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Compatibility Between Platelet Histogram with IP Message and Platelet Morphology in Thrombocytopenia Patients

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ABSTRACT The aim of this study was to evaluate the compatibility of platelet histogram results with IP Message on the hematology analyzer Sysmex XN-1000 for the platelet morphology of peripheral blood smears of thrombocytopenia patients. This was a quantitative descriptive study of 54 samples taken using the saturation sampling technique at Grati Pasuruan Hospital from April 1–30 2023. The results showed that the histograms that often appeared were abnormal heights on the PU Flag and the IP Messages that often appeared were "PLT Clumps?", "PLT Abn Distribution", and "Thrombocytopenia", with a compatibility level of platelet morphology on peripheral blood smear examination reaching 90.7%. The study concluded that there was a high compatibility between the platelet histogram results accompanied by the IP Message and the platelet morphology of the peripheral blood smear of thrombocytopenia patients, which could improve the accuracy and efficiency of platelet count examination.

INDEX TERMS IP message; peripheral blood smear; platelet histogram; thrombocytopenia

I. INTRODUCTION

Laboratory examination is a multiphase process, starting from patient preparation, specimen collection, examination, and reporting test results. Examinations that can be performed in the laboratory include hematology, clinical chemistry, microbiology, parasitology, immunology, anatomical pathology, and other fields related to individual health interests [1]. One of the examinations that is often carried out is a hematological examination, one of which is a platelet count. Platelet count examination is one of the examinations that is often carried out because it has a role in diagnosis enforcement efforts [2]. One of the purposes of a platelet count examination is to identify platelet disorders such as thrombocytopenia. Thrombocytopenia is a condition where the platelet count is below the normal value [3]. There are several ways to check platelet counts, namely manual and automatic methods, and with the increasing demand for platelet count checks, the manual method is deemed less able to meet these needs [2].

Along with the times and technology, hematology examinations, including platelet counts, have now progressed from the use of manual methods to automated methods and tools called Hematology analyzers. A hematology analyzer is an automatic tool for complete blood count (CBC) that has high speed and accuracy and has more parameters that can be examined, such as leukocyte type count, erythrocyte index, and platelet index [4], [5]. The use of automated tools will have a direct impact on the accuracy of the results and the efficiency of the examination time [6]. The sophistication of hematology analyzers can also produce histograms of Complete Blood Count results [7], [8]. A histogram is a graphical representation of the results of automated hematology analysis, where cell size will be plotted on the X-axis and the number of cells will be plotted along the Y-axis so that the morphology and the number of normal cells and abnormal cells will be known. Unfortunately, for several reasons, blood cell histograms are still not popular and are often ignored [9]–[11].

Some advanced hematology analyzers are able to show what types of abnormal cells are detected in the blood cell examination, but it is still not as good as the manual method because, considering the principle of the hematology analyzer, it is to detect signal messages proportional to the size of the cell [12]. Where this cell size is automatically set on the device according to the type of cell, for example, for platelet size, the range is 2-6 fL and 12-30 fL, so that when

giant platelets are found, they can be detected as erythrocytes, which will affect the examination results marked by the appearance of a signal flag or Interpretive Program Message on the examination results [7]. Therefore, platelet examination with peripheral blood smear is needed to confirm the platelet histogram issued by the device. Platelet count examination with peripheral blood smear has advantages, including an easy to do, cheaper price, and can determine the size and morphology of platelets [13].

Confirmation using peripheral blood smears is done to find out whether the results issued by the tool are correct or because they are influenced by several factors. Carol stated that most analyzers will detect the presence of platelet clumps and produce a sign, but the sensitivity and specificity of the sign vary between instruments, failure to produce a sign will result in a low number of platelets reported [14]. The above statement is also supported by Jiankai, who states that the presence of platelet clumps can cause pseudothrombocytopenia [15]. Previous researchers have conducted research to compare the results of a CBC examination accompanied by a histogram with a peripheral blood smear examination. Ghupta showed that there was agreement on platelet upper (platelet count limit with the largest size) and disagreement on platelet lower (platelet count limit with the smallest size) [16].

Based on this description, the researcher wants to know and analyze how compatible the platelet histogram results with the IP message on the hematology analyzer Sysmex XN-1000 are with the platelet morphology of peripheral blood smears of thrombocytopenia patients. This study can help laboratory staff regarding the reading and utilization of histograms accompanied by IP messages.

II. METHOD

This type of study is a quantitative descriptive study, which is to determine the suitability of platelet histogram results with IP Message on hematology analyzer tools for peripheral blood smear platelet morphology of thrombocytopenia patients. The population in this study were hospitalized patients with thrombocytopenia with samples in the form of EDTA blood. This study is a clinical study with a non-random sampling method based on the consideration that the number of thrombocytopenia patients is unknown, so with this consideration, the sampling technique that is very supportive to use is saturation sampling. Saturation sampling is a sampling method by including all members of the population as research samples based on the research time, namely for one month starting from April 2023.

This study was conducted at Grati Hospital, Pasuruan Regency during April 1-30, 2023. The instrument used in this study was the Sysmex XN-1000 tool. This tool uses the principle of hydrodynamic focusing (HD Detection) to calculate platelet parameters, this method improves the accuracy and reproducibility of blood cell counts and prevents the generation of abnormal cell signals because blood cells pass through the center of the aperture in one direction. The EDTA blood examination was carried out using the Sysmex XN-1000 tool and they identified the Complete Blood Counts results. If an abnormal histogram curve was found accompanied by an IP Message, it was necessary to conduct a confirmation examination using peripheral blood smears. The smear is made by dripping blood on the tip of the glass object, then placing the cover glass on top at a 45-degree angle and pulling it to form a tongue and then drying the smear. After the smear dries, it is stained using Wright, and then the peripheral blood smear is read using a microscope at the tail or thin part to observe cell morphology.

This study uses primary data obtained from the results of the CBC examination and evaluation using peripheral blood smears. Data from the CBC examination results will be recorded and adjusted to the data found in the peripheral blood smear examination. The data obtained is presented in the form of a distribution table with grouping using scores or codes, namely code 1 (compability) and code 2 (incompability). The indicators used in coding in this study are:

- There is a compatibility between the platelet histogram of the abnormal curve and the appearance of the IP message.
- There is a compatibility between the histogram of abnormal curve platelets and platelet morphology on the peripheral blood smear.
- There is a compatibility between the histogram of abnormal curve platelets and fragments and/or microerythrocytes on the peripheral blood smear.
- There is compatibility between the IP message and platelet morphology on peripheral blood smears.

Giving a code aims to facilitate analysis using the percentage formula to determine how much conformity there is between the results of the tool and the results of confirmation using peripheral blood smears. The formula used is as follows:

$$\text{Percentage (compatibility)} = \frac{\text{total score obtained}}{\text{score maximum}} \text{ x } 100\% \quad (1)$$

III. RESULT

From the results of the CBC examination conducted within 1 month from April 1 to April 30, 2023 using the Sysmex XN-1000 tool at RSUD Grati, Pasuruan Regency, 54 samples were obtained which showed platelet histograms accompanied by IP Message.

TABLE 1
Histogram of platelets with IP message

Histogram of Platelets with IP Message		Total
PLT UD Error	PLT Clumps?	31
	PLT Abn Distribution	21
	Thrombocytopenia	9
Normal	PLT Clumps?	5
	Thrombocytopenia	3

TABLE 1 shows the results of platelet histograms accompanied by the appearance of IP Messages on CBC examinations, from 54 samples it is known that there are 41 samples with single IP Messages and 13 samples with combined IP Messages so that over all there are 69 IP Messages that appear. Samples that showed histograms accompanied by IP Message were then subjected to confirmation examination using peripheral blood smears. This examination aims to confirm the results issued by the tool and whether it really matches the patient's condition.

TABLE 2
Peripheral Blood Smear Result

Peripheral Blood Smear Result	Found in Total Sample
Normal platelets	54
Large platelets	24
Giant platelets	40
Platelet clump	14
Normal erythrocytes	54
Fragment erythrocytes	39
Microerythrocytes	43

TABLE 2 shows the results of evaluation using peripheral blood smear examination, it is known that the cells found are normal platelet cells, large platelets, giant platelets, platelet clumps, normal erythrocytes, fragments, and aja microerythrocytes. Furthermore, to determine the percentage distribution of suitability, you can use the percentage formula. Where the percentage of conformity will compare the results of platelet histograms accompanied by IP Message with the results of platelet morphology reading on peripheral blood smears. Frequency distribution is a list that contains an arrangement of data grouped according to certain categories. The data that has been obtained is then categorized with a code in the form of numbers. The coding for the percentage level of conformity in this study is:
a. Code 1 for "compatibility" level of conformity
b. Code 0 for the level of suitability " incompatibility "

TABLE 3
Sample suitability table

Description	Total
Compatibility	49
Incompatibility	5

Based on table 3, it is known that the total number of scores obtained is 49 and the maximum score is 54 (number of samples), then the calculation results are as follows:

$$\text{Percentage (compatibility)} = \frac{\text{total score obtained}}{\text{score maximum}} \text{ x } 100\%$$
$$= \frac{45}{54} \text{ x } 100\%$$
$$= 90{,}7\ \%$$

$$\text{Percentage (incompatibility)} = \frac{\text{total score obtained}}{\text{score maximum}} \text{ x } 100\%$$
$$= \frac{5}{54} \text{ x } 100\%$$
$$= 9{,}3\ \%$$

Based on the results of the percentage calculation, it is known that for the results of the tool in the form of a platelet histogram accompanied by an IP Message after being confirmed with a peripheral blood smear examination, there is a conformity of 90.7% (n = 49) and there is a discrepancy of 9.3% (n = 5) so that it can be stated that the results of the platelet histogram accompanied by an IP Message have conformity with platelet morphology on peripheral blood smears.

IV. DISCUSSION

This study was conducted to determine the compatibility of platelet histogram results with IP Message on the hematology analyzer for the platelet morphology of peripheral blood smears with thrombocytopenia. Blood samples that show CBC results with platelet levels below the normal value range (thrombocytopenia) will be confirmed using peripheral blood smears with the Wright staining method, and further smears will be observed under a microscope to observe platelet morphology.

A histogram is a graphical representation of the results of automated analysis on a hematology analyzer where cell size is plotted on the X-axis and cell count is plotted on the Y-axis [9], [11]. Histograms show the frequency of cells by size, so that cell subpopulations are clearly visible [17]. The platelet histogram results in this study are accompanied by an IP Message, which is a notification of abnormalities in the sample or the possibility that the sample is not normal, such as the discovery of giant platelets, large platelets, and platelet clumps. IP Message has the advantage of being able to know the type of abnormal cells detected in blood samples before confirmatory examination with blood smears [16]. Platelet count results that show an IP Message need to be confirmed using a peripheral blood smear [18], [19].

Confirmation checks using peripheral blood smears need to be done, given that the measurement principle of the hematology analyzer is to detect signal messages proportional to cell size [20]. Where this cell size is automatically set on the device according to the cell type, for platelet size is 2 fL-30 fL with the lower discriminator division set in the range of 2fL-6 fL and the upper discriminator in the range of 12 fL-30 fL [7]. Abnormal cell size will affect the examination results, such as false low platelets which can be caused by giant platelets and platelet clumps, and false high platelets caused by erythrocyte or microerythrocyte fragmentation [15], [21]–[23]. Peripheral blood smear examination aims to see whether there are really abnormal cells in the patient's blood or caused by other factors. Other factors that can affect platelet examination results are pre-analytical stages such as the comparison of blood samples with inappropriate anticoagulants or improper sampling methods [24].

Based on the results of the CBC examination using the Sysmex XN-1000 Hematology Analyzer, a total of 54 samples were obtained showing patient platelet levels of less

than 150,000 µL. The mean platelet level of the samples was 90,000 µL, with a range of 28,000 to 139,000 µL. This is in accordance that patients with thrombocytopenia have platelet levels below the normal value range [3].

Based on TABLE 1, it is known that there are 2 types of histograms that appear, namely the histogram PU Flag or the histogram that does not end at the base line of as many as 49 samples, and a normal histogram of 5 samples. In this study, 41 samples showed a single IP Message and 13 samples showed a combination of IP Message with the information that the IP Message "PLT Clumps?" was 36 samples, "PLT Abn Distribution" was 21 samples, and "Thrombocytopenia" was 11 samples, so the total IP Message that appeared in this study was 69 IP Messages.

Histograms with abnormal heights in the PU Flag were found in 49 samples and the results of the peripheral blood smear examination, they found giant platelets, large platelets, platelet clumps, microerythrocytes, and fragments. This is in accordance with the Sysmex guidebook that curves that do not end at the base line are generally caused by giant platelets, platelet clumps, or microerythrocytes and erythrocyte fragments [19]. The difference between giant platelets and large platelets is based on their size. Giant platelets have a size of 8-20 fL while large platelets are 4-7 fL [25]. The appearance of this histogram is accompanied by IP Messages "PLT Clumps?", "PLT Abn Distribution", and "Thrombocytopenia". For the IP Message "PLT Clumps?", 31 samples were found, of which 10 samples showed concordance with the results of examination using peripheral blood smears, 16 samples showed false positive results, while the other 5 samples showed discordance. In the normal histogram, the IP Message "PLT Clumps?" was also found in 5 samples, where 2 samples showed conformity with the results of the examination using peripheral blood smears and 3 other samples showed false positive results.

IP Message "PLT Clumps?" is an IP Message that has a high risk of giving false-positive results. This can be caused by the presence of other blood cell abnormalities that can cause increased signals on the same channel, such as giant platelets, erythrocyte fragmentation, and leukocyte clumps [26], [27]. Based on its type, the IP Message "PLT Clumps?" is included in the suspect message category along with the IP Message Giant Platelets, which is a message that appears if there is a possibility that the sample has abnormal cells [7]. The presence of IP Message Giant Platelets will make it easier for the tool to distinguish giant platelets and platelet clumps so that it will reduce the frequency of false IP Message "PLT Clumps?" appearing, but unfortunately, the availability of this Giant Platelet flag depends on system settings, and this flag is still rarely activated [19].

In the peripheral blood smear examination results for samples accompanied by IP Message PLT Abn Distribution, 21 samples were found and declared entirely in accordance with the peripheral blood smear examination results, because large platelets, giant platelets, microerythrocytes, and erythrocyte fragments were found. This is in accordance with the Sysmex guidebook regarding flagging interpretation, which states that the PLT Abn Distribution IP Message can be triggered when certain parameters (PDW, P-LCR, MPV, PU discriminator, LR discriminator) cannot be evaluated or exceed the specified threshold [19]. The appearance of the IP Message "PLT Abn Distribution" is often associated with a decrease in platelet levels in blood samples [28].

Histograms PU Flag accompanied by IP Message Thrombocytopenia were found in 9 samples and were declared to be in accordance with the results of the examination using peripheral blood smears. IP Message Thrombocytopenia also appeared on normal histograms with low peaks in 3 samples and showed concordance with the examination using peripheral blood smears. Samples that showed IP Message Thrombocytopenia were also accompanied by platelet levels below the normal range, which in this study had levels below 150,000 µL. In the examination using peripheral blood smears, it was found that the patient's platelet levels were lower than the results of the tool, this could occur because there were microerythrocytes and erythrocyte fragments that could be read as platelets by the tool [29]–[32].

The results showed that there was a high compatibility of 90.7% between the platelet histogram results with IP Message and the platelet morphology of the peripheral blood smear of thrombocytopenia patients.This is in line with previous research conducted by Ghupta et al. in 2017, which states that there is 100% (n = 7) conformity with the platelet upper flag histogram.

This study has limitations in the tool used, namely Sysmex XN-1000 at RSUD Grati Pasuruan, which is not equipped with the Giant Platelet flag so that it allows the appearance of a false "PLT Clumps?" IP Message. This study found 24 samples, of which 19 were giant platelets and/or erythrocyte fragments that could affect the appearance of the IP Message "PLT Clumps?" while the other 2 samples were not giant platelets or erythrocyte fragments.

V. CONCLUSION

Based on this study, it can be concluded that there was a high compatibility of 90.7% between the platelet histogram results accompanied by the IP Message and the platelet morphology of the peripheral blood smear of thrombocytopenia patients, which could improve the accuracy and efficiency of platelet count examination. In the future, this research is expected to be a reference for further researchers on the compatibility of platelet histograms with IP messages for platelet morphology on blood smears. For further researchers, it is recommended to use a tool that has been equipped with "IP Message Giant Platelets".

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