

Multiple lymphoid nodules in bone marrow biopsy in immunocompetent patient with cytomegalovirus infection: an immunohistochemical analysis

Múltiplos nódulos linfóides na medula óssea de paciente imunocompetente com infecção pelo citomegalovírus: uma análise imunohistoquímica

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Abstract In Brazil, a high prevalence of cytomegalovirus (CMV) infection has been documented. In immunocompetent adults CMV infection is usually asymptomatic and therefore morphologic and immunophenotypic bone marrow changes have rarely been described. The authors report the case of a previously healthy patient who developed fever of undetermined origin. The diagnosis of acute CMV infection was based on serological testing. A computed tomographic scan showed mediastinal lymphadenopathy. A bone marrow biopsy revealed a hypercellular haematopoiesis with eosinophilia and large mixed T- and B-cell lymphoid aggregates. In spite of bcl-2 positivity, their reactive nature was demonstrated. Polymerase chain reaction (PCR) and immunohistochemistry were unable to detect CMV-DNA in paraffin-embedded bone marrow sections. Much like in other systemic disorders, the lymphoid nodules in this case seemed to be caused by immunological mechanisms, possibly due to cytokines released in response to the systemic infectious process.

Key-words: Cytomegalovirus. Bone marrow. Lymphoid nodules.

Resumo Uma elevada prevalência de infecção pelo citomegalovírus (CMV) está documentada no Brasil. Em adultos imunocompetentes a infecção pelo CMV é, em geral, assintomática e, portanto, as alterações morfológicas e imunohistoquímicas na medula óssea têm sido raramente descritas. Relatamos o caso de um paciente previamente assintomático que desenvolveu febre de origem obscura. O diagnóstico de infecção aguda pelo CMV foi baseado em estudo sorológico. A tomografia computadorizada do tórax mostrou linfadenopatia mediastinal. A biópsia óssea revelou medula hiper celular com eosinofilia e grandes agregados linfóides mistos de células B e T. Apesar da positividade para bcl-2, a sua natureza reacional foi demonstrada. A reação em cadeia da polimerase (PCR) e a imunohistoquímica não detectaram DNA viral nos cortes de medula óssea em parafina. Assim como em outros distúrbios sistêmicos, os nódulos linfóides no nosso caso parecem ser causados por mecanismos imunológicos, possivelmente relacionados a citocinas liberadas em resposta ao processo infeccioso sistêmico.

Palavras-chaves: Citomegalovirus. Medula óssea. Nódulos linfóides.

Cytomegalovirus (CMV) is a common cause of viral infection and a major cause of morbidity and mortality in immunocompromised patients. Both primary infection and reactivation of endogenous latent virus owing to acquired immunodeficiency or immunosuppressive treatment can cause disease. The infection is associated with non-specific bone marrow changes including myelosuppression and a florid haemophagocytic syndrome, especially in severe immunodeficiency⁴.

Nonimmunosuppressed patients rarely manifest clinical infection although CMV-induced thrombocytopenia and haemolysis have been reported^{1,4}. CMV seroprevalence in adults ranges from 40% to 100% depending on geographical and socioeconomic conditions⁴. In Brazil, a high frequency of seropositivity has been reported⁵. Like other herpes virus, primary infection is followed by persistence of the virus in a latent form. It has been proposed that bone marrow progenitor cells and peripheral blood monocytes play a major role in the pathogenesis and latency of CMV¹⁵.

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Reactive lymphocytic aggregates may be observed in marrow biopsies from patients with a wide range of infectious processes and immunological disorders. However, the distinction between benign and malignant proliferation based on morphologic criteria alone may be difficult. Although general guidelines for making this distinction are proposed, when

lymphoid nodules are relatively large or numerous, immunohistochemical analysis has been shown to be useful in demonstrating their reactive nature¹³.

The present report describes the histopathological and immunohistochemical features of lymphoid aggregates in bone marrow of an immunocompetent patient who developed symptomatic CMV infection.

CASE REPORT

A 38-year-old man, previously healthy, reported bouts of fever without chills and fatigue in the last two months. The patient had a weight loss of 9kg. On physical examination no abnormality was found, except fever (37.9°C). Laboratory findings included haemoglobin of 12.5g/dl, a white blood cell count of $4.2 \times 10^9/L$ with a normal differential count and platelet number. Erythrocyte sedimentation rate was 96 mm. Anti-nuclear antibody testing was negative. Serologic analysis were negative for human immunodeficiency virus, Epstein-Barr virus, and for hepatitis A, B, and C. Infection with CMV was indicated by detection of markedly elevated CMV-specific IgM using enzyme-linked immunosorbent assay (ELISA). In peripheral blood, B-lymphocytes were

6% (N: 15-30%) and T cells were 74% (N: 65-74%). Blood cultures for bacterial organisms were negative. A computed tomographic scan of the chest showed slight mediastinal lymphadenopathy (<1cm). The bone marrow biopsy revealed a hypercellular haematopoiesis with increase in eosinophils and multiple lymphoid nodules, including a poorly demarcated, large lymphoid expansion composed of small mature lymphocytes in the immediate paratrabecular area (Figure 1). No histological viral inclusions were seen. Immunohistochemical analysis of these aggregates was performed using the avidin-biotin-peroxidase complex, and monoclonal antibodies from DAKO, Denmark. A strong central positivity with CD20 and a positive

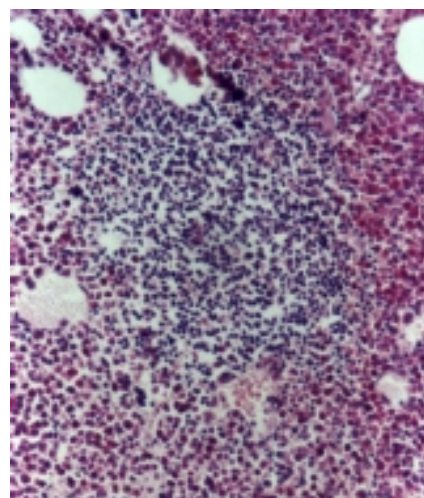
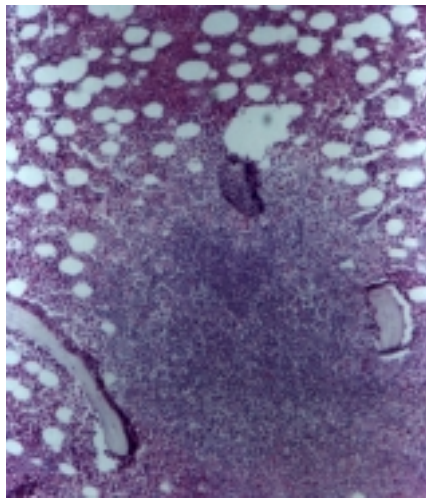


Figure 1 - Bone marrow biopsy section showing a poorly demarcated, large lymphoid aggregate in the immediate paratrabecular area (left) and a well circumscribed proliferation composed of small mature lymphocytes (right).

dispersed reaction with CD3 and CD45RO were observed (Figure 2). Staining with monoclonal antibody to bcl-2 was weakly positive. To investigate the possible presence of CMV-DNA the paraffin-embedded bone marrow was analysed by immunohistochemistry using primary monoclonal antibody against CMV delayed antigen. CMV-DNA was also determined using a nested polymerase chain reaction (PCR) with two pairs of primers, major immediate-early antigen (MIE) and immediate-early (IE), which correspond to conserved

regions of the CMV genome. Specific primers for b-globin genes were used to ascertain the integrity of the extracted DNA and to detect any inhibition of polymerization in the extract. Both methods were unable to demonstrate the presence of CMV-DNA. One month later bouts of fever disappeared and white blood cell count raised to $7.3 \times 10^9/L$. No specific therapy was given and the patient was still well 18 months after bone marrow examination.

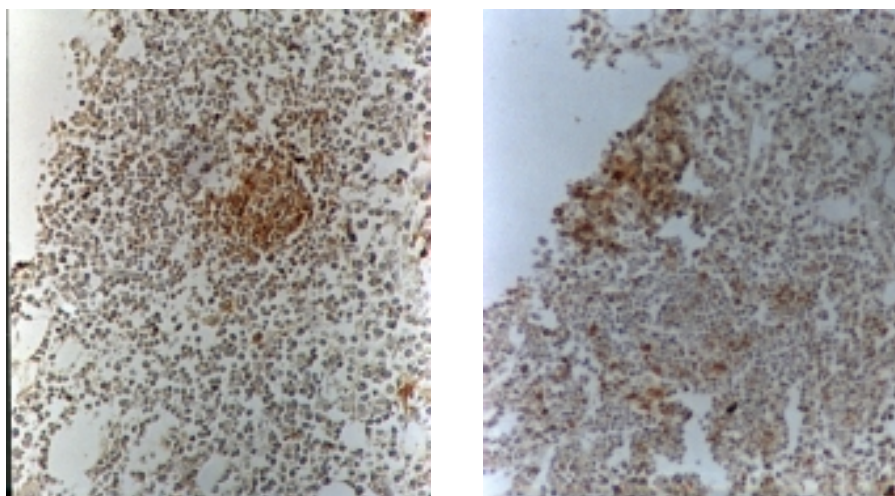


Figure 2 - Immunohistochemistry of bone marrow section, immunoperoxidase technique with hemotaxilin counterstain (left) Strong and central marking of lymphoid aggregate with monoclonal pan-B antibody (CD20) (right) Positive dispersed staining of lymphoid nodules with monoclonal pan-T antibody (CD45RO)

DISCUSSION

Infection with CMV rarely causes disease in immunocompetent individuals, except for a mononucleosis-like illness in some people⁷. However, in immunocompromised patients, most notably transplant-recipients and HIV-infected persons, CMV infection remains an important cause of illness associated with considerable morbidity and mortality⁴. Viral infection has been observed to cause suppression of haematopoiesis that may be mediated by CD8+ lymphocytes exposed to CMV, direct infection of myeloid progenitor cells, impairment of stromal cell function and decreased production of haematopoietic growth factors leading to leukopenia and thrombocytopenia⁴.

Lymphoid aggregates may be observed in bone marrow biopsy in a wide variety of viral infections but also in immune-related diseases and lymphomas. In the present case the diagnosis of acute CMV infection was based on clinical manifestations and serological testing. The patient presented a slight reversible leukopenia. However, bone marrow examination showed a hyperplastic granulopoiesis, with conspicuous increase in eosinophils. The large lymphoid aggregates observed were poorly circumscribed, had no germinal centre, and were mostly intertrabecular in position. Immunohistochemical analysis detected the presence of B and T lymphocytes, favouring their benign nature. Bcl-2 was weakly positive.

Differential diagnosis between benign and malignant lymphoid infiltrates may be difficult. In cases with a florid bone marrow lymphoid reaction, lymphoid aggregates may reach or line the bone trabeculae. Neoplastic lymphoid aggregates are usually composed of homogeneous B cells but reactive T cells may be observed in B-cell lymphomas⁸. In this topic,

morphological features, including in particular histotopography and content of reticulin fibres, associated to immunohistochemistry are useful in differential diagnosis¹³. It is worth mentioning that demonstration of immunoglobulin light chain restriction as a criterion of B-cell neoplasia using immunohistochemical analysis of paraffin tissue sections is of limited value. It has been primarily used in determining the monoclonal nature of plasma cell proliferation¹⁰. Molecular analysis, in spite of not always feasible, is helpful in proving monoclonality. Immunogenotypic approach, using the polymerase chain reaction (PCR) analysis of the unique immunoglobulin heavy chain on DNA extracted from routinely fixed trephine biopsies, can provide additional arguments in favour of lymphoid malignancy. However, because of the incomplete correlation between PCR data and immunomorphological findings resulting in low sensitivity rate, many authors have continued to consider morphology as a gold standard in evaluating bone marrow involvement by malignant lymphoma^{2 3 10}. Therefore, immunogenotypic analysis is considered to be a useful and valuable complementary adjunct to morphology in the differential diagnosis of equivocal cases. In our case, the reactive nature of the lymphoid nodules was presumed by morphological and immunophenotypic features. On the other hand, the clinical manifestations completely reversed without specific therapy and the patient was followed for 18 months with no evidence of malignant disease. In agreement with previous reports, the bcl-2 expression was not a discriminatory criterion for distinction between benign and malignant nature^{6 12}.

The high sensitivity and specificity combined with rapid diagnosis make the molecular biology methods useful adjuncts to histology for detection of CMV-DNA¹¹. However, in our case, both immunohistochemical and PCR studies performed on paraffin-embedded bone marrow specimen were negative. Therefore it seemed improbable that the changes found in bone marrow examination were due to a direct action of CMV on the haematopoietic tissue.

It has been demonstrated that CMV infection disturbs the balanced cytokine network that controls the proliferation and differentiation of haematopoietic cells⁹. The biologic significance of marrow lymphoid aggregates in the majority of patients is unknown. Their presence in our case seemed to represent a systemic response to the viral CMV infection.

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