J Bras Patol Med Lab, v. 51, n. 4, p. 224-228, August 2015

10.5935/1676-2444.20150037

Evaluation of semi-automated cells counting in peritoneal fluid

Avaliação de semiautomação para contagem de células em líquido peritoneal

Daikelly I. Braghirolli[1]; Patricia Pranke[1-3]; Luciane N. Calil[4]

1. Laboratório de Hematologia e Células-Tronco, Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul. 2. Programa de Pós-Graduação em Fisiologia Universidade Federal do Rio Grande do Sul. 3. Instituto de Pesquisa com Células-Tronco, Porto Alegre. 4. Laboratório de Citologia, Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul.

ABSTRACT

Introduction: Currently, the cytological analysis of biological fluids, such as peritoneal fluid, is performed by manually cells counting in Fuchs-Rosenthal chamber. However, this method has a number of limitations. Because of these limitations, automatic counters have been evaluated for cell counting in this type of sample in order to make it faster and more reliable test. **Objective:** The aim of this study is to compare the manual and semi-automated leukocytes and erythrocytes counting in peritoneal fluid. **Materials and methods:** The samples were analyzed manually and using the Countess™ (Invitrogen). **Results:** The results showed that although there is a correlation between the two counting methods, the correlation is relatively low, for both leukocytes and erythrocytes analysis. **Conclusion:** The results suggest that peritoneal fluid should continue to be analyzed in Fuchs-Rosenthal chamber. However, further studies should be conducted with a greater number of samples to investigate the possibility of using automated cells counting in serous fluids and, thus, provide greater speed and quality of results.

Key words: peritoneal fluid; ascites; cell count; body fluids.

INTRODUCTION

Ascites is a condition characterized by the accumulation of serous fluid between peritoneal membranes. The most frequent cause of this clinical condition is liver disease, such as cirrhosis, peritonitis and cancer[1, 2]. The study of serous fluid, also referred to as peritoneal ascites fluid, provides important information about differential diagnosis of stroke and disease status monitoring. Routine laboratory investigations of peritoneal fluid include physical, cytological, biochemical and bacteriological analyses[3]. This may also be complemented by other techniques, such as cytochemistry, immunocytology, cytogenetics and molecular biology. These analyses contribute to differentiate the fluid between exudate and transudate, evaluate the presence of tumor markers and detection of bacterial infections. They are, therefore, very important for clinical and therapeutic decisions-making[4-6].

Cytological analysis is the microscopic examination of the fluid, where total count and differential cell is performed[7]. Currently, most clinical laboratories perform cell count in serous fluid and cerebrospinal fluid (CSF) by manual microscopy using counting chambers, such as Fuchs-Rosenthal[8, 9]. However, this method has several limitations. Besides being a time-consuming technique, low accurate and has considerable inter and intra-operator variability, requiring highly skilled and experienced technicians[8-10]. Based on these facts, different automated devices have been evaluated for cell counts in different biological fluids[8, 11, 12]. It is believed that the use of automation in this examination would help in obtaining more reliable results, better organization and manipulation of the samples, and optimization of time, human resources, space and material[13]. The semi-automated Countess™ (Invitrogen) is used for cell count, mainly in research laboratories. The present study has aimed to evaluate the semi-automated method for cells counting

First submission on 26/02/15; last submission on 09/06/15; accepted for publication on 22/06/15; published on 20/08/15

in peritoneal fluid, compared with manual counting in Fuchs-Rosenthal chamber.

MATERIALS AND METHODS

Obtaining samples

The study was performed on 30 samples of peritoneal fluid. The samples were from the hematology unit of the Hospital de Clinicas of Porto Alegre and were collected from April to May 2012. All the samples were stored between 4ºC and 6ºC until the time of analysis, not exceeding 24 hours.

Microscopic examination using Fuchs-Rosenthal chamber

Manual cell count was performed using Fuchs-Rosenthal chamber with a phase-contrast microscopy, using eye set at 10-fold increase in 40× magnification objective lens, resulting in a total magnification of 400 times. Before counting, the Fuchs-Rosenthal chamber was cleaned with 70% ethanol and covered with a thin glass coverslip, leaving a space of 0.2 mM between the chamber and the coverslip. The coverslip was positioned to cover the two chamber reticles. After homogenization, the samples were carefully placed in the chamber with the aid of a capillary, avoiding bubbles formation.

The chamber was then placed under a microscope and all the quadrants were evaluated to verify the homogeneous distribution of cells, and thus, continue the count of the erythrocytes and leukocytes. The cells were counted in at least four quadrants of Fuchs-Rosenthal chamber; however, when the number of cells was less than 20 in each quadrant, cell counting was performed in each chamber (3.2 microl) to ensure high-quality of analysis.

Each sample was separately processed and analyzed by two biochemical professionals. In cases of major differences between the two assessments, a third count was performed by a professional third-party. The average of these results was used for comparison with the results obtained by the semi-automated device.

Cell counts using a semi-automated device

To quantify cells in Countess™ device, two protocols for counting cells were created in the apparatus, one for leukocytes and the other for erythrocytes. In these protocols, the parameters for inclusion and exclusion of each cell type (**Table**) were defined.

For analysis of the samples, firstly a solution containing 20 µl of biological fluid to be counted, and 20 µl of trypan blue dye (Gibco) were prepared. Then, 10 µl of this mixture was transferred to the chamber of the specific counting device (Countess cell counting chamber slides – Invitrogen). The chamber was then placed on the counter, the brightness and focus were adjusted and the erythrocyte and leukocyte count was performed.

TABLE – Parameters in cell counting using Countess™

	Minimum size	Maximum size	Circularity	Sensitivity
White blood cells	9 µm	21 µm	85%	7
Red blood cells	4 µm	8 µm	95%	5

Statistical analysis

Statistical analyses were performed in Prism 5 software. The study of distributions was carried out using Kolmogorov-Smirnov test. The cell counts obtained in Fuchs-Rosenthal chamber and semi-automated were compared using the Spearman correlation coefficient. The correlation was considered significant when $p < 0.05$.

RESULTS

Correlation analysis of cell counts in the total net

The results obtained for the two methods of counting white blood cells and erythrocyte evaluations are shown in **Figures 1** and **2**. For both cell types, a significant positive correlation between semi-automated and manual counting in a Fuchs-Rosenthal chamber was found. The factor using Spearman correlation was 0.60 ($p = 0.0005$) for white blood cells (WBC) count, and 0.53 ($p = 0.0026$) for red blood cells (RBC) count.

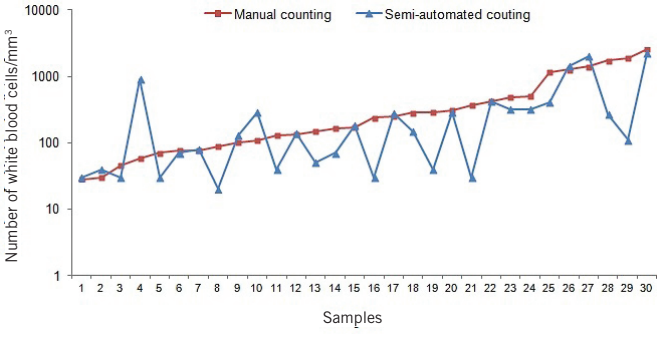

FIGURE 1 – *Comparison between manual (Fuchs-Rosenthal chamber) and semi-automated white blood cells count in peritoneal fluid*

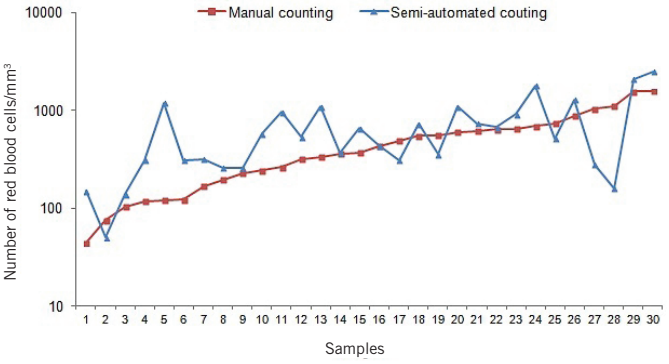

FIGURE 2 – *Comparison between manual (Fuchs-Rosenthal chamber) and semi-automated red blood cells count in peritoneal fluid*

DISCUSSION

For diagnosis of ascites in patients, cytological examination of peritoneal fluid is a very important test. RBC and nucleated cells counting in the liquid allows classification of stroke as either transudate or exudate, and helps to defining the ascites etiology. It also enables future clinical evaluation[1]. Even today, the identification and quantification of cells in biological fluids, including peritoneal fluid, are performed by in manual counting chamber. Because of the limitations associated with manual counting, several studies have been conducted in order to replace this practice by automated counters[8, 10, 13-15]. Since the analysis of biological fluids and emergency tests are extremely important for a conclusive diagnosis, it is believed that the use of automated devices could improve the quality and reproducibility of results, as well as provide a speedy diagnosis. Thus, these devices can contribute to a more accurate diagnosis, resulting in a better prognosis for recovery of the patient[13].

Previous studies have evaluated whether automation employed in urinalysis or the equipment used in hematology could be applied for CSF or serous fluid analysis. The study of Buoro *et al*. (2012) evaluated the performance of urine analyzer Sysmex UF-1000 for counting cells in peritoneal fluid samples, comparing it to manual microscopic analysis. Their results showed that there was a good correlation between the two methods, with a correlation factor of 0.988 for WBC count, and 0.995 for total nucleated cells. When the quantification of mesothelial cells and macrophages was assessed, lower correlation was obtained (r^2 = 0.706) between the two methods, but it was still satisfactory[1]. In another study, the equipment Iris iQ®200 Elite, initially designed for cell count in the urine, was evaluated for the analysis of serous fluid and cerebrospinal fluid. The results demonstrated that this equipment produced blood counts and total nucleated cells similar to that of manual counting, with a correlation coefficient ranging from 0.82 to 0.96. However, when reproducibility was assessed, automation showed a higher coefficient of variation than manual counting. Furthermore, there was no difference in analysis time for the two methods evaluated[16]. Other situations in which automated cell counting has a low performance for analyzing serous liquids and in CSF samples are when the samples present low cellularity and then show the presence of cell clusters. In the first case, automation has low specificity, detecting counts that are not observed in the manual microscopic examination[8]. In the existence of cell clusters, the number of nucleated cells is falsely lowered and in such cases, manual counting is a more accurate method because the human eye is able to discern between individual cells and cell aggregates[11, 16]. Furthermore, some automation requires a greater volume of fluid so that cell count can be accomplished by rejecting samples with insufficient volume[12]. In a study conducted by Keuren et al. employing the CELL-DYN Sapphire hematology analyzer, the authors mention that the standard Sapphire algorithm showed substantial deviations from the reference microscopic differentiation: polymorphonuclear cell counts were too high because they contained some monocytic cells. However, when the optimized manual gating strategy is used, a good correlation and negligible bias are found[17].

Thus, in some situations, automation still has flaws, failing to completely replace the manual counting chamber. The cell counting Countess™ is based on image analysis for cell count. A measurement of the number of cells is performed, based on parameters dictated by the operator, such as size and the roundness of the cell to be analyzed. Thus, the protocol design to be employed by Countess™ is of importance for the specificity of the method to be guaranteed. From the defined cell characteristics, algorithmic image analysis is performed. Based on their operational characteristics, the use of this counter has proved to be interesting for the analysis of biological fluids, such as peritoneal fluid to avoid operational error. In addition to performing the cell count, it is possible with the Countess™ to measure cell viability using trypan blue dye. Although this evaluation is not important in biological fluids, the dye was used in the samples to ensure better contrast, brightness and focus of images, ensuring a good performance of the equipment.

The results of this study showed that Countess™ has a significant positive correlation with manual counting for quantification of both the leukocytes and erythrocytes. Moreover, the equipment time analysis was reduced, taking about 30 seconds for reading and demonstration of results of the cell counts. Despite this favorable and significant data, the correlation between the semi-automated and manual count was relatively low. As can

be seen in Figure 1, semi-automated counting for analyzing leukocytes showed lower sensitivity than the manual counting. In some sample of liquids, the Countess™ equipment has detected a smaller number of cells than the manual counting. This probably occurred because of the small number of cells in the samples, insufficient to be accurately detected by the semi-automated method. As for leukocytes analyzing, semi-automated generated a higher frequency of false negative results; for erythrocytes analyzing, higher variability of results was observed (Figure 2). For this cell type, semi-automated showed counts both above and below those obtained using Fuchs-Rosenthal chamber. Since it is a smaller cell, little differentiation by the automation regarding erythrocytes and cellular debris, granules or dye precipitates, may lead to great discrepancy between counts.

Thus, the results obtained in this study showed that semi-automated has not been able to ensure specificity of the analysis, failing to replace manual counting in the microscopic analysis of peritoneal fluid. The study methodology may show false-positive results, as well as false negative results, impairing diagnostic interpretation and therefore, the treatment of patients. However, there are still problems associated with manual counting. As with analysis of biological fluids, tests of an urgent nature must be performed quickly, with quality and reproducibility of results assurance[13]. Accordingly, further studies should be performed using automation based on other analysis methods, in order to obtain an effective and reduced level of imprecision and inaccuracy for analysis of peritoneal fluid and other serous fluids or CSF samples.

CONCLUSION

The results of this study demonstrate that semi-automated counting method showed positive and significant correlation with manual cells counting in peritoneal fluid. However, this correlation was not high and this method can generate false-positive or false negative results. Thus, further studies with increased sampling should be performed, with this and other types of equipment, to assess the possibility of automating the analysis of peritoneal fluid. Techniques with greater specificity and reproducibility than the manual count should be sought so that more reliable results can be obtained, favoring the effectiveness of diagnosis and monitoring of patients.

RESUMO

Introdução: *Atualmente, a análise citológica de líquidos biológicos, como líquido peritoneal, é realizada por meio da contagem manual de células, em câmara de Fuchs-Rosenthal. Porém, esse método apresenta uma série de limitações. Com isso, contadores automáticos têm sido avaliados para a contagem de células nesse tipo de amostra a fim de tornar esse exame mais rápido e confiável.* ***Objetivo:*** *Comparar a contagem manual e semiautomatizada de leucócitos e eritrócitos em líquido peritoneal.* ***Materiais e métodos:*** *As amostras foram analisadas manualmente e no contador de células Countess™ (Invitrogen).* ***Resultados:*** *Os resultados mostraram que apesar de existir correlação entre os dois métodos de contagem, essa correlação é relativamente fraca, tanto para análise de leucócitos como para de eritrócitos.* ***Conclusão:*** *Esses resultados sugerem que o líquido peritoneal deve continuar a ser analisado em câmara de Fuchs-Rosenthal, contudo novos estudos devem ser realizados, com maior número de amostras, para investigar a possibilidade do uso de automação na contagem de células em líquidos serosos e, assim, proporcionar maior agilidade e qualidade no resultado.*

Unitermos: *líquido peritoneal; ascite; contagem de células; fluidos corpóreos.*

REFERENCES

1. Buoro S, Gustinetti R, Dominoni P, et al. Analytical evaluation of Sysmex UF-1000i for flow cytometric analysis of peritoneal fluid. Clin Biochem. 2012; 45: 1263-5.

2. Runyon BA. Care of patients with ascites. N Engl J Med. 1994; 330(5): 337-42.

3. Burgess LJ. Biochemical analysis of pleural, peritoneal and pericardial effusions. Clin Chim Acta. 2004; 343: 61-84.

4. Lippi G, Cattabiani C, Benegiamo A, et al. Evaluation of white blood cell count in peritoneal fluid with five diferente hemocytometers. Clin Biochem. 2013; 46: 173-6.

5. Lippi G, Cattabiani C, Benegiamo A, et al. Evaluation of the Fully Automated Hematological Analyzer Sysmex XE-5000 for flow cytometric analysis of peritoneal fluid. J Lab Autom. 2013; 18(3): 240-4.

6. Kaleta EJ, Tolan NV, Ness KA, O'Kane D, Algeciras-Schimnich A, et al. CEA, AFP and CA 19-9 analysis in peritoneal fluid to differentiate causes of ascites formation. Clin Biochem. 2013; 46(9): 814-8.

7. Strasinger SK, Lorenzo MS. Urinálise e fluidos corporais. 5 ed. São Paulo: Livraria Médica Paulista Editora; 2009.

8. Zimmermann M, Ruprecht K, Kainzinger F, Heppner FL, Weimann A. Automated vs. manual cerebrospinal fluid cell counts: a work and cost analysis comparing the Sysmex XE-5000 and the Fuchs-Rosenthal manual counting chamber. Int J Lab Hematol. 2011; 33(6): 629-37.

9. de Jonge R, Brouwer R, van Rijn M, van Acker BA, Otten HJ, Lindemans J. Automated analysis of pleural fluid total and differential leukocyte counts with the Sysmex XE-2100. Clin Chem Lab Med. 2006; 44(11): 1367-71.

10. de Jonge R, Brouwer R, Smit M, et al. Automated counting of white blood cells in synovial fluid. Rheumatology (Oxford). 2004; 43(2): 170-3.

11. Nanos NE, Delanghe JR. Evaluation of Sysmex UF-1000i for use in cerebrospinal fluid analysis. Clin Chim Acta. 2008; 392(1-2): 30-3.

12. Walker TJ, Nelson LD, Dunphy BW, Anderson DM, Kickler TS. Comparative evaluation of the Iris iQ200 body fluid module with manual hemacytometer count. Am J Clin Pathol. 2009; 131(3): 333-8.

13. Bonavigo AG, Gelinski V, Costa GFM, Plewka J, Costa MA. Comparação entre a contagem manual e automatizada de células no líquido cefalorraquidiano. RBAC. 2009; 41: 47-50.

14. Danise P, Maconi M, Rovetti A. Cell counting of body fluids: comparison between three automated haematology analysers and the manual microscope method. Int J Lab Hematol. 2013; 35(6): 608-13.

15. van de Geijn GJ, van Gent M, van Pul-Bom N, Beunis MH, van Tilburg AJ, Njo TL. A new flow cytometric method for differential cell counting in ascitic fluid. Cytometry B Clin Cytom. 2014.

16. Goubard A, Marzouk M, Canoui-Poitrine F, Launay S, Le Thuaut A, Legrand P. Performance of the Iris iQ(R)200 Elite analyser in the cell counting of serous effusion fluids and cerebrospinal drainage fluids. J Clin Pathol. 2011; 64(12): 1123-7.

17. Keuren JF, Hoffmann JJ, Leers MP. Analysis of serous body fluids using the CELL-DYN Sapphire hematology analyzer. Clin Chem Lab Med. 2013; 51(6): 1285-90.

MAILING ADDRESS

Luciane N. Calil

Laboratório de Citologia; Faculdade de Farmácia; Universidade Federal do Rio Grande do Sul; Avenida Ipiranga, 2752; Rio Branco; CEP: 90610-000; Porto Alegre-RS, Brazil; e-mail: luciane1011@gmail.com.