ISSN 2744-1636
Bioengineering Studies
Original Research
Vol. 6, No. 1, April 2025, pp.11-23
https://doi.org/10.37868/bes.v6i1.id280

Comparison of Sysmex DI-60 digital optical microscopy and manual optical microscopy in leukocyte differentiation

Nermina Klapuh-Bukvić[1,2*], Zehra Kurtanović[3,4], Damir Šeper[3,5]
[1]Clinical Center University of Sarajevo, Sarajevo, Bosnia and Herzegovina
[2]University of Sarajevo, Faculty of Health Studies, Sarajevo, Bosnia and Herzegovina
[3]University of Sarajevo, Faculty of Pharmacy, Sarajevo, Bosnia and Herzegovina
[4]Public Institution Health Center of Sarajevo Canton, Sarajevo, Bosnia and Herzegovina
[5]Public Institution General Hospital "Prim.dr. Abdullah Nakas" Sarajevo, Bosnia and Herzegovina

*Corresponding author: klapuhbukvicnermina@gmail.com

Received Mar. 29, 2025
Revised Apr. 28, 2025
Accepted Apr. 29, 2025

Abstract

Manual microscopic differentiation of leukocytes is the primary tool for the diagnosis and monitoring of various diseases. Recently, digital optical microscopy has become a more common method, being an alternative to the conventional one, and therefore, there is a need to investigate its compatibility in more detail. The objective is to compare the results of digital and manual microscopy in the analysis of different leukocyte types and to assess the linearity, systemic and proportional differences between the methods. 109 samples were analyzed by manual and digital microscopy (Sysmex DI-60), and the comparison of the obtained results was performed by Passing-Bablok and Bland-Altman analysis. The linearity of the methods is satisfactory in all cells, except basophils and blasts. A statistically significant systemic difference was detected in segmented neutrophils, immature granulocytes, and lymphocytes, and correlations between the methods range from very strong to moderate, depending on the type of cells. Significant deviations were observed in leukocytosis and leukopenia. The results of the conducted analysis indicate a good correlation between digital and manual microscopy, but the identified systemic and proportional differences indicate the significance of the reclassification offered by the analyzer. In cases of severe leukopenia and leukocytosis, it is recommended to use manual microscopy as an additional check.

Keywords: Sysmex DI-60; Digital microscopy; Leukocyte differentiation; Optical microscopy; Artificial intelligence;

1. Introduction

Leukocytes, known as white blood cells (WBCs), represent a heterogeneous group of cells that contain a nucleus, and are formed in the bone marrow by a specific process of hematopoiesis known as leukopoiesis. Leukopoiesis is a complex, multi-phase process in which a pluripotent stem cell becomes a unipotent stem cell committed to leukocytes and further differentiation produces mature leukocytes that are released into the blood [1]. They are divided into two basic groups: granulocytes (neutrophils, eosinophils and basophils) and agranulocytes (lymphocytes and monocytes).

The main role in the body is to participate in an immune reaction that can be caused by various pathogens (viruses, bacteria, parasites), while actively participating in the recognition, neutralization and destruction of the pathogen itself. In a healthy organism, the total number of leukocytes, as well as the percentage share of certain types of leukocytes, is clearly defined, and immature forms of leukocytes are not present in circulation. Any deviation from these performances indicates the existence of a disturbance. Leukocyte disorders can generally be divided into two groups: quantitative and qualitative. Quantitative disorders imply an increase or decrease in the total number of leukocytes accompanied by an increase or decrease in the specific leukocyte population, while qualitative disorders indicate the presence of abnormal cells in circulation. All registered changes reflect some pathological condition in the body. Therefore, leukocyte differentiation is a primary and one of the key diagnostic procedures in clinical medicine and further diagnostic and therapeutic procedures depend on the accuracy of the differentiation itself [2].

The leukocyte differentiation process itself can be performed using automatic hematology analyzers or manually, by optical examination. Modern automatic analyzers ensure high accuracy and precision results in a short time, which significantly increases the number of analyses per unit of time. Different technologies such as optical, impedance or fluorescent technology as well as combinations thereof are used in the quantification and classification of leukocytes. The good performance of these analyzers ensures their daily application in routine work [3]. However, in laboratories of secondary and tertiary level health care, where there is a very large share of pathological samples in the total number of analyzes, differentiation of leukocytes is done by manual or optical microscopy in addition to automatic analyzers. This method is a traditional method of differentiation, which requires significantly more time per sample, longer preparation of blood smear and a highly educated staff that analyzes the preparation itself. The advantages of this type of differentiation are reflected in the fact that in this way, in addition to the quantitative status of the subpopulation of leukocytes, morphological abnormalities of cells and the possible presence of abnormal cells can be observed at the same time, which some hematology analyzers will not detect [4].

Modern hematology analyzers provide the possibility of digital optical microscopy, the use of which significantly shortens the analysis time. In a special part of the analyzer intended for digital optical microscopy, there is a part in which a blood smear is automatically prepared and painted, and then using a motorized light microscope and a digital camera, an appropriate number of cells are imaged and classified using software that works on the principle of artificial intelligence and predefined algorithms [5].

The main objective of this study is to compare the primary results obtained by digital optical microscopy, the Sysmex XN-3100 analyzer, manufactured by Sysmex Corporation (Kobe, Japan) and the results of manual optical microscopy, and on this basis evaluate this analyzer in terms of the safety of applying its primary results in everyday work, and determine in which cases it is necessary to reclassify and verify the results by manual optical microscopy.

2. Material and methods

Comparison of the methods was performed using whole blood samples collected during routine analyses at the Department of Clinical Biochemistry and Laboratory Medicine of the Clinical Center University of Sarajevo (UKCS), Sarajevo, Bosnia and Herzegovina. Whole blood was taken by venipuncture in vacuum containers with EDTA anticoagulant. A total of 109 samples were analyzed, which were divided by gender into two groups: men (n=55) and women (n=54). The age structure of the samples included four groups: younger than 18 years (n=12), 19-40 years (n=13), 41-65 years (n=36) and older than 65 years (n=48). Samples were also classified according to white blood cell count into three groups: leukopenia (n=31), leukocytosis (n=35), and white blood cell count reference interval (n=43). The entire evaluation process was carried out with the approval of the UKCS Ethics Committee, in accordance with all principles of the Helsinki Declaration.

All samples were analyzed on the Sysmex XN-3100 automatic analyzer and by manual optical microscopy, and the obtained results of both methods were collected and statistically processed. Manual optical microscopy involves the analysis of peripheral blood smears using a light microscope, and the process consists of several

stages. First, a thin smear of blood is made on the glass, followed by drying and then staining the smear with MGG (May-Grunwald-Giemsa) paint. The resulting smear is observed under a light microscope at 100x magnification on Olympus CX33, manufactured by Olympus Corporation, Tokyo, Japan. The analysis is carried out by professional staff who assess the morphological characteristics of different types of leukocytes, including the size, shape and color of the nucleus, the presence or absence of granulations in the cytoplasm, the nucleus/cytoplasm ratio, and other relevant characteristics, on the basis of which differentiation is performed. 100 leukocytes are differentiated, and the results are expressed in number or percentage.

Sysmex XN-3100 is an automatic multifunctional hematology analyzer whose use significantly shortens the analysis time, thus increasing the sample processing capacity in everyday work. The operation of this analyzer is based on a combination of fluorescent flow cytometry and light scattering method. Fluorescent flow cytometry involves the specific binding of fluorescent dyes to nucleic acids within a cell that are excised by passing through a beam of laser light, resulting in a fluorescent emission that is detected, as a side fluorescence light (SFL) signal. The fluorescence intensity directly depends on the amount of nucleic acids within the cell itself so that cells containing a higher amount of nucleic acids have a higher SFL. In addition, a light scattering method is used for the morphological characterization of cells, in which the intensity of the scattered light is measured when the cell is in a beam of laser light. Depending on how the light is scattered, basic information about the size, shape, structure and other morphological characteristics of the cell itself is obtained. In this method, scatter detection systems are positioned at different angles, which also ensures different types of output signals. Forward Scatter (FSC) measures scattering at small angles (between 0° and 5°) and this parameter actually gives cell size data, where larger scattering is given by larger cells. The Side Scatter (SSC) measures the scattering at a 90° angle, and the intensity of the scattering in this case depends on the granularity and complexity of the internal structure of the cell itself [6].

By combining the results of these three parameters and with pre-set algorithms based on artificial intelligence, the analyzer automatically performs leukocyte differentiation. In all conditions where abnormalities are detected, the analyzer gives a specific warning in the form of flags [7], and makes a recommendation for digital optical microscopy.

The digital microscopy process includes the parts of the analyzer designated as SP-50 (Slide Preparation) and DI-60 (Digital Imaging). SP-50 is a specific part of the analyzer in which the preparation and staining of the blood smear is performed according to clearly defined guidelines created on the basis of hematocrit. The dyeing of the preparation is carried out by standardized dyeing methods (May-Grünwald-Giemsa, Wright-Giemsa), which can be adapted to the specific requirements of the laboratory. The DI-60 analyzer unit contains a motorized light microscope and a color digital camera. At least 110 cells are localized in the preparation, which are observed with a microscope and recorded with a digital camera. The analysis of localized elements is performed on the basis of the obtained photos and previously defined algorithms incorporated into the CellaVision software, whereby the classification of leukocytes into subgroups is performed [6]. This creates a primary data set that is labeled as preclassification. The obtained data reclassify the specialist's approach that confirms and/or corrects the primary results, thus creating secondary data of digital microscopy analysis that are labeled as reclassification.

The obtained data of the primary analysis (preclassification) of the Sysmex XN-3100 hematology analyzer were compared with the data of manual optical microscopy, according to the guidelines given in the CLSI (Clinical & Laboratory Standards Institute) guide[8], and the statistical analysis was done in the MedCalc statistical program (Version 20.218-64-bit, Ostende, Belgium). Seven cell groups were analyzed: segmented neutrophils, eosinophils, basophils, lymphocytes, monocytes, blasts and immature granulocytes (Immature Granulocytes group representing the sum of bands, metamyelocytes, myelocytes and promyelocytes). According to the CLSI guidelines, the comparison of the two methods was made by Passing-Bablok and Bland-Altman plot analysis.

Passing-Bablok regression analysis is used to evaluate the agreement of two methods where the values of intercept (a) and slope (b) as well as the confidence interval (CI) for these parameters are monitored in the analysis. The regression equation is represented by the formula y=a+bx. Slope represents the degree of method compliance and shows how much change in one method is reflected in the change in another method. The ideal

value for slope is 1. The intercept shows the difference between the methods when the value from the first method is zero, that is, the intercept represents the initial difference between the methods. Ideally, the intercept is equal to 0. If slope=1 and intercept=0, the regression equation takes the form y=x, so it can be said that there is a linear and proportional relationship between the methods. If the slope value is ≠1 and the intercept is ≠0, then there are linear but not proportional methods, i.e. there is a system error between the methods. Within this analysis, it is important to consider both slope and intercept confidence intervals. These intervals show the limits within which the true value of these parameters is located. If the slope interval includes a value of 1 and if the confidence interval includes a value of 0 for the intercept, it can be said that there is no statistically significant difference between the compared methods [9], [10].

The Bland-Altman plot analysis provides a very useful graphical assessment of the consistency of the two methods. On the X-axis, the mean value between the two methods for each measurement is represented, and on the Y-axis, the difference between the results of the two methods for each individual measurement is represented. In this method, a mean difference (bias) ideally equal to 0 is monitored, indicating that there is no significant systemic error between the methods. A non-zero value indicates the existence of a system error, which means that one method can consistently give greater or lesser values than another method. With this method, we also get data related to the stacking limits. These are defined as bias ±1.96 x standard deviation and actually represent the limits within which 95% of the results of the analyses were conducted. If the limits are wide this means that there is a large variation in the differences between the methods, while narrow limits indicate that the differences between the methods are small. From the graph, differences in the distribution of data can be observed, which can indicate the variability of the method in different ranges of measurements [11], [12].

3. Results

The analyzed blood samples were classified on the basis of three criteria: age, gender and white blood cell count. The number by groups and the percentage share in the total number are shown in Table 1, and a graphical presentation of the same data is given in Fig. 1 and Fig. 2.

Table 1. Classification of analyzed samples by age, sex and number of leukocytes

Age			**Age/Gender:**			**Number of leukocytes**		
GROUP	N	%	GROUP	N	%	GROUP	N	%
0-18	12	11,01	Male	55	50,46	Normal	43	39,45
19-40	13	11,93				Leukocytosis	35	32,11
42-65	36	33,03	Female	54	49,54	Leukopenia	31	28,44
>65	48	44,04						
Total	109			109			109	

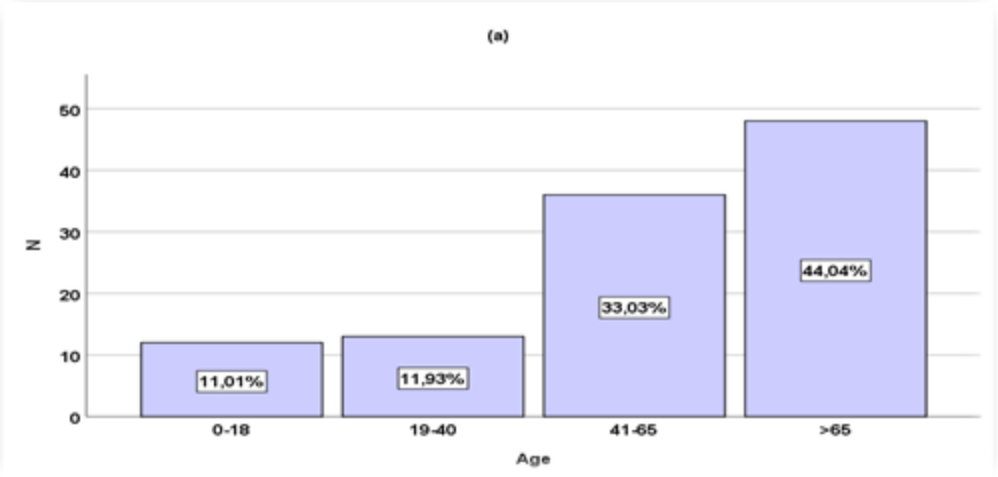

Figure1. Percentage share in the total number of samples by groups (a-age)

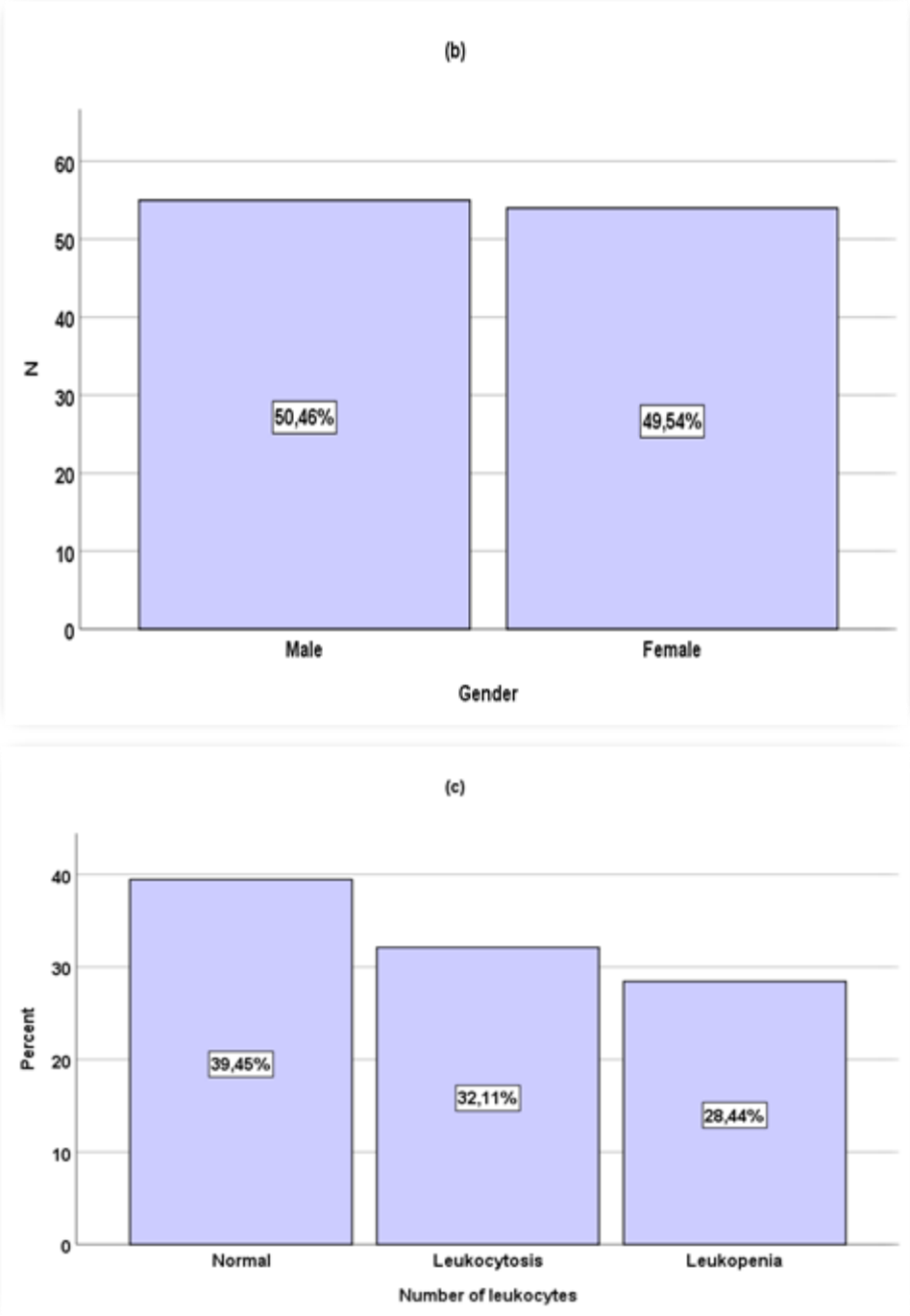

Figure 2. Percentage share in the total number of samples by groups (b-gender, c-number of leukocytes)

The results of the Passing-Bablok regression analysis regarding the linearity of the compared methods indicate that linearity is satisfactory in all analyzed groups of cells, except basophils, for which a statistically significant deviation from linearity is evident. Given this, the results of the Passing-Bablok analysis for basophils will not be interpreted. In addition, by analyzing the blast using this method, results were obtained that indicate that the selected method is not suitable for a given group of cells.

The values of intercept (a) and slope (b) and their confidence intervals (CI) are different in different types of leukocytes:

- In neutrophil granulocytes, the value of the intercept is 3,113 with slope 1,093. The value of the intercept indicates that there is a systemic difference between the methods, whereby the digital optical microscopy method yields systemically higher values than manual microscopy. In doing so, the confidence interval for the intercept does not include 0, which shows that the systemic difference is present and statistically significant. The slope value is positive and slightly above the ideal value, and the confidence interval includes 1, indicating that there is a minimal proportional difference between the methods whereby digital microscopy provides proportionally slightly higher values compared to manual microscopy.
- For eosinophils, the value of the intercept is 0 with slope 0,9, indicating that there is no systemic difference in the baseline values between the methods, which is also contributed by the confidence interval for the intercept involving 0. The slope value is slightly below the ideal value, and the confidence interval includes 1, indicating that the digital microscopy method gives proportionally lower values than manual microscopy, but this proportionate difference is not statistically significant.
- In lymphocytes, the value of the intercept is -4,8, and the slope is 1,057. The value of the intercept implies the existence of a systemic difference, which means that the methods are constantly different for the value of the intercept, which is supported by the confidence interval for the intercept that does not include 0. The slope value is greater than 1 indicating that there is a proportional difference between the methods, wherein the digital optical microscopy method provides proportionally higher values compared to manual optical microscopy for the slope value.
- In monocytes, the value of the intercept is 0,02 and the slope is 0,911. The value of the intercept is very close to the ideal value, but there is still a small systemic difference in the initial values between the methods. In doing so, the confidence interval includes a value of 0 which may even imply that this systemic difference may be negligible. Regarding the slope value, which is very close to the ideal value with a confidence interval that includes 1, it can be said that there is a minimum proportional error between the methods, i.e. the digital optical microscopy method gives proportionally slightly lower values for monocytes compared to manual microscopy.
- In immature granulocytes, the value of the intercept is 0,955 and the slope is 1,023. These data indicate the existence of a systemic difference between the methods whereby the positive value of the intercept implies that the digital microscopy method systematically gives more values than manual microscopy. In doing so, the confidence interval for the intercept does not include 0, indicating that this system shift is statistically significant. The slope value is slightly higher than the ideal value, indicating that there is a minimal proportional difference between the methods, with the digital microscopy method giving proportionally slightly higher results compared to manual microscopy.

Spearman's correlation coefficient [13] was analyzed in the type of leukocytes in which the linearity condition was met and the following results were obtained:

- Segmented neutrophils (0,932)- very strong correlation
- Eosinophils (0,535)- moderate correlation
- Lymphocytes (0,853)- strong correlation
- Monocytes (0,817)- strong correlation
- Immature granulocytes (0,627)- moderate correlation

In further work, statistical data processing implies a Bland-Altman plot analysis. Within this analysis, the differences between the arithmetic means of the methods are observed, starting from the null hypothesis according to which these differences are equal to 0. All values at which $p<0,05$ actually represent statistically significant models, indicating that the systemic difference between the methods is statistically significant. The analysis of individual types of leukocytes by this method gave the following results:

- Segmented neutrophils: mean difference is -6,522, with p<0,0001, which shows that the difference between the methods is statistically significant, which is also contributed by a confidence interval that does not include 0. The manual optical microscopy method gives lower values compared to digital microscopy by 6,52 units on average. By reviewing the graphical presentation of the analysis, two significant deviations can be observed, outside the stacking area, in which a significantly lower number of neutrophil granulocytes is detected in manual microscopy compared to digital microscopy. Both cases were detected in the leukopenia group.

- Basophils: mean difference is 0,21 with p=0,301 (p>0,05), the confidence interval includes 0, indicating the existence of a small difference between methods that is not statistically significant. The digital microscopy method gives slightly higher values than manual microscopy. From the graphical representation of the analysis, two points of deviation can be observed that are outside the area of agreement. Both cases were detected in severe basophilia.

- Eosinophils: mean difference is 0,368 with p=0,052 (p>0,05), the confidence interval includes 0, indicating the existence of a small difference between methods that is not statistically significant. The digital microscopy method gives slightly higher values than manual microscopy. From the graphical representation of the analysis, five points located outside the stacking area can be observed. Deviations are significant in cases of leukocytosis with pronounced eosinophilia.

- Lymphocytes: mean difference is -0,692 with p=0,616 (p>0,05), the confidence interval includes 0, indicating the existence of a small difference between methods that is not statistically significant. This means that the manual microscopy method produces on average lower results than digital microscopy by approximately 0,69 units. From the graphical representation of the performed analysis, deviations can be observed by 8 points outside the stacking area. The largest number of deviations was detected in cases of leukopenia.

- Monocytes: mean difference is 0,122 with p=0,863 (p>0,05), the confidence interval includes 0, indicating the existence of a small difference between methods that is not statistically significant. On average, the method of digital microscopy produces slightly higher results compared to optical microscopy. From the graphical presentation of the performed analysis, deviations can be observed for two points that are outside the area of agreement, and both cases are related to the state of leukopenia.

- Immature granulocytes: mean difference is -0,67 with p=0,207 (p>0,05), confidence interval includes 0, indicating the existence of a small difference between methods that is not statistically significant. The manual microscopy method produces on average lower results than the digital microscopy method by 0,67 units. The graphical representation of the analysis shows deviations for three points located outside the stacking area, all of which were detected in pronounced leukocytosis.

- Blasts: mean difference is -0,251 with p=0,416 (p>0,05), the confidence interval includes 0, indicating the existence of a small difference between the methods that is not statistically significant. The manual microscopy method produces on average lower results than the digital microscopy method by 0,251 units. The graphical representation of the analysis shows a deviation of one point located outside the stacking area, which is related to the case of pronounced leukocytosis.

The results of the Passing-Bablok and Bland-Altman plot analysis are shown in Fig. 3, and the graphical representation is given in Fig.4. A graphical representation of the conducted Bland-Altman plot analysis is shown in Fig. 5.

	SEG% O	SEG% P	BASO% O	BASO% P	EOS% O	EOS% P	LYM% O	LYM% P	MONO% O	MONO% P	IG% O	IG% P	BLAST% O	BLAST% P
Sample size	109													
Lowest value	1	0,9	0	0	0	0	2	2,7	0	0	0	0	*	*
Highest value	83	92,5	62	50	42	30	95	90,5	39	78,2	39	31,9	*	*
Arithmetic mean	36,973	43,495	1,596	1,386	1,697	1,329	36,661	37,352	8,780	8,658	5,257	5,927	*	*
Median	37	45,5	0	0	1	0	37	33	6	6	2	4	*	*
Standard deviation	24,084	25,843	8,137	6,329	4,600	3,290	22,686	25,887	9,178	12,332	7,866	5,658	*	*
Standard error of the mean	2,307	2,475	0,779	0,606	0,441	0,315	2,173	2,480	0,879	1,181	0,753	0,542	*	*
Passing-Bablok regression														
Regression Equation	**y = 3,113 + 1,093 x**		**y = 0,000 + 0,900 x**		**y = 0,000 + 0,900 x**		**y = -4,800 + 1,057 x**		**y = 0,020 + 0,911 x**		**y = 0,955 + 1,023 x**		*	
Systematic differences														
Intercept A	3,113		0		0		-4,8		0,02		0,955		*	
95% CI	1,553 to 5,300		0,000 to 0,000		0,000 to 0,000		-6,518 to -2,073		-0,750 to 0,700		0,371 to 1,400		*	
Proportional differences														
Slope B	1,093		0,9		0,9		1,057		0,911		1,023		*	
95% CI	1,027 to 1,147		0,806 to 1,800		0,600 to 1,000		0,982 to 1,147		0,800 to 1,025		0,800 to 1,429		*	
Random differences														
Residual Std. Deviation	6,472		1,073		1,214		10,289		5,587		3,967		*	
± 1.96 RSD Interval	-12,685 to 12,685		-2,103 to 2,103		-2,380 to 2,380		-20,167 to 20,167		-10,949 to 10,949		-7,775 to 7,775		*	
Linear model validity														
Cusum test for linearity	No significant deviation from linearity (P=0,58)		Significant deviation from linearity (P=0,01)		No significant deviation from linearity (P=0,14)		No significant deviation from linearity (P=0,30)		No significant deviation from linearity (P=0,97)		No significant deviation from linearity (P=0,20)		*	
Spearman rank correlation coefficient														
Correlation coefficient	0,932		0,347		0,535		0,853		0,817		0,627		0,455	
Significance level	P<0,0001		P=0,0002		P<0,0001		P<0,0001		P<0,0001		P<0,0001		P<0,0001	
95% CI	0,902 to 0,953		0,170 to 0,503		0,385 to 0,657		0,792 to 0,897		0,744 to 0,871		0,497 to 0,729		0,292 to 0,592	
Bland-Altman plot														
Arithmetic mean	-6,522		0,210		0,368		-0,692		0,122		-0,670		-0,251	
95% Confidence interval	-8,285 to -4,759		-0,191 to 0,611		-0,003 to 0,738		-3,416 to 2,033		-1,277 to 1,522		-1,715 to 0,376		-0,859 to 0,358	
P (H_0: Mean=0)	<0,0001		0,301		0,052		0,616		0,863		0,207		0,416	
Lower limit	-24,719		-3,930		-3,457		-28,816		-14,326		-11,465		-6,530	
95% Confidence interval	-27,741 to -21,698		-4,617 to -3,242		-4,092 to -2,822		-33,486 to -24,146		-16,725 to -11,927		-13,258 to -9,673		-7,573 to -5,487	
Upper limit	11,675		4,350		4,192		27,433		14,570		10,126		6,029	
95% Confidence interval	8,653 to 14,697		3,663 to 5,037		3,557 to 4,828		22,763 to 32,103		12,171 to 16,969		8,333 to 11,918		4,986 to 7,072	
*- Passin-Bablok regression analysis is not a predictive model for this group of cells														

Figure 3. Passing-Bablok and Bland-Altman plot analysis results

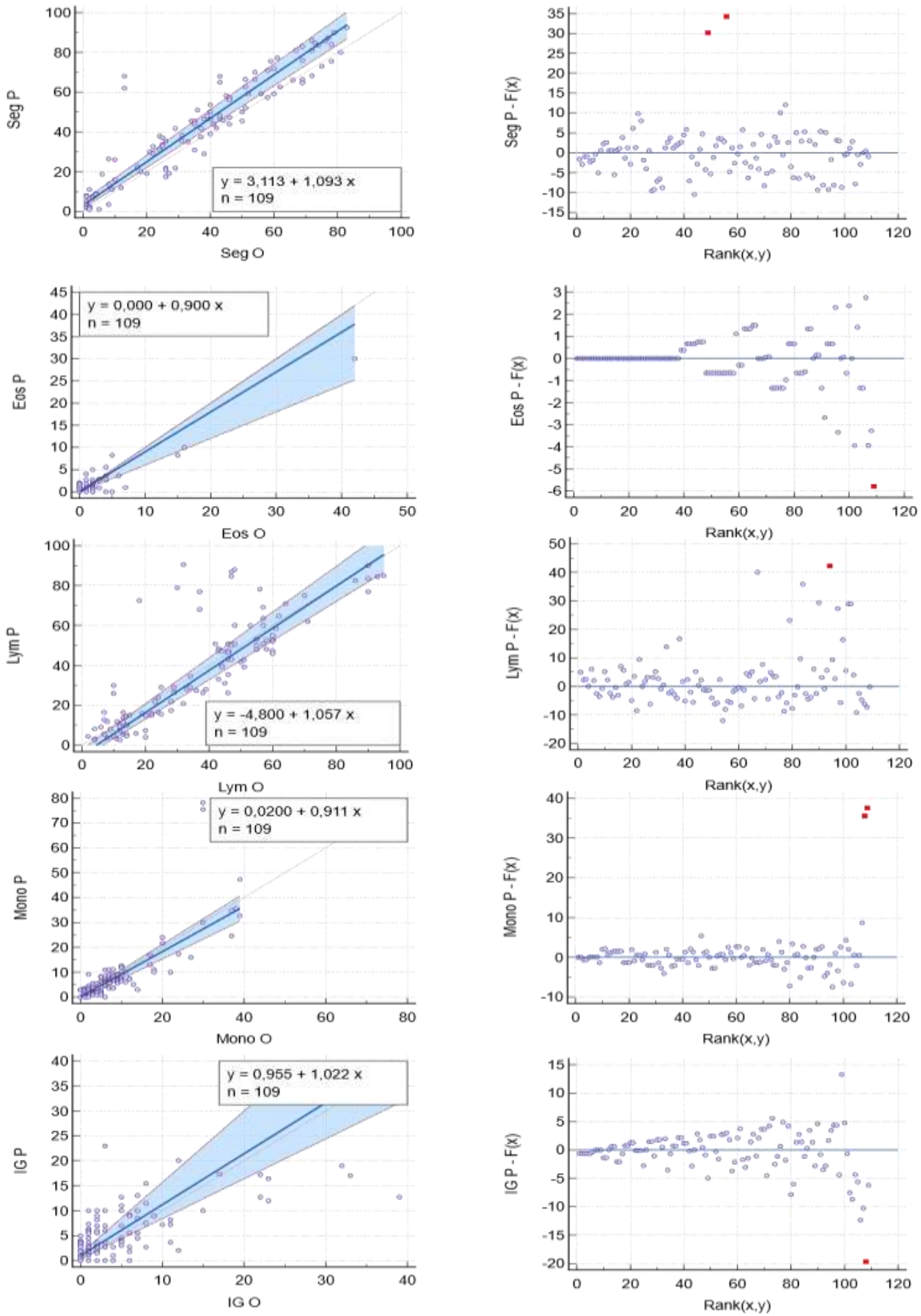

Figure 4. Graphical representation of Passing-bablok regression results for segmented neutrophils (Seg), eosinophils (Eos), lymphocytes (Lim), monocytes (Mon) and immature granulocytes (IG)

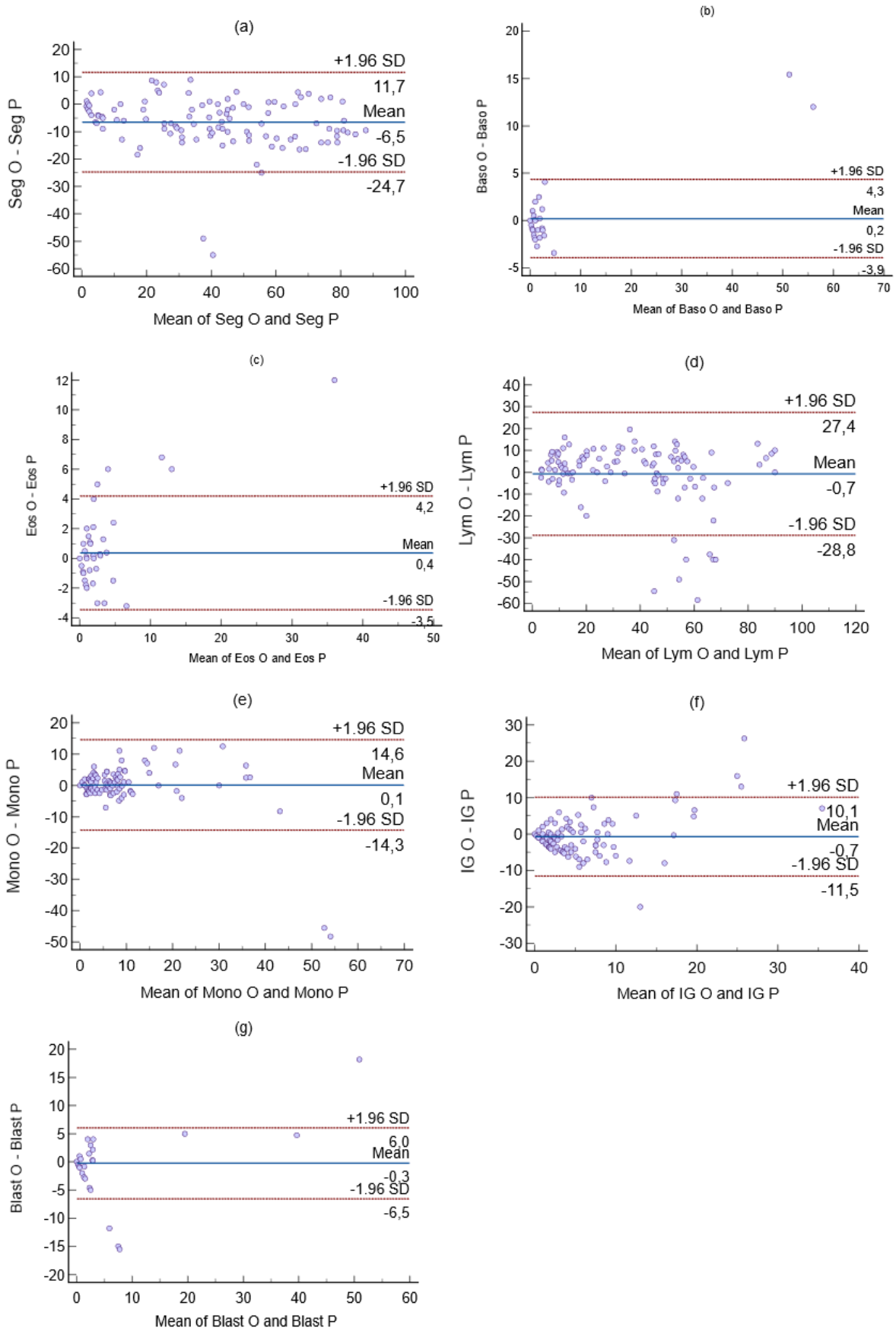

Figure 5. Graphical representation of the results of the Bland-Altman plot analysis for (a) -segmented granulocytes, (b) -basophils, (c) -eosinophils, (d) -lymphocytes, (e) -monocytes, (f)- immature granulocytes and (g)-blasts. O-manual microscopy, P-digital microscopy

4. Discussion

The obtained results of the analysis indicate the need for discussion within the linearity of the methods, systemic and proportional differences between the methods, and the correlation between digital and manual optical microscopy.

Linearity between methods, analyzed through Passing-Bablok regression analysis, was satisfied for all cells except basophils and blasts. Systemic and/or proportional differences were detected between the methods depending on the type of cell analyzed, as indicated by the values of the intercept and slope.

A statistically significant systemic difference was observed in segmented neutrophils, lymphocytes and immature granulocytes. Positive intercept values in segmented neutrophils and immature granulocytes indicate that digital microscopy provides statistically significantly higher values compared to manual microscopy. Kweoon et al (2022) also demonstrated the presence of a significant difference in the field of neutrophils [14]. Lackova (2022) performed a comparative analysis of immature granulocytes where the results of digital and manual microscopy were compared and, unlike our research, did not find a statistically and clinically significant difference between the methods [15].

On the other hand, the negative value of the intercept in lymphocytes indicates that digital microscopy gives statistically significantly lower values compared to manual microscopy.

A statistically significant proportional difference between the methods was observed in segmented neutrophils. A slope value greater than 1 (segmented neutrophils) indicates that digital microscopy provides proportionally higher values compared to manual microscopy.

Spearman's correlation coefficient shows a different degree of agreement between the methods, with a very strong correlation in segmented neutrophils (r=0,932), a strong correlation in lymphocytes (r=0,853), while correlations in eosinophils (r=0,535) and immature granulocytes (r=0,627) are moderate. These results suggest that, although there is a strong correlation between methods for most types of leukocytes, deviations become more pronounced in specific conditions such as leukopenia and leukocytosis, which was also observed through the Bland-Altman analysis. The Zhao et al study (2024) shows concurrent results with our study, where it was shown that the digital (DI-60) and manual microscopy method had a high correlation for segmented neutrophils (r=0,94), lymphocytes (r=0,85); moderate correlation for eosinophils (r=0,63) and immature granulocytes (r=0,56), while data for basophils did not allow for Passing-Bablok regression analysis. The only difference from our study is in monocytes where Zhao et al demonstrated a low correlation for monocytes (r=0,45) [16].

The same study showed limited use of digital microscopy in routine work in moderate and severe leukocytosis and leukopenia where manual microscopy is preferred.

On the other hand, in the study by Kweoon et al (2022), monocytes showed a good correlation between the two methods (r=0,76), which we also proved in our research (r=0,817), and there is also a proven insufficient possibility of using digital microscopy for basophil differentiation[14].

The conducted Bland-Altman analysis confirms the existence of a systemic difference between the methods. It is statistically significant only in segmented neutrophils, where manual microscopy gives lower values compared to digital microscopy, thus confirming the result obtained by Passing-Bablok analysis. The analysis of graphical representations shows deviations that are mainly related to the conditions of leukopenia or leukocytosis. This implies that specific states may affect the accuracy and precision of digital microscopy relative to manual microscopic analysis.

5. Conclusion

Digital microscopy (Sysmex DI-60) shows a high correlation with manual microscopy in the analysis of most types of leukocytes. There are less proportional and systemic differences that are not statistically significant. However, the systemic differences observed in segmented neutrophils, lymphocytes and immature granulocytes, cause higher values for the analyzed cell types by digital microscopy compared to manual, indicating the need for additional reclassification, enabled by the analyzer. Of course, in order to fully use this analyzer in routine work, it is necessary to compare the results of reclassification and manual microscopy on different groups of

samples in relation to the total number of leukocytes, and on a significantly larger number of samples. This will give a clearer picture of the capabilities and performance of digital microscopy. Verification of specific pathological conditions that are characterized by an extremely high or low white blood cell count should remain under the supervision of manual microscopy, due to the pronounced deviations detected by this analysis.

Declaration of Competing Interest

All authors declare that they have no conflict of interest in this work.

Funding information

No funding was received from any financial organization to conduct this research.

Author contribution

Nermina Klapuh-Bukvić: study design; Zehra Kurtanović, Damir Seper: analysis and interpretation of results; All authors: draft preparation. All authors read and approved the final version of the manuscript

Ethical approval statement

The present study was approved by the Ethics Committee of the Clinical Center University of Sarajevo. All procedures were performed in accordance with the ethical standards of the Institutional Review Board and the Declaration of Helsinki.

References

[1] Chapman, Joseph, and Yaoping Zhang. "Histology, hematopoiesis." 2018.
[2] Blumenreich, M. S., H. K. Walker, W. D. Hall, and J. W. Hurst. "Clinical methods: the history, physical, and laboratory examinations." *Third ed1990*, 1990.
[3] Daves, Massimo, Vincenzo Roccaforte, Fortunata Lombardi, Rossella Panella, Stefano Pastori, Marta Spreafico, Erika Jani, and Andrea Piccin. "Modern hematology analyzers: beyond the simple blood cells count (with focus on the red blood cells)." *Journal of Laboratory and Precision Medicine* 92024).
[4] Lynch, Edward C. "Peripheral blood smear." Clinical Methods: The History, Physical, and Laboratory Examinations. *3rd Edition*,1990.
[5] Kratz, Alexander, Szu-hee Lee, Gina Zini, Jurgen A. Riedl, Mina Hur, Sam Machin, and International Council for Standardization in Haematology. "Digital morphology analyzers in hematology: ICSH review and recommendations." *International journal of laboratory hematology* 41, no. 4,437-447, 2019.
[6] Klapuh-Bukvić, Nermina. "Possibilities and limitations of digital microscopy of blood smear of the modern hematological analyzer Sysmex XN-3100 in leukocyte differentiation." *Journal of Medical Biochemistry*,2025.
[7] Sysmex Corporation, "XN-Series Flagging Interpretation Guide," Apr. 2022. [Online]. Available: www.sysmex-europe.com
[8] Cesana, Bruno Mario, Paolo Antonelli, and Simona Ferraro. "Critical appraisal of the CLSI guideline EP09c "measurement procedure comparison and bias estimation using patient samples"." *Clinical Chemistry and Laboratory Medicine (CCLM)* 63, no. 3, 507-514, 2025.
[9] Bilic-Zulle, Lidija. "Comparison of methods: Passing and Bablok regression." *Biochemia medica* 21, no. 1,49-52, 2011.
[10] MedCalc, "Passing-Bablok regression," 2024. [Online]. Available: www.medcalc.org
[11] D. Giavarina, "Understanding Bland Altman analysis," *Biochem Med (Zagreb)*, vol. 25, no. 2, pp. 141– 151, 2015

[12] P. Kaur and J. Stoltzfus, “Bland–Altman plot: A brief overview,” *Int J Acad Med*, vol. 3, no. 1, p. 110, 2017

[13] P. Schober, C. Boer, and L. A. Schwarte, “Correlation Coefficients: Appropriate Use and Interpretation,” *Anesth Analg*, vol. 126, no. 5, pp. 1763–1768, 2018.

[14] O. J. Kweon, Y. K. Lim, M.-K. Lee, and H. R. Kim, “Red and white blood cell morphology characterization and hands-on time analysis by the digital cell imaging analyzer DI-60,” *PLoS One*, vol. 17, no. 4, p. e0267638, 2022.

[15] Vytisková, Soňa, Mária Lacková, Jana Klinerová, Ludmila Bourková, and Miroslav Penka. "Immature Granulocyte Count by the Sysmex XN-Series Haematology Analyser compared to the Sysmex DI-60 Digital Morphology Analyser and to Manual Microscopy." 2022.

[16] Zhao, Yan, Yingying Diao, Jun Zheng, Xinyao Li, and Hong Luan. "Performance evaluation of the digital morphology analyser Sysmex DI-60 for white blood cell differentials in abnormal samples." *Scientific Reports* 14, no. 1,14344, 2024.