal subject (A) and patients with UHV "Brussels": mother (B) and son (C). The flag "WDF abnormal scattergram" is present in case B

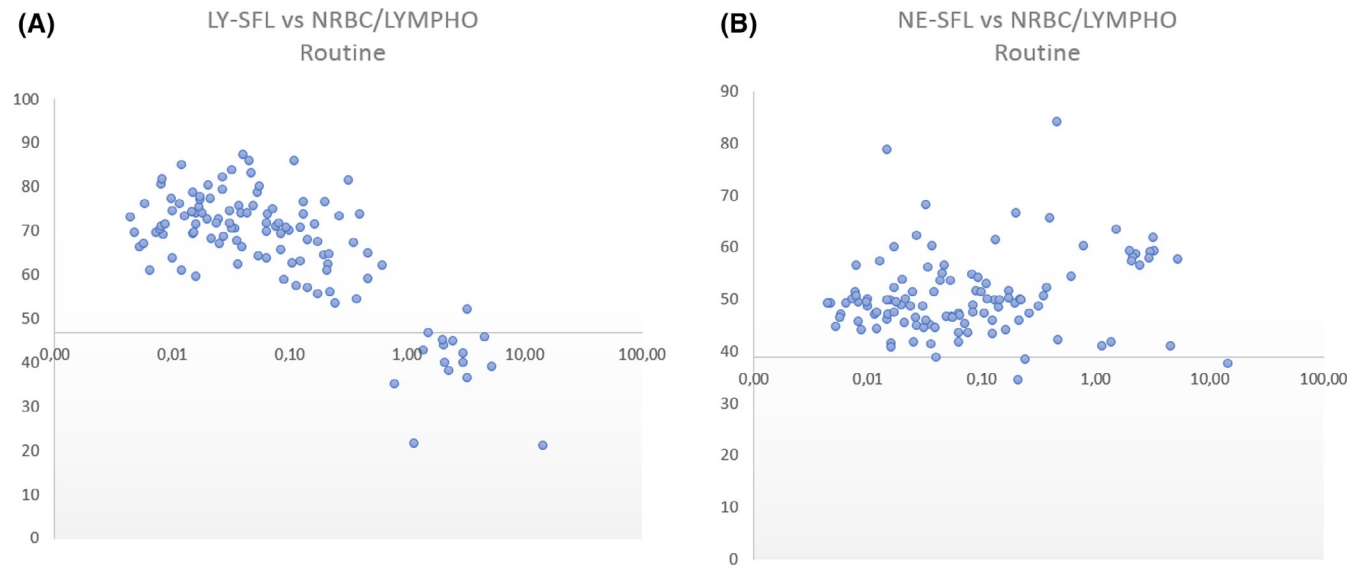
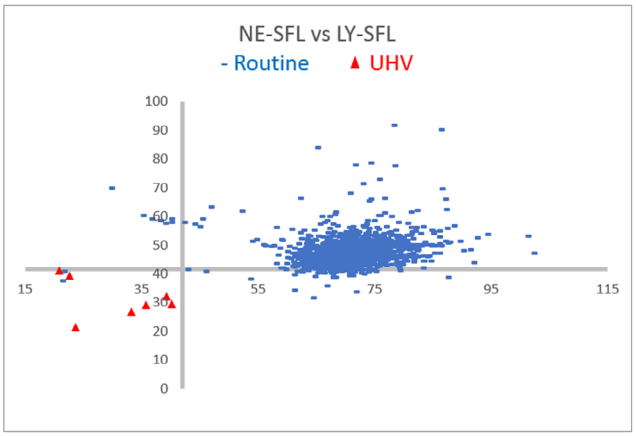


FIGURE 2 Influence of erythroblasts (NRBC)/lymphocytes ratio on lymphocytes-side fluorescence light (LY-SFL) (A) versus neutrophils-side fluorescence light (NE-SFL) (B) in a routine laboratory population

2020.3.1). RBC parameters did not demonstrate any significant difference between both groups apart from the absolute reticulocyte count. Conversely, Lymphocytes - Side Fluorescence Light (LY-SFL) and Neutrophils-Side Fluorescence Light (NE-SFL) were decreased in all UHV patients, as already previously reported.[2] The receiver operating characteristic (ROC) curves of the LY-SFL parameter and NE-SFL parameter for UHV patients show an area under the curve equal to 0.997 [0.995–1.000] and 0.996 [0.991–1.000], respectively. The LY-SFL parameter provides the best sensitivity (100%), but thanks to the comparison of this parameter with the routine population, we highlight another reason for the decrease of the LY-SFL: the presence of erythroblasts (NRBC) in the blood sample. On the other hand, the NE-SFL, whose sensitivity is slightly less than the LY-SFL, is not affected by the presence of erythroblasts (Figure 2). Its specificity is therefore better. Thus, the optimal threshold combination of both parameters to specify UHV is below 42 for LY-SFL and below 41.2 for NE-SFL together. With the association of LY-SFL and NE-SFL, 100% of UHV patients and 99.9% of other patients are well classified (sensitivity = 100%, specificity = 99.9%, positive predictive value = 81.8%, negative predictive value = 100%; Figure 3).

Overall, when an abnormally low fluorescent signal is present on the Sysmex XN-10 analyser, the presence of a UHV can be suspected. The LY-SFL and NE-SFL demonstrate excellent sensitivity and specificity, which allowed us to well classify 100% of the UHV patients. Even if the pathology is uncommon, its detection and correct classification are very important, especially because UHV are usually undiagnosed. The impact of UHV on LY-SFL and NE-SFL parameters is rather unexpected since these are two leukocyte parameters. Some authors hypothesise an interference between polymethine and UHV. Indeed, UHV released when RBC are lysed disturbs the leukocyte staining by binding to polymethine with higher affinity. Thereby, the WBC staining is reduced which leads to abnormally low fluorescence levels in the WDF channels. It is necessary to remain vigilant in the



	Value	Low Limit (95%)	High Limit (95%)
Sensitivity	1.000	0.650	1.000
Specificity	0.999	0.995	1.000
PPV (Positive Predictive Value)	0.818	0.590	1.000
NPV (NegativePredictive Value)	1.000	1.000	1.000

FIGURE 3 Association of neutrophils-side fluorescence light (NE-SFL) and lymphocytes-side fluorescence light (LY-SFL) for the classification of UHV patients versus a routine laboratory population. Sensitivity, specificity, PPV and NPV are mentioned below

clinical context since this WDF scattergram phenomenon can also be seen if the reagent is not correctly installed in the analysers. Moreover, myelodysplastic syndromes can also show a slight decrease of NE-SFL but without any effect on LY-SFL. Therefore, in case of suspicion of UHV, a reticulocyte count and a blood smear should be added.

Of course, some limitations of our study can be highlighted. The number of UHV is not very high and our population does not consider the possible overlap between the different UHV. However, this abnormal scattergram was already described in earlier reports by different authors.[3] We therefore suggest, in the same approach as the

previous article on Detection of Southern Asian Ovalocytosis with Sysmex XN-10,[4] completing the RBC algorithm previously described.[5] The sample is considered as a suspect for UHV disease if both LY-SFL and NE-SFL are lower than 42 and 41.2 respectively. The applicability of this algorithm for standardised UHV screening with the Sysmex XN-10 should be verified on a larger scale.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

Correspondence

Vanessa Nivaggioni, Laboratoire d'hématologie biologique, Hôpital de la Timone, 264 Rue Saint Pierre, 13385 Marseille cedex 05, France.

Email: vanessa.nivaggioni@ap-hm.fr

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