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Original Article

The discrepancy between manual and computerized leukocyte and thrombocyte counts

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ABSTRACT

Background Discrepancy between results of leukocyte and thrombocyte count by computerized and manual examination may exist

Objective To determine the discrepancy between computerized and manual leukocyte and thrombocyte count.

Methods The design was a randomized sampling cross sectional study. The blood sample was examined with computerized Cell Dyn 1400 instrument for the leukocyte and thrombocyte count. For manual examination, blood smear was performed to measure thrombocyte while leukocyte was measured in Improved Neubauer hemocytometer. The results of computerized examination were used as gold standard. Sensitivity, specificity, predictive values of manual count were calculated. The agreement of Kappa and Mc Nemar test were determined

Results Blood specimens drawn from 100 patients with different kinds of diagnoses were examined using computerized and manual methods. In computerized group, 66% had normal leukocyte and 55% had normal thrombocyte count. In the manual group, 78% of subjects had normal leukocyte and 82% had normal thrombocyte count. From leukocyte examination, the sensitivity of manual count was 87.9%, specificity was 41.2%, and positive predictive value was 74.36 with the agreement of Kappa of 0.32 and Mc Nemar value of 0.036. From thrombocyte examination, the sensitivity was 96.4%, specificity was 35.6%, and positive predictive value was 64.6 with the agreement of Kappa of 0.41 and Mc Nemar value of 0.41.

Conclusion The result of manual thrombocyte count was in accordance with computerized with the agreement of Kappa of 0.41. On the other hand, there was a discrepancy between manual in favor of computerized leukocyte count with the agreement of Kappa of 0.32 **[Paediatr Indones 2003;43:95-98].**

Keywords: *leukocyte count, thrombocyte count, manual method, computerized method, discrepancy*

Careful assessment of blood elements is often the first step in assessment of hematological function and diagnosis of diseases. Many hematological disorders are defined by specific finding gleaned from tests of the blood. Blood examination often yields important diagnostic information and allows broad differential diagnosis impressions to be performed, indicating further specific testing. Therefore, careful examination of cellular morphology and quantification of each type of blood elements, as well as evaluation of a variety of parameters relating to cellular size and shape, are required. [1] Until the last of previous decade the examinations still used manual method but by the first last decade, automated method has also been used in daily examinations. Automated counters have brought a high degree of precision and reproducibility to what was once a very tedious task in the laboratory. The speed makes it possible to handle large numbers of blood samples and the quality of the results make suitable for even relatively small laboratories with few technicians. Indeed, the only contraindication apart from cost is remoteness of a laboratory from access to service engineer since breakdown is not infrequent.[1,2] Even with the widespread use of electronic cell counters, the manual methods are still the reference method for calibrating these counters.[2]

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Either manual methods or automated hematology analyzers enumerate leukocyte count. Leukocytes are counted following dilution of blood in diluents that lyses the red blood cells (usually acid or detergent). Less dilution of the blood is required to greatly lower number of leukocytes than is needed for the red blood cell counts. Manual counts, as with the red cells counts, have more inherent error, with coefficients of variation ranging from 6.5% in cases with normal or increased white cell counts to 15% in cases with decreased white cell counts. Automated methods characteristically yield coefficients of variation in 1 to 3% of range.[1,3] Thrombocytes are counted in automated hematology analyzers once red cells have been removed by sedimentation or centrifugation, as well as by techniques using whole blood. These give highly reliable platelets numbers when compared with manual methods of counting using a hemocytometer. False low platelets counts may be caused by the presence of platelet clumps, platelet agglutinins, or adsorption of platelets by leukocytes.[4,5] The purpose of this study was to determine the discrepancy of leukocytes and thrombocyte counts between computerized and manual blood examination.

Methods

The study design was cross sectional analysis. Randomization was performed using computer with Epi info system. Ethical clearance was obtained and patients had to sign informed consent prior to the study. Blood specimens were drawn from patients with different kinds of diagnoses hospitalized in the Department of Child Health, Soetomo Hospital during February 2002.

The blood sample were examined with computerized Cell Dyn 1400 instruments for the leukocytes and thrombocyte count which enumerates cells in small aperture by measuring changes in electrical resistance as the cell passes through the orifice. For manual examination, blood smear was performed to measure thrombocytes while leukocytes were measured in Improved Neubauer hemocytometer. Blood smear were usually stained with Giemsa stain.

Microscope Olympus CH 30-FN 18 and Olympus CH 20-FN 18 were used in Pediatric Hematology Laboratory. The normal value for thrombocyte count was 150,000-350,000/cmm and leukocyte count was 4,000-11,000/cmm. The laboratory results were divided in two groups, normal and abnormal (out of normal limit). The results of computerized examination were used as gold standard.

Data analysis was performed with a computer assisted statistical package (SPSS version 10). Sensitivity, specificity, predictive value was calculated. The agreement of Kappa and Mc Nemar test were determined. A *p* value less than 0.05 was considered significant. Interpretation the agreement of Kappa: [6] $K>0.75$ means the agreement is very good; $0.4\leq K\leq 0.75$ means the agreement is good; $0\leq K<0.4$ means the agreement is weak

Results

During the study period, blood specimens were drawn from 100 patients with different kinds of diagnoses. **Table 1** shows that false positive leukocyte count was 59% and false negative was 12% with efficiency of test was 72%.

TABLE 1. CROSS TABULATION OF COMPUTERIZED AND MANUAL LEUKOCYTE COUNTS

Count		Leukocytes computerized Normal	Abnormal	Total
Leukocytes manual	Normal	58	20	78
	Abnormal	8	14	22
Total		66	34	100

TABLE 2. CROSS TABULATION OF COMPUTERIZED AND MANUAL THROMBOCYTE COUNTS

Count		Thrombocytes computerized Normal	Abnormal	Total
Thrombocytes manual	Normal	53	29	82
	Abnormal	2	16	18
Total		55	45	100

Table 2 shows the evidence of false positive thrombocyte count was 64.4%, false negative was 3.64%, and efficiency of test was 69%.

TABLE 3. SENSITIVITY, SPECIFICITY, PREDICTIVE VALUE OF MANUAL LEUKOCYTE AND THROMBOCYTE COUNTS

	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Leukocytes	87.9	41.2	74.4	63.6
Thrombocytes	96.4	35.6	64.6	88.9

Manual leukocyte count showed the agreement of Kappa was 0.32 with *p* value of Mc Nemar test of 0.036.

Manual thrombocyte count showed the agreement of Kappa was 0.41 with *p* value of Mc Nemar test was 0.41. As shown in **Table 3**, manual thrombocyte and leukocyte count have high sensitivity but not specific.

Discussion

Cell counts remain the basis for many of the parameters used in evaluating the blood. Cell counts may be determined either manually or by automated hematology analyzers. Whether they are performed by manual or automated means, accuracy and precision of the counts depends on proper dilution of the blood sample and precise sample measurement. Errors in cells counts are caused primarily by errors in sample measurement, dilution, and enumeration of cells. The highest degree of precision occurs when a very large number of cells are enumerated. Clearly automated methods provide the best means for counting large numbers of cells and minimizing statistical error. [1]

Either manual methods or automated hematology analyzers may be used to enumerate leukocytes. Manual counts have more inherent error, with coefficients of variation ranging from 6.5% in cases with normal or increased white cell counts to 15% in cases with decreased white cell counts. [3]

This study showed that manual leukocyte count was sensitive but not specific, it means that manual leukocyte count have the ability to measure samples with normal leukocyte count but cannot determine samples with abnormal leukocyte count precisely. The agreement of Kappa showed the discrepancy between manual and computerized methods was less than 0.4, which means that the agreement was weak. (K = 0.32)

The enumeration of platelets has now become a routine component of the complete blood cell count because of automation. In cases of severe thrombocytopenia or whenever cellular abnormalities may be spuriously affecting the automated count, a manual count should be performed. [7]

This study showed that manual leukocyte count and thrombocyte count had high sensitivity but the specificity was low. Based on the result of the agreement of Kappa, the manual thrombocyte count was in accordance with the computerized one.

This study suggested that the precision of manual cell count was not better than computerized count although the sensitivity of thrombocyte count was better than that of the leukocyte count.

There are many sources of error in performing manual cell counts, one of the most important being that too few cells counted, particularly in comparison with electronic cell counters. The more the cells counted, the more valid the result, however, this approach needs more time. A scrupulous technique is vital; counting errors are due to ***dilution*:** The potential error is large as only a small volume of blood is diluted in a large volume of diluents. The blood must be precisely aliquot and the cells evenly distributed within the sample. Adequate mixing of the diluted sample is essential; thrombocytes take longer to mix than erythrocytes or leukocytes do ***sampling*:** The blood sample must be thoroughly mixed, the aliquot obtained for dilution should be measured precisely, the diluted sample itself must be adequately mixed, and the transfer of fluid to the counting chamber should be smooth, uninterrupted and stopped at the right moment ***counting*:** The cells must be recognized and counted by the operator, and care taken to count all the cells in the appropriate area of the grid. Observer error in counting might be common, particularly between individual observers, such as debris and dust may be mistaken for white cells by the inexperienced technicians. Phase contrast microscope provides better resolution for thrombocyte counting, and thrombocytes can be better differentiated from dust and cellular debris.

In conclusion, manual leukocyte count was sensitive but not specific with the positive predictive value of 74.4% and the negative predictive value of 63.3%. There was discrepancy between manuals in favor of computerized leukocyte count with the agreement of Kappa of 0.32. Manual thrombocyte count had high sensitivity, but not specific with positive predictive value of 64.6%

and the negative predictive value was 88.9%. The result of manual thrombocyte count was in accordance with computerized count with the agreement of Kappa of 0.41.

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