Figure 2 The duplicate lymphocyte.

atypical or plasma cell (fig 1E). For seven cells, four different subtypes were mentioned (fig 1F,G), and to one cell, five different assessments were attributed (fig 1H).

One cell was shown twice in the PowerPoint presentation: in 210/671 the same cell was classified by the same observer as another subtype.

Discussion

Even in the age of molecular analysis and sophisticated laboratory tests, the blood smear remains an important diagnostic tool.¹ The recognition of abnormal lymphocytes in blood smears can contribute to a rapid diagnosis of various diseases, both reactive and (pre)malignant) neoplastic, and enables rapid therapeutic intervention. Some rare diseases may be diagnosed faster by a critical review of the lymphocytes, such as T-cell disease.¹³ The need for recognition of abnormal lymphocytes should not be underestimated. The morphology of lymphocytes is complex; cell size, amount and colour of cytoplasm, as well as shape and chromatin structure of the nucleus, must be combined to come to a good characterisation of the lymphocyte. Diagnosis of infectious mononucleosis may be based on Hoagland's criteria. However, previous studies have questioned the percentage of patients with serological evidence using these criteria.¹⁴ Unfortunately a uniform definition of abnormal lymphocytes is lacking. A confused terminology is used for abnormal lymphocytes, for example, variant lymphocytes, atypical lymphocytes and even combinations of several cells are used (e.g. monocytoid lymphocytes and plasmacytoid lymphocytes). In this study we showed the variability in classification of abnormal lymphocytes and would recommend abnormal lymphocytes should be interpreted and reported with care. One cell was shown twice in the PowerPoint presentation (fig 2), and remarkably, 31% of the morphologists were not able to reproduce their previous classification. This illustrates the problem of uniform and consistent reporting of the subtypes of abnormal lymphocytes even more clearly.

In spite of highly qualified technologists who spend large amount of time on manual microscopy, clinicians often base their diagnosis and treatment on other information. This is because they have access to physical findings, radiographs, cultures etc.¹⁵ We recommend provision of additional clinical information, which should lead to a better interpretation of blood cell morphology. However, additional information from automated haematology analysers could lead to overestimation of abnormal lymphocytes.⁵

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"In situ" detection of human cytomegalovirus infection of bone marrow in a patient previously treated for B-prolymphocytic leukaemia

Infection of immunosuppressed patients by human cytomegalovirus (CMV) may be responsible for a wide range of symptoms, depending on the affected organ. Iatrogenic immunosuppression is an important contributing factor secondary to organ transplantation or to treatment for haematological malignancies. In these conditions appropriate diagnosis of infection by CMV has practical relevance for the management of the patients.¹ Detection of CMV in bone marrow by the pathologist has been reported as a rare event, although pancytopenia may be indicative of such infection.^{2–5} The purpose of this report is to present a case of a patient who had been treated for a B-cell prolymphocytic leukaemia five years before the onset of a relapse of the disease accompanied by CMV infection of bone marrow. It is intended to stress the possibility of this rare feature that can be missed by the histopathologist at routine diagnosis.

Case report

We report the case of a woman in her 70s in whom a B-cell prolymphocytic leukaemia had been diagnosed. She was successfully treated with fludarabine and Endoxan (cyclophosphamide). In 2005, five years after initial diagnosis, she was referred to our hospital to investigate a recent weight loss, accompanied by fever and pancytopenia. The possibility of Richter's syndrome was suspected, but a bone marrow trephine biopsy was performed to rule out the diagnosis of tuberculosis, for which the patient had been treated recently.

Materials and methods

The bone marrow specimen was fixed in Duboscq-Brazil (alcoholic based Bouin), decalcified in 10% EDTA, and embedded in paraffin. Histological sections stained with H&E were reviewed. Immunohistochemical staining was performed using a Ventana immunostainer (Ventana-Biotech, Tucson, Arizona, USA). The panel included antibodies to CD20, CD79a, CD3, CD5, CD10, CD21, CD23, cyclin D1, and to an immediate-early (IE-1) CMV antigen. The marker for CMV was included because morphology showed suspicious atypical cells (see Discussion). A streptavidin-biotin-peroxidase detection system and staining with diaminobenzidine was used.

Results

At morphological evaluation, the bone marrow was moderately infiltrated by lymphoma cells (fig 1). A few large atypical cells suspicious of viral infection could also be seen, but no typical cytological feature of viral infection was evidenced. Immunophenotyping on paraffin sections confirmed the diagnosis of bone marrow infiltration by the same lymphoid neoplasia, most cells being positive for CD20 and CD79a; reactive T-lymphocytes expressing CD3 and CD5 were sparse. Neoplastic cells did not express CD5, CD10, CD21, CD23 or cyclin D1. CMV infection was confirmed by immunoreactivity in rare atypical cells in areas not infiltrated by the neoplasm (fig 2). Furthermore, CMV infection was confirmed by serology and PCR. During investigation,

hepatopathy was diagnosed, interpreted as superimposition of CMV and hepatitis C virus (HCV) infection. The patient was treated with antiviral therapy, foscarnet and ganciclovir, but the clinical course was unfavourable: she developed encephalopathy, pneumopathy and renal failure, dying few weeks after onset of therapy.

Discussion

Immunophenotyping of bone marrow allowed the confirmation of both the diagnosis of relapse of the B-cell prolymphocytic leukaemia and of CMV infection. The marker for CMV was included because it is known that: CMV infection may be also responsible for fever and pancytopenia; this infection must always be suspected in immunocompromised patients; and the virus may be detected in the analysis of precursor blood cells. This diagnostic procedure providing evidence for a CMV infection, was important for adequate treatment planning.² Careful analysis of blood films are also reported to allow the recognition of infected cells, which may be confused with neoplastic cells, as often happens in histopathology.⁴ In addition, examination of the nature of lymphoid infiltrates by immunohistochemistry may provide an important tool for the differentiation between immunological reaction to CMV infection and infiltration of bone marrow by malignant lymphoma, as has already been discussed elsewhere.⁶ In the present case, the neoplastic nature of the lymphoid infiltrate of bone marrow was given first morphologically,

by the density and irregularity of lymphoid nodules, and the polymorphism of the nuclei. The neoplastic nature of this infiltrate was confirmed by the striking predominance of CD20 and CD79a expression by the lymphoma cells.

The present case illustrates a rare immunoeexpression of CMV antigen in atypical cells infiltrating the bone marrow along with lymphoma infiltration, emphasising the role of the pathologist in this diagnosis. It also stresses the importance of an early diagnosis of this viral infection, for establishment of proper treatment, especially in a patient who is debilitated because of the underlying lymphoproliferative neoplasm. The fact that therapy has not been successful does not diminish the importance for the pathologist to establish the correct diagnosis as early as possible. The difficulty in treating CMV infection in such patients is recognised.¹ However, a simple and rapid method, such as immunohistochemistry, may be useful in diagnosing CMV infection in addition to neoplastic infiltration using the same specimen, allowing prompt initiation of antiviral therapy, which may increase the chance of a successful outcome.

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Storiform collagenoma as a clue for Cowden disease or PTEN hamartoma tumour syndrome

Cowden disease (CD) is a rare autosomal dominant disease with variable expression, affecting a number of systems in the form of multiple hamartomatous neoplasms of ectodermal, mesodermal, and endodermal origin¹ (multiple hamartoma neoplasia syndrome). CD usually presents in late adolescence and is caused by a germ mutation in the PTEN gene.² Classically, the mucocutaneous features of CD are trichilemmomas, oral fibromas, acral keratoses, palmar pits, and gingival and palatal papules. These mucocutaneous signs are important markers for systemic findings; in particular breast carcinoma (20%), which is often bilateral,^{3,4} thyroid carcinoma (8%) and endometrial carcinomas.² Various manifestations of the central nervous system (mental retardation,⁵ seizures, ganglioneuromas), musculoskeletal system (craniomegaly, kyphoscoliosis and high arched palate) and gastrointestinal system (multiple hamartomatous polyps, adenocarcinomas arising in the polyps) are well documented. In addition, ovarian cysts and menstrual abnormalities are known to occur in the female genital tract.⁶

Bannayan–Ruvalcaba–Riley syndrome has many clinical features in common with CD, including mucocutaneous manifestations and an increased risk of gastrointestinal polyps and malignancy.⁷ Several other similar phenotypes caused by mutations in the PTEN gene have been described. After studying 64 unrelated families with Cowden-like features, Marsh *et al* proposed that these different entities lie within a spectrum; they referred to these conditions as the PTEN hamartoma tumour syndrome (PHTS).^{7–9}

We report a case of an 18-year-old man of Fitzpatrick type 1 skin and freckles who developed a non-tender, 10 mm erythematous nodule on his back over few months. The clinical differential diagnosis included an amelanotic melanoma, vascular tumour or a dermatofibroma. His past medical history included Pierre Robin syndrome (the triad of glossoptosis (downward displacement of the tongue), micrognathia (small jaw) and cleft palate), moderately severe learning difficulties and dyspraxia.

The nodule was excised. Microscopic examination (fig 1) showed a paucicellular (although a cellular variant has been reported¹⁰) well-circumscribed dermal nodule composed chiefly of collagen bundles with a distinctive interweaving, storiform pattern with prominent elongated clefts between them, typical of a storiform collagenoma.¹¹ Tinctorial stains showed absence of elastic fibres (Orcein and EVG) within the lesion and reticular fibres were seen prominently (Reticulin). Immunohistochemistry showed negative staining with CEA, EMA or keratin staining. Most of the cells within the nodule stained positively for vimentin, and factor XIIIa stain highlighted scattered dermal dendritic cells.¹¹ On the basis of similar fibrotic changes observed in dermatofibroma, it has been speculated that storiform collagenoma may have diverse origins. This issue has been resolved by showing that type 1 collagen synthesis indicates fibroblastic origin of storiform collagenoma.¹² Furthermore, storiform collagenoma is distinguished from dermatofibroma by an epidermis overlying amorphous, eosinophilic bundles in a

Figure 1 Hypercellular area of bone marrow, infiltrated by atypical small lymphoid cells, diagnosed as B-prolymphocytic leukaemia (H&E, 400x).

Figure 2 Area with normal cellularity, where an infected CMV-positive cell can be seen (immunoperoxidase, anti-CMV, 400x; inset 1600x).