Comments

According to a new classification by the World Health Organization, the term "neuroendocrine tumours" is recommended instead "carcinoid". Neuroendocrine tumours belong to a heterogeneous group of tumours affecting almost the entire alimentary tract as well as other organs such as the lungs and thymus.² The traditional term "carcinoid tumour" is restricted to the subtype of serotonin-producing neuroendocrine tumours. Neuroendocrine tumours are neoplasms that arise from mature endocrine cells or their stem cells. Our patient had neuroendocrine tumour cells identified in the wall of a splenic artery aneurysm. The diagnosis was based on characteristic morphological and immunohistochemical findings. This tumour type is obviously extremely rare, as a MEDLINE search did not reveal any previously published case of neuroendocrine tumour cells in the wall of an artery nor in an artery aneurysm.

Aneurysms of visceral arteries are rare, often of unknown aetiology. In contrast to other intra-abdominal aneurysms, atherosclerosis is rarely the primary causative factor for splenic artery aneurysm, but considerable evidence exists to implicate hormonal and local haemodynamic factors in the development of these aneurysms.³ An association between carcinoid tumours and thoracoabdominal aneurysm has been reported previously.⁴

It can only be speculated whether neuroendocrine tumour cells could produce products that initiate local changes in the wall of the artery with subsequent aneurysm development. However, the most likely explanation of the finding in our patient is that the neuroendocrine tumour cells represent a micrometastasis of a still undiagnosed neuroendocrine tumour. The future strategy in this case will be reinvestigation.

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SHORT REPORTS

The divergent morphological classification of variant lymphocytes in blood smears

An examination of a blood smear may be requested by physicians or initiated by laboratory staff as a diagnostic aid when a lymphoproliferative disorder or mononucleosis is suspected.¹ The classification of lymphocyte disorders is complex because there are various manifestations of these disorders.^{2,3} Three major groups can be distinguished: reactive lymphocytosis, premalignant neoplastic disorders of lymphocytes and neoplastic disorders of lymphocytes.⁴ Lymphocyte disorders may be accompanied by abnormal lymphocyte morphology; therefore it is important to recognise and report abnormal lymphocytes, such as atypical lymphocytes and lymphoblasts. The ability of individual technicians, however, to recognise abnormal lymphocytes is consistently quite poor.⁵ There are no standardised definitions regarding the morphology of the various cells, and interpretation is based on individual experience and dependent on the availability of additional clinical information. Often, transitional forms between lymphocytes and plasma cells are seen in the blood of patients with viral infections. These cells are variously known as atypical lymphocytes, lymphocytoid plasma cells or plasmacytoid lymphocytes.⁶ The so-called atypical lymphocyte is a non-neoplastic lymphocyte seen in the peripheral blood, and appears to be a non-specific response to stress from a variety of disorders.⁷ Small lymphocytes become larger in size and become capable of dividing. These atypical lymphocytes vary in morphology and surface markers.^{8,9} Hoagland developed criteria for diagnosis of infectious mononucleosis comprising $\geq 50\%$ of the white blood cell differential, with atypical lymphocytes accounting for $\geq 10\%$ of the total white blood cell count.¹⁰ The morphology of lymphoblasts is even more complicated, as morphological appearance varies from small cells with scanty cytoplasm and

coarse chromatin to large cells with an abundance of vacuolated cytoplasm and fine chromatin.¹¹

The morphological assessment of the peripheral blood smear remains a valued diagnostic tool,¹ despite the considerable interobserver variation in interpretation. New techniques such as automated recognition systems may reduce the microscopic review of blood films in the near future.¹² Nevertheless, the relevance of the morphological evaluation of abnormal lymphocytes can be questioned, considering the large variation in reported results.

In this letter we show the general inconsistency in reporting whether an abnormal lymphocyte is (non-neoplastic) reactive, neoplastic or an artefact.

Methods

From a blood smear of an orthopaedic patient (female, age 3 years) with a lymphocytosis which was normal for a child of that age, 100 white blood cells were randomly selected, microphotographed and processed in a PowerPoint presentation. This PowerPoint presentation was sent to 157 different hospital laboratories in The Netherlands and a leucocyte differential was requested. All participants were asked to differentiate the lymphocytes into normal lymphocytes, atypical lymphocytes, plasma cells, prolymphocytes or (lympho)blasts. Participants did not have any information of the patient.

All results were processed in Excel and for each laboratory and individual technician the mean differential was calculated. For each lymphocyte subtype the degree of concordance was calculated.

Results

Of the 157 laboratories, 114 responded (73%) with a total of 671 individual results. The overall mean lymphocyte count (including sub-cell types) was 56%. For 7 cells there was a concordance of $>90\%$ for normal lymphocytes (fig 1A), which means that more than 90% of the individual observers called these cells normal lymphocytes. For the other 49 lymphocytes there was no concordance in subtyping. In the majority, lymphocytes (29 cells) were classified as normal or atypical (fig 1B). One cell (fig 1C) was classified as a normal lymphocyte or a plasma cell. Nine cells were classified as normal or atypical or prolymphocyte (fig 1D) and two cells as normal or

Figure 1 Examples of the different lymphocytes. Lymphocytes classified as: (A) lymphocyte with $>90\%$ concordance; (B) normal or atypical; (C) normal or plasma cell; (D) normal, atypical or prolymphocyte; (E) normal, atypical or plasma cell; (F) normal, atypical, prolymphocyte or plasma cell; (G) normal, atypical, prolymphocyte or blast; (H) normal, atypical, prolymphocyte, plasma cell or blast.

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,