Automated Evaluation of Synovial and Ascitic Fluids With the Advia 2120 Hematology Analyzer

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ABSTRACT

Objectives: The purpose of this study was to determine if the Advia 2120 hematology analyzer (Siemens Healthcare Diagnostics, Deerfield, IL) can accurately quantify both WBCs and the proportion of neutrophils from synovial and ascitic body fluids.

Methods: We analyzed 60 samples on the Advia and compared the results with manual counts and smear reviews. We also assessed the effect of adding hyaluronidase to the samples. WBC counts and the proportion of neutrophils reported by the hematology analyzer were harmonized and highly correlated with manual counts and fluid smear reviews.

Results: The addition of hyaluronidase to the synovial fluid consistently increased the WBC counts on both manual and automated analysis (P < .001).

Conclusions: We conclude that the Advia hematology analyzer can be used for WBC and neutrophil counting of cells in synovial and ascitic fluids. Hyaluronidase should be added before manual or automated counting of cells in synovial fluids.

Materials and Methods

We chose 18 and 42 samples sent for synovial and ascitic fluid analysis, respectively. The fluid samples were sent to the laboratory in Vacuette K3 ethylenediaminetetraacetic acid 3-mL tubes (Greiner Bio-One, Frichenhausen, Germany). All tests were analyzed within 4 hours of sampling. Manual counts were done using a Bright-Line hemocytometer.

Analyzers routinely used for a complete blood count can potentially replace manual counting and fluid smear review for various body fluids. The Advia 2120 hematology analyzer (Siemens Healthcare Diagnostics, Deerfield, IL) has received Food and Drug Administration approval for analyzing pleural and ascitic fluid on a special channel of the analyzer. This channel counts total nucleated cells (TNCs), but has not been approved for synovial fluid analysis, and does not determine the proportion of neutrophils, which is thought to be important in differentiating septic joints and bacterial peritonitis from less emergent conditions. Therefore, even for the analysis of ascitic fluid, a manual smear review is still required to determine if the absolute neutrophil count is elevated. Automated analyzers are usually not used to analyze synovial fluids because of the presence of substances that increase the viscosity of the fluid that can clog the flow cell and result in a malfunction.

In the following study, we examined ascitic and synovial fluids, comparing manual counts and differentials to those determined with the Advia 2120 analyzer. Furthermore, we also assessed the effect of adding hyaluronidase to the synovial samples.
(Sigma-Aldrich, St Louis, MO). Automated analysis was done twice on an Advia 2120, once using the regular channel and once using the channel that checks only for TNCs. For the TNC, the basophil/lobularity and peroxidase channel cytograms are modified specifically for the light scatter characteristics of the cells in body fluid samples. The WBC count from the peroxidase channel (WBCP) is compared with the TNC count from the basophil/lobularity channel. When the TNC does not agree with the WBCP count within limits specific to the body fluid application, the TNC count is flagged with an asterisk. The synovial fluid tests were done once with and once without the addition of 1 mg of hyaluronidase (from bovine testes type VIII, 683.6 units/mg, lyophilized powder, Sigma-Aldrich) to a final concentration of more than 250 cells/mL. The samples were inverted 10 times and not incubated. The proportion of neutrophils on the Advia 2120 was compared with counts obtained on fluid smear review (prepared after centrifugation at 1,500g for 5 minutes, smeared and stained with Wright-Giemsa). Fluid smears for ascitic fluid were reviewed only if the total WBC count was more than 250 cells/µL, the upper limit of the reference interval for polymorphonuclear cells. Concordance was examined using a predetermined cutoff value of 1,999 cells/µL for synovial fluid, and 249 cells/µL for ascitic fluid. Median values with 25% to 75% ranges were compared because the test results were not normally distributed. The Pearson coefficient was used to test for correlations if the relationships were linear, and had high correlations on the Spearman rank test.

The data for this study are part of our quality management system. Use of the data is in accordance with the ethical standards established by Clalit Health Services.

Results

Sixty samples had results from Advia’s regular and TNC channels, and from manual counting. Because the values were nearly identical, with a high correlation coefficient for the 2 Advia channels \( r = .998 \), we subsequently compared the hemocytometer counts and fluid smear reviews only with the regular channel results that also reported the proportion of neutrophils. The Advia regular channel results were nearly identical to those obtained with manual counting Table 1 for both synovial and ascitic fluids. The proportion of neutrophils on the Advia was harmonized and highly correlated with counts on the fluid smear review. Results using the Spearman rank correlation were high, similar to Pearson correlation coefficients, and the relationships were linear.

Eight values were concordantly high and 32 concordantly low for ascitic fluid, with only 2 misclassifications: 250 cells/µL compared with 240 cells/µL, and 220 cells/µL compared with 290 cells/µL for manual and Advia analyzer counts, respectively. Results were also concordant for neutrophil differential counts (fluid smear review compared with the Advia analyzer for 5 samples: 49% vs 44%, 6% vs 9%, 11% vs 17%, 25% vs 26%, and 4% vs 11%, respectively).

For synovial fluid, the values counted on the hemocytometer were lower if no enzyme was added in 18 of 18 cases \( (P < .001) \). The median values increased from 68 cells/µL to 515 cells/µL after the addition of hyaluronidase. Nevertheless, at an upper limit of the reference interval of 1,999 cells, 11 of 11 had low-low values and 6 of 6 had high-high values. In 1 discordant case, 432 cells/µL increased to 4,800 cells/µL after the addition of hyaluronidase. This case demonstrated the same discordance with the Advia analyzer. After adding hyaluronidase, no discordant values were found between the Advia and the hemocytometer. Results were also concordant for the proportion of neutrophils (Table 1). A cutoff value of less than 90% neutrophils has been used to rule out septic arthritis.8 We had only 3 cases with more than 90% on the fluid smear review (91%, 91%, and 92%), with 92%, 87%, and 91%, respectively, determined on the Advia analyzer.

Three samples had a high number of RBCs. To count the cells manually, the RBCs were hemolyzed. The manual counts were nearly identical to those found with the Advia. All the other samples had less than or equal to 20,000 cells/µL.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Manual Hemocytometer Counts and Smear Review vs Advia Analyzer Results With and Without Hyaluronidase</th>
</tr>
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<tbody>
<tr>
<td>Variable</td>
<td>Median Manual Counts (25%-75%)</td>
</tr>
<tr>
<td>Synovial fluid</td>
<td></td>
</tr>
<tr>
<td>WBC (n = 18)</td>
<td>515 (150-6,560)</td>
</tr>
<tr>
<td>WBC (n = 18)</td>
<td>68 (17-560)</td>
</tr>
<tr>
<td>Neutrophils (n = 18)</td>
<td>25 (12-77)</td>
</tr>
<tr>
<td>Neutrophils (n = 18)</td>
<td>ND</td>
</tr>
<tr>
<td>Ascitic fluid</td>
<td>145 (80-212)</td>
</tr>
<tr>
<td>WBC (n = 42)</td>
<td>ND</td>
</tr>
<tr>
<td>Neutrophils (n = 42)</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND, not determined.

\( a \) \( P < 0.001 \) compared with samples analyzed with hyaluronidase.

\( b \) See “Results” section.

Discussion

We found that the Advia analyzer’s regular channel is harmonized and reliable for identifying WBCs and the proportion of neutrophils in synovial and ascitic fluids. Hyaluronidase, however, is needed to prevent falsely low values on automated and manual counting. This might explain part of the interlaboratory variation in synovial fluid analysis,1 and the inability of the synovial fluid WBCs to differentiate...
those with and without septic arthritis.\textsuperscript{6-9} The other advantage of using hyaluronidase is that it prevents the clogging of flow cells of certain analyzers.\textsuperscript{2}

Only 3 samples were tinged with blood whereas nearly all the other samples had 0 to 10,000 cells/µL; a few samples had up to 20,000 cells/µL. We comment only on the visual presence of blood, without quantifying the concentration of microscopic RBCs. We are unaware of reference intervals for microscopic RBCs or the demonstration of clinical usefulness of reporting microscopic blood in the synovial or ascitic fluid.

Our results are consistent with others reported previously.\textsuperscript{2} Sugiuchi et al\textsuperscript{2} studied 47 synovial fluid samples within 24 hours of sample collection from inpatients and outpatients with arthritis of the knee who had undergone operations.\textsuperscript{2} They incubated the synovial fluid after the addition of hyaluronidase for 10 minutes at 37°C and analyzed the fluid using a Sysmex XE-2100 analyzer (Sysmex, Kobe, Japan). They found that the results on the analyzer were highly correlated with those obtained manually ($r= .998$). Extending these results to the Advia 2120 analyzer, we found that there was no need for incubation after inverting the tubes 10 times. This standardized method might be easily adopted in laboratories that analyze only a few samples per month.

We found that hyaluronidase also increased the total WBC found with manual counting, consistent with results from an older study.\textsuperscript{4} It is unclear why this method has not been adopted. Earlier studies also found that hyaluronidase facilitated a good cytospin preparation, and found significant differences in the differential counts on fluid specimens that did not contain an anticoagulant\textsuperscript{5} usually recommended to prevent clotting.

The upper limit cutoff value for WBC count in the diagnosis of septic arthritis is uncertain,\textsuperscript{7} but the likelihood of the disease increases as the total WBC count increases.\textsuperscript{8} One report retrospectively determined that the best differentiating values were 15,000 to 20,000 cells/µL, with a sensitivity of 83% and a specificity of 67%. Commonly a higher value (50,000 cells/µL) is used as the cutoff, but this approach lacks the sensitivity required to be clinically useful in ruling out infectious arthritis.\textsuperscript{9} It is important to realize that there is a great deal of clinical uncertainty when the diagnosis of septic arthritis is based on WBC counts.

Our results should be interpreted with caution. They should not be extrapolated to other analyzers before making comparisons with manual counts for such equipment. Furthermore, our numbers were small, and we did not have a cohort with diagnosed septic arthritis. Still, our results suggest that hyaluronidase should be added to joint fluids analyzed on the Advia analyzer or before manual counting to prevent a negative bias.

We conclude that the Advia analyzer can be used to determine the concentrations of WBCs and neutrophils in synovial and ascitic fluids. For synovial fluids, hyaluronidase should be added before manual counting or analysis on the Advia.

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References