



# Accuracy of Sysmex XN immature granulocyte percentage compared to manual microscopy

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**Background:** We evaluated autovalidation limit for immature granulocyte percentage (IG%) measured with Sysmex XN (Sysmex Co., Kobe, Japan) to optimize microscopy review for white blood cell (WBC) differential samples. The purpose was to determine if the autovalidation limit can be increased from IG 3%.

**Methods:** We analysed WBC differential with Sysmex XN and manual microscopy in samples with IG >3% and no other analyser flagging (N=557). The reference samples (N=245) had IG <3% and no analyser flagging. The comparison was based on IG% subgroups. The effect of total WBC and absolute neutrophil count were examined.

**Results:** Sysmex XN gave median positive error compared to manual microscopy with all IG% levels. The error was higher in subgroups with IG >3% compared to IG <3% (P<0.001). Within IG 3–5.9% subgroups there was no statistically significant difference. The error increased above IG 6%. Neutropenia or neutrophilia did not affect the degree of difference in IG% between the methods (P=0.296). In samples with low WBC, the IG% difference was slightly higher (P<0.001) compared to samples with normal or high WBC.

**Conclusions:** The positive difference for Sysmex XN IG% is acceptable and clinically not significant up to IG 6%. This autovalidation limit is adequate for reporting IG as a separate parameter (six-part WBC differential). Samples with higher IG% should be reviewed. Autovalidation limit of IG 3% should be used for five-part differential to detect and report neutrophil left shift.

**Keywords:** Sysmex XN; immature granulocytes (IG); immature granulocytes percentage (IG%)

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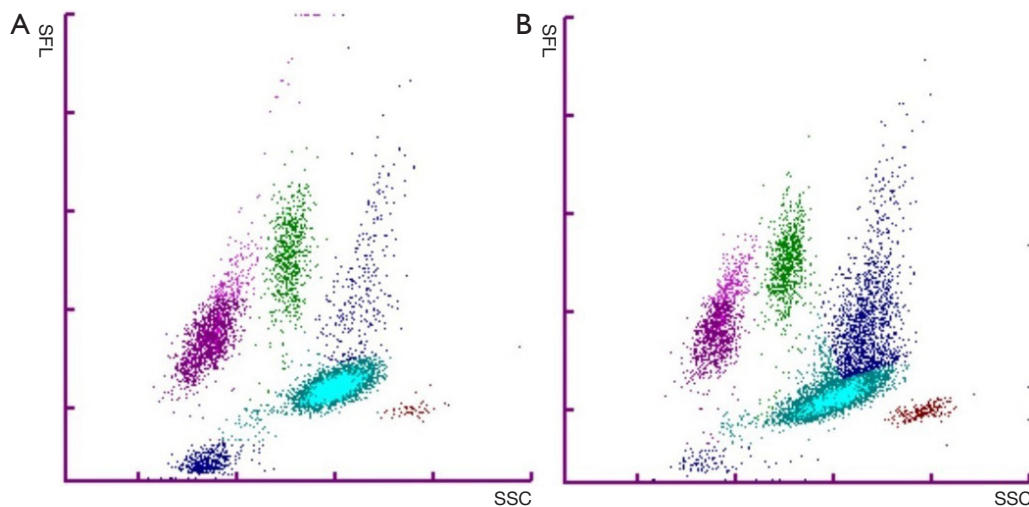
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## Introduction

Immature granulocytes (IG) are neutrophilic cells produced by bone marrow in response to inner or outer stimuli, e.g., infection, inflammation or physiological stress (1). Metamyelocytes, myelocytes and promyelocytes are classified as IG, while segmented and band form neutrophils are considered mature. Blast cells are not included in the IG

class. Neither are eosinophilic or basophilic immature cells classified as IG. Under normal physiological conditions IG% is low, less than 1% (2). Higher IG% is linked to several medical conditions needing urgent treatment, such as bacterial sepsis, pancreatitis and appendicitis (3-8). Modern haematology analysers are able to classify IG based on the size, granularity and nucleic acid content of

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**Figure 1** An example of Sysmex XN white blood cell differential (WDF) channel scatter gram with side-scatter (SSC) versus side fluorescence (SFL) light, a. immature granulocyte (IG) 3.9% and total white blood cell (WBC) count  $8.4 \times 10^9/L$ , and (B) IG 18% and WBC count  $8.2 \times 10^9/L$ . Different cells are indicated with colours, purple: lymphocytes, green: monocytes, turquoise: neutrophils (+basophils), red: eosinophils. Events coloured in blue above the neutrophil cloud are IG. The events on low right corner are debris. Basophils are enumerated separately on white cell nucleated (WNR) channel.

the cells (9). For white blood cell (WBC) analysis Sysmex XN analysers lyse red blood cells and use flow cytometry with a semiconductor laser (633 nm) to detect cells based on forward-scattered (FSC), side-scattered (SSC), and side-fluorescent (SFL) light. Lysis reagent causes morphological changes in the cells and a fluorescent stain reacts based on individual properties of the WBC types enabling their classification based on scatter grams. Basophils are detected and enumerated, together with possible nucleated red blood cells, using white cell nucleated (WNR) channel with SFL *vs.* FSC. The analysers use the WNR channel also to enumerate total WBC. White blood cell differential (WDF) channel with SSC *vs.* SFL gram is used to differentiate and enumerate neutrophils, eosinophils, lymphocytes, and monocytes, and to detect atypical lymphocytes and abnormal cells. IG form a cluster of events above the mature neutrophil cell cloud in the WDF channel (9) (*Figure 1*).

Most research regarding the usefulness of IG% as a marker of inflammation relies on analyser data, not on manual microscopy. Some studies have found an automated haematology analyser (Sysmex XE-2100) to be more precise than manual microscopy in enumerating IG (10). However, Sysmex XN is also described to systematically give higher IG percentage compared to manual microscopy (11). Sysmex XN analyser algorithms may produce interpretive program messages (flagging) based on scatter grams, particle size, and numerical data. Samples marked with flagging should be

reviewed by the user, while samples without any flagging may be autovalidated. Sysmex XN manufacturer, as well as certain studies, have recommended to use IG 3% as the cut-off for analyser flagging and microscopy review (11). Some studies have set the limit to 5% and even showed that automated analysers should be used as the reference method instead of manual microscopy (10,12). Still manual microscopy remains the gold standard for WDF, in spite of high inter-individual variation and rather low reproducibility (13). Digital microscopy has improved the work flow with the samples requiring review after analyser flagging. Despite of this, the review process can cause delay in patient care, reducing the value of IG as an inflammation marker.

The data regarding the reliability of Sysmex XN IG remains somewhat controversial, while this parameter is increasingly used as a clinical inflammation marker. In this study we investigated the reliability of Sysmex XN IG% in comparison to manual microscopy. We evaluated whether the IG% cut-off for microscopy review could be raised from the recommended 3% in order to reduce the amount of manual work, and compared the data from Sysmex XN to manual microscopy at different IG%, absolute neutrophil count (ANC) and WBC levels. For the reference, we used samples with a special request for manual microscopy WBC differential, but with no analyser flagging and IG <3%. We present the following article in accordance with the MDAR reporting checklist (available at <https://dx.doi.org/10.21037/>

jlpm-21-33).

## Methods

### Samples

The samples in this retrospective study were anonymised routine analysis whole blood samples collected in K2EDTA tubes and stored at room temperature at the Turku University Hospital Laboratory, Turku, Finland. The samples were analysed and the blood films were prepared within 8 hours after sample taking. The study comprised of 557 samples, 242 (43%) from female, overall median age 67 years (IQR with 25<sup>th</sup>–75<sup>th</sup> percentile 54–74 years) with a request for WBC differential, collected Dec 21, 2018 to Jul 31, 2019. The reference samples, 245 with 81 (33%) females, were from babies (<1 year old) with a special request for manual microscopy WBC differential, collected in Apr 3, 2018 to Feb 27, 2019. Without the special manual microscopy review request, these samples would have been autovalidated. This study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). No patient permission or evaluation from the ethics committee were needed for this retrospective study with anonymised samples.

### Laboratory tests

All samples were analysed with Sysmex XN-10 or Sysmex XN-20 (Sysmex Co. Kobe, Japan) analysers with WNR and WDF channels. The total leukocyte count (WNR channel), IG percentage (WDF channel) and ANC (WDF channel) were recorded. The analyser flagging for IG was set to 3%. No samples with other or additional analyser flagging, i.e., notifications regarding other WBC abnormalities, were included. No samples with interference in WBC count or IG measurement leading to an unreliable WBC enumeration or WBC identification were included in the study. The reference samples had IG <3% and no analyser flagging. All samples were single analysis according to routine protocol. The precision was 7% (at IG 0.74%) for intra-assay and 4% (at IG 11%) for inter-assay IG analysis, the WBC reportable range was (0–440) ×10<sup>9</sup>/L, and the WBC reference range (3.4–8.2) ×10<sup>9</sup>/L for adults and (6.0–17) ×10<sup>9</sup>/L for babies <1 years.

The blood films were made with Sysmex SP-10 (Sysmex Co., Kobe, Japan) and dyed with May-Grünwald-Giemsa – dye (MGG, RAL Diagnostics, Martillac, France) or prepared manually and dyed with MGG (Reagent, Toivala, Finland)

according to established protocols (14,15). With manual microscopy 200 WBC were evaluated. Promyelocytes, myelocytes, and metamyelocytes were classified as IG, and band form and segmented neutrophils as mature (15). The inter-individual variation for manual microscopy IG has varied up to CV 77% at IG% <3 and occasionally exceeded the accuracy limits (13), while the CV for manual mature neutrophil percentage has been <5% in our laboratory.

### Statistical analysis

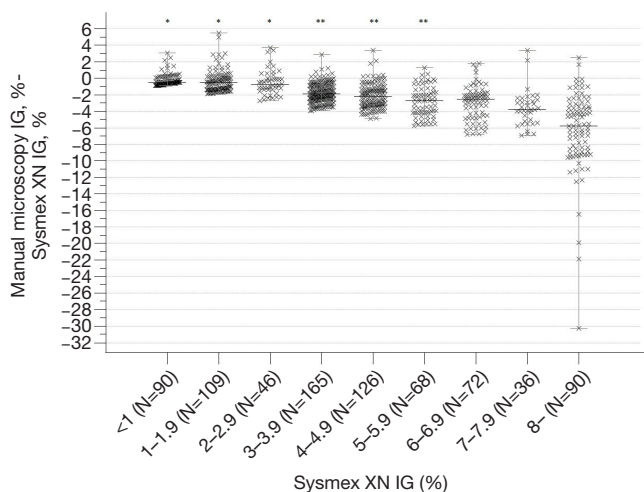
To evaluate the differences in the analyser and microscopy IG% we compared the methods in samples with IG <3% (N=245) and >3% (N=557). With all the samples combined (N=802), we divided the samples into nine subgroups with IG% <1 (N=90), 1–1.9 (N=109), 2–2.9 (N=46), 3–3.9 (N=165), 4–4.9 (N=126), 5–5.9 (N=68), 6–6.9 (N=72), 7–7.9 (N=36) and >8 (n=90) (see *Figure 2*). We further compared the differences in IG% based on total WBC <3.4×10<sup>9</sup>/L (N=53), (3.4–8.2) ×10<sup>9</sup>/L (N=255), and >8.2 ×10<sup>9</sup>/L (N=494), and ANC <1.5×10<sup>9</sup>/L (N=48), (1.5–6.7) ×10<sup>9</sup>/L (N=404), and >6.7×10<sup>9</sup>/L (N=350) (see *Table 1*). MedCalc 19.7 with nonparametric Shapiro-Wilk test, Regression model with 95% confidence and 95% prediction intervals for the regression line, and Kruskal-Wallis H test with Dunn post-hoc method were used due to not normally distributed data.

## Results

The analyser IG% and manual microscopy IG% fitted only moderately in the regression model both in the reference samples with IG <3% (R<sup>2</sup>=0.1319, *Figure 3*) and in samples with IG above 3% (R<sup>2</sup>=0.3273, *Figure 4*). The deviation increased with higher analyser IG%, Sysmex XN giving positive error.

We compared the degree of difference between analyser IG% a manual microscopy IG% at different Sysmex XN IG thresholds (*Figure 2*). In the subgroups above 3%, the median positive error for the analyser IG% was higher than in IG <3% (P<0.001). The positive error and the degree of difference between the methods further increased above IG 6%. When the subgroups were further compared, within subgroups IG <1, 1–1.9, and 2–2.9%, and again within subgroups IG 3–3.9, 4–4.9, and 5–5.9%, there was no statistically significant difference, but subgroups with IG >6% did differ statistically significantly from the other groups (P<0.001).

Neutropenia or neutrophilia, compared to samples with

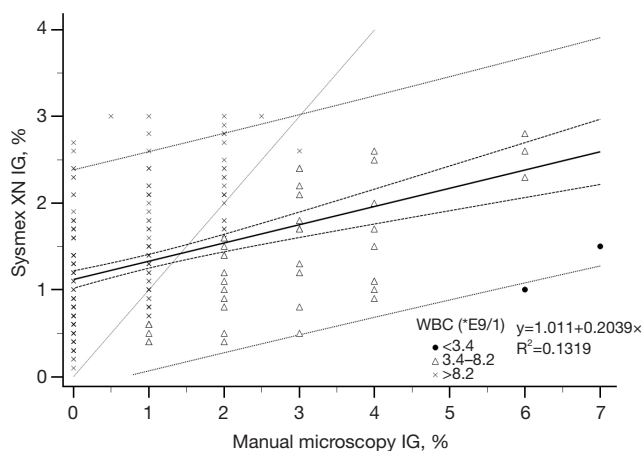


**Figure 2** Difference of the immature granulocyte percentage (IG%) analysed with manual microscopy and Sysmex XN at different levels of Sysmex XN IG% (N=802). The horizontal line shows the median of the deviation between the methods, the whiskers represent the range, and the marks individual results. The deviation was statistically significantly different with IG <3% and subgroups with IG >3% (P<0.001). Within subgroups marked with \* or \*\* there was no statistically significant difference. Subgroups with IG >6% differed statistically significantly from lower IG% groups (P<0.001).

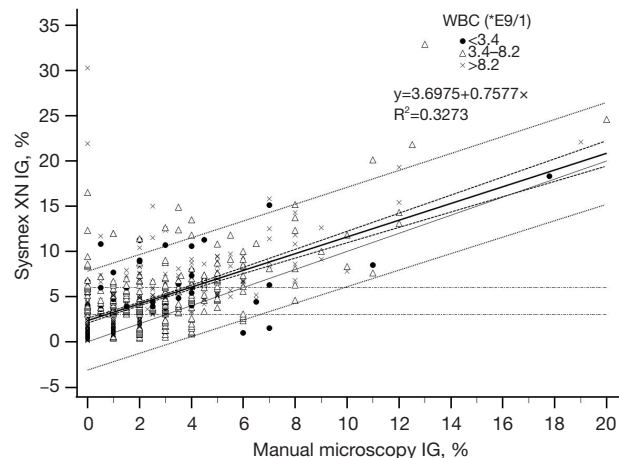
**Table 1** Immature granulocyte (IG%) measured with Sysmex XN and manual microscopy did not differ in samples with different absolute neutrophil counts (ANC) (P=0.296)

Group	N	Manual microscopy- Sysmex XN IG%, median (IQR)	Sysmex XN IG%, median (IQR)
<b>ANC</b>			
<1.5×10 <sup>9</sup> /L	48	-1.4 (-3.4 to -0.25)	3.9 (1.2-4.8)
(1.5-6.7) ×10 <sup>9</sup> /L	404	-1.8 (-3.5 to -0.60)	4.0 (2.2-6.1)
>6.7×10 <sup>9</sup> /L	350	-1.7 (-3.1 to -0.60)	3.8 (2.0-5.3)
<b>WBC</b>			
<3.4×10 <sup>9</sup> /L	53	-2.9* (-5.5 to -1.375)	5.3 (3.9-7.4)
(3.4-8.2) ×10 <sup>9</sup> /L	255	-2.2 (-3.8 to -0.33)	4.6 (3.3-6.7)
>8.2×10 <sup>9</sup> /L	494	-1.5 (-2.8 to -0.60)	3.5 (1.4-5.1)

The method difference was slightly higher in samples with low white blood cell (WBC) count (\*, P<0.001) compared to normal or high WBC samples. IQR, interquartile range.



**Figure 3** Immature granulocyte percentage (IG%) analysed with Sysmex XN and manual microscopy in the samples with Sysmex XN IG <3%. The data was grouped based on total white blood cell (WBC) count. The thick solid line shows the regression, dashed lines 95% confidence, and fine dashed lines 95% prediction intervals for the regression line. The line of equality is thin solid. The horizontal line shows cut-off level (IG 3%).



**Figure 4** Immature granulocyte percentage (IG%) analysed with Sysmex XN and manual microscopy. The data was grouped based on total white blood cell (WBC) count. The thick solid line shows the regression, dashed lines 95% confidence intervals, and fine dashed lines 95% prediction intervals for the regression line. The line of equality is thin solid. The horizontal lines show two cut-off levels (IG 3% and 6%).

normal ANC, did not affect the degree of difference in IG% between Sysmex XN and manual microscopy ( $P=0.296$ ). In samples with low WBC, the IG% difference between the two analysis methods was slightly higher ( $P<0.001$ ) compared to samples with normal or high WBC (*Table 1*).

## Conclusions

Manual microscopy is the gold standard and the worldwide confirmatory method for WBC differential after an automated haematology analyser, such as Sysmex XN. In general, Sysmex XN analyser WBC differential correlates well with manual microscopy results in samples with no abnormal cells (8,9), but analyser specific cut-off limits have been debated and further studied (16-18). The recommendation for Sysmex XN haematology analysers is to review WBC differential samples with IG above 3% with manual microscopy to achieve higher accuracy. Samples with IG <3% may be autovalidated. Some studies have showed that IG 5% for Sysmex XN analysers is an adequately accurate cut-off instead of 3% (12,19). Possible variations between an automated haematology analyser and manual microscopy result are often explained by inaccuracy of the microscopy review with only 200 WBC. However, increasing the WBC amount counted in microscopy review, even with digital microscopy, is practically impossible in hospital laboratories due to time and labour demand.

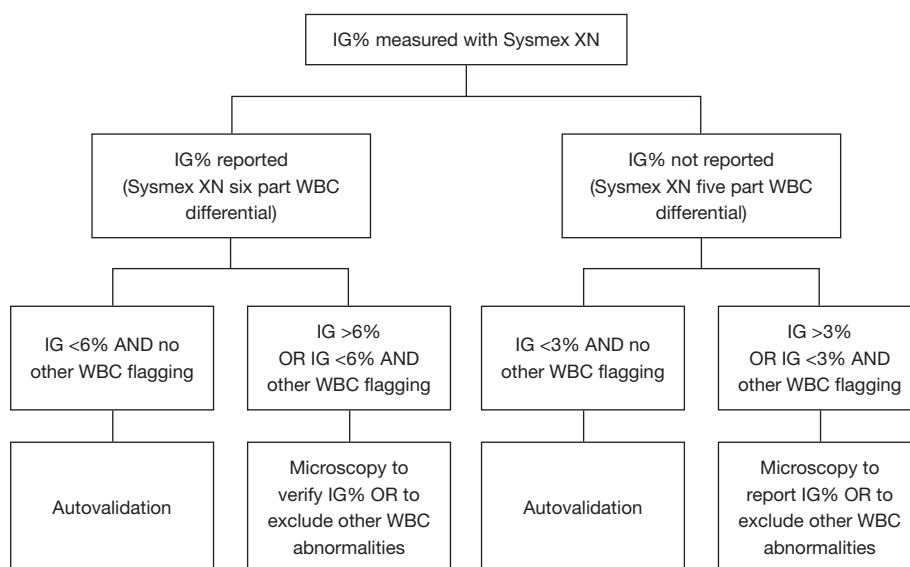
In this study, Sysmex XN gave positive error for IG% at every IG level, even in autovalidated samples (IG <3%). Also occasional lower IG% compared to manual microscopy were recorded. As seen with these data, we would accept the differences exceeding the widely adopted limits from Rümke (13) in the autovalidated Sysmex XN results below IG 3%, were they reviewed by microscopy. Similar deviation as with IG <3%, but with slightly higher median positive error, was seen in samples with IG up to 6%. There was no statistically or clinically significant difference between the methods with IG 5% compared to IG 6% in this study. It could also be claimed that the difference in IG% results between Sysmex XN and manual microscopy was not clinically significant up to IG 7%, even when the IG subgroup 6-7% did differ statistically significantly from the lower IG% subgroups. Deviation of single results, however, increased with IG above 6% and there were samples with a high analyser IG% and no, or miniscule, amount of IG with manual microscopy.

In the samples with Sysmex XN IG above 15% and manual microscopy IG% very low, the majority of the

WBC were neutrophils (84–96%) and Sysmex XN showed a distinct IG cloud above mature neutrophils. In these cases, it is possible that the deviation between the methods is caused by incorrect microscopy review, although this cannot be confirmed retrospectively. Possible explanations are high amounts of ghost cells on the blood film causing deviation in the cell classes or morphologically challenging samples with, for example, abnormal neutrophil granulation. It is also possible that the analyser data is erroneous if algorithms misclassify IG among highly granulated neutrophils in neutrophilic samples or if there is merging of neutrophil and IG cell clouds confusing algorithm-based identification (see *Figure 1B*). It is important to note that the inter-individual CV% of IG in manual microscopy in our accredited laboratory has been up to 77% (at IG <3%) while for neutrophils it has been remarkably lower. For the Sysmex XN intra- and inter-assay CV has been under 10% with low and higher IG%, putting the value of the manual microscopy IG as the reference method under doubt in accordance to some previous studies (10).

In the samples with low WBC, the IG% difference was slightly higher than in samples with normal or high WBC. Human error might explain deviation in low WBC samples due to low amount of representative WBC evaluated in manual microscopy. In comparison, ANC did not affect the accuracy of the Sysmex XN compared to manual microscopy IG%, nor did it explain the differences between the analysis methods. IG% can thereafter be reported in neutropenic samples according to the same criteria as normal ANC samples. In general, it can be claimed that automated haematology analysers enumerating thousands of cells are more accurate compared to manual microscopy. It is possible that IG% measured with Sysmex XN reflects a more truthful physiological situation in cases with still rather low circulating IG. This hypothesis should be verified with consecutive samples, e.g., after leukocyte growth factor administration.

In this study, there were samples with Sysmex XN IG 3–6% and manual microscopy IG 0%. Similarly, there were samples with Sysmex XN IG <1% and manual microscopy IG 6%. The trueness of microscopy IG% cannot be verified based on these data. The clinical significance of these differences can, however, be considered minimal. A positive error in IG measured with Sysmex XN could lead to suspicion of infection or inflammation, but in patient care this can be confirmed with consequent samples and other clinical chemistry parameters such as C-reactive protein or procalcitonin, were not investigated in this study. Falsely



**Figure 5** Summary of actions in measuring immature granulocyte percentage (IG%) parameter with Sysmex XN automated haematology analyser based on this study. WBC, white blood cell; IG, immature granulocyte.

low IG, on the other hand, might lead to missed acute inflammatory conditions. If the IG is reported as a WBC parameter (six-part WBC differential), we suggest that the cut-off IG 6% for manual microscopy is adequate when clinical significance of the possible inaccuracy is concerned. If the cut-off is used only for directing the samples for microscopy review and the IG parameter is not reported as such, the focus is on reporting the left shift of neutrophils in general and the IG cut-off 3% is more adequate. Reporting immature neutrophil forms with manual microscopy or with an automated haematology analyser is of clinical significance and this would be missed with an IG cut-off above 3% and five-part differential only. Based on these data we suggest to consider actions described in *Figure 5* when analysing IG% with Sysmex XN.

In our university hospital laboratory, IG cut-off 3% causes about 30% of all Sysmex XN interpretive program WBC messages, or flagging, leading to manual microscopy. In this study, 65% of the routine analysis samples had IG under 6%. No clinically significant deviation from manual microscopy IG was seen in this study doubting the need of sample review below this IG cut-off. Reporting Sysmex XN IG% and rising the cut-off to 6% would reduce the need for WBC microscopy review by 20%. This can be considered a significant reduction on monthly and yearly basis. Faster reporting of the analyser IG would benefit patient care and possibly promote using IG%, or absolute IG amount not evaluated in this study, as an inflammatory marker. The data

presented here still requires further confirmation, but is of value for laboratories considering to evaluate the IG% cut-off for Sysmex XN.

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### Footnote

*Reporting Checklist:* The authors have completed the MDAR reporting checklist. Available at <https://dx.doi.org/10.21037/jlpm-21-33>

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*Conflicts of Interest:* All authors have completed the ICMJE uniform disclosure form (available at <https://dx.doi.org/10.21037/jlpm-21-33>). The authors have no conflicts of

interest to declare.

**Ethical Statement:** The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). No patient permission or evaluation from the ethics committee were needed for this retrospective study with anonymised samples.

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