

Peer Review File

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Reviewer A

Introduction.

Comment 1: Characterize the measurement methods by supplementing the data with abbreviations of the parameters included in the WDF scategram.

Reply: We added data regarding measurement principles along with WDF parameters in Introduction.

Changes in the text: Introduction, Paragraph 1, Page 3, Line 52 forward.

Comment 2: Indicate the described group of cells on the graphical result (WDF).

Reply: We added a figure with two different immature granulocyte percentage levels showing WDF scatter gram with white blood cell differentiation.

Changes in the text: Figure 1.

Comment 3: Briefly describe the flow cytometry method.

Reply: We added a description of the flow cytometry method/Sysmex XN into Introduction part.

Changes in the text: Introduction, Page 3, Line 52 forward.

Comment 4: Specify the term "flagging" of the automatic result.

Reply: We added description of the term flagging in Introduction and Methods/Laboratory tests.

Changes in the text: Introduction, Paragraph 2, Page 4, Line 69 forward and Methods, Page 5, Laboratory tests, Line 112 forward.

Methods Samples

Comment 5: Was your study a retrospective or prospective study? Include this information.

Reply: The study was a retrospective study. We added this to Methods/Samples.

Changes in the text: Added to Methods, Page 5, Samples, Line 95

Comment 6: Information on linearity for WBC, IG and neutrophils in the table.

Reply: We suggest keeping this in the text format.

Changes in the text: None.

Comment 7: More precisely characterize the colors of cell elements in the applied staining and the morphology of cells belonging to the IG group.

Reply: MGG staining is an established method for white blood cell morphology staining. The method has been used for decades. For this reason, we suggest to leave out any detailed description of the method. We added two references for the staining method. White blood cell differentiation with microscopy according to morphological characteristics is based on widely agreed criteria that are followed in the laboratory where the study was carried out. We suggest to leave out detailed description, but we added a reference for WBC classification.

Changes in the text: We added references for WBC staining and classification, Methods, Page 6, Laboratory tests, Line 123.

Comment 8: The division into groups depending on the percentage of IG present on the graph.

Reply: Presenting the data in a table is space demanding. We suggest keeping the data in the text format. We added a reference to Figure 3, where the data is presented in a graphic format.

Changes in the text: Methods, Page 6, Statistical analysis, Line 135 and 138.

Comment 9: Complete the manuscript with an analysis of data on inflammatory markers (C-reactive protein, procalcitonin and correlation with IG).

Reply: This study compared only methodological differences in IG measurement, not usefulness of IG as an inflammatory marker. Therefore, unfortunately, further data on inflammatory markers was not available. We stated that they were not evaluated in this study.

Changes in the text: None. Stated in Conclusions, Page 10, Line 221.

Comment 10: Complete the manuscript with the absolute number for IG (#IG).

Reply: This study was a method comparison study. Only proportional values for WBC classes are obtained with manual microscopy, also for IG. The purpose of this study was specifically to investigate the Sysmex XN threshold of IG% for manual microscopy review and thereafter evaluate the performance of Sysmex XN analyser in IG identification. For these reasons, no absolute IG values were obtained or investigated in this study. We suggest keeping only IG%. We stated that absolute IG

was not evaluated.

Changes in the text: None. Stated in Conclusions, Page 11, Line 241.

Comment 11: Describe in detail the leukocyte counting method. Indicate if there are any interference in the measurement of IG and leukocytes.

Reply: The leukocyte counting method is described in the introduction. The effect of possible interferences to leukocyte counting is now described in Methods.

Changes in the text: Introduction, Paragraph 1, Page 3, Line 52 forward and Methods, Page 5, Laboratory tests, Line 112.

Results

Comment 12: Figures should include p-value.

Reply: We rephrased the results regarding figures and added P-values to figure 4.

Changes in the text: Page 7, Results, Line 148 forward. Figure 4 and figure text.

Comment 13: Specify the term "flagging" of the automatic result.

Reply: The term flagging is not mentioned in Result. It is clarified in Introduction, Methods and further in Conclusions.

Changes in the text: None in Results. Introduction, Page 4, Line 69 forward and Methods/Laboratory tests, Line 112 forward. Conclusions, Page 10, Line 224.

Comment 14: Add the absolute number of the entire manuscript to the% immature granulocytes value.

Reply: This study was a method comparison study. Only proportional values for WBC classes are obtained with manual microscopy, also for IG. The purpose of this study was specifically investigate the Sysmex XN threshold of IG% for manual microscopy review and thereafter evaluate the performance of Sysmex XN analyser in IG identification. For these reasons, no absolute IG values were obtained or investigated in this study. We suggest keeping only IG%. We stated that absolute IG was not evaluated.

Changes in the text: None. Stated in Conclusions, Page 11, Line 241.

Conclusions

Comment 15: Rephrase the conclusions and refer to more scientific manuscripts related to the automatic and manual leukocyte counting, then focus on automatic and manual IG analysis.

Reply: We added references (Ref. no. 16-18) and revised the conclusions.

Changes in the text: Thorough revision of conclusions.

Reviewer B

In this study, the authors investigated the accuracy of Sysmex XN Immature Granulocyte Percentage. They enrolled 802 subjects and analyzed the correlation between hematology analyzer and microscopy. The work is of high clinical relevance. I have some comments.

Comment 1: I suggest the authors use a flowchart to summarize the major findings of this study.

Reply: We added a flowchart with a summary of actions in measuring immature granulocytes with Sysmex XN according to this study.

Changes in the text: Table 1.

Comment 2: I suggest the authors use Bland-Altman analysis and Passing-Bablok regression. Please refer to the following reference: Clin Chem Lab Med. 2021;59(5):857-867; Biochem Med (Zagreb). 2021;31(2):020707; Am J Clin Pathol. 2011;136(1):20-29.

Reply: We chose not to use Bland-Altman plot analysis or Passing-Bablok regression model as the data was not normally distributed and fitted poorly on linear relationship assumption. To our understanding these methods can not thereafter be used with these data. We agree that otherwise these methods were the best choice. However, we remain open for further suggestions regarding data analysis and presentation.

Changes in the text: None

Comment 3: Results section: In the last paragraph of the Results section, the authors reported the effect of ANC and WBC on the degree of deviation. More details concerning this issue should be reported. I suggest the author use a table to describe it.

Reply: We rephrased the results to a more clear form and presented the results in a table. We also revised the conclusions accordingly.

Changes in the text: Page 7, Results, Line 156 forward. Table 1. Thorough revision of conclusions.