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Comparison of manual and automatic cell count methods for synovial fluid: A prospective study

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Ethics Committee Approval
Ethics committee permission for this study was granted by Hitit University Faculty of Medicine Clinical Research Ethics Committee on 19.12.2017, with the decision number 2017-199. All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

Conflict of Interest
No conflict of interest was declared by the authors.

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Abstract

Background/Aim: Cell count measurement methods in the synovial fluid are still a current problem in orthopedic practice. Knowing the cell count in the synovial fluid is important for the assessment of a variety of orthopedic and rheumatologic diseases. We aimed to assess the correlation of WBC and RBC results obtained with a complete blood count analyzer with that obtained by a manual cell count.
Methods: The WBC and RBC count in the synovial fluid of 43 patients undergoing gonarthrosis surgery were determined by a Mindray BC-6800 hematology analyzer. The study groups were defined as manual cell count (MC), hemogram mode (HM), and body fluid mode (BFM). All samples were analyzed twice consecutively, and the mean results were calculated. Cell counting was performed using different methods in the same samples and compared statistically.
Results: The mean age of the patients was 60.9 years, and there were 17 males (39.5%) and 26 females (60.5%). The WBC and RBC counts in the synovial fluid samples were determined using manual cell count, and the HM and BFM on a Mindray BC-6800 automatic hematology analyzer. WBC counts significantly differed between MC and BFM, and RBC counts significantly differed between HM-MC and HM-BFM (P=0.001, P=0.001, P=0.001, respectively). There was a significant positive correlation between BFM and MC in WBC counts (r=0.633, P<0.001), with no statistically significant correlations identified between other methods. For RBC counts, there was a significant positive correlation between BFM and MC results (r=0.363, P=0.032).
Conclusion: While the body fluid mode in hematology analyzers can be recommended for obtaining an RBC count in the synovial fluid, the hemogram mode may be recommended for the WBC count.

Keywords: Synovial fluid, Hematology analyzer, Cell count, WBC, RBC

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Introduction

Synovial fluid cell analysis is important to assess a variety of diseases, such as inflammatory disorders, infection, hemorrhage, and malignancy and may be used for differential diagnosis and treatment monitoring [1-6]. The manual cell count is the gold standard method for the assessment of cell counts in body fluids [7]. Cell counts in the synovial fluid show the degree of inflammation in the joint [8, 9]. Hematology analyzers provide accurate results in the synovial fluid, though the results are better for other body fluids [10]. However, the use of hematology analyzers for synovial fluids is not common [1, 11]. Additionally, recently developed hematology analyzers have the potential to take the place of the manual cell count [12].

The gold standard method for cell count, manual cell counting, is time-consuming and not common due to the lack of qualified personnel trained in the field. Additionally, the repeatability between the analyzers is low. For this reason, automated methods were developed [13]. However, problems are encountered during the analysis of synovial fluid with automated methods due to high viscosity linked to hyaluronic acid [1, 2, 14, 15].

Different analyzers and automated methods were trialed for cell counts in body fluids. The Mindray BC-6800 used in this study is an automatic hematology analyzer equipped with a special module [1, 15]. This study aimed to use the Mindray BC-6800 hematology analyzer in the hemogram and body fluid modes for obtained synovial fluid cell count and perform manual cell counts to determine compatibility.

Materials and methods

Study design

Ethics committee permission was granted by Hitit University Faculty of Medicine, Clinical Research Ethics Committee, dated 19.12.2017, with the decision number 2017-199. This study included 52 voluntary patients who underwent gonarthrosis surgery in the orthopedics and traumatology clinic from January 2019-December 2019. The synovial fluid samples of the patients who provided informed consent were used.

The samples were collected in tubes containing K_3EDTA, inverted gently 6-8 times, and did not undergo any preliminary processing. Every sample reaching the laboratory was analyzed within one hour. Bloody or cloudy samples were diluted 1/10 before manual and automatic analysis due to high concentrations of RBC and/or WBC. Each sample was analyzed twice consecutively, first with a manual count, then in the BFM and HM modes of the Mindray BC-6800, automated hematology analyzer, and the mean results were obtained.

Cell count with Neubauer slide

A Neubauer slide was used for manual cell counts. Two count areas are determined on the Neubauer slide and separated from each other with a hollow. Each count area contains four sections of 16 squares with 1 mm^2 in the corners for leukocyte count and a section of 25 squares with 1 mm^2 in the central section for erythrocyte count.

In our study, all manual cell counts were performed by the same researcher. A clean coverslip was placed on the Neubauer slide, 10 µL of the synovial fluid sample was taken with calibration-certified automatic pipettes and pipetted onto the Neubauer slide under the coverslip without any air bubbles. Synovial fluid rapidly filled the area between the coverslip and the gridded area on the Neubauer slide. The cells were left for five minutes to settle onto the slide. With a microscope, the homogeneous distribution of cells was checked at 100X magnification, and the microscope was set to 400X for the count. Leukocytes were counted in one of the 4 sections with 16 squares at the edges of the Neubauer slide. Erythrocytes were counted from the section comprising 25 squares in the center of the slide. At the end of counts, cell numbers were multiplied by 10 and the cell numbers in 1 mm^3 volume were calculated (cells/mm^3). Mesothelial cells were not included in WBC counts. The macroscopic images of samples and microscopic leukocyte morphologies and aggregation were assessed.

Cell count with Mindray BC-6800 automated hematology analyzer

After the targeted blood cells enter the reaction, scattered laser light coming from two angles and fluorescent signals are used for three-dimensional counting. The three-dimensional scatter diagram is especially important to better identify and differentiate blood cell populations and can determine abnormal cell populations that are not identified with other techniques. The Mindray BC-6800 analyzer uses BFM-SF cube technology and assesses WBC count, as well as the distribution of cells and nucleated cells. The targeted cells undergo 3D analysis with the information from the fluorescence flow cytometry signals and from a laser scanner that is illuminated at two angles. BFM can directly perform WBC and total nuclear cell counts from the DIFF channel. After studying each blood sample with the hematology analyzer, a blank sample is studied to minimize the carry-over effect. The study was performed according to the CLSI document numbered H56-A6 and International Council for Standardization in Hematology (ICSH) recommendations and abided by the Helsinki Declaration [1, 16].

Statistical analysis

Statistical analysis was completed using the SPSS IBM Version 23.0 (SPSS Inc Chicago, IL, USA). The normal distribution of the data was assessed with the Shapiro Wilk test. Normally distributed continuous variables were presented as mean ± standard deviation, while non-normal data were presented as median (25^{th}-75^{th} percentile). Descriptive statistics for the categorical data are given in number and percentage. Inter-group comparisons were performed with the Wilcoxon signed-rank test. The correlation between measurement methods was researched with Spearman's correlation coefficient. $P<0.05$ was accepted as the level of statistical significance. A power analysis yielded the minimum sample size as 34.

Results

Of the patients, 17 were males (39.5%) and 26 were females (60.5%). The median values of WBC and RBC count in the synovial fluid, detected with the hemogram and body fluid modes of the Mindray BC-6800 automatic hematology analyzer, and the manual cell counts are shown in Table 1.

For WBC counts in synovial fluid, the results obtained from MC are nearly twice that obtained from HM, while BFM

counts are nearly half. For RBC counts, HM was thirteen times more than the manual count; however, MC and BFM results were close (Table 1).

Table 1: Comparison of the hemogram mode, body fluid mode, and manual cell count results

	WBC		RBC	
	Median (Q1-Q3)	*P*-value	Median (Q1-Q3)	*P*-value
HM (1)	100 (50-400)	1-2: 0.957	20,000 (10,000-30,000)	1-2: <0.001
MC (2)	175 (100-366)	1-3: 0.077	1,540 (340-4,000)	1-3: <0.001
BFM (3)	275 (136-450)	2-3: 0.001	2,000 (1,000-4,000)	2-3: 0.734

HM: Hemogram Mode, MC: Manual count, BFM: Body fluid mode, WBC: White Blood Cell, RBC: Red Blood Cell

According to two-way group comparisons with the Wilcoxon signed-rank test, there were significant differences between MC and BFM in terms of WBC counts (P=0.001) and between MC-HM and HM-BFM for RBC counts (P<0.001 and P<0.001, respectively, Table 1).

According to Spearman's correlation analysis, the BFM and MC results of the WBC counts were significantly correlated (r=0.633, P<0.001), while the results of the other methods were not. For RBC counts, there was a significant correlation between BFM and MC counts (r=0.363, P=0.032), but no significant correlation between HM and MC and between BFM and HM results (Table 2).

Table 2: The correlation of WBC and RBC values with the three methods

	WBC		RBC	
	r	*P*-value	r	*P*-value
HM-MC	0.083	0.595	0.171	0.395
BFM-MC	0.633	<0.001	0.363	0.032
BFM-HM	0.009	0.956	0.297	0.132

HM: Hemogram Mode, MC: Manual count, BFM: Body fluid mode, WBC: White Blood Cell, RBC: Red Blood Cell

Discussion

Limited studies are comparing the gold standard for cell count in the synovial fluid with the performance of automatic analyzers. In this study, a BC-6800 Mindray (Mindray, Shenzhen, China) automated hematology analyzer was used to determine the cell counts in the synovial fluid with cell counts performed in hemogram and body fluid modes. Considering the manual count results as a reference, the compatibility between the two automatic cell counts with the manual cell count was researched. The body fluid mode of the hematology analyzer was significantly correlated with the manual method results in terms of WBC and RBC count. However, the hemogram mode on the hematology was not.

Cell counts in the synovial fluid still pose a significant problem for clinical laboratories because manual assessment is difficult and time-consuming. Additionally, there may be intra- and inter-observer variability, and repeatability is low. This problem increased the need for automatic analyzers. Despite the increased sensitivity and accuracy of automatic analyzers, reduced variability between the observers, getting the results within a short time and a good correlation with manual counts, debates about whether automatic cellular analyzers can be used instead of a manual cell count continue [3, 17-19]. Most automated hematology analyzers have a body fluid mode. Nearly all cell count studies performed with different hematology analyzers use the body fluid mode for different body fluids [1, 3, 7, 10, 12, 13, 16, 18-21]. However, some hematology analyzers in clinical laboratories only have a hemogram mode. This means that the analysis of body fluids is performed in the hemogram mode. Additionally, different cell counts are obtained with the use of automatic analyzers in different modes. For this reason, it is very important to determine the correlation of different cell count methods with the manual count and find a standard cell count method.

Cho et al. assessed RBC, WBC, neutrophil, eosinophil, basophil, and polymorphonuclear cell counts using the manual method and three different automatic analyzers (Beckman Coulter UniCel DxH 800, Sysmex XN-350, and Sysmex UF-5000) for five synovial fluids and different body fluid samples. They identified a significant correlation between all cell counts with the UniCel DxH 800, except for the RBC count in cerebrospinal fluid samples [3]. Lim et al. counted WBC, RBC, mononuclear, polymorphonuclear cells, and differentiated cells in full blood mode and high-fluorescence body fluid (HF-BF) mode with a Sysmex XN-350 device. They concluded that the HF-BF mode will be beneficial for screening abnormal cells in body fluids [22].

Jiwon et al. [23] counted total cells, WBC, RBC, polymorphonuclear leukocytes (PMN), mononuclear leukocytes (MN), neutrophils, lymphocytes, monocytes, and eosinophils in body fluids using an XN-350 hematology analyzer. Their results very strongly correlated with the manual count of total cells, WBC, RBC, PMN, and MN, strongly correlated in terms of neutrophil and lymphocyte percentages, and weakly correlated in terms of eosinophil percentages.

As hematology analyzers normally study blood samples with much higher cell densities, acceptable cell density is important. According to published data, counts are not reliable in body fluids with the available automatic analyzers below 3 cell/μL for WBC and 1,000 cell/μL for RBC. Additionally, manual counts may not be definite for low cell densities. Due to the high inconsistency in low cell counts, cell numbers above 50 cell/μL for WBC and 3,000 cell/μL for RBC are recommended [13].

In synovial fluid samples obtained from healthy individuals, the WBC count is less than 200 per mm³. A WBC threshold of 2000 cell/mm³ is needed to differentiate inflammatory and non-inflammatory diseases [11]. In our study, synovial fluids which are considered normal were used, so the WBC count cut-off was determined as 200 cell/mm³, and we could not identify a suitable cut-off value for RBC.

Fuster et al. [7] reported no significant difference between the median WBC counts in the three fluids analyzed with the BC-6800 body fluid mode and a manual count, and there was a good correlation between them in a study assessing peritoneal dialysis fluid, ascites, and pleural fluids. A correlation between results obtained with automated methods and manual cell counts in these fluids are expected, as pleura and peritoneal fluid are non-viscous body fluids. However, synovial fluid has high viscosity due to hyaluronic acid. The increase in viscosity may be another cause of the false low values for WBC and RBC counts with both the automatic and manual methods. Samples may be treated with hyaluronidase to prevent these erroneous values and reduce viscosity. Hyaluronidase prevents the reduction in cell flow in automatic analyzers [1, 15]. A study by Kerolus et al. [24] performing manual cell counts did not use hyaluronidase; however, samples were only diluted with 3% saline solution, and they reported that the low cell counts were not due to dilution but due to the slow investigation.

A study by Buoro et al. [1] stated that the body fluid mode in the BC-6800 device may provide a rapid and accurate assessment of WBC and PNL counts in synovial fluid. They identified a high correlation between samples undergoing pretreatment with hyaluronidase during analyses of synovial fluid cell counts in samples treated and not treated with hyaluronidase.

The Sysmex XE-2100 has two different WBC count modes. The first is the WBC/BASO channel, which performs total WBC count and selective basophil count. The second is the DIFF channel and is used to count neutrophils, lymphocytes, monocytes, and eosinophils. In terms of WBC counts, there is a weak correlation between WBC/BASO channel and manual counts. Contrarily, the DIFF channel and manual reference method are highly correlated in WBC counts. In this study, there was a high correlation between WBC counts in diluted and undiluted synovial fluid samples analyzed with the DIFF channel on the hematology analyzer. For this reason, the dilution procedure is not necessary to investigate synovial fluid samples in the DIFF channel. The reason for obtaining false low WBC counts with the WBC/BASO channel is the mucin clotting and hyaluronate polymerization linked to the low pH (pH=3.4) of the inorganic surfactant used in the WBC/BASO channel. When the WBC/BASO channel sample is treated with hyaluronidase, the WBC count significantly increases and equalizes with the WBC count in the DIFF channel. As the surfactant used in the DIFF channel is not acidic (pH=7.3), mucin clotting does not occur, and the WBC count is accurate [20]. The BF mode of the BC-6800 Mindray automatic hematology analyzer used in our study uses the DIFF channel, so we did not consider it necessary to process with hyaluronidase. We concluded that the body fluid mode and manual count were not affected for WBC and RBC. However, we think false low WBC and RBC values were obtained because of hemolysis and disrupted cell flow, because of high viscosity. Under these circumstances, low WBC and RBC counts were encountered on automatic counts. However, the manual counts of samples with hemolysis yielded much better cell counts and differentiation. Additionally, the human eye can differentiate new and old cells with clinical significance. These features are the superior aspects of manual count compared to automatic devices. Though there are studies performed with RBC, WBC, and other cell counts in different body fluids in the literature, there does not appear to be any study with RBC counts in synovial fluid. For this reason, we think it is important to detect RBC values in synovial fluids.

Limitations

Limitations of our study include the lack of the use of different automatic analyzers and counts for leukocyte subclasses, as well as the manual counts not being performed by several individuals and the lack of use of staining techniques.

Conclusion

Body fluid mode in hematology analyzers may be recommended as a cell count method for synovial fluid as results show a high correlation with manual cell counts. The development of a standardized method for cell counts in the synovial fluid is an open issue. The selection of hematology analyzers with a BF mode by the laboratories may contribute to the diagnostic power of the test by increasing awareness about BF mode among laboratory personnel.

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