Review

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Clinical relevance and contemporary methods for counting blood cells in body fluids suspected of inflammatory disease

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Abstract: In many inflammatory diseases, the cellular components in body fluids [cerebrospinal fluid (CSF), serous fluids] are increased, rendering essential diagnostic information. The diagnostic value of the total white blood cell count (WBC) and differential count has been evaluated extensively over the years, and a remarkable amount of knowledge has been gained; yet, there is a great deal of clinical uncertainty whether the diagnosis should be based solely on these variables. In some diseases, such as peritonitis, the total WBC and differential count has high sensitivity; whereas, in differentiating pleural effusions, it lacks the sensitivity required to be clinically useful. Nevertheless, many guidelines consider these tests as cornerstone parameters, and in combination with clinical variables, they can successfully guide clinical decision making in initiating or postponing a treatment course for infection and/or inflammatory diseases while awaiting culture results. Although other methods are available for detecting and differentiating WBCs in body fluids, manual microscopy is still considered the gold standard despite its many limitations. During the last decade, automated analyzers have become a popular method for first line screening. Continued progress in their design has led to major improvements including their speed, improved accuracy and lower variability compared with microscopy. Disadvantages of this method include high imprecision in low ranges (depending on the method) and interfering factors. In a time where automation is at the front line in clinical laboratories, it is essential the results obtained are precise, accurate and reproducible. This review provides an overview of the relevance for cell counting in a variety of diagnostic body fluids, and highlights the current technologies used.

Keywords: ascites; automated analyzers; cerebrospinal fluid; differential count; inflammatory disease; pleural fluid; synovial fluid; white blood cells (WBC).

Introduction

Body fluids [cerebrospinal fluids (CSF), serous fluids, synovial fluids (SFs)] contain a variety of nucleated cells including white blood cells (WBCs), nucleated red blood cells (NRBC), lining cells (mesothelial cells, synoviocytes, ependymal cells etc.) and other non-hematopoietic cells (malignant cells) which are reported as the total nucleated cell count (TNC) [1]. Various inflammatory diseases are characterized by the presence of abnormal numbers of WBCs in body fluids (BFs) other than blood; therefore, the total WBC and the differential (lymphocytes, monocytes, eosinophils, neutrophils) count is of substantial diagnostic value in these conditions, and may guide therapy. The diagnostic value of WBCs in various BFs has been studied extensively, but results remain controversial. Nonetheless, the role of WBCs as important diagnostic parameters has undoubtedly been recognized, and is currently included in many clinical diagnostic guidelines. Improper techniques and inaccurate results may lead to the under- or over-diagnosis of threatening inflammatory disorders, such as meningitis and peritonitis.

Currently, three methodologies prevail to generate data for counting and differentiating cells in BFs. These methods are manual microscopy, automated flow cytometry, and automated impedance technology. Traditional manual microscopy is the gold standard; however, in the

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last decade, automated analyzers using flow cytometry and/or impedance technology have become mainstream in clinical laboratories. To date, there is an ongoing discussion about the use of automated cell counters versus conventional microscopy; however, each technique comes with its own limitations. In selecting between methodologies, many factors such as technical capability, sample volume, type of BF and patient category come into play. Each laboratory should make a choice based on its own situation; however, profound knowledge is required of the advantages and disadvantages of each available technology.

The first part of this review focusses on the diagnostic significance of the total WBC and differential count in various BFs during inflammatory diseases. The second part describes the advantages and disadvantages of the different techniques for measuring these cells in BFs.

Methods

To identify relevant articles, a literature search was conducted in Pubmed and Medline up to May 2014 using varied Mesh and free-text terms such as "cerebrospinal fluid", "synovial", "CAPD", "peritoneal dialysis", "ascites fluid", "pleural fluid", "erythrocyte", "leukocyte", "neutrophil", "PMN", "MN", "hematology guidelines", "hematology analyzers", "automated analyzers", "meningitis", "peritonitis", and "cell count". The number of initial hits was reduced to 143 by including only full research articles and reviews written in English, and are based on human studies with no restrictions to the year of publication. Furthermore, the reference list of relevant individual papers was examined for other articles of interest.

Cerebrospinal fluid (CSF)

CSF is located within the cerebral ventricles and subarachnoid spaces of the brain. The composition (WBC, glucose, protein etc.) of CSF in the ventricles is different compared to the lumbar region in physiological and in pathological conditions [2]. The majority of CSF samples sent to the laboratory for cellular analysis are from patients suspected of an infectious or inflammatory disease of which meningitis is the main suspect.

Meningitis, which is mainly caused by bacteria and viruses, is the most common and severe infection affecting the CNS. Microbiological culture technique is the gold standard for confirmation of the causative organism; however, results are usually delayed (up to 72 h for bacteria, and 6 weeks for *Mycobacterium)*. In approximately 80% of the cases, culture results will reveal the causative agent; however, in patients receiving antibiotics prior to CSF analysis, results may be obscured, and the test sensitivity decreases by approximately 20% [3]. This prompted the search for other rapid and reliable markers to predict and differentiate between bacterial and non-bacterial meningitis, and to aid in reducing the administration of unnecessary antibiotics.

Early reports in the 1970s indicated the total WBC and differential counts to be helpful in distinguishing bacterial meningitis from other meningitis [4]. WBC counts $>1000\times10^6$/L and >50% polymorphonuclear (PMN) cells were seen in more than half of the cases with bacterial compared to viral meningitis [4–7]. In a large retrospective study, the group with bacterial meningitis resulted in a higher WBC count (median: 1195×10^6/L vs. 100×10^6 WBC/L) and%PMN (median: 86% vs. 33%) compared to the viral meningitis group [7]. However, in 45% of the bacterial cases, the WBC count was $<1000\times10^6$/L, and even $<250\times10^6$/L at times. The authors concluded that no single parameter could rule out the presence of bacterial meningitis, nor could it differentiate between bacterial and viral meningitis because of the wide range of overlapping results between the two diseases. However, the combined use of the predictors (age, CSF-blood glucose ratio, month of the year, and CSF PMN count) could predict the likelihood of bacterial vs. viral meningitis with optimal diagnostic accuracy in the test sample (AUC: 0.968) and the validation set (AUC: 0.985) [7]. The diagnostic accuracy of this prediction model was confirmed in a retrospective validation study (n=500) [8], but these authors proposed a new diagnostic model (AUC close to 1) based on four different independent variables (CSF protein, CSF PMN, blood glucose and blood WBC). These findings evoked the interest of other groups to validate the various diagnostic prediction models, and also to introduce new self-computed models that showed varying degrees of sensitivity (77%–98%) and specificity (49%–98%) for predicting bacterial meningitis in neonates, children and adults [9–14].

Unfortunately, a large variability between studies in the methodological section (i.e., study design, laboratory criteria, patient inclusion criteria, and isolated pathogen) was found, which made it difficult to interpret and compare the results of the different models. All prediction models for differentiating bacterial from viral meningitis include a combination of parameters (WBC, PMN, protein, LDH, CRP, lactate, age, month, glucose, etc.) as opposed to one single parameter [2, 15–20].

Apart from bacteria and viruses, meningitis can be caused by fungi and parasites. Fungal meningitis predominantly affects immune compromised, and in particular HIV/AIDS patients, and is usually caused by *Cryptococcus neoformans* [21]. In CSF, WBCs range from as low as 20 to a maximum of 2000×10^6/L, with a preponderance (>50%) of lymphocytes [22]. Tuberculous meningitis represents about 1% of all tuberculosis cases and remains the most lethal form of extra-pulmonary tuberculosis [23]. The WBC findings were similar to those described in fungal meningitis, ranging from 10 to 3900×10^6/L, with a predominance of lymphocytes (80%); however, it was not unusual to find patients with PMN predominance [24–27]. Eosinophilic meningitis accounts for <2% of all meningitis cases, and is mainly correlated with parasitic and fungal infections or allergic reactions to shunt material. Eosinophilic meningitis is defined by the presence of $>10\times10^6$ eosinophils/L, or when >10% of total WBCs are eosinophils [28, 29].

In summary, the evaluation of the total WBC and differential count in CSF is an important aspect of laboratory diagnosis of meningitis. Some are of the opinion that the WBC and differential counts are indispensable diagnostic parameters [5], while others tend to deny this [6]. The majority of proposed guidelines use the traditional combination of CSF parameters including an elevated WBC count ($>1000\times10^6$/L) with a predominance of PMNs (Table 1), elevated protein and decreased glucose to be characteristic of bacterial meningitis [42]. Conversely, WBCs between 10×10^6/L and 1000×10^6/L with a lymphocytic pleocytosis, normal to elevated protein and normal glucose is more representative of viral meningitis [43]. Of note, in the acute stage of viral meningitis, WBCs can be $>1000\times10^6$/L with neutrophilic predominance [44]; however, as the disease progresses, the balance shifts, and lymphocytes start to predominate [45]. Similar to viral meningitis, WBCs in tuberculous and fungal meningitis range between 10×10^6/L and 1000×10^6/L with lymphocytic predominance [46]. Importantly, when interpreting biochemical and cytological CSF results, CSF collection site (ventricular drainage or LP) should be considered, because CSF composition varies between the two especially during CNS infection/inflammation, and can produce higher or lower results [47]. Based on the reviewed literature, no single finding (WBC or differential) was absolutely conclusive in differentiating bacterial from non-bacterial conditions; therefore, should not be used as a sole criterion. However, when clinical condition will not allow waiting for confirmation by microbiological culture results, the CSF WBC and differentials (relative proportion of PMN to MN) can give the best indication of the degree and type of inflammation, and may guide initial treatment choices.

Table 1: Overview of studies evaluating the diagnostic accuracy of WBC counts in inflammatory disorders and the current guidelines.

Fluid type		Study criteria [ref]	Counting method	No. patients included	Sensitivity, %	Specificity, %	PPV, %	NPV, %	ROC	Current guidelines	Ref.
CSF	BM	WBC >1500 [6]	Manual	710	51	99	–	–	–	WBC: 1000–10,000	[3]
		WBC >1000			61	98				PMN: 80%–90%	
Pleural	Effusions	WBC >1000 [30]	Manual	150	–	–	–	–	–	Exudate:	[31]
		WBC <1000								WBC: >1000	
										Transudate:	
										WBC: <1000	
Ascites	SBP	WBC >1000 [32]	Manual	10	100	89	63	100	–	PMN: ≥250	[34]
		WBC >500 [33]		46	91	88	–	–	–		
		PMN >500 [35]		14	86	98	92	96	95		
		PMN >250 [36]		6	100	94	67	100	–		
SF	Inflammatory	WBC >2000 [37]	Manual	100	84	84			91	WBC: 2000–50,000	[38]
		PMN >70%			75	92			94	PMN: >50%	
	Septic	WBC >50,000 [39]		44	72	92	–	–	–	WBC: >50,000	
		PMN >80%			93	59				PMN: >75%	
CAPD	Peritonitis	WBC >100 [40]	Manual	48	–	–	–	–	–	WBC: >100	[41]
		PMN >50%								PMN: >50%	

BM, bacterial meningitis; NPV, negative predictive value; PMN, polymorphonuclear cells; PPV, positive predictive value; Ref, references; ROC, receiver operating curve; SBP, spontaneous bacterial meningitis; WBC, white blood cells.

Reference range CSF

To distinguish normal from abnormal, the reference range must be known. Normal CSF WBC reference values have been mentioned in many scientific publications from old textbooks to recent references [48–50]. In 1931, normal CSF WBCs were reported to be $<2.6\times10^6$/L [51]. Presently, there is a general consensus that WBCs in CSF is age-dependent, and normal adult CSF should contain $<5\times10^6$ WBC/L [52], infants $<7\times10^6$ WBC/L [53] and neonates $<30\times10^6$ WBC/L [54], based on manual microscopic analysis.

There are only three peer-reviewed papers providing reference values for automated cell counters in CSF. By using the Sysmex XE-5000 BF mode, our group [55] reported normal CSF (n=87) reference ranges to be $<7\times10^6$ WBC/L in adult patients who underwent spinal anesthesia before an orthopedic, gynecological, surgical or urological surgery; whereas, Sandhaus et al. [56] found values ranging between 20×10^6 WBC/L and 30×10^6 WBC/L (n=200) in patients with (non) hematological malignancies, cerebral hemorrhage and suspected infection/inflammation disorders. Differences in the patients included may be responsible for the observed discrepancy. The adult patients included in our study had no history of neurological diseases; whereas, Sandhaus et al. [56] included samples from both children and adults with known CNS disorders. More recently, new reference ranges for CSF samples (n=80) of neurologically healthy patients undergoing orthopedic surgery were measured on the Advia 2120i, and were reported to be $<7\times10^6$/L [57], which are in concordance with our study [55].

In summary, interpretation of automated CSF WBC counts requires established reference values. Though, ethical considerations prohibit subjecting healthy individuals to invasive procedures solely for research purposes; consequently, normative values are determined from diagnostic LPs, preferentially from patients with non-inflammatory disorders. The current reference ranges referenced by manufacturers and textbooks were established decades ago using manual microscopy. However, with many laboratories transitioning from manual to automated methods, old reference ranges should be verified because higher reference ranges have been reported using automated methods [55–57].

Ascites

Ascites fluid is defined as an abnormal accumulation of fluid in the peritoneal space. The most common cause of ascites is liver cirrhosis which accounts for 80%, and the remaining 20% may be caused by congestive heart failure, tuberculosis, cancer or other causes.

Spontaneous bacterial peritonitis (SBP) is defined as an infection of pre-existing ascites without any obvious intra-abdominal source of infection [58]. Bar-Meir et al. [33] reported the total WBC and differential count to be of paramount importance in diagnosing SBP in cirrhotic patients. As culture results were not available within 24 h, and guidelines to diagnose SBP were lacking at that time, this group recommended treating patients suspected of SBP if one of the following criteria were present: WBC $>1000\times10^6$/L (sensitivity 89%) or PMN $\geq500\times10^6$/L (sensitivity 87%). Moreover, this group proposed treating patients with symptoms compatible with SBP if the WBCs are $>500\times10^6$/L (sensitivity 91%) or the PMNs $>250\times10^6$/L (sensitivity 92%). Several studies investigated the proposed criteria, and compared them with the gold standard (positive bacterial culture). The ascites PMN count had the highest diagnostic test accuracy (>92%) for SBP, and could potentially be used in diagnosis [32, 35, 59]. Sensitivity varied from 86% to 100% and specificity between 93% and 98% when PMNs were $>500\times10^6$/L [35, 59]. Further attempts to increase the diagnostic ability of the PMN count led to higher sensitivity (90%–100%) and slightly lower specificity (86%–100%) when the cut-off criterion was set to $\geq250\times10^6$ PMN/L [36, 60].

In summary, PMN $\geq250\times10^6$/L shows optimal sensitivity to detect SBP; whereas, PMN $>500\times10^6$/L is more specific (Table 1). Taking the severity of this disease, coupled with the high mortality rate if left untreated, the number of false negatives should be at a minimum (high sensitivity). In 2000, a panel of experts formed by the International Ascites Club suggested the best criteria for diagnosing SBP is based on $\geq250\times10^6$ PMN/L in ascitic fluid, which should immediately initiate empiric antibiotic treatment [34].

Pleural fluid

Pleural effusions result from an excessive accumulation of fluid in the pleural cavity, and are associated with a wide variety of disorders. When pleural effusions are discovered, the primary question is whether it is a transudate or an exudate. Common causes of exudates include malignancy, inflammation and infection; whereas, congestive heart failure and osmotic pressure are amongst the many diseases causing transudative effusions [61].

A WBC count $\geq1000\times10^6$/L was three times more frequent in infected compared to non-infected fluids [62].

Similar findings were reported by Light et al. [30] who noticed a higher tendency for WBCs to be $<1000\times10^6$/L in transudative effusions compared to exudative effusions (WBC $>1000\times10^6$/L); although, these differences alone were not significant enough to be of diagnostic value (Table 1). The same authors [63] reported the WBC differential count to be quite helpful in the differential diagnosis. For example, the presence of PMNs (≥50%) in pleural fluids is suggestive of acute pleural inflammation. In the same way, the presence of lymphocytes (>50%) was predominantly found in tuberculous exudates, but this was also a trademark for malignant exudates [64–66]. The presence of >10% eosinophils is correlated with eosinophilic pleural effusion, and found in a wide range of diseases including infection, drug reactions and malignancy [67].

In summary, controversial results regarding the diagnostic value of WBCs in pleural effusions remain. Several authors [64, 68] consider the total WBC and differential count to be useful, while others [31, 69–71] concluded these test are of limited diagnostic value. However, none of the reported studies have assessed the diagnostic accuracy (sensitivity/specificity) of the WBC and differential count. Based on the above literature, there is insufficient evidence supporting the use of WBCs as the sole diagnostic criterion in pleural fluid; however, in combination with biochemical parameters, this data can provide additional information in assisting the separation of transudates (WBC $<1000\times10^6$/L) from exudates (WBC $>1000\times10^6$/L). Furthermore, the WBC differential count can be used to narrow down the diagnostic possibilities in exudative effusions. During the past four decades, Light's criteria have been the most common criteria used for differentiating pleural effusions. It relies on the measurement of biochemical parameters solely (protein and LDH levels) in pleural fluid and serum, and not on cellular parameters [30]. The validity of Light's criteria proved to be robust, and has a high (90%) diagnostic accuracy [72].

Synovial fluid (SF)

SF is an ultra-filtrate of plasma combined with hyaluronic acid secreted by joint tissue. The analysis of SF is strongly recommended as a vital tool in diagnosing patients with joint effusions, and to classify the fluid as non-inflammatory, inflammatory or septic [73, 74].

The SF WBCs and PMNs were recommended as diagnostic parameters to differentiate between various forms of inflammatory disorders [75, 76]. Shmerling et al. [37] prospectively studied 100 SFs, and found the WBC count and %PMN to be significantly higher in the inflammatory group (median: 16100×10^6 WBC/L, 90%PMN) compared with the non-inflammatory group (median: 400×10^6 WBC/L, 13%PMN). The diagnostic accuracy obtained from the ROC curve for %PMN (AUC=0.94) was beyond that of the WBC (AUC=0.91) and the LDH (AUC=0.81) in classifying the process as inflammatory or non-inflammatory. Furthermore, they reported the %PMN to be more specific (92%) than the WBC count (84%) in diagnosing inflammatory arthritis [37]. Various studies concluded that the WBC count alone is insufficient to reliably discriminate between infectious and inflammatory arthritis because of the tremendous amount of overlap between the groups [38, 39, 77, 78]. As an example, one study showed 70% of patients with culture-confirmed infections had a WBC count $>50{,}000\times10^6$/L together with a preponderance of PMN (>90%); whereas, 26% of their patients with inflammatory diseases (gout and rheumatoid arthritis) had WBC counts in the same range [79].

In patients with knee or hip prosthetic joint infections (PJI), the criteria for SF WBC and differential count are different compared to patients without prostheses. One study reported a cut-off value of $>50{,}000\times10^6$ WBC/L (specificity: 99%, sensitivity: 36%) and >90% neutrophils (specificity: 85%, sensitivity: 89%) to be highly indicative for knee PJI, which is similar to non-prosthetic infections [80]. However, a more recent study reported lower cut-off values for WBC (1590×10^6/L, sensitivity: 0.89, specificity: 0.91, AUC: 0.99) and neutrophils (65%, sensitivity: 0.89, specificity: 0.86, AUC: 0.95) in diagnosing PJI with higher sensitivity compared to previous criteria [81].

In summary, evidence regarding the use of the WBC and differential count in SF analysis in literature is mixed. The findings of total WBC and its differential in SFs varied markedly, but in general, the majority of textbooks and studies emphasize that the combination of SF WBCs and PMNs are important diagnostic markers in the immediate discrimination of non-inflammatory, inflammatory and infectious disorders. However, the WBC alone and the %PMN alone are limited in distinguishing among the specific disease categories within inflammatory groups because of the wide range of overlapping results. Some suggest the SF WBC count to be the better predictive marker in inflamed joints; while others consider the %PMN to be more sensitive than the WBC count, especially in the case of septic arthritis [39, 77, 79]. Today, many textbooks and publications quote the following traditional classification system composed by the American Rheumatism Association: normal (WBC $<200\times10^6$/L, PMN<25%), non-inflammatory (WBC $<2000\times10^6$/L, PMN <25%), inflammatory

(WBC 2000–50,000×10⁶/L, PMN >50%) and infectious (WBC >50,000×10⁶/L, PMN >75%) [38, 82].

Continuous Ambulatory Peritoneal Dialysis (CAPD) fluid

Patients with end stage renal diseases may be treated with continuous ambulatory peritoneal dialysis (CAPD). Peritonitis, a major complication of CAPD, occurs in 10%–25% of patients undergoing dialysis, and it is the most frequent cause of technique failure leading to significant morbidity [83].

In the past, diagnosis of peritonitis was predominantly based on clinical symptoms and microbiology results. This view shifted in the early 1980s when Rubin et al. [84] and others [85–88] reported that the WBC and PMN counts were early indicators of peritonitis. In 43 infected CAPD patients, the WBC count exceeded 100×10⁶/L, and the PMNs accounted for more than 50% in all but one patient (PMN 37%) [40]. In this study, the %PMN in uninfected patients never exceeded 50%, indicating that the %PMN was a better predictive marker for discriminating infected from non-infected, even when the WBCs are <100×10⁶/L. Similar results were observed in a retrospective study by our group (unpublished data). In our study, the neutrophilic PMN count was a better predictor of infectious peritonitis compared to the WBC count in 64 infected-related peritonitis cases.

Fungal peritonitis (FP) is rare, but is coupled to a high morbidity and mortality rate, and accounts for 3%–6% of all peritonitis episodes [89]. The clinical symptoms of FP are similar to those of bacterial peritonitis, which makes diagnosis difficult. In a large number (n=804) of peritonitis episodes, approximately 6% were caused by fungi, and WBCs ranged from 150 to 9000×10⁶/L with a preponderance of PMNs, which is a similar finding for bacterial peritonitis [90]. Given the lack of specificity in accurately differentiating bacterial from FP based on cellular analysis, culture results together with clinical judgment remain important parameters in confirming FP [90].

Eosinophilic peritonitis (EP) is usually benign. It is either caused by an allergic reaction to some constituents of the peritoneal dialysis system or from intraperitoneal air introduced at the time of catheter placement [91]. Treatment regimen includes clinical observation without antibiotic treatment [92]. In 1967, Lee and Schoen [93] were the first to describe the presence of elevated eosinophils in a patient undergoing peritoneal dialysis with consistent negative culture results. EP was later defined as either an eosinophilic count greater than 10% of the WBC count, provided the absolute eosinophilic count exceeded 40×10⁶/L [94], or when the absolute eosinophilic count was >100×10⁶/L [95]. Eosinophils should always be reported when present.

In summary, a review of the literature revealed surprisingly few studies reporting on the diagnostic accuracy of the WBC and PMN count in peritonitis; however, all studies report the WBC, PMN and eosinophilic count to be of paramount importance in diagnosing dialysate peritonitis, and should be included in the diagnostic workup. According to the International Society for Peritoneal Dialysis, the effluent WBC (>100×10⁶/L) and PMN (>50%) count, together with patient symptoms, are supportive of early peritonitis diagnosis (Table 1). Also, when more than 100×10⁶ eosinophils/L, or >10% of effluent WBCs are eosinophils, EP should be considered [41].

Laboratory techniques for hemocytometric analysis

Pre-analytical phase

The pre-analytical phase (specimen collection, transport and processing) of BFs has a great importance on the analysis technique, and can affect the reliability of the results. In compliance with CSLI H56-A guidelines [1], CSF samples should be collected in plain tubes and transported at room temperature to the laboratory immediately. The time delay between CSF collection and laboratory entry should be to a minimum to minimalize/avoid cell loss which could result in erroneous WBC/differential counts. One study showed that up to 40% of lymphocytes decayed in native CSF after 90 min; moreover, 90% of monocytes and neutrophils were lost after the same timeframe [96]. Other BFs (serous fluids, SFs, CAPD) are collected in anticoagulant tubes (EDTA tubes) to prevent cell clumping and to prolong cell stability; however, samples should be administered to the laboratory immediately after collection.

Manual microscopy

The hemocytometer, also referred to as the counting chamber, is the most frequently used method for determining WBCs and RBCs in BFs. The Neubauer-Improved and Fuchs-Rosenthal chambers are the most common, and both utilize the same basic principles, but differ in dimensions

(counting grids and depth). The hemocytometer consists of a special optical glass slide with a rectangular indentation that creates a chamber. The addition of Samson or Türk staining reagent may facilitate the total WBC and differential count [31]. Accuracy of this device strongly depends on a number of variables including correct loading of sample volume, appropriate dilutions and the number of squares and cells counted. Other methodological factors contributing to the disadvantage of this technique include: high imprecision in samples with low cellularity, the process is tedious, and variability between technicians is high. Variation coefficients for hemocytometers were reported to be as high as 45% [56]; moreover, variability between technicians is even more pronounced, and range from 2.5% to 116% for WBCs ($>300\times10^6$/L) and up to 141% for RBCs ($>300\times10^6$/L) [97, 98]. However, if this technique is performed by an experienced observer, disadvantages previously mentioned will be minimalized or eliminated.

Stained cytocentrifugation is considered the best preparative method for differentiating cells in BFs [99–101]. Major concerns with this procedure are the loss of cells during centrifugation [96, 102], aberrant cell morphology due to the forces of centrifugation [103], and clustering of macrophages or mesothelial cells (which can be mistaken for malignancy, especially in the case of pleural effusions and ascites fluids) [104]. To overcome these difficulties, studies recommend specimens to be fresh and processed within a few hours to limit cellular degradation [103], immediate addition of a serum contaning medium to CSF tubes to protect against degradation and cellular distortion [105], and to carefully validate centrifugation speed and processing time because these can influence the quality of the slide [104].

Apart from manual differentiation, BF cytocentrifuged slides can be automatically analyzed using the BF module on the Cellavision digital microscopy system (DM96) [106]. The DM96 is FDA cleared, and enables automatic recognition and pre-classification of eosinophils, neutrophils, lymphocytes and macrophages and other cells. The DM96 accurately pre-classified cells in 90% of the CSF cases and in 83% of the other BFs, and is recommended as a reliable and accurate system [106].

In summary, the combination of hemocytometry and stained cytocentrifugation is considered the gold standard for counting and differentiating cells in BFs despite their disadvantages. The reasons are: 1) in small laboratories cell counts are sporadic and automation is not justified; or 2) automated counters still misidentify specific cell types. The results of the gold standards must be interpreted in the context of the method used, specimen type, and technical problems should be considered.

Automated hematology analyzers

Nowadays, automated hematology analyzers are employed for counting blood cells in CSF, pleural fluid, ascites fluid, CAPD, pericardial fluid and SF amongst others. These machines are designed to be faster, more precise and easier to use compared to traditional manual methods. Many manufactures have adapted flow cytometry and impedance technique for counting and differentiating cells. The principles of these methods are well known and are available in various textbooks and publications [107]. To briefly summarize, flow cytometry relies on three main properties: size of the particle (forward scatter), granularity or internal complexity of the cell (side scatter) and DNA/RNA binding (fluorescence intensity). After sample incubation and staining with a fluorescence marker, light signals are collected and digitized for computer analysis, and displayed as scattergrams or histograms to provide information about cell populations within a sample. The impedance method requires cells to pass through an aperture where an electrical current is passing. This results in a change in the electrical resistance, which is proportional to the cell volume. With these analyzers, a fixed volume of sample is first mixed and incubated with specific reagents, followed by counting. Automated analyzers aspirates more sample volume compared to the counting chamber. This leads to more cells being counted to enhance precision and accuracy. Common difficulties encountered with these analyzers are: high imprecision in the low range, inability to detect malignant cells and interference from non-cellular particles (crystals, bacteria, fat globules and yeast) which leads to false increased WBC or RBC results [108, 109].

At present, there are a variety of manufacturers (Siemens, Sysmex, Abbott and Beckman Coulter) on the market with analyzers suited for routine BF analysis, and the majority is FDA cleared (see Table 2 for summary of the manufacturer's performance specification for each analyzer).

Advia analyzers (Siemens Healthcare Diagnostics, Deerfield, IL, USA)

The ADVIA 120/2120 has a dedicated CSF application. This semi-automated instrument uses direct flow cytometry to count and differentiate cells according to their size and granularity. First, samples are manually pretreated with special CSF reagent to fix and sphere cells, followed by a minimum incubation period of 4 min. The following CSF parameters are reported: RBC, WBC, three-part differential (neutrophils, lymphocytes, monocytes), PMN and mononuclear (MN) cells. The eosinophil count is available

Table 2: Overview of manufacturer's specification for automated analyzers.

Manufacturer	Model	Analysis principles	Volume, µL	Throughput samples/hour	Reportable Parameters	Linearity WBC×10⁶/L	Linearity RBC×10⁶/L	Carry over, %	Precision WBC, CV%	Precision RBC, CV%	FDA	
											Cleared	BFs
Hematology analyzers												
Sysmex	XE-5000 BF mode	Fluorescence Flow cytometry Impedance	130	38	WBC, RBC MN, PMN TC-BF	0–10,000	0–5000	–	–	–	Yes	CSF Peritoneal-dialysate Serous fluids Synovial fluids
	XN-Series BF mode		88	40				≤0.3	≤7. 5	≤4.00		
Siemens	120/2120 CSF mode	Flow cytometry	175	120	WBC, RBC MN, PMN Neut, lymph Mono	0–5000	0–1500	<0.4	≤15	≤10	Yes	CSF Peritoneal-dialysate Serous fluids Synovial fluids
	2120 BF mode				TNC, RBC	20–400	100–6760					
Abbott	CELL-DYN 3500	Flow cytometry Impedance	130	90	WBC, RBC Neut, lymph	0.4–250	0.22–750	–	≤2.7	≤1.5	No	–
	CELL-DYN Sapphire		117	105	Baso, Mono EO							
Beckman Coulter	LH 750 CSF mode	Impedance	200	110	WBC/TNC RBC	0–3570	0–129,000	<0.64	≤4.4	≤6.6	Yes	CSF Serous fluids Synovial fluids
Urinalysis analyzers												
Sysmex	UF-1000*i*	Flow cytometry	800	100	WBC, RBC, EC, Bacteria	1–5000	1–5000	≤0.1	≤10	≤10	No	–
	UF-1000*i* BF mode		550	28	WBC, TNC RBC	2–10,000	5–99,999	<0.01	≤30	≤30		
Iris	iQ200 BF mode	Digital Imaging Auto-recognition	500	–	TNC, RBC	0–10,000	0–10,000	–	–	–	Yes	CSF Peritoneal-dialysate Serous fluids Synovial fluids

Specification information was taken from the online published food and drug administration (FDA) decision summary reports 510(k) and from manufacturer's online website's. Baso, basophil; EC, epithelial cells; Lymph, lymphocytes; Mono, monocytes; Neut, neutrophils; RBC, red blood cells; TNC, total nucleated count; WBC, white blood cells. Values are taken from online publications of the FDA.

as research parameter. For serous fluids and peritoneal dialysis fluids, the TNC and RBC counts are reported. The ADVIA does not provide morphology flags to indicate the presence of abnormal cells.

Comparison studies between conventional microscopy and the ADVIA CSF assay showed acceptable results in CSF, serous fluids and SFs [110–119]. Common difficulties observed amongst studies were: 1) absence of morphological flagging (making it difficult to detect abnormality in samples); 2) presence of high RBC (>1500×10^6/L) counts, (leading to falsely increased WBCs because of overflow in the fixed WBC clusters); and 3) incorrect classification of malignant cells, macrophages, atypical lymphocytes and degenerated neutrophil cells (leading to imprecise differential counts).

In samples containing <20×10^6 WBC/L, the differential count is not reported [111]. The best correlations (r=0.98) were observed when RBCs were low (<100×10^6/L) and the WBCs were high (≥100×10^6/L) [112]. The ADVIA CSF reagent (mixture of formaldehyde and glutaraldehyde) causes cells to shrink and appear smaller in size [112]. As flow cytometry is partially based on the detection of cell size, this phenomenon may hamper the differential results; however, this is yet to be confirmed by other studies. SFs are highly viscous in nature. Pretreatment of these fluids with hyaluronidase is necessary to prevent a strong negative bias on the ADVIA because of the polymerization in the acidic environment [116].

In summary, the ADVIA CSF assay shows promising results for BF analysis making it a suitable analyzer for preliminary evaluation. Some recommend limited use of the Advia for analyzing CSF samples [112] while others promote their use [111]. An overview of the Advia's advantages, disadvantages and recommendations based on published literature is summarized in Table 3.

Sysmex analyzers BF mode (Sysmex Corporation, Kobe, Japan)

Sysmex analyzers (XE-5000 and XN-Series) are fully automated and contain dedicated BF modes. While the total WBC and its differentials are determined by forward and sideward scattered light (size and inner complexity) and fluorescence intensity (DNA/RNA content) measurements, the RBCs are measured by electrical impedance. The XE-5000 reports RBC, WBC, MN/PMN and (TC-BF). High fluorescence body fluid (HF-BF) cells are reported as research parameter. The HF-BF cluster can consist of macrophages, mesothelial and malignant cells, and are not included in the WBC differential count in contrast to most other BF modes of other manufacturers. Apart from these parameters, the XN-Series reports a four-part differential (lymphocytes, monocytes, eosinophils and neutrophils) as research parameters. Both analyzers generate one flag ("WBC Abn Scattergram") in the presence of abnormality.

XE-5000

The XE-5000 is the most commonly evaluated analyzer for BFs analysis. Good results were demonstrated for analyzing CSF, serous, CAPD and SFs; although, the results were not always superior [55, 56, 119–125, 140–142]. The most encountered problems were associated with high imprecision of the XE-5000 in the low WBC range (<20×10^6/L), and the inability to detect or specifically flag malignant cells.

The XE-5000 is shown to have slightly higher WBC counts than manual methods, especially in the lower concentration range [121]. More pathological cell counts (>5×10^6/L) were detected by the XE-5000 (sensitivity 100%, specificity 75%) compared to manual counts [120, 124]. This could be partially explained by the imprecision of both methods in the lower range [56] or the slightly higher reference values for automated methods [55]. Another explanation might be the presence of cell debris or interfering fragments, causing the overestimation of WBCs/PMNs on the XE-5000. This phenomenon was more pronounced in CSF samples with low cell counts (WBC <20×10^6/L) and in samples taken from ventricular drainage systems [55, 121]. To solve this problem, Sysmex upgraded its software on the XE-5000 by improving gating algorithms; however, a positive bias remained for PMNs with low WBCs [142]. The lower limit of quantitation (LoQ) is defined as "the lowest concentration in a sample that can be quantitatively determined with acceptable imprecision (coefficient of variation: 20%)" [143]. The LoQ of the XE-5000 is approximately 10×10^6 WBCs/L [55]. As the XE-5000 lacks precision in the lower range, it was recommended that samples with low WBCs (5–20×10^6/L) be manually recounted [121], and in samples containing <10×10^6 WBCs/L, the differential count should not be reported [55].

The accuracy of the "WBC Abnormal Scattergram flag" was evaluated in CSF samples and showed poor sensitivity (60%) but good specificity (96%) [56]. Of the eight false negative cases, four cases had lymphocytosis with few reactive lymphocytes, three cases had neural tissue, and one case was suspected for malignancy. The HF-BF

Table 3: Summary of the advantages, disadvantages and recommendations of automated analyzers.

Counting method	Advantages	Disadvantages	Recommendations	Ref.
Hemocytometry (Fuchs-Rosenthal & Neubauer chamber)	–Inexpensive – Low sample volume – TNC/WBC/RBC	– High imprecision – High inter-observer variability – Time consuming	– Use only in doubt of results analyzed on automated analyzers and from samples suspected of malignancy	[31, 98, 111]
Sysmex (XE-5000 BF-mode & XN-Series BF-mode)	– Reduced TAT – No sample preparation – Low sample volume – Low detection limit (~5×10^6/L)→ XN-BF – 2-part diff (MN/PMN) – 4-part diff (research parameters) – Extended counting (count more cells) – 1 flag to notify abnormality – Commercially QC material available	– Lowest reportable value RBC: 1000×10^6/L – Overestimation of PMNs in low range (WBC $<20\times10^6$/L) → XE-BF – High imprecision in the low WBC range (CSF) → XE-BF – Unable to detect malignant cells	– Critically review scattergrams and histograms to detect abnormality and follow up with manual microscopy – Not to analyze samples from onco-hematology – patients – Do not report Diff when WBC $<10\times10^6$/L → XE-BF	[55, 56, 119–126]
Siemens (Advia 120/2120 CSF mode & BF mode)	– Longer cell stability in samples – Low detection limit (~2×10^6/L) – 3-part diff (lymph, mono, neutro) – Eosinophils (research parameter) – Detection of Cryptococcus in CSF – samples (distinct cytogram pattern) – Commercially QC material available	– Samples containing ↑ RBCs or incomplete lysis of RBCs leads to false elevated WBCs – Do not provide morphology flags – Requires manual predilution – Incorrect classification of malignant cells and atypical lymphocytes – High imprecision in the low range (CSF)	– Pretreat SFs with hyaluronidase – Samples should be diluted when RBCs 1500×10^6/L or use special hemolysis reagent – Not to analyze samples from onco-hematology patients – Cytospin slide in the presence of abnormality – Reports WBC Diff only when WBCs 20×10^6/L	[110–112, 115–118]
Abbott (CELL-DYN 3500 & CELL-DYN Sapphire)	– 5-part diff (lympho, mono, neutro, eo, baso) – Extended count mode (count more cells) – Possible to detect malignant cells by using monoclonal antibodies – 3 flags to notify WBC abnormality	– Detection limits WBC 50×10^6/L and RBCs 3000×10^6/L – Overestimation PMNs in serous fluids – No flagging for abnormality – Not FDA cleared	– Only report counts greater than the detection limits – Do not analyze samples from onco-hematology patients – Run blank sample after blood sample to eliminate carryover	[127–130]
Beckman Coulter (LH 750 BF mode)	– Reduced TAT – WBC (TNC)/RBC	– Do not report diff counts – Overestimation of PMNs in serous fluids – Detection limit WBC (>200×10^6/L) and RBC (>$10{,}000\times10^6$/L) – No detection of malignant cells	– Restricts CSF and peritoneal fluids analysis – Only analyze samples greater than detection limits – Pretreat SFs with hyaluronidase	[98, 131, 132]
Sysmex (urinalysis) (UF-1000*i* & UF-1000*i* BF mode)	– Report semi-quantitative bacteria counts (urine mode) – Reports WBC/RBC/TNC (BF mode)	– Do not report Diff counts – Requires high sample volume – Overestimation WBCs in serous fluids (BF mode) – Not FDA cleared	– Critically review scatterplots to detect – Abnormality especially in serous fluids	[133–135]
Iris (urinalysis) (iQ200 BF-mode)	– Reduced TAT – Reports RBC/TNC	– Semi-automated (requires 2 manual dilutions) – Decreased WBC counts in serous fluids due to cell clumping – Do not report DIFF counts	– Critically review plots to detect cell clumping – Dilute samples based on appearance	[136–139]

Diff, differential; QC, quality control; SF, synovial fluid; TAT, turnaround time; TNC, total nucleated count.

count should not be used as a diagnostic test (sensitivity: 75%, specificity: 73%) for identification or exclusion of neoplastic meningitis [123]. In cases where a high percentage of HF cells are incidentally present, further evaluation by manual microscopy is necessary [55, 121, 122].

RBCs are counted in a separate RBC channel using impedance technique on the XE-5000. Unlike the Advia CSF mode, blood stained fluids do not interfere with the WBC/differential count on Sysmex analyzers. However, the lowest reportable value for RBCs on the XE-5000 is 1000×10^6/L.

XN-BF mode

The XN-BF mode is relatively new, which explains the low quantity of published data. We evaluated this mode and found good agreement with manual microscopy for counting cells in CSF, serous and CAPD samples [126]. The XN-BF mode overestimated the CSF PMN count compared to microscopy. This could possibly be explained by the fact that cells break down during cytospin preparation, and because CSF WBCs are known to decay easily due to their fragile nature. Other fluids (CAPD, ascites and pleura fluid), investigated in the same study did not show an overestimation of PMNs. Furthermore, the sensitivity (100%) and specificity (97%) on the XN-BF mode were good for predicting abnormality in CSF (WBC $>6\times10^6$/L) [126].

In summary, Sysmex analyzers have proven to be accurate and sensitive for processing BF samples while offering advantages, such as cost effectiveness and faster turnaround times. Literature reveals contrasting data on the analysis of CSF on these analyzers. Although similar results were found in studies, the interpretations and recommendations differed. An overview of the advantages, disadvantages and recommendations, based on published literature are summarized in Table 3.

CELL-Dyn (Abbott diagnostics, Abbott Park, IL, USA)

The CELL-DYN Sapphire hematology analyzer and its predecessor, the CELL-DYN 3500, are fully automated and primarily designed for measuring cells in blood samples. They do not contain any application for separate BF analysis. Nevertheless, investigators have evaluated their performance for counting cells in BFs.

The CELL-DYN analyzers enumerate and differentiate WBCs by dual technologies (multi-angle polarized scatter separation combined with impedance method). The total WBC and a five-part differential (lymphocytes, monocytes, neutrophils, basophils and eosinophils) are reported. RBCs on the CELL-DYN 3500 are measured by impedance technique; whereas, on the CELL-DYN Sapphire, they are measured by impedance as well as optically. Three flags are generated when WBC abnormality is suspected in blood.

The CELL-DYN 3500 should not be used for counting cells in CSF [113, 127] because CSF samples containing high numbers of lymphocytes and atypical lymphocytes resulted in false elevated WBCs on the 3500 [127]. Abbott's newer generation, the CELL-DYN Sapphire, was evaluated for counting cells in CSF and serous fluids [128]. No significant differences were found when concentrations exceeded the detection limits ($>50\times10^6$ WBC/L and $>3000\times10^6$ RBC/L); however, samples below these values differed significantly. Furthermore, the Sapphire significantly overestimated the PMN count, and underestimated the MNC count in serous fluids compared to manual microscopy. Apparently, macrophages and mesothelial cells are clustered as PMNs, making the automated differential counts unreliable in these fluids. To overcome this problem, new software with optimized gating algorithms specifically for BF analysis was developed, and its performance was evaluated in serous fluids. Results were greatly improved between microscopy and the optimized method compared with the standard algorithm. Despite the use of the improved software, the Sapphire was unable to correctly identify monocytic cells [129].

The Sapphire has a high diagnostic ability (sensitivity and specificity >90%) to distinguish normal from abnormal samples in serous and CAPD samples; whereas, for CSF samples (cut-off limit WBC $>5\times10^6$/L), sensitivity was poor (45%) [128]. This could be expected because of analyzers high detection limits. Furthermore, the ability of the Sapphire to detect malignant cells resulted in low sensitivity (20%) but high specificity (94%). The Sapphire correctly flagged two out of the seven samples flagged for malignancy, but failed to flag eight other samples with metastatic cells. However, when an immunophenotypic assay (using monoclonal antibodies) on the Sapphire was used, sensitivity increased to 75% [130].

In summary, CELL-DYN analyzers are easy to use, and with exception to CSF samples, they are suitable as a screening tool for BF analysis. Like other hematology analyzers, the Sapphire is unable to detect or generate a flag for the presence of malignant cells. However, unlike other analyzers, this instrument offers the possibility to detect such cells by using specific monoclonal antibodies. An overview of advantages, disadvantages and recommendations based on published literature are summarized in Table 3.

LH 750 (Beckman Coulter, Miami, FL, USA)

The LH 750 is fully automated and contains a separate BF application. The LH 750 is based on impedance technology combining direct current (size of cells), conductivity (internal structure) and light scatter (cell surface and granularity) to count and report WBC/TNC and RBC counts.

The LH 750 reports inaccurate results when concentrations are below the manufacture's detection limits (WBC: 200×10^6/L and RBC: $10{,}000\times10^6$/L). However, when samples contained counts greater than the reportable limits, correlation coefficients improved and no statistical differences were observed between automated and manual method [98, 131, 132, 136]. As approximately 90% of CSF samples have WBC counts lower than the detection limits of this analyzer, it is recommended not to use this instrument for CSF and peritoneal fluid analysis, and to analyze these samples manually instead to ensure accuracy [98, 136]. The LH 750 was able to discriminate normal from abnormal samples with high sensitivity (>85%) in serous fluids and SFs [136]. For CSF samples, the LH 750 misclassified 64% of samples according to clinical threshold (WBC $>5\times10^6$/L) [136].

The LH 750 was compared with the FC500 flow cytometry (Beckman Coulter) for counting WBCs (differential) in ascitic fluids [144]. The methods correlated well for WBCs (r=0.99) and moderately for neutrophils (r=0.82). In the majority of cases, the LH 750 counted higher neutrophils than flow cytometry. It seems as if macrophages were being misclassified as neutrophils on the LH 750, which led to the overestimation.

In summary, the LH 750 BF application has been proven to be accurate and precise in measuring all types of BF samples containing $>200\times10^6$ WBC/L and $>10{,}000\times10^6$ RBC/L. Beckman Coulter new-generation hematology analyzers (D×H 800) contains a body fluid mode. Beckman states that the BF mode is able to report WBCs down to 20×106/L; though, this is yet to be confirmed by studies. An overview of advantages, disadvantages and recommendations, based on published literature, are summarized in Table 3.

Other analyzers

Apart from hematology analyzers, urinalysis analyzers such as the Sysmex UF-Series and Iris iQ200 have grown in popularity for BF analysis.

iQ200 (Iris Diagnostics, Chatsworth, CA)

The Iris iQ200 is a semi-automated analyzer with a BF module. The TNC and RBC counts are determined by using flow cell digital imaging and auto particle recognition. Comparative studies between the automated iQ200 and microscopy showed good to excellent correlation (0.84–0.99) for WBCs and RBCs in various BFs including CSF [137–139]. Some concluded that the precision of the iQ200 may not be adequate for CSF analysis because of its detection limit (35×10^6 WBC/L) [138], while others report the iQ200 to have high precision in the lower range, deeming it acceptable for CSF analysis [137, 139]. Furthermore, WBCs in serous fluids were falsely decreased on the iQ200 because of nucleated cell clumping in these samples [137].

Sysmex UF-1000*i*

The Sysmex UF-Series is fully automated, and uses hydrodynamic focusing and flow cytometry technique to count particles. It reports WBCs and RBCs along with other urinalysis parameters including bacteria and yeast counts. In 2014, Sysmex urinalysis division launched its first application for BF analysis on the UF-1000*i*. It employs the same techniques as the urine mode, but contains new gating's and modified algorithms to enhance cell counting. The UF-1000*i* BF mode reports TNCs, WBCs and RBCs.

Good agreement between the UF-1000*i* urine mode and manual microscopy for RBCs and WBCs was found [133, 134, 145–147]. We observed that the optical RBC count on the UF outperformed the impedance count on the XE analyzers (unpublished data). The UF-1000*i* urine mode contains improved algorithms (ability to classify lymphocytes as WBCs) compared to its predecessor (UF-100) [145]. This solved the problem of WBC underestimation in the presence of elevated lymphocytes on the UF-100 [134]. Furthermore, the diagnostic accuracy of the UF-1000*i* for differentiating between pathological and normal CSF (cut-off: 4×10^6 WBC/L) showed 100% sensitivity and 84% specificity [133].

The UF-1000*i* BF mode was evaluated in our laboratory using CAPD and serous fluids [135]. Agreement between the UF-BF mode and the counting chamber was acceptable. The UF overestimated the WBCs in serous fluids. A possible explanation could be the presence of macrophages and mesothelial cells which are being counted as WBCs. In addition, the diagnostic accuracy of the UF-BF showed excellent sensitivity (100%) and a specificity of 78% and 83%, respectively, to detect WBCs in CAPD fluid ($>100\times10^6$/L) and ascites ($>250\times10^6$/L).

In summary, the analysis of various BFs on urinalysis analyzers showed satisfactory agreement for rapid and accurate screening of total WBCs and RBCs. Like hematology analyzers, conflicting evidence remain for measuring CSF samples on these analyzers because of their detection limits. Compared to hematology analyzers and manual methods, urinalysis analyzes require far more sample volume, which can be problematic for CSF. However, CSF samples containing low volumes can be manually prediluted (1:5) and measured on Sysmex UF-1000*i* urine mode [134]. Naturally, the LoQ will increase accordingly with the dilution factor and accuracy is also compromised, which hampers counting in CSF with low WBC counts. Results obtained by urinalysis analyzers are mostly restricted to WBCs and RBCs. This limits their use because most guidelines rely on the combination of WBC and differential counts to differentiate between infectious and non-infectious diseases. Despite the fact that these analyzers do not report a differential count, they have the ability to detect and semi-quantitate bacteria and yeast cells. A future possibility to report bacteria counts along with the total WBC and differential count simultaneously would be meaningful for clinicians in the early diagnosis of peritonitis and bacterial meningitis.

Man vs. machine in the diagnosis of inflammation

The hematology and urinalysis analyzers reviewed in this study showed promising results when compared with manual microscopy (Table 4). Different studies confirmed many of their benefits, such as improved proficiency and workflow productivity, and recommend them as suitable alternatives for conventional microscopy (Table 3). With this in mind, one can predict that it is only a matter of time before all laboratories make the leap from manual to automated methods for BF analysis to diagnose infection and inflammation. However, there are still some unresolved issues regarding these analyzers. First, the most common literature discussions pertain to the inability of some analyzers to accurately count cells in the lower concentration ranges. Samples containing low WBCs $<30\times10^6$/L can be problematic for some of these analyzers due to their relative high LoQ (Table 4) which limits their utility for CSF analysis. Analyzers, such as the CELL DYN and LH 750 using impedance technology, have high background counts which prevent or hinder accuracy in detecting low cell counts (Table 2). In contrast, a LoQ of 5×10^6 WBC/L for all fluids on the Sysmex XN-Series BF mode, and 2×10^6 WBC/L for CSF on the Advia 120/2120 probably make these analyzers more suitable for low WBC counts in CSF. An ongoing challenge will be for manufacturers to develop analyzers with extremely low detection limits and high precision, especially for CSF. Second, automated analyzers are unable to reliably differentiate between hematological and non-hematological cells. Serous fluids are characterized by their unique composition of biological matrix, and by the presence of cellular elements, such as macrophages and mesothelial cells. Though these cells are generally larger (15–21 µm) than WBCs (10–20 µm), there is a window of overlap which causes them to be mistakenly counted as WBCs. The presence of macrophages and mesothelial cells in serous fluids can lead to falsely elevated WBCs, and furthermore, mistakenly assigned to MNs and/or PMNs [55, 128, 135, 144]. The acquisition of these false positive data can result in misdiagnosis, and have adverse implications for therapy in SBP, for example. Unfortunately, not all analyzers report TNC and WBC counts separately (Table 2), and often, non-WBCs (included in the TNC count) are counted as WBCs or the TNC and WBC count are similar. The Sysmex BF-mode on the XE and XN-Series analyzers excludes HF-BF cells, such as macrophages and mesothelial cells, from the WBC differential count, limiting their positive interference [55]. Other analyzers, containing dedicated BF modes such as the Beckman 750, Siemens 2120 BF mode and the Iris iQ200, reports the WBC (TNC) count, hence the TNC count is equal to the WBC count for these analyzers, which is not always the case. According to CSLI H56-A guidelines [1], the TNC should include all nucleated cells including nucleated WBCs, NRBCs, lining cells and other non-hematological cells. In the majority of cases, it's the WBC/WBC differential count that reflects different stages of the inflammation/infection; therefore reporting accurate results for the WBC count is of utmost importance. Manufacturers should improve this interference in the WBC count to be able to deliver reliable and fast total WBC and differential counts to aid the diagnosis of inflammation. Furthermore, all automated analyzers could not accurately identify or flag malignant cells. This is clearly reflected in the low sensitivity values found [123]. These cells, if present, were being misclassified, mainly as lymphocytes. In light of these limitations we suggest: 1) malignant body fluids should always be analyzed by manual microscopy; and 2) doubtful automated results of inflamed BFs, such as abnormal scatterplots, suspected interferences or many large cells should always be followed up by manual/digital microscopy to reveal the cause.

The diagnostic accuracy of automated analyzers to differentiate between normal and abnormal conditions showed

Table 4: Published results on performance evaluations on automated analyzers for CSF analysis.

	Number of samples in study	Parameters	Slope, r	Sen/ Spec, %	Linearity WBC×10⁶/L	Linearity RBC×10⁶/L	LoQ WBC×10⁶/L	Carry over WBC%	Refs
XE-5000 BF mode	69	WBC	1.15[a]	–	0–13,000	0–5000	10	<0.17	[55]
		RBC	–						
		PMN	2.40[a]						
		MN	1.08						
XN-1000 BF mode	67	WBC	1.06[a], r=0.96	100/97	0–12,000	0–8000	5	<0.05	[126]
		RBC	0.99, r=0.99						
		PMN	1.48[a], r=0.99						
		MN	1.04, r=0.93						
ADVIA 120 CSF assay	60	WBC	0.83, r=0.98	–	0–9850	0–20,000	2[b]	–	[118]
		RBC	–						
		PMN	0.83, r=0.98						
		MN	0.83, r=0.98						
CELL-DYN 3500	73	WBC	1.11[a], r=0.92	–	5–900	–	–	–	[127]
		RBC	–						
CELL-DYN Sapphire	30	WBC	1.04, r=1.00	97/45	10–900	3000–90,000	50	–	[128]
		RBC	1.06, r=1.00						
		PMN	0.87, r=1.00						
		MN	0.89, r=1.00						
LH 750	148	WBC	0.89, r=0.99	–	200–3570	10,000–129,000	200	<0.64	[132]
		RBC	1.05, r=0.84						
UF-1000*i*	77	WBC	0.93[a], r=0.99	100/84	4–278	–	–	<0.3	[133]
UF-100	256	WBC	r=0.88	–	–	–	–	–	[145]
		RBC	r=0.91						
iQ200	66	WBC	1.10, r=0.99	–	0–2558	0–43,829	35	0	[137]
		RBC	0.81, r=0.99						

The r-values demonstrate the correlation between automated analyzers and manual microscopy. [a]Significant; LoQ, lower limit of quantitation at a CV of 20%, [b]CV=30%. Ref, references. For the LH750, results are for samples correlating above the detection limits. Results were considered normal or abnormal based on counting chamber (WBCs <6×10⁶/L).

good results. Though the sensitivity was nearly always 100%, the specificity ranged between 70% and 85%. This means patients with negative results can be spared antibiotic treatment; however, there is a higher chance of reporting false positive results compared with manual counts.

In summary, it is our experience, in common with those of others, that automated analyzers are suitable for BF analysis of suspected inflammatory disease by providing preliminary rapid results for clinical action which is likely to benefit patients' care. Deciding on which method to use is dependent on the laboratory's preference, experience and an assessment of the advantages and disadvantages each analyzer has to offer.

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