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 guidelines¹ 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^{2,3} these specimens should be treated as potentially HIV infectious.

We have been mixing equal volumes of cerebrospinal fluid with 10% (10 g NaH₂PO₄, 16.2 g Na₂HPO₄ 0.25 l formalin, diluted in H₂O to 2.5 l) 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 inactivation⁴ 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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Hyperferritinaemia 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.¹ To this list Brownell *et al*² have now added vaso-occlusive 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 elsewhere³) 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,^{4,5} were found to be greatly increased at 758 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).⁶ 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)).⁷ The 24 hour urine total free desferrioxamine was 291 mg, a normal frac-

tion 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 anticoagulation 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.⁸ 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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