CLINICOPATHOLOGICAL CONFERENCE

Progressive intrathoracic lymphadenopathy: EBV associated non-Hodgkin’s lymphoma

R F Miller, E L Jones, M J Duddy, M Shahmanesh

A 30 year old man presented with late stage HIV disease and intrathoracic lymphadenopathy. Histology of a mediastinal biopsy suggested infective follicular hyperplasia or a peripheral T cell lymphoma. Subsequently, Epstein-Barr virus (EBV) infection was demonstrated in lymphocytes in the biopsy. Later, hepatosplenomegaly and peripheral lymphadenopathy developed. Histology of a cervical lymph node biopsy showed EBV associated diffuse large B cell (non-Hodgkin’s) lymphoma.

CASE PRESENTATION

(Pl Shahmanesh)

A 30 year old white homosexual male was admitted to hospital on 20 October 2000 following a positive test for HIV-1 antibodies. He had been investigated by his general practitioner for progressive weight loss and dysphagia. A chest radiograph had shown bulky hilar, parenchymal shadowing in both mid zones and sepal lines. These radiographic abnormalities had raised the possibility of a diagnosis of sarcoidosis.

On admission, further inquiry revealed a history of 21 kg weight loss over 9 weeks, a 16 month history of a draining abscess in the left axilla, dysphagia, and a 4 week history of cough and mild exertional dyspnoea. In the past he reported mild exertional asthma. He had travelled to southern Spain on holiday in 1999. On examination he was pyrexial (temperature 38°C), felt faint on standing but had no postural hypotension. Plasma cortisol = 325 mmol/l. The findings were in keeping with inappropriate ADH secretion.

The patient began co-trimoxazole 960 mg once daily as primary prophylaxis against pneumocystis pneumonia. The candidiasis was treated with fluconazole. Nasogastric feeding was commenced. The patient remained pyrexial and on day 5 of admission the axillary abscess was opened out and packed. Histology of excised tissue showed inflammatory changes only. By day 10 of admission the patient remained unwell. He became apyrexial; the discharging sinus dried up and other fungi.

amoxyclillin and metronidazole were given for the axillary infection and the patient rapidly became apyrexial; the discharging sinus dried up and began to heal. A bone marrow aspirate and trephine were performed on day 12. This showed a hypercellular marrow. Special stains and culture were negative for bacteria, including Bartonella spp, fungi, protozoa including Leishmania spp, and mycobacteria. On day 17 a mediastinal biopsy was performed and histology was reported as showing loss of nodal architecture with loss of residual follicles. Residual CD 23+ follicular dendritic cells were seen focally surrounded by large sheets of CD 3+ T cells occupying much of the node. These findings were interpreted as being consistent with infective follicular lysis or peripheral T cell lymphoma. T cell receptor gene rearrangement

CASE PRESENTATION

(Dr Shahmanesh)

At fibroptic bronchoscopy the subcarinae were splayed, suggesting subcarinal lymphadenopathy. The bronchi looked oedematous consistent with external compression. Bronchoscopic endobronchial biopsies showed non-specific granulation tissue. Staining and culture revealed only Candida spp. Special stains and culture were negative for mycobacteria, cytomegalovirus (CMV) bacteria, and other fungi.

Blood results revealed Hb = 11.0 g/dl, WBC = 12.2 × 10^9/l (neutrophils = 8.2 × 10^9/l) and platelets = 633 × 10^9/l. Urea and electrolytes were normal except for hyponatraemia Na^- = 129 (normal 136–145) mmol/l. Liver function tests were abnormal, alkaline phosphatase = 1786 (normal 70–320) IU/l, AST = 116 (normal <43) IU/l, γGT = 232 (normal = 9–50) IU/l, and albumin = 24 (normal 34–55) g/l. Serological tests showed CMV IgG was positive, Epstein-Barr virus (EBV) NA IgG, and VCA IgG were positive; VCA IgM was negative. Prolonged culture of blood and urine was negative for mycobacteria. A baseline CD4 count = 170 cells × 10^9/l and plasma HIV viral load = 89 000 copies/ml.

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atypical for tuberculosis; the absence of intrapulmonary nodules is against Kaposi's sarcoma and the ground glass shadowing may represent *P. carinii* or another fungal infection. As the CD4 count is >150 cells ×10^9/l this appearance is unlikely to be due to CMV. The bronchoscopic findings are important. The non-specific granulation tissue could be caused by general medical diseases such as Wegener's granulomatosis or sarcoidosis, or to HIV related processes such as tuberculosis or lymphoma. The mediastinal biopsy findings are unusual. I wonder if this is infective follicular hyperplasia. I also wonder whether it may be virally driven—for example, by EBV, or by HHV8 (human herpes virus 8). I would be keen to see the results of immunostaining or in situ hybridisation for these viruses. I'd also still be wondering about HHV8 and the plasma cell variant of Castleman's disease (angiofollicular hyperplasia). If this is a malignant T cell lymphoma then this process is clearly not HIV associated; however, there is a suggestion that T cell lymphoma may be occurring more frequently in HIV infected patients. It may be difficult to distinguish between infective follicular hyperplasia and T cell lymphoma on histological appearances alone. I think then I would like some more details about the mediastinal biopsy—if this is not possible, then I would consider re-biopsying the patient to obtain further tissue.

**CASE PRESENTATION**

(Prof E L Jones)

Immunohistological staining and in situ hybridisation for EBV and HHV8 were not carried out on the mediastinal biopsy. On day 21 of admission the patient began antiretroviral therapy with zidovudine, lamivudine, and efavirenz. He was discharged the next day. When reviewed in the outpatient department a week later, the antiretrovirals were stopped as they had induced severe nausea. He appeared generally unwell, was pyrexial, and had new splinter haemorrhages under his fingernails. An echocardiogram showed equivocal aortic valve thickening, no vegetations, and was otherwise normal and blood cultures were negative.

Over the next few days he developed severe left sided chest pain, a spiking temperature, and dyspnoea on walking 100 metres. He was readmitted to hospital 9 days after discharge. Investigations revealed Hb = 8.6 g/dl and WBC = 12.2 × 10^9/l (neutrophils 9.6 × 10^9/l). He was transfused with blood, antiretrovirals were restarted—stavudine replacing the zidovudine, and he was discharged. A follow up chest radiograph in late November 2000, a month after the original presentation, showed the extensive bilateral perihilar lymphadenopathy was unchanged; new findings were right middle lobe consolidation with loss of volume and a right pleural effusion.

The patient was reviewed in the outpatient department in early December 2000. At this time he had severe chest pain, requiring opiate analgesia. Investigations showed CD4 = 220 cells ×10^9/l and HIV viral load <50 copies/ml. Just before Christmas the patient was admitted to hospital and a Hickman line was inserted in preparation for commencing chemotherapy as treatment for peripheral T cell lymphoma. A repeat abdominal CT scan was performed and further information became available following review of the mediastinal biopsy.

**IMAGING**

(Dr Duddy)

The abdominal CT showed hepatomegaly. Within the liver and spleen there were multiple, up to 1 cm, nodular foci of reduced enhancement. There was left para-aortic lymphadenopathy.

**PATHOLOGY**

(Professor E L Jones)

The original lymph node biopsy specimen was reviewed. There was partial nodular effacement with paracortical expansion of small lymphocytes with small numbers by larger lymphoid cell follicles. There were multiple collections of lympho-plasmacytoid cells. The plasma cells were negative for EBV and HHV8.

**DISCUSSION**

(Dr R F Miller)

This patient has presented with late HIV disease, a positive travel history, non-specific symptoms, and significant intrathoracic radiographic abnormalities. Faced with such a case, the questions to ask are, firstly, is the presentation due to an HIV associated problem or is it unrelated and, secondly, could there be more than one disease process co-existing? The travel history is important as the patient is at risk of leishmaniasis and the negative bone marrow findings do not rule out this possibility. The chest radiographic abnormalities have a wide differential diagnosis; knowing the patient is HIV infected means that tuberculosis, lymphoma and Kaposi's sarcoma, and multicentric Castleman's disease need to be considered. Non-HIV related possibilities include a “sarcoid” reaction to a testicular tumour, small cell lung cancer, and again mediastinal multicentric Castleman's disease. The thoracic CT scan provides additional information and is very useful following review of the mediastinal biopsy.

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Figure 1 Chest radiograph in October 2000, showing mediastinal widening and bilateral hilar lymphadenopathy.

Figure 2 CT scan of thorax in October 2000 (lung window setting) showing parenchymal “ground glass” lung opacity and air space consolidation.

What are your initial thoughts on this case?
“blasts.” Immunohistological staining showed a predominant population of CD3+ T cells (fig 3) with a marked predominance of CD8+ cytotoxic T cells. A smaller population of CD20+ B cells were identified with a few larger cells noted. Light chain staining showed a polyclonal population of cells with lambda chain predominating. A few larger cells expressed EBV latent membrane protein-1 (LMP-1) and a few cells showed nuclear EBERs (Epstein-Barr virus encoded small RNAs) by in situ hybridisation. These appearances are of a reactive B cell hyperplasia, probably EBV driven with a marked cytotoxic T cell response. There was no evidence of lymphoma.

CASE PRESENTATION
(Prof Shahmanesh)
In view of the further report on the mediastinal biopsy it was decided to defer chemotherapy. What are your thoughts now and what would you have done next?

DISCUSSION
(Dr Miller)
I wonder what is going on in the liver and the spleen? These appearances have a wide differential but I would put lymphoma top of my list. Tuberculosis, fungal infection including *Candida* spp and *Aspergillus* spp, histoplasmosis and coccidioidomycosis, bacillary angiomatosis, and even extrapulmonary pneumocystosis can produce these findings.10-11 I think I would go for an ultrasound or CT guided biopsy of one of these lesions. The pleural effusion has a wide differential diagnosis and so this finding is very non-specific.12 The finding of EBV in lymphocytes from the mediastinal lymph node is interesting. Mature B lymphocytes express the surface antigen CD21, which acts as a receptor for EBV. In HIV infected immunosuppressed individuals EBV infection is latent and EBV viral load is low, cell lysis does not occur. Instead, EBV infection in these cells causes B cell proliferation. In contrast with infectious mononucleosis, this EBV induced B cell proliferation is not ultimately halted by cytotoxic T cells and so proliferation proceeds unchecked. This creates a favourable environment for activation of the proto-oncogene c-myc to occur and, as result, a neoplastic B cell clone may eventually develop.13 The mediastinal biopsy findings suggest a polyclonal B cell expansion as both kappa and lambda light chains are expressed by B cells. If this was a monoclonal B cell expansion, then I would expect light chain restriction. In terms of treatment strategy, I would continue antiretroviral therapy. You have a good immune/virological response and there may be further benefit from immune reconstitution on the B cell expansion. Some groups have tried giving aciclovir to treat the EBV in an attempt to reduce the “drive” on the B cells in this situation.

CASE PRESENTATION
(Prof Shahmanesh)
We also hoped that antiretroviral therapy would have a beneficial effect. In addition, we gave the patient valaciclovir 1 g twice daily. With empirical antibiotics (clarithromycin and ceftazidine) the patient’s temperature fell promptly, rose rapidly once again after 2 weeks when the course was finished, and finally settled with a further course. The patient was readmitted to hospital on 10 January 2001, complaining of sudden onset left sided chest pain. On examination there were new lymph nodes in the axillae and also the cervical chain. In the chest there were bilateral inspiratory crackles, more marked on the right. Investigations showed alkaline phosphatase = 2335 IU/l and AST = 198 IU/l. The full blood count showed Hb = 10.7 g/dl and WBC = 19.5 × 10^9/l (neutrophils = 15.4 × 10^9/l). Blood cultures grew *Staphylococcus aureus*.
D-Dimers were negative making a diagnosis of pulmonary embolism very unlikely. An abdominal ultrasound showed no progression of the appearances previously noted on CT. The Hickman line was removed and the Staph aureus sepsis treated.

A liver biopsy was performed but histology was non-diagnostic. Special stains were negative for mycobacteria and CMV.

A follow up CT scan of the chest was performed on 22 January 2001 and a cervical lymph node biopsy was performed on 24 January.

Before the findings from these investigations are presented, what do you think is the likely diagnosis?

**DISCUSSION**

(Dr Miller)

I think that this is going to turn out to be a B cell non-Hodgkin’s lymphoma. I am still a bit concerned about a possible second diagnosis, and even though cultures of the mediastinal biopsy, liver biopsy, blood, and bronchoalveolar lavage have been negative for mycobacteria, I think we need to keep the possibility of tuberculosis in the back of our minds.

**IMAGING**

(Dr Duddy)

CT showed a bulky increase in the mediastinal/hilar lymphadenopathy with central low attenuation “necrotic” or “caseous” change (fig 4). Within the lungs, there were focal parenchymal abnormalities and areas of consolidation. The spleen was enlarged further and heterogeneous in attenuation.

**PATHOLOGY**

(Professor Jones)

The cervical lymph node showed extensive areas of eosinophilic necrosis with the nodal architecture effaced by sheets of pleomorphic, large lymphoid blast cells with a high mitotic rate (fig 5). These large cells stain as B cells (fig 6) and co-express the activation marker CD30. Most of these large cells show strong cytoplasmic expression of EBV LMP-1 and
nuclear EBERs (fig 7). The appearances are those of EBV-associated diffuse large B cell lymphoma.

CASE PRESENTATION
(Dr Shahmanesh)
The patient began cyclical combination chemotherapy with vincristine, cyclophosphamide, and doxorubicin. Subsequently, this was modified with addition of methotrexate and prednisolone. Initially there was a good clinical and radiological response. Subsequently, the patient’s condition deteriorated with worsening hepatomegaly and development of bone pains.

IMAGING
(Dr Duddy)
CT of the abdomen and pelvis in May 2001 confirmed the clinical findings of hepatosplenomegaly. In addition, there was moderate ascites and a solitary lytic destructive lesion of the right iliac blade with a rim enhancing soft tissue component projecting into the gluteal muscle.

CASE PRESENTATION
(Dr Shahmanesh)
The chemotherapy was modified to a regimen of etoposide, carmustine, cytarabine, and melphalan. However, the patient died soon after in June 2001.

Final diagnosis was disseminated large B cell non-Hodgkin’s lymphoma.

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References