Cholesterol Crystals Causing Falsely Elevated Automated Cell Count

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Abstract

This is a report of 3 cases of body fluid containing numerous cholesterol crystals that caused falsely elevated cell counts on an automated cell counter. Two of the cases were pleural effusion fluid from patients with long-standing rheumatoid arthritis. Fluid in the third case was from upper extremity cystic lesions of a patient with squamous cell carcinoma of the head and neck. Microscopic examination revealed abundant cholesterol crystals in all fluid samples. In all 3 cases, initially, the automated cell counter reported very elevated WBC and RBC counts that were much higher than those from the manual count. This interference by cholesterol in the automated cell counter is discussed. In addition, possible pathophysiology of cholesterol formation in the body fluid is discussed and chylous and pseudochylous (chyliform) effusions are reviewed. Finally, the use of automated instruments in the evaluation of body fluid is reviewed.

Cholesterol crystals in body fluids, particularly pleural fluids, are rare but well described, with characteristic morphologic features revealed by microscopic examination. They are described as rectangular plates with notched edges and birefringence under polarized light.1 Manual hemocytometers and automated cell counters have been used in the evaluation of body fluid. Recently, manufacturers of hematology instruments also have obtained US Food and Drug Administration (FDA) approval for performance of body fluid cell counts using instruments primarily intended for whole blood CBC counts. These instruments use, among several technologies, impedance-based technology to enumerate events based on cell size. Numerous interfering factors are possible. However, the effect of cholesterol crystals on results from automated cell (particle) counters has not been well described. We report 3 cases of marked interference in the automated cell count by the presence of cholesterol crystals and the implication of this interference.

Materials and Methods

All body fluids submitted by clinical services for hematology and cell count evaluation during the period July 1, 2001, through June 30, 2005, at Eastern Colorado (Denver) Veterans Administration Medical Center, Denver, CO, were reviewed. Cases with cholesterol crystals were identified.

Automated Cell Count Method

Body fluids were diluted and cells counted using the Coulter Z1 particle count and size analyzer (Beckman Coulter, Fullerton, CA). In our institution, this analyzer is dedicated to
body fluid cell counting and is not used for CBC counts. Owing to the linearity of the instrument (cell counts, 200-93,365/μL), samples with cell counts lower than 300/μL are counted in the manual counting chamber per laboratory protocol. Briefly, the background count is obtained by obtaining a cell count from a suspension in Isoton II (diluent; Beckman Coulter) and Isoton II with Zap-O Globin (RBC lysing reagent, Beckman Coulter). Background counts of less than 100/μL and 200/μL for Isoton II and Isoton II with Zap-O Globin, respectively, should be ascertained. For the total cell count (sum of WBC and RBC counts), 40 μL of specimens and 20 mL of Isoton II are mixed and counted in the Coulter Z1. If the total count is less than 1,000/μL, the background count is subtracted from it. To obtain the WBC count, 1 drop of Zap-O Globin is added and the cell count is recorded. To obtain the RBC count, the WBC count is subtracted from the total count.

Manual Cell Count Method

The hemocytometer cell count is determined by dispensing the body fluid, with appropriate dilution if necessary, into the standard counting chamber (Improved Neubauer hemocytometer, depth of 1/10 mm, 3 × 3 mm grid area, American Optical, Buffalo, NY). After 1 minute to allow the cells to settle, the WBC count is obtained by counting cells in 4 corners of the counting chamber, including cells that touch the lower and right inner line, under microscopic examination. Each corner consists of a 1 × 1 mm area. The RBC count is obtained by counting within the counting chamber center square that contains 25 small squares. (Each small square consists of a 1/5 × 1/5 mm area.) RBCs are counted in the 4 corners and the center of the 25 small squares. WBCs and RBCs are counted in duplicate. Calculation for the WBC count (μL) is the number of cells (in 4 corners) × dilution × 2.5; for the RBC count (μL), the calculation is the number of cells (in 5 small squares) × dilution × 50. For very low WBC or RBC cell counts, cells within all 9 (1 × 1 mm) squares are counted. Low cell count are calculated as the number of cells obtained by counting within the counting chamber center square that contains 25 small squares. (Each small square consists of a 1 × 1 mm area. The RBC count is recorded. To obtain the RBC count, the WBC count is subtracted from the total count.

Microscopic Examination

Cytocentrifuged slides of the body fluid are prepared and Wright-Giemsa stained. A 100-cell differential (neutrophils, lymphocytes, mononuclear cells) is manually determined. All slides are reviewed by pathologists.

Results

Three cases of cholesterol crystals were found in 3,798 accessioned body fluid evaluations submitted to the hematology laboratory.

Description of the Cases

Case 1

The patient was a 57-year-old man with a 29-year history of rheumatoid arthritis who was admitted to the hospital with respiratory failure. Owing to the deforming nature of his disease, he was confined to a wheelchair. On admission, a chest radiograph revealed bilateral pleural effusions. During video-assisted thoracic surgery to obtain a lung and lung biopsy, pleural fluid was removed and sent to the laboratory for analysis. Of interest, the pleural biopsy showed pleural fibrosis with mild chronic inflammation; the lung biopsy showed histologic evidence of diffuse alveolar damage and organizing pneumonia.

Case 2

The patient was a 68-year-old man with a 15-year history of rheumatoid arthritis who came to the chest clinic for evaluation of a left-sided pleural effusion that had been diagnosed by computed tomography. The effusion had been present for 11 years and was asymptomatic. A thoracentesis was performed, and a sample of pleural fluid was sent to the laboratory for analysis.

Case 3

The patient was a 57-year-old man with a history of squamous cell carcinoma of the head and neck who was undergoing chemotherapy and radiation. He had multiple cysts in his right upper arm. Ultrasonography confirmed 3 cystic lesions, ranging in size from 4.2 to 6.1 cm in maximum dimension. Fluid was aspirated from one of the cysts and sent to the laboratory for analysis. No morphologic evidence of squamous cell carcinoma was present in the submitted fluid.

Morphologic Evaluation and Cell Count

In all 3 cases, the initial automated instrument cell count, based on impedance principles, reported very elevated cell counts |Table 1| and |Table 2|. Gross examination of the fluid revealed it to be opaque or cloudy |Image 1|. Microscopic evaluation of the fluid in all 3 cases revealed numerous cholesterol crystals recognizable as rectangular plates with notched edges |Image 2| and |Image 3|. The manual cell count revealed a much lower cell count, by orders of magnitude, than the instrument count (Table 2). To our knowledge, this is the first report of cholesterol crystals causing spuriously elevated cell counts by an automated cell counter.

Discussion

The presence of a high concentration of cholesterol in pleural fluid, with low levels of triglycerides and absence of
Cholesterol crystals influence cell count

Chyle (lymph), is referred to as a *pseudochylous effusion* and also is known as a *chyliform* or *cholesterol effusion*. This is the result of a chronic, long-standing (usually several years) pleural effusion, as can be seen in rheumatoid arthritis and tuberculosis. Over time, the effusion can become enriched with cholesterol (usually >200 mg/dL). Our cases, in particular cases 1 and 2 with typical long-standing rheumatoid arthritis, are consistent with cholesterol effusion.

**Table 1** Summary of Patient Data, Including Initial Uncorrected Automated Cell Counts

<table>
<thead>
<tr>
<th></th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC count (/µL)</td>
<td>68,500</td>
<td>1,900</td>
<td>12,600</td>
</tr>
<tr>
<td>RBC count (×10^3/µL)</td>
<td>20.4</td>
<td>6.9</td>
<td>1,400</td>
</tr>
<tr>
<td>Volume (mL)</td>
<td>5</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Appearance</td>
<td>Opaque</td>
<td>Cloudy</td>
<td>Cloudy</td>
</tr>
<tr>
<td>Comment</td>
<td>Abundant cholesterol crystals present</td>
<td>Abundant cholesterol crystals present</td>
<td>Abundant cholesterol crystals seen</td>
</tr>
<tr>
<td>Body fluid levels</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total protein (g/dL)</td>
<td>2.8</td>
<td>5.1</td>
<td>NA</td>
</tr>
<tr>
<td>LDH (IU/dL)</td>
<td>1,677</td>
<td>352</td>
<td>NA</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>30</td>
<td>49</td>
<td>NA</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>254</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Serum cholesterol level, mg/dL (mmol/L)</td>
<td>NA</td>
<td>149 (3.85)</td>
<td>NA</td>
</tr>
</tbody>
</table>

LDH, lactate dehydrogenase; NA, not available.

**Table 2** Automated and Manual Cell Counts

<table>
<thead>
<tr>
<th></th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC count (/µL)</td>
<td>Automated 68,500</td>
<td>1,900</td>
<td>12,600</td>
</tr>
<tr>
<td></td>
<td>Manual No identifiable cells</td>
<td>15</td>
<td>1,150</td>
</tr>
<tr>
<td>RBC count (×10^3/µL)</td>
<td>Automated 20.4</td>
<td>6.9</td>
<td>1,400</td>
</tr>
<tr>
<td></td>
<td>Manual No identifiable cells</td>
<td>4.7</td>
<td>37</td>
</tr>
</tbody>
</table>

**Image 1** Gross examination of pleural fluid from case 1 revealed it to be opaque or cloudy.

**Image 2** Cholesterol crystals (×500).

**Image 3** Cholesterol crystals (×1,000).
Cholesterol effusions are relatively rare. The last major review by Coe and Aikawa\(^3\) included a total of 101 cases from the world’s literature up to 1961. The average reported time for evolution of pleural effusion into a cholesterol effusion was 5 years, although intervals of 11 to 15 years were not uncommon. Consistent with that, cases 1 and 2 had 29- and 15-year histories, respectively, of rheumatoid arthritis.

A true chylous effusion results from leakage of chyle from the thoracic duct following trauma or malignancy. The onset of this type of pleural effusion usually is sudden, in contrast with the gradual onset of a pseudochylous effusion.\(^1,2\) Triglyceride levels usually are high, and cholesterol levels are low (usually <150 mg/dL).\(^5\) Microscopically, lymphocytosis is seen in the chylous effusion. See Table 3 for comparison of chylous and pseudochylous effusions.

Although the pathogenesis of chyliform effusions is not clear, there are several speculations about the mechanism involved in the accumulation of cholesterol. Coe and Aikawa\(^3\) divided these speculations into 2 classes, the general metabolic theory, and the local process theory.

The general metabolic theory proposes that cholesterol effusions are merely a manifestation of systemic hypercholesterolemia. Studies consistently have shown, however, that this is not the case, because many patients with chyliform effusions have normal blood cholesterol levels.\(^3,6\) Available data show that the serum cholesterol level was not elevated in case 2 (Table 1).

The most accepted local process theory suggests that the cholesterol is derived from the cell membranes of degenerating RBCs and WBCs in the pleural fluid. The diseased pleura slows the egress of cholesterol, which then can accumulate to high concentrations.\(^1,3,4,6,7\) A case report of cholesterol in the synovial fluid in patients with rheumatoid arthritis without generalized lipid abnormalities also points to a local origin of cholesterol.\(^8\)

In case 3, the cholesterol may have formed as a result of degeneration of cells in the soft tissue of the arm. The patient had squamous cell carcinoma of the head and neck and had no evidence of autoimmune disorders. There is 1 case report of a synovial cyst with cholesterol formation in seronegative arthritis.\(^9\) This suggests that the cholesterol formation process might occur outside the context of rheumatoid arthritis as well.

These 3 cases serve to remind us that automated cell counters do not always yield accurate results. The automated cell counter, the Coulter Z1 particle count and size analyzer, relies on the impedance method of counting cells based on the detection and measurement of changes in electric resistance. These resistance changes occur when a particle or cell suspended in a conductive liquid moves through a small aperture. As each particle or cell traverses the aperture, a voltage change is detected and registered as a counted event. The magnitude of the voltage change reflects the cell size. Multiple interfering factors are possible. These factors are listed in the operating manual of the Coulter Z1, and include the following: RBCs that resist lysing, nucleated RBCs, fragmented WBCs, large aggregated platelets, and any unlysed particles greater than 45 µL.\(^10\)

The particle counter using the impedance principle also can be used in enumerating the RBCs and WBCs of animals\(^10\) such as cats, cattle, dogs, and sheep. Other biologic uses include evaluation of semen,\(^11,12\) cells in milk,\(^13,14\) hematopoietic stem cells,\(^15\) and muscle and fat stem cells.\(^16\) Other particulate substances in aqueous electrolyte solution also can be analyzed by the impedance-based counter. (Coulter’s manual includes substances such as asphalt emulsion, clay, and iron oxide.) In Body Fluids, when describing synovial fluid cell counts, Kjeldsberg and Knight\(^1\) say: “automatic counters may give spuriously high cell counts by also counting extracellular material (crystals and fat globules).”\(^1\)

There are very few materials that cannot be analyzed by using the impedance technique.\(^10\) The few types of materials not amenable to counting by the impedance method include organic compounds such as pesticides and drugs that are too soluble in electrolyte solution, or if the particles are dispersed in mineral oil.\(^10\)

Numerous examples of using automated cell counters to enumerate cells in body fluids other than whole blood are reported in the literature.\(^17-20\) These include bronchioalveolar fluid,\(^21\) synovial fluid,\(^22-25\) cerebrospinal fluid,\(^26,27\) peritoneal fluid,\(^28\) and pleural fluids.\(^29\) In addition, animal specimens have been used in the automated instrument.\(^30-32\) Several

**Table 3**

Distinguishing Features in Chylous and Pseudochylous Effusions*

<table>
<thead>
<tr>
<th>Chylous</th>
<th>Pseudochylous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Etiology</td>
<td>Damage or obstruction to thoracic duct</td>
</tr>
<tr>
<td>Common causes</td>
<td>Trauma, malignancy, congenital</td>
</tr>
<tr>
<td>Onset</td>
<td>Sudden</td>
</tr>
<tr>
<td>Appearance</td>
<td>Milky white or yellow-bloody</td>
</tr>
<tr>
<td>Microscopic examination</td>
<td>Lymphocytosis</td>
</tr>
<tr>
<td>Triglyceride level, mg/dL (mmol/L)</td>
<td>&gt;110 (&gt;1.24)</td>
</tr>
<tr>
<td>Chronic effusion</td>
<td>Tuberculosis, rheumatoid pleuritis</td>
</tr>
<tr>
<td>Gradual</td>
<td>Milky or green, metallic sheen</td>
</tr>
<tr>
<td>Mixed cellular reaction, cholesterol crystals</td>
<td></td>
</tr>
<tr>
<td>&lt;50 (&lt;0.56)</td>
<td></td>
</tr>
</tbody>
</table>

*Adapted from Kjeldsberg and Knight.\(^1\) Used with permission.
manufacturers of automated hematology instruments have obtained FDA approval for performing cell counts on body fluids (for example, Coulter LH750, Beckman Coulter, approval No. 510(K)050057; Sysmex XE-2100, Sysmex, Mundelein, IL, approval No. 510(K)040073; and Advia 120 [cerebrospinal fluid WBC, WBC differential, and RBC counts], Bayer, Tarrytown, NY, approval No. 510(K)022331).

Although the manual cell count is regarded as the "gold standard" in body fluid cell counting, there is operator-dependent variability. Barnes et al.18 recently found that manual chamber cell counting among 4 technologists can have a high coefficient of variation (4%-116% for WBC counts and 2.5%-141.4% for RBC counts). On the other hand, the precision of the automated instrument has been reported to be 6.3% to 44.2% for total nucleated cell counts and 2.5% to 17.1% for RBC counts.19 The correlation between manual and instrument cell counts seems excellent. For example, the coefficient of correlation has been reported to be as high as 0.9955 (using the CELL-DYN 3200,19 Abbott Diagnostics, Abbott Park, IL) and 0.9833 (using the LH75018) for WBC counting and 0.9961 (using the CELL-DYN)19 and 0.9616 (using LH75018) for RBC counting.

The linearity of the instrument cell counting is important, particularly in evaluating cerebrospinal fluid, in which the paucity of cells requires the linearity of cell counting to extend to zero. (There is one report of RBC and WBC extending to zero in the Bayer Advia 120 CSF Assay27; one report of WBC extending to zero by the Abbott CELL-DYN 3200.19) Barnes et al.18 reported the policy of using the automated cell count on cerebrospinal fluid only if it appears grossly bloody or cloudy and would allow other body fluids to be analyzed by automated instrument if the WBC count was more than 0.3 × 10⁹/L and the RBC count more than 0.03 × 10⁹/L. In the evaluation of very low cell counts, many laboratories like ours (see the "Materials and Methods" section) also require subtraction of background counts. This may not be necessary with certain modern instruments.18

The use of automated hematology instruments in the enumeration of body fluid cells may require modification of hardware or software (such as LH750 software version 2B). Larger sample volume and specimen preparation (eg, viscous synovial fluid may require treatment) also may be required for use of an automatic instrument for body fluid cell counting. However, the advantages of automatic cell counting may be reduced labor requirements18,19 and fewer counting errors. With these issues in mind, the use of automated instruments in the evaluation of body fluids likely will increase over time. Therefore, it is more important now to consider possible interfering factors in body fluids that might affect the cell count.

To our knowledge, there have been no specific reports of cholesterol crystals causing falsely elevated WBC counts in an automated cell counting instrument. In 3 cases, we demonstrated examples of the interference with automatic cell counting when abundant cholesterol crystals are present. Direct microscopic visual evaluation revealed the presence of cholesterol crystals and the discordant cell counts; manual recounts then revealed that the automated cell counts had been spuriously high.

**Conclusion**

The presence of abundant cholesterol crystals in the body fluid can cause spuriously elevated WBC and RBC counts in impedance-based automated cell counters. The recognition of cholesterol crystals is important to avoid reporting falsely elevated cell counts. In addition, outside the context of rheumatoid arthritis, cholesterol crystals also might be observed.

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**References**

5. Hamm H, Pfalzer B, Fabel H. Lipoprotein analysis in a cholesterol crystal counting instrument. In 3 cases, we demonstrated examples of the interference with automatic cell counting when abundant cholesterol crystals are present. Direct microscopic visual evaluation revealed the presence of cholesterol crystals and the discordant cell counts; manual recounts then revealed that the automated cell counts had been spuriously high.

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