JCP Online First, published on March 25, 2014 as 10.1136/jclinpath-2014-202223

LETTER TO THE EDITOR

Immature granulocyte count in peripheral blood by the Sysmex haematology XN series compared to microscopic differentiation

Recent advances in automation of haematology analysers have significantly shortened turn-around-times for reporting leukocyte differential and count in the peripheral blood. Due to the introduction of improved flagging rates, the amount of microscopic peripheral blood slide reviews can be minimised.[1] Having access to an enormous amount of data, one has to find the balance between productivity and clinical quality. Reliable automated blood cell characterisation and quantification remain challenging in pathological samples, whereas slide reviews due to unnecessary flagging should be avoided in normal samples. An important feature of the automated haematology cell counters is their ability to identify and quantify immature granulocytes (IG) in a peripheral blood sample.

Recently, the Sysmex XN series was introduced and its performance was evaluated.[2] It provides the possibility to automatically count the IG in the peripheral blood. After applying a specific lysing agent (Lysercell WDF), the IG are measured in the WDF channel and differentiation is made based on granularity (side scatter) and nucleic acid content (side fluorescence by the Fluorocell WDF reagent). The IG cluster is found right above the neutrophil cluster in the biparametric histogram side scatter/fluorescence. The IG fraction includes promyelocytes, myelocytes and metamyelocytes (blasts and band cells are not included). In the present study, the relative and absolute amount of IG counted by the XN series was compared with automated microscopic differentiation of 500 white blood cells (WBC). Peripheral blood samples from 103 consecutive patients were analysed for IG on the XN series and simultaneously, a blood smear was made for automated microscopic differentiation of 500 WBC using the DM-96 automated microscope (leukocyte count range $4.1–39.7 \times 10^9$/L). Samples showing the 'abnormal WBC scattergram' flagging on the XN were excluded from the analysis. Microscopic classification of the WBCs was independently performed by two haematology experts and mean values were used. The classification was based on the definitions published by the College of American Pathologists.[3] We have compared the IG on the XN with the sum of the promyelocytes, myelocytes and metamyelocytes counted using the automated microscope.

The relative and absolute amount of IG was significantly higher when measured by the XN analyser (5.1%; 0.739×10^9/L) compared to the microscopic differentiation (2.6%; 0.357×10^9/L; $p<0.0001$; Wilcoxon test for paired samples). The Bland and Altman plot illustrates the systematic positive bias on the XN compared to automated microscopy (figure 1). The mean difference in %IG and absolute IG count between the XN and the microscopic differentiation is respectively 2.5% and 0.382×10^9/L. In figure 2 the XN %IG and microscopic %IG are compared with the optimal regression line ($y=x$) and corresponding CI for microscopic differentiation based on 500 WBCs obtained from Rümke's table.[4] The XN %IG measured in 59% of the samples falls outside the 95% CI obtained from Rümke's table for the microscopic enumeration of the %IG, indicating a significant difference between the %IG on XN and by microscopic differentiation. The correlation between the microscopic %IG count and the %IG count on XN was 0.83 (Pearson's correlation coefficient). The linear regression curve has a slope of 1.2 and an intercept at 1.9%IG. In-depth analysis revealed that the systematic error is

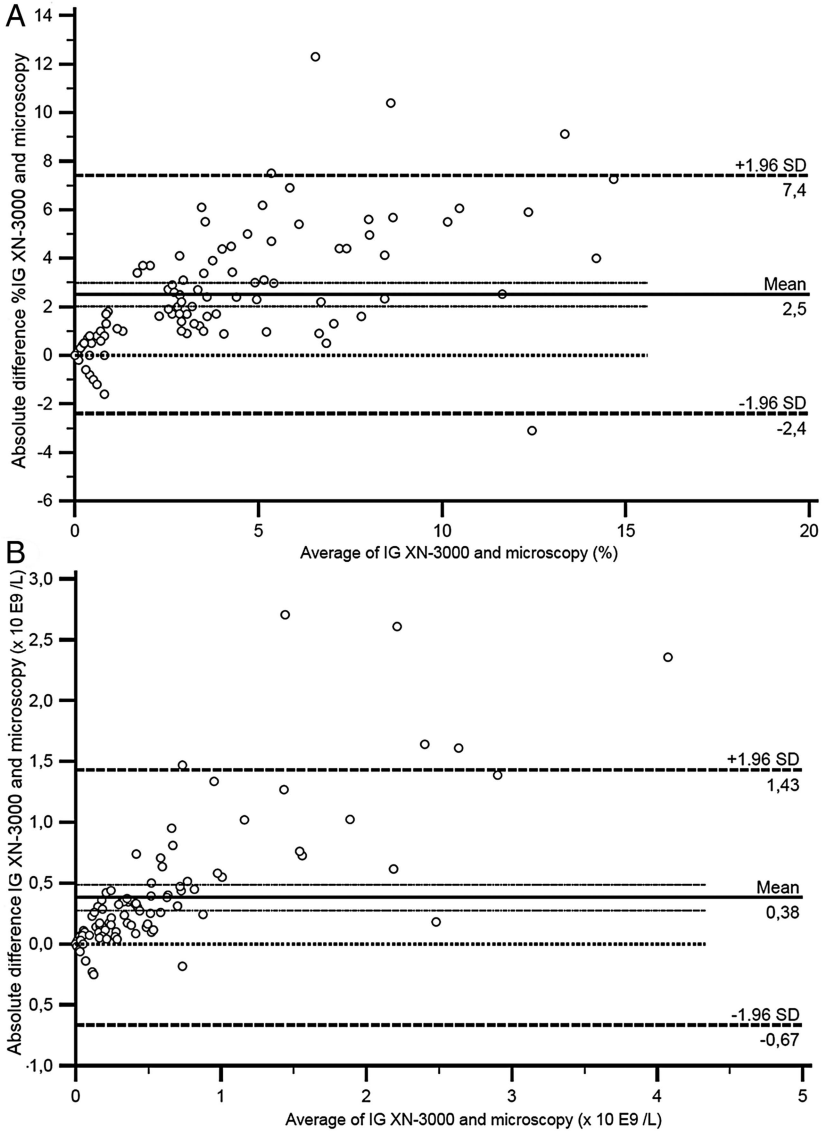

Figure 1 Bland-and-Altman plot comparing the percentage (A) and the absolute count (B) of the immature granulocytes obtained by the XN-3000 and by microscopic differentiation on 500 leukocytes. The bold line indicates the mean absolute difference. The dot-dashed line indicates the 95% CI of the mean difference. The large dashed line indicates the ±1.96SD of the mean difference. The small dashed line indicates the optimal mean difference of 0.

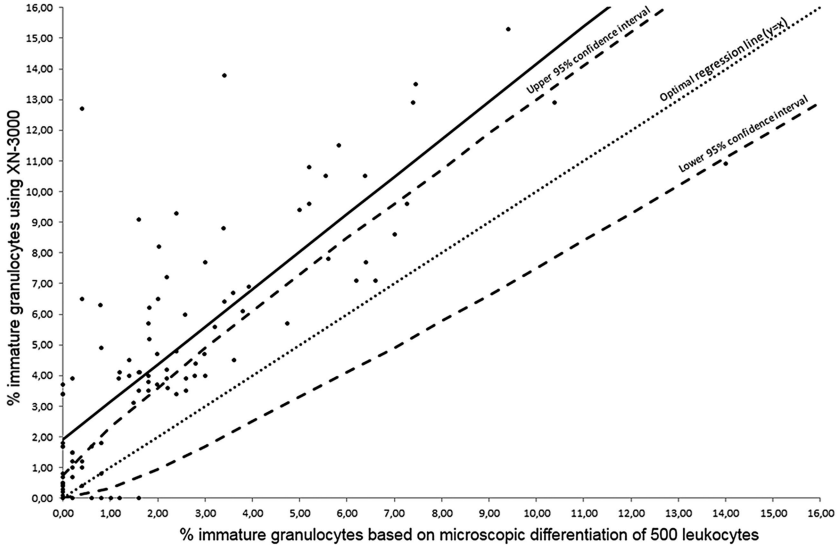

Figure 2 Linear regression analysis and dot plot comparing the percentage of immature granulocytes counted by the XN-3000 and by microscopic differentiation on 500 leukocytes. The bold line indicates the regression line of the linear regression. The dotted line indicates the optimal regression line. The dashed line indicates the upper and lower 95% CIs of the microscopic differentiation.

especially present in samples showing >3% IGs on the XN instrument. Evaluation of the qualitative analysis for the presence of IG using the XN-3000 compared to microscopic differentiation showed that the XN-3000 reports false positive results in 6/103 samples (5.8%) and false negative results in 5/103 samples (4.8%).

These results indicate that counting the IG using the XN series shows a systematic positive error compared to microscopic morphology count. Previous reports have suggested that automated IG count can replace the microscopic morphology count for IG counting in the clinical laboratory.[5] However, as the correlation of automated IG count and microscopic morphology count is only moderate, complete replacement of the microscopic morphology count for IG is not advisable.[6] The weak correlation is often explained in different ways, including the smaller number of cells counted microscopically (generally 100 cells), the subjectivity involved in the morphologic classification (especially the difficulty in separating metamyelocytes and band cells) and the inhomogeneous repartition of cells on blood smears. However, as in this study, the microscopic count is performed on 500 leukocytes and on a well-standardised manner (automated microscope), the systematic error cannot be neglected or explained by the factors described above.

A better (and often applied) strategy is using the %IG of the XN as a flagging for morphological smear review. As %IG results are not interchangeable between the two methods in samples showing >3% IG, slide review should be recommended in all peripheral blood samples of IG >3%. This is in concordance with earlier reports on other Sysmex instruments (XE-2100 and XE-5000).[1] Increasing the slide review threshold to 5% IG,[6] has been suggested and could decrease the slide review by 30%.[7] In our laboratory, increasing the slide review cut off from 3%IG to 5%IG would mean a total decrease of 8% of all slide reviews. Although increasing the review threshold probably will only have a minimal impact on the clinical decision-making process, our findings show that this is not advisable, as the XN clearly overestimates the amount of IG present in samples with >3% IG. In conclusion, microscopic confirmation for the presence and the amount of IG in all peripheral blood samples showing >3% IG on XN should be advised.

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Competing interests None.

Provenance and peer review Not commissioned; externally peer reviewed.

To cite Maenhout TM, Marcelis L. *J Clin Pathol* Published Online First: [*please include* Day Month Year] doi:10.1136/jclinpath-2014-202223

Accepted 6 March 2014

J Clin Pathol 2014;**0**:1–2. doi:10.1136/jclinpath-2014-202223

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doi: 10.1136/jclinpath-2014-202223