Letters to the Editor

Treatment of cerebrospinal fluid with formalin from patients infected with human immunodeficiency virus before diagnostic microscopy

Central nervous system infections are a well recognised complication of acquired immune deficiency syndrome (AIDS). Many different aetiological agents have been described, including Cryptococcus neoformans. Microscopic examination of cerebrospinal fluid plays an important part in the rapid diagnosis and management of these infections.

The Advisory Committee on Dangerous Pathogens (LAV/HTLVIII) revised guidelines advise that the clinical laboratory examination of human immunodeficiency virus (HIV) specimens may take place within a containment level 2 laboratory and that no microbiological safety cabinet is required (unless the virus is to be propagated, concentrated, or dispersed by processes into the air). Clearly, where possible, it remains desirable to inactivate potentially infectious specimens. As HIV has been isolated from the cerebrospinal fluid of patients with AIDS these specimens should be treated as potentially HIV infectious.

We have been mixing equal volumes of cerebrospinal fluid with 10% (10g NaH₂PO₄, 16·2g Na₂HPO₄ 0·25l formalin, diluted in H₂O to 2·5l) buffered formalin solution (4% available formaldehyde) to give a final concentration of 2% formaldehyde for 10 minutes at room temperature before microscopy. Both leucocytes and erythrocytes remain morphologically intact so that a cerebrospinal fluid cell count can be performed. It is also possible to perform the India ink test (to detect the capsule of Cryptococcus neoformans) on cerebrospinal fluid after treatment with formalin. Formalin does not change the capsular morphology, and our laboratory has diagnosed cryptococcal meningitis in patients with AIDS using this technique.

HIV is sensitive to 0·5% paraformaldehyde inactivation under comparable conditions (10 minutes, 21–25°C 0·35 g/100 ml protein concentration). The treatment of cerebrospinal fluid by the method described above should inactivate HIV before microscopic examination. We would recommend this method for the handling of cerebrospinal fluid from suspected and confirmed patients infected with HIV.

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References


Hyperferritinemia associated with splenic infarction

High values for serum ferritin concentration, disproportionate to the size of iron stores, may be seen with inflammation, neoplasia, and liver damage. To this list Brownell et al have now added vasocclusive sickle cell crisis, in which they suggest that ferritin may be released into circulation as a result of bone marrow infarction. The case described below (to which we briefly refer elsewhere) illustrates that damage to other ferritin rich tissues may also result in extremely high serum ferritin concentrations and a spurious diagnosis of iron overload.

Case report

A 53 year old woman presented with a short history of severe dyspnoea and ankle swelling and was found to be in predominantly right sided cardiac failure. She had an 11 year history of asthma and untreated mild hypertension. An isotope lung scan suggested pulmonary emboli. Echocardiography showed poor cardiac movement, large ventricles without muscle hypertrophy, and a left ventricular ejection fraction of only 18%. Serum bilirubin concentration was 1.7 mg/dl (29 μmol/l) and serum aspartate aminotransferase activity 236 IU/l. The possibility of a haemochromatotic cardiomyopathy was considered, and the serum iron and iron binding capacity, using ICSH methods, were found to be greatly increased at 755 μg/dl (135 μmol/l) and 970 μg/dl (173 μmol/l), respectively. The serum ferritin concentration was >20 000 μg/l (normal 10–200 μg/l). Intramuscular injection of 500 mg of desferrioxamine produced a 24 hour urine iron excretion of 1·7 mg (normal 1·0 (SD 0·5 mg/l)). The 24 hour urine total free desferrioxamine was 291 mg, a normal fraction of the injected dose (unpublished observations), indicating no impairment of renal clearance of the drug. Six days after admission the serum iron had fallen to 155 μg/dl (28 μmol/l), the serum total iron binding capacity to 478 μg/dl (85 μmol/l), and the serum ferritin to 2000 μg/l. Despite anticogulation and supportive treatment the patient died seven days later. At necropsy the lungs contained a number of recent (days) pulmonary infarcts. The heart weighed 450 g with no histological evidence of excess iron, and idiopathic cardiomyopathy was diagnosed. The liver showed small amounts of iron in macrophages (Kupffer cells) but no parenchymal iron deposits. The chemical iron content of the liver was at the upper limit of normal at 0·14% of the dry weight. The spleen showed a 3 cm in diameter infarct, which was organising.

Discussion

The cardiomyopathy and high serum ferritin concentration initially raised a suspicion of tissue damage due to iron overload—that is, haemochromatosis. The presence of an unsaturated iron binding capacity of over 200 μg/dl, however, and the limited iron excretion after desferrioxamine contradicted the possibility of enlarged parenchymal iron stores. The unusually high initial concentrations of serum iron, iron binding capacity, and ferritin, which fell rapidly over the first few days, suggested a transient release into the circulation of iron rich ferritin from damaged tissues. The necropsy findings indicated that this release was likely to have been from the splenic infarct. Extraordinarily high serum ferritin concentrations should suggest tissue necrosis, and in these circumstances a desferrioxamine excretion test may be a helpful non-invasive aid to determining whether parenchymal iron overload is truly present.

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References

Pseudoleucocytosis and pseudothrombocytosis due to cryoglobulinaemia

Erroneously high blood counts, measured by the Coulter Model S plus phase IV blood counter, may be caused by artefactual particulate matter, such as platelet clumps, epidermal cells, dust or small air bubbles. Cryoglobulins are immunoglobulins of one or more classes that precipitate on cooling below 37°C to form globules. This phenomenon is most prominent at 4°C, though it occurs to a lesser degree at higher temperatures. Pseudoleucocytosis due to cryoglobulinaemia has long been recognised, but the phenomenon of pseudothrombocytosis due to cryoglobulinaemia has not been previously reported.

Case report

A sixty year old West Indian woman presented with menorrhagia in March 1986. A full blood count on a Coulter Counter S plus phase IV showed a haemoglobin concentration of 13.5 g/l, with an increased leucocyte count of 46.9 × 10⁹/l and a platelet count of 646 × 10⁹/l. The red cell count was within normal limits at 5.24 × 10¹²/l. Examination of the peripheral blood film and manual white cell and platelet counts did not corroborate the automated results. The peripheral blood film showed small translucent globules dispersed among the red cells. Cryoglobulinaemia was suspected, and the sample was reprocessed at varying temperatures. This showed a progressive increase in the leucocyte and platelet counts when the sample cooled on standing (figure). The plasma viscosity using a Luckham clinical viscometer was 2.9 cps, at 37°C but was greater than 5cps at 4°C. Donath-Landsteiner and direct Coomb’s tests yielded negative results. The total serum protein concentration was 83 g/l with an albumin concentration of 40 g/l. Electrophoresis showed the presence of a monoclonal band of IgM containing λ light chains. IgM measured at 37°C was 16.0 g/l, and at 4°C it was 10.0 g/l. There was no immune suppression. Urinary Bence-Jones protein reaction was negative. Bone marrow aspirate and a trephine biopsy showed gross infiltration with small lymphoid cells, with some plasmacytic differentiation consistent with Waldenstrom’s macroglobulinaemia.

### Table 1: Comparison of cell counts on plasma and serum with falling temperature

<table>
<thead>
<tr>
<th>Temperature</th>
<th>White cell count (× 10⁹/l)</th>
<th>Platelet count (× 10⁹/l)</th>
<th>Red cell count (× 10¹²/l)</th>
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<tr>
<td></td>
<td>Plasma</td>
<td>Serum</td>
<td>Plasma</td>
</tr>
<tr>
<td>37°C</td>
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<td>90</td>
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<td>4°C</td>
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<td>58.5</td>
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</table>

### Discussion

Cryoglobulins form particles of various sizes, ranging from 3–24 μm when they precipitate. The Coulter Counter Model S plus phase IV is an electronic blood counter, which operates on the principle of an impedance change being produced by particles passing through a small aperture. It usually measures particles ranging from 2μl to 450μl (Instruction Manual, Coulter Model S, 1983.) Our findings showed interference in whole blood counts in the 2μl and 35μl regions, the smaller particles being counted as platelets and the larger ones as leucocytes. As expected, the spurious leucocyte and leucocyte counts were greater in plasma than in serum. Emori et al described a case of pseudoleucocytosis due to cryoglobulinaemia, in which they attributed the spuriously increased leucocyte count to the particle formation between cryoglobulin and fibrinogen.

Previous reports of pseudoleucocytosis caused by cryoglobulinaemia have been on counts performed on older models of blood counters without the ability to report platelets. With the advent of the Coulter S plus series and the additional availability of automated platelet counts, pseudoleucocytosis and pseudothrombocytosis should now also be detected. Leucocytosis and thrombocytosis, unsubstantiated by examination of a peripheral blood film and manual counts, should raise the suspicion of cryoglobulinaemia.