Technical Note

Automated flow cytometry analysis of peritoneal dialysis fluid

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Abstract

Background. Recently, the Sysmex UF-100 flow cytometer has been developed to automate urinalysis. We have evaluated this instrument to explore the possibilities of flow cytometry in the analysis of peritoneal dialysis fluid (PD) and have compared the obtained data with those of counting chamber techniques, biochemical analysis and bacterial culture.

Methods. UF-100 data were correlated with microscopy and biochemical data in 135 PD samples. Microbiological analysis was performed in 63 suspected cases of peritonitis.

Results. Good agreement (P < 0.001) was obtained between UF-100 and microscopy data for leukocytes (r = 0.825). UF-100 bacterial count correlated (P < 0.001) with UF-100 leukocyte count (r = 0.549). UF-100 bacterial counts were unreliable in samples where interference by blood platelets was observed. Another major problem was the UF-100 ‘bacterial’ background signal in sterile PD samples. Yeast cells were detected by the flow cytometer in spiked samples.

Conclusions. Flow cytometry of PD with the UF-100 offers a rapid and reliable leukocyte count. Sensitivity of the ‘bacterial’ channel count in predicting positive culture exceeds the sensitivity of conventional Gram stain. Furthermore, additional semi-quantitative information is provided regarding the presence of yeasts.

Keywords: flow cytometry; infection; microorganisms; peritoneal dialysis; UF-100

Introduction

Peritoneal dialysis (PD) is a widely accepted treatment for end-stage renal disease [1,2]. Peritonitis, a frequent and major complication of PD, is associated with high risk of mortality and morbidity [3,4], is one of the most frequent causes of peritoneal catheter loss and discontinuation of PD [5] and leads sometimes to a serious complication like sclerosing peritonitis [6]. Peritonitis-free dialysis remains an important goal for the long-term use of the peritoneum as a dialytic membrane [7]. The diagnosis and effective treatment of peritonitis depends on clinical evaluation and correlation with laboratory examination of the dialysate. Diagnostic criteria of peritonitis in PD patients include any two of the following: cloudy or turbid effluent containing >100 leukocytes/µl, abdominal pain and a positive fluid culture [8,9]. Previous reports have demonstrated problems associated with the diagnosis of peritonitis based solely on these indicators [9]. Various techniques have been used to facilitate the recovery of microorganisms from dialysate, among them the use of selected broth media, processing of large volumes of dialysis effluent by concentration techniques or total volume culture. Nevertheless, microorganisms are not always recovered from dialysate during peritonitis [10,11]. Numerous non-infectious causes of cloudy peritoneal dialysate are known [12]. Fungal causes should be ruled out as early as possible [12]. Other markers have been described [13,14], sometimes not being specific to peritonitis [14].

Microscopy has been the gold standard for counting leukocytes [white blood cells (WBC)] in PD fluid. However, it is imprecise and has wide interobserver variability. Moreover, it is labour-intensive and time consuming. Automation seems the answer to improve both accuracy and productivity of PD fluid analysis.

A flow cytometer-based instrument (UF-100) that performs automated microscopic analysis has been developed. Until now, this instrument has been evaluated for urinalysis [15–17] and analysis of CSF [18] and saliva [19]. Since flow cytometry allows accurate and precise quantitative analysis of cells, we aimed to explore the possibilities of the instrument to analyse PD fluid. In this study, flow cytometric data from PD fluid were not only compared with Fuchs-Rosenthal chamber counting but also with biochemical and microbiological data.
Subjects and methods

Patients and samples

We studied 135 routinely collected PD fluid samples. Diagnosis of peritonitis could be suspected when symptoms such as cloudy fluid, fever, abdominal pain and rebound tenderness were present. All samples consisted of a collection in a sterile container for routine biochemical analysis and an accompanying dialysis bag for bacteriological analysis. We obtained samples from 13 (35%) male and 24 (65%) female patients with an age distribution of 2–75 years (median: 55 years) admitted to the renal division of the University Hospital, Ghent. Suspected peritonitis was the most important reason of admission. All analyses were performed within 8 h after collection. The dialysis fluids contained NaCl (5.7 g/l), sodium lactate (3.9 g/l), CaCl₂ (257 mg/l), MgCl₂ (152 mg/l) and glucose (13.6, 22.7 and 38.6 g/l) with an osmolality range of 275–494 mOsm/l.

Sysmex UF-100

The Sysmex UF-100 (TOA Medical Electronics, Kobe, Japan) uses argon laser flow cytometry and measures the sample conductivity. Particles are analysed by electrical impedance for volume, forward light-scatter for size and by fluorescent dyes for DNA (phenanthridine) and membranes (carbocyanine). Pulse intensity and pulse width of the forward scattered light and fluorescence light are measured. From the data, together with the impedance data, the formed particles are categorized by multi-parametric algorithms on the basis of their size, shape, volume and staining characteristics. The results are displayed in scattergrams, histograms and as counts/µl. The UF-100, initially developed for urinalysis, automatically detects and counts erythrocytes [red blood cells (RBC)], WBC, bacteria, yeast cells, crystals, epithelial cells, small round cells, sperm cells and casts. Particles that cannot be classified are counted as ‘other cells’.

Biochemical and microscopic investigations

Total PD fluid protein concentration was measured using a pyrogallol red assay (Sopachem, Brussels, Belgium) on a Hitachi 917 analyser (Roche Diagnostics, Mannheim, Germany). Manual microscopic examination of leukocytes was performed in Fuchs-Rosenthal counting chambers. In each sample at least 20 random microscopic fields were examined at 40×10 magnification and the mean WBC cell count was calculated.

Microbiological investigations

Handling of the injection ports and the fluid exchange system was according to standard hospital hygienic rules: injection ports were disinfected with methanol and were allowed to dry for 2 min. Dialysate was then aspirated into a separate sterile container and sent to the laboratory for routine chemical investigation. Microbiological investigations were performed in 63 samples (49%).

The dialysis fluid bags were tested for the presence of bacteria. Before adding brain–heart infusion broth (10 times concentrated) for enrichment, two sterile tubes (50 ml) were sampled from this bag, centrifuged (1000 g, 10 min) and the sediment was inoculated to several media: 5% sheep blood agar, chocolate agar, thioglyconate broth (with paraffin), Schaedler agar (anaerobical incubation), Sabouraud agar, Candida 1D agar (Biomerieux) and tryptic soy agar with incorporated Tween 80 for disruption of WBCs to obtain a higher bacterial recovery. Identification of isolates was by standard bacteriological methods. The fluid bags and all cultures were incubated and examined daily for 7 days. This way, most organisms are discovered with an easy to perform method [10].

Gram stain was also performed on the sediment. Slides were examined by light microscopy under immersion oil at 100×10 magnification.

Performance and interference studies

To evaluate the linearity in the UF-100 bacterial count channel, we analysed isonotic saline solutions (5 ml) containing one colony from patient isolates of Escherichia coli (n = 3) and Streptococcus agalactiae (n = 3). Platelet-rich plasma, obtained after centrifugation of sterile citrated blood for 10 min at 200 g (n = 3; average platelet count: 485 × 10³/µl), was used to study suspected interference of platelets in the bacterial count.

Three physiological saline solutions (5 ml) containing one colony of Cryptococcus neoformans were used to evaluate the UF-100 yeast cell count.

Statistics

Data are presented as median and interquartile range (range between 25th and 75th percentile). Agreement between automated cell counts and microscopic data was examined by Spearman rank analysis. Statistical significance was considered at the level of P < 0.05. To assess the diagnostic accuracy of WBC, bacteria and total protein, we used ROC-curves and calculated the areas under curves (AUCs) for comparison.

Results

Leukocytes and erythrocytes

The distributions of automated (UF-100) cell counts in negative and positive cultures are summarized in Table 1. Median overall UF-100 WBC and RBC counts were 7 WBC/µl (interquartile range: 4–32 WBC/µl) and 5 RBC/µl (interquartile range: 2–25 RBC/µl), respectively. After logarithmic transformation, good agreement (P < 0.001) was found between UF-100 and microscopic counts for WBC (r = 0.825) (Figure 1).

Bacteria

Median overall UF-100 count was 31 bacteria/µl (interquartile range: 13–95 bacteria/µl). Similar to UF-100 analysis of cerebrospinal fluid, a ‘bacterial’ background signal was detected by the instrument in PD fluid samples with negative bacterial culture.
Bacterial cultures were positive in 27 of 63 cultured specimens (43%) and showed coagulase-negative staphylococci (n = 5), *Staphylococcus aureus* (n = 5), *Streptococcus viridans* (n = 3), *Stenotrophomonas maltophilia* (n = 3), *Corynebacterium* sp. (n = 3), *Candida albicans* (n = 1) and mixed infections (n = 7). Figure 2 represents the automated count in function of the culture result. In contrast to the flow cytometric data, Gram stain followed by microscopy only allowed to detect six of 27 positive cultures (22%).

A moderate correlation was found between the bacterial and WBC counts on the flow cytometer: log(bact; bacterial count/µl) = 1.19 + 0.38 (r = 0.549; P < 0.001) (Figure 3).

### Epithelial cells

Epithelial cells are also measured by the UF-100. No significant difference was observed in epithelial cell count between culture-positive and culture-negative cases.

### Yeast cells

In none of the samples, UF-100 yeast cell counts were above the manufacturer-defined cut-off value (10 cells/µl). However, *C. neoformans* yeast cells were correctly categorized by the UF-100 in three physiological saline solutions to which one colony of *C. neoformans* was added (mean yeast cell count: 28 cells/µl).

### Interference studies

Interference studies focused on the UF-100 bacterial count. In 58 of 64 (90.6%) samples with a bacterial count above the 75th percentile, Gram stain and/or culture remained negative.

We assumed possible interference of cell debris in the UF-100 bacterial count. In diluted sterile K_2_ EDTA blood (1/100 to 1/1000) samples, UF-100 bacterial counts were high (>100 bacteria/µl). Further analysis of platelet-rich plasma (n = 3) showed that platelets were exclusively categorized as bacteria by the instrument.

### ROC-curve analysis

Figure 4 represents a ROC-curve, based on UF-100 analysis, for early prediction of positive PD fluid culture.

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**Table 1. Distribution of automated (UF-100) RBC, WBC, bacterial, epithelial cell counts and total protein**

<table>
<thead>
<tr>
<th>Percentile</th>
<th>Culture positive (n = 27)</th>
<th>Culture negative (n = 33)</th>
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<tbody>
<tr>
<td></td>
<td>RBC (µl)</td>
<td>WBC (µl)</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>5</td>
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<tr>
<td>25</td>
<td>3</td>
<td>9</td>
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<tr>
<td>50</td>
<td>9</td>
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<td>310</td>
</tr>
<tr>
<td>90</td>
<td>30</td>
<td>1367</td>
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</table>

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**Fig. 1.** Correlation (line in full) between flow cytometry (UF-100) and microscopy counts of WBC: log(microscopy WBC; cells/µl) = 0.0067 + 0.97 log(flow cytometry WBC; cells/µl) (r = 0.825; P < 0.001). The dashed line indicates the ideal relationship.

**Fig. 2.**
At a cut-off level of 58 WBC/μl, a sensitivity of 50.0% and specificity of 78.9% was observed. The corresponding AUC was 0.655, which was slightly better than the AUC for bacteria (0.634) and total protein (0.605). Addition of other analytes (bacteria, protein) in the model resulted in slightly improved diagnostic performance with a sensitivity of 75.0%, a specificity of 72.2% and an AUC of 0.743 (Figure 4).

Discussion

We have evaluated the use of a flow cytometer (Sysmex UF-100) in the routine analysis of PD fluid. A good agreement was obtained between WBC counts by the UF-100 and the counting chamber. Comparison with counting chamber techniques, the ‘gold standard’, is difficult as the latter technique has several steps that may contribute to imprecision and inaccuracy. Especially in the high WBC range, accuracy of microscopic counting can be poor.

As the UF-100 has initially been developed for urinalysis, flow cytometric gating for the detection of leukocytes is focused on the neutrophils, which predominate in peritonitis [10,20]. In most cases and especially when low cell counts are encountered, flow cytometry offers a rapid and reliable WBC count. It has been shown that neutrophils and monocytes are properly classified as leukocytes [18].

Because the UF-100 also reports data on bacteria, it might be tempting to use bacterial counts in reporting probabilities for peritonitis. However, two major

![Fig. 2. Box-and-whisker plots of automated UF-100 bacterial count in samples with positive and negative culture results.](https://academic.oup.com/ndt/article-abstract/19/2/463/1863880/466)

![Fig. 3. Correlation between bacterial and WBC counts on the flow cytometer: log(bact; bacterial count/μl) = 1.19 × log(WBC; leukocyte count/μl) + 0.38 (r = 0.549; P < 0.001).](https://academic.oup.com/ndt/article-abstract/19/2/463/1863880/467)
Flow cytometric analysis of PD fluid

Fig. 4. ROC curve for early prediction of positive PD fluid cultures. Combining UF-100 WBC count, bacterial channel count and total protein measurement results in a sensitivity of 75.0% (47.6–92.6%), a specificity of 72.2% (46.5–90.2%) respecting a criterion of 3.15.

points of concern are involved. First, as was the case in the analysis of cerebrospinal fluid [18], a ‘noise’ was detected in the bacterial channel, possibly representing cell debris, which cannot be distinguished from bacteria. This is a major concern for flow cytometric analysis of PD fluid, as this body fluid is sterile in normal conditions. Secondly, this parameter has no added value in distinguishing peritonitis because of the wide spreading of the data (Figure 2).

Sensitivity and specificity of the bacterial count in predicting culture were 43 and 77.8%, respectively, which is better than the traditional Gram stain where bacteria were only seen in 22% of positive cultures, a figure which is comparable with previous publications [10,20]. This illustrates the difficulties in culturing PD fluid: the concentration of bacteria is usually low and there is the possibility of pathogens located in the white blood cells [10].

In vitro supplementation of PD fluid with peripheral blood resulted in a small apparent increase in the bacterial channel count despite negative Gram stain and culture. We postulate that interfering particles (probably cell fragments) are measured in the bacterial channel. Cell fragments and bacteria share similar flow cytometric characteristics (low forward scatter, low phenanthridine and carbocyanine fluorescence). Moreover, we demonstrated that blood platelets are exclusively categorized as ‘bacteria’ by the UF-100.

The additional capacity of the UF-100 to detect yeasts was demonstrated in spiked samples and might help the clinician in the early diagnosis of peritonitis caused by yeasts [12].

In conclusion, flow cytometric analysis is a useful additional tool for PD examination, especially in the emergency setting. It provides rapid (36 s) and accurate data on WBC content of PD fluid. The apparent bacterial count is more sensitive than the conventional Gram stain in predicting positive bacterial cultures and results of bacterial channel count and total protein only have small additional value in the ROC-curve analysis. Absolute flow cytometric bacterial counts should be interpreted with caution since they do not solely represent bacteria. The background ‘bacterial’ signal in sterile PD fluid is a major point of concern. The possibility to detect yeast cells in spiked samples, suggests that the instrument might help in the diagnosis of fungal peritonitis.

Conflict of interest statement. None declared.

References


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