Clinico-pathological Conference

Disseminated histoplasmosis in patients with acquired immunodeficiency syndrome

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Case reports (Dr R F Miller)

Case 1: A 43 year old heterosexual Afro-Caribbean man was admitted to his local hospital in the West Indies at the end of February 1993 complaining of the acute onset of central chest pain. Previously he was a heavy smoker of more than 40 cigarettes a day but had stopped 10 years previously: he did not drink alcohol and there was no prior family history or past medical history. Examination at that time revealed no abnormal physical signs. He was thought initially to have a myocardial infarction but serial ECG and cardiac enzymes were normal. A chest radiograph showed a 3cm diameter coin lesion in the periphery of the right upper zone. At thoracotomy a necrotic granuloma was resected, but histological examination did not identify the underlying cause. Post-operatively a right sided pneumothorax required tube drainage. A month later, on vacation in London, he became acutely dyspnoeic and was admitted as an emergency to the Middlesex Hospital. He had sustained a further right sided pneumothorax which was treated with an intercostal tube drain. Following re-expansion of the lung he remained hypoxic and his chest radiograph showed marked bilateral interstitial shadowing.

Fibreoptic bronchoscopy with broncho-alveolar lavage and transbronchial biopsies confirmed a diagnosis of *Pneumocystis carinii* pneumonia. This was treated with intravenous high dose co-trimoxazole and methylprednisolone.

He was counselled about the possibilities of HIV infection but initially denied any risk activity. Subsequently he reported unprotected sexual intercourse with multiple female partners over the last nine years throughout the Caribbean and United States of America. He also reported weight loss of more than 20 kg over the previous 12 months. His HIV test was positive; a CD4 count was $0.02 \times 10^9/l$ (normal $0.35-2.2 \times 10^9/l$).

Despite treatment for pneumocystis pneumonia the patient's condition deteriorated and he became increasingly dyspnoeic and hypoxic and so was transferred to the Intensive Care Unit for mechanical ventilation. In view of the granuloma resected from the lung it was thought possible that the patient had occult mycobacterial or fungal infection in addition to pneumocystis pneumonia so empirical anti-tuberculous therapy (rifampicin 600mg, isoniazid 300mg and ethambutol 800mg, each once daily via a

nasogastric tube) and anti-fungal treatment (amphotericin B 1mg/kg IV once daily) were added. A bone marrow aspirate was performed and staining was negative for acid and alcohol-fast bacilli (AAFB) and fungi (as was subsequent culture). Review of lung granuloma showed a necrotic mass with focal calcification and a granulomatous and fibrotic rim; whilst the fungi were invisible on haematoxylin and eosin stain, a Grocott's silver stain revealed abundant yeasts of *Histoplasma capsulatum* (figs 1 and 2). The patient continued to deteriorate with the development of a further pneumothorax requiring tube drainage and died 14 days after admission. An autopsy was performed.

Autopsy (Dr S B Lucas)

The body was that of thin but well nourished black male. The recent surgical scar on the right chest wall had healed. The lungs were consolidated and in the upper zones, emphysematous; the other organs were macroscopically normal. Histology confirmed the centri-acinar emphysema and showed *Pneumocystis carinii* pneumonia in all five

Figure 1 Case 1: Lung resection. Lower power view of the necrotic granuloma, with a sharply defined rim of fibrous tissue. (haematoxylin and eosin)

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Figure 2 Case 1: Lung resection. High power view of necrotic granuloma; left hand panel shows calcified amorphous material. (Haematoxylin and Eosin) Right hand panel shows yeasts of *Histoplasma capsulatum*. (Grocott's silver stain) $\times 200$

lobes. There was no residual histoplasmosis in the lung scar tissue, but yeasts were present in small scars in the hilar lymph nodes. Thus the patient had an old primary complex lesion of histoplasmosis. Cytomegalovirus (CMV) inclusions were present in small bowel and adrenals, but no other organs showed any significant abnormalities. The brain and spinal cord were normal.

Pathological diagnoses:

1. *Pneumocystis carinii* pneumonia
2. Previous and residual thoracic *Histoplasma capsulatum* infection
3. CMV ileitis and adrenalitis.
4. Emphysema (? tobacco-related).

Dr R F Miller

Case 2: A 38 year old homosexual Venezuelan man had lived in the United Kingdom since 1972. He originally presented to this Hospital in February 1986 with a 6 weeks history of right sided pleuritic chest pain, dyspnoea and fever with chills together with anorexia. At this time the patient was found to be hepatitis B immune and HIV 1 antibody positive. He had signs of a right pleural effusion confirmed on a chest radiograph. A pleural aspiration and biopsy were performed and culture of both pleural fluid and the pleural biopsy confirmed *Mycobacterium tuberculosis*: this was treated with rifampicin, isoniazid and ethambutol for two months, continuing for a further seven months with rifampicin and isoniazid. The patient was notified to the Local Public Health Department.

The patient then returned to Venezuela for a holiday and completed nine months chemotherapy. On his return to the United Kingdom he was well and continued to be followed up regularly in the outpatient department. In view of a falling CD4 count and the presence of oral candida he began zidovudine and fluconazole. He was intolerant of co-trimoxazole (it produced a rash) and so he received monthly nebulised pentamidine as primary prophylaxis against

P. carinii pneumonia. CD4 counts in October 1991 and April 1992 were 0.03 and $0.01 \times 10^9/l$ respectively. The patient was admitted to hospital in April with a 10 months history of low grade fever and 12kg weight loss. In the five weeks before admission he had developed a non productive cough with worsening exertional dyspnoea and an exacerbation of his fever. On examination on admission he was pyrexial 38.5°C and had marked seborrhoeic dermatitis on the face. In the chest there were bilateral bibasal mid-inspiratory crackles and in the abdomen there was a 4cm liver edge and an obviously enlarged spleen. In the heart there were clinical signs of mitral regurgitation and in the nervous system the only abnormality was cotton wool spots in the right fundus. In the peripheral nervous system the ankle jerks were absent and knee jerks were diminished; sensation was impaired below the knees bilaterally. Investigations showed him to be profoundly anaemic with a haemoglobin of 5.9g/dl , MCV = 93, a white blood count of 0.9 (neutrophils = 0.5) $\times 10^9/l$ and a platelet count of $97 \times 10^9/l$, INR 1.0. Urea and electrolytes were normal and liver function tests showed normal albumin and bilirubin but an AST of 232 (normal <45) IU/l and an alkaline phosphatase of 457 (normal <280) IU/l. The blood gases taken with the patient breathing air showed $\text{PaO}_2 = 8.9\text{kPa}$ and $\text{pCO}_2 = 5.8\text{kPa}$. Ultrasound of the abdomen showed an homogeneously enlarged liver and a 17cm diameter enlarged spleen. A chest radiograph showed diffuse interstitial shadowing (fig 3) and CT of the chest showed mediastinal lymphadenopathy and diffuse interstitial shadows. Fibreoptic bronchoscopy and bronchoalveolar lavage were negative for *P. carinii* and other pathogens, including fungi. An indium labelled human polyclonal immunoglobulin scan¹ showed diffuse intrapulmonary accumulation of tracer, suggesting an infective pathology. Repeat fibreoptic bronchoscopy seven days later was again negative. Atypical serology was negative for legionella, chlamydia and mycoplasma; a test

Figure 3 Case 2: Chest radiograph taken on presentation in April 1992, showing diffuse bilateral interstitial shadowing

for serum cryptococcal antigen was also negative. Histoplasma complement-fixation and immunodiffusion tests were both negative. Cultures of blood were negative for fungi but positive for AAFB; subsequently, *Mycobacterium avium-intracellulare* was grown on prolonged culture. The same organism was also grown from bronchoalveolar lavage fluid. Cranial MRI showed minor atrophy and nerve conduction studies revealed a mild sensory neuropathy. An echo-cardiogram showed mitral regurgitation.

The patient was transfused with whole blood and a diagnosis of presumptive pneumocystis pneumonia was made. This was treated with IV pentamidine and zidovudine was stopped (as it was felt that it might have contributed to the pancytopenia). After six days of IV pentamidine the serum creatinine rose and so treatment was changed to clindamycin 600mg four times daily and primaquine 15mg once daily. With this change the creatinine returned to normal but there was little impact on the symptoms and the arterial blood gases. In view of the abnormal liver function tests and enlarged liver, a liver biopsy was performed. This showed small non-necrotic granulomata containing some *Histoplasma capsulatum* yeasts (fig 4); AAFB were not seen. On culture of the liver biopsy both *Histoplasma capsulatum* and *M avium-intracellulare* were identified. A stained peripheral blood film was negative for fungi. Treatment with amphotericin B (1mg/kg IV once daily) was begun. This produced a further rapid deterioration in renal function and so treatment was changed to itraconazole initially 400mg twice daily, subsequently 400mg daily. This treatment produced a rapid lysis of fever. The patient was discharged after 27 days in hospital and continued to receive itraconazole 400mg daily and monthly intravenous pentamidine as secondary prophylaxis against *P carinii* pneumonia.

The patient was readmitted five weeks later with a five days history of high fever with

sweats and a non-productive cough. There were no focal signs in the chest but the liver and spleen were further enlarged. Arterial blood gases and the chest radiograph were now normal. The haemoglobin level was 11.0g/dl, the white blood count was $3.5 \times 10^9/l$, and the platelet count was $95 \times 10^9/l$. Urea and electrolytes and liver function tests were normal. Blood cultures were negative for bacteria and fungi and no AAFB were seen. In the absence of another diagnosis and the known disseminated *M avium-intracellulare* infection he was treated with a combination of rifampicin 600mg once daily, ciprofloxacin 200mg twice daily and ethambutol 800mg once daily; a two weeks induction course of IV amikacin (7.5mg/kg once daily) was also given. The itraconazole dose was doubled because of the known interaction with rifampicin. His fever persisted and so he was given a short course of oral steroids which rapidly abolished it. He was then discharged to outpatient follow up and remained well, attending as a day case on a monthly basis as a day case for IV pentamidine.

In October 1992 he developed swelling of the right leg and lymphadenopathy was noted in the right groin. A lymph node biopsy in December 1992 showed Kaposi's sarcoma for which he received local radiotherapy. In January 1993 Kaposi's sarcoma was noted on the gums and hard palate. The patient re-presented at this stage with a four weeks history of fever and cough and a two weeks history of anorexia and exertional dyspnoea. Examination revealed him to be pyrexial 39.5°C , tachycardic (124 beats per minute) and to have extensive Kaposi's sarcoma on the gums and hard palate. The chest was clear and the auscultatory findings in the heart were unchanged. In the abdomen, the liver and spleen were further enlarged. Investigations included an ultrasound which showed smooth heterogenous hepatomegaly and homogeneous splenomegaly. Arterial blood gases (breathing air) showed a $\text{pO}_2 = 13.5\text{kPa}$, $\text{pCO}_2 = 2.8\text{kPa}$, $\text{pH} = 7.35$, bicarbonate of 11mmol/l and a base excess of -11.8 , (that is, a compensated metabolic acidosis). The serum potassium and sodium were normal but the urea was 28.3 (normal <8.5) mmol/l and the creatinine was 407.3 (normal <125) $\mu\text{mol/l}$. Haemoglobin was 4.9g/dl , the white count was $0.2 \times 10^9/l$ and the platelet count was $82 \times 10^9/l$. A chest radiograph showed bilateral Kerley's B lines (indicating interstitial oedema) and right upper zone shadowing (fig 5) which was thought to be due to either mycobacterial infection or to pulmonary Kaposi's sarcoma. It was felt that he was septic and had developed acute renal failure. He was transfused and following culture of urine, blood and stools commenced on empirical broad-spectrum antibiotics. Despite treatment his condition deteriorated and he died 36 hours after admission. Blood cultures obtained at this time subsequently grew *Staphylococcus aureus* and *M avium-intracellulare*. An autopsy was performed.

Figure 4 Case 2: Liver biopsy. High power view showing small granulomata containing yeasts of *Histoplasma capsulatum* staining red. (Periodic Acid Schiff stain) $\times 400$.

Figure 5 Case 2: Chest radiograph taken on representation in January 1993, showing right upper zone shadowing and Kerley's B lines (indicating interstitial oedema)

Autopsy (Dr S B Lucas)

The body was of a thin white male. Kaposi's sarcoma was not visible on the skin but was present on the gums and the palate in the mouth; Kaposi's sarcoma also involved the bronchi and pleura. The lungs were oedematous. Within the liver, which weighed 2000g, there were purple deposits of Kaposi's sarcoma extending from the portal tracts into the parenchyma. The spleen was enlarged and weighed 1280g; the cut surface was congested and dotted with 1mm white spots. The hepatic and mesenteric lymph nodes were prominent. The brain showed subarachnoid haemorrhage and swelling, due to a large right frontal intracerebral haemorrhage.

On histology Kaposi's sarcoma was confirmed in hepatic nodes, lung, liver and mouth. Despite the microbiological evidence, mycobacterial infection consistent with *M avium-intracellulare* was found only in one mesenteric lymph node. Yeasts of *Histoplasma capsulatum*, however, were found in the macrophages in many organs; spleen, liver, abdominal lymph nodes and bone marrow (fig 6). The cerebral haemorrhage was not associated with infection or tumour and is presumed to have been spontaneous.

Pathological diagnoses:

1. Spontaneous intracerebral haemorrhage
2. Disseminated histoplasmosis
3. Pulmonary oedema from Kaposi's sarcoma
4. Mycobacteriosis

Discussion (Professor A J Pinching)

Histoplasmosis denotes the range of diseases caused by *Histoplasma capsulatum* usually acquired by inhalation of spores. The fungus is dimorphic and is found in soil with a high nitrogen content, especially if contaminated by bird or bat faeces; bats, but not birds, may also be colonized or infected by *H capsulatum*. It has a mycelial phase, but develops yeast forms at the body temperature of man and mammals. *H capsulatum* is endemic in large parts of North America, especially the mid-West and Texas. Other endemic areas include the north part of South America—Venezuela, Colombia, Peru, Brazil and southern Mexico, most of the Caribbean, and south-east Asia—Burma, Indonesia and the Philippines, Turkey, Israel and Australia; a related but distinct organism *Histoplasma duboisii* is seen in parts of Central Africa.

The primary infection is usually shortlived with fever, headache, weakness and malaise: cough, chest pain and chills may also occur.² The intensity of exposure correlates with the severity of symptoms, and patients previously exposed to *H capsulatum* have milder illness. The chest radiograph may be normal or show widespread interstitial shadowing with hilar and mediastinal lymphadenopathy. In the majority of patients primary infection is self-limiting, often having multiple small calcified lesions visible on chest radiography. Following a primary infection the organism may however remain dormant for many years and become "reactivated" during subsequent immunosuppression, whether iatrogenic or due to HIV. The clinical presentation of chronic symptomatic histoplasmosis may resemble tuberculosis, with classic granuloma formation. This occurs without evident immunosuppression but is often associated with underlying lung disease such as emphysema; disease can affect the lung apices, with cavities, mimicking tuberculosis.²

In the context of HIV infection, *H capsulatum* has emerged as a classic intracellular pathogen with disseminated disease in which macrophages throughout the reticuloendothelial system become parasitised. It typically presents in patients with established HIV disease, frequently usually those with CD4 counts below $0.2 \times 10^9/l$; disseminated histoplasmosis in an HIV infected individual is an AIDS-defining illness. The severity of clinical disease depends on the degree of underlying immunosuppression and the extent of macrophage parasitisation. In the United Kingdom this infection is rare, only being seen in people who have spent time in endemic areas, so clinicians may not think of it in their differential diagnosis. It is clearly

Figure 6 Case 2: Bone marrow. High power views; left hand panel shows diffuse macrophage infiltrate (Haematoxylin and Eosin) right hand panel shows abundant yeasts of *Histoplasma capsulatum*. (Grocott's silver stain) $\times 200$.

Table Clinical features of disseminated histoplasmosis in AIDS patients¹

	Number of patients (%)
(a) Symptoms	
fever	81
weight loss (> 4.5kg)	52
cough	21
night sweats	19
diarrhoea	19
dyspnoea	13
(b) Signs	
splenomegaly	31
hepatomegaly	19
lymphadenopathy	19
rash	10
(c) Investigations	
abnormal chest radiograph (infiltrates or lymphadenopathy)	52
thrombocytopenia	27
anaemia	23
leucopenia	21

¹From Johnson PC *et al*, *Am J Med* 1988;85:152-8

essential to take a detailed travel history from HIV-infected patients to define the range of possible pathogens such as this. Typical symptoms and signs of disseminated histoplasmosis are shown in the table.³ In essence, these comprise fever and constitutional symptoms, prominent and rapidly developing lymphadenopathy and hepatomegaly, pulmonary disease and associated pancytopenia.^{3,4} Of course many infections in HIV positive patients can present in a similar way, and specific diagnostic measures are needed. A rather distinctive rash occurs in approximately 10% of cases and may be helpful in diagnosis. It may be morbilliform or resemble folliculitis. These may develop into larger verruca-like lesions with central necrotic “dimples”, similar to lesions encountered with *Cryptococcus neoformans* or *Penicillium marneffei* infection; biopsy is usually diagnostic, with intracellular organisms readily seen on special strains. Untreated, the infection may advance to resemble septicaemia, with development of respiratory failure, shock, hepatic and renal failure and disseminated intravascular coagulopathy.

The diagnosis can be made non-invasively in up to half of cases by careful examination of a peripheral blood film (*Wright’s stain*) to identify intracellular yeast. Fungal blood cultures may also be positive. Otherwise the diagnosis can be made by examination of bone marrow, liver, lymph node aspirate or biopsy, as well as skin biopsy.⁴ Biopsies should always be sent for fungal culture as well as for histology. Because of the B cell defects in AIDS, serological tests are rarely helpful in making the diagnosis, a negative result not excluding infection.

Turning to your cases, in the first case, bronchoalveolar lavage and lung biopsy, bone marrow and blood cultures were all negative for *H capsulatum* but organisms were found in lymph nodes at autopsy. In the second case I think you had several clues to the infection. The size of the spleen and liver, and the pancytopenia. It is always very easy to blame zidovudine for pancytopenia in such patients but in this instance the anaemia was very severe. I would have had a very low threshold for doing a bone marrow in a patient present-

ing in this way as disseminated *M avium-intracellulare* infection and lymphoma would also need to be considered in the differential diagnosis. You took the alternative route, performing a liver biopsy, which gave you the diagnosis and also identified *M avium-intracellulare*.

Disseminated infection, if diagnosed promptly, responds well to treatment. There are, however, a substantial number of early deaths, especially in patients who are severely ill at presentation, despite antifungal therapy. Amphotericin B is the “gold standard” in initial treatment at a dose of 1mg/kg per day intravenously. Some centres advocate even higher doses during the initial period, but toxicity is more likely^{5,6}; theoretically, liposomal amphotericin could be used to avoid toxicity with high-dose therapy. Oral itraconazole has been used as initial treatment,^{7,8} and it may be especially acceptable in patients presenting with skin disease alone. It has been shown to be of value as maintenance therapy,^{5,7,8} and is obviously much more convenient to administer than long term intermittent IV amphotericin B which has also proved effective.⁶ Itraconazole appears to be very effective, with very few relapses.⁴ Some relapses appear either to be due to drug interactions (drugs such as rifampicin induce cytochrome P450 metabolism and so reduce serum concentrations of itraconazole), or to problems with compliance. Fluconazole appears to be less effective against *H capsulatum*⁷; in one study of its use as primary prophylaxis in an endemic area there were several instances of breakthrough infections.⁹ There is also some evidence to suggest that ketoconazole, while useful, may not be as effective as itraconazole in non-immunosuppressed patients with disseminated histoplasmosis.¹⁰ However, anecdotally, we have successfully used ketoconazole for maintenance therapy in an HIV positive patient who became intolerant of itraconazole.

In summary, for physicians in non-endemic areas such as the UK, the first priority is to think of the diagnosis in patients who have visited endemic areas, even many years before. As with other infections in HIV-infected patients, diagnostic tests for the organisms themselves should be performed at appropriate sites—in this case, blood smears or samples from bone marrow, skin, liver or lymph node. Prompt aggressive induction therapy with high-dose amphotericin, followed by maintenance or secondary prophylaxis with itraconazole is the current standard.

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