## Human herpesvirus 8 and epstein-barr virus coinfection in localized castleman disease during pregnancy

**Castleman's disease is a rare disorder characterized by two distinct entities with similar histology but different time course and therapeutic response. Multicentric plasma cell variant is highly associated with infection by human herpesvirus 8 (HHV-8), but the pathogenesis of the hyaline vascular variant is currently unknown. We report a pregnant patient who develops a localized axillary hyaline-type Castleman's disease in which HHV-8 DNA sequences were detected in the lymph node lesions by nested PCR. In addition, the PCR multiplex also showed positivity for EBV. Immunohistochemical studies confirmed the presence of both viruses. Our results provide the first evidence of the presence of HHV-8 and EBV sequences in localized Castleman's disease, suggesting a possible role of the association of these herpes virus in the pathogenesis of this type of disorder. This case highlights that searching for HHV-8 and EBV sequences in cases of localized Castleman's disease is strongly advised.**

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Angiofollicular or giant lymph node hyperplasia was first described in 1954 by Benjamin Castleman as a mediastinal lymphadenopathy.[1] Traditionally, the disease has been subdivided on clinical grounds (localized or multicentric) and pathological findings, including a hyaline vascular pattern, plasma cell predominance, and a plasmablastic variant. It is now enough evidence that there are different etiologies for each of these different subtypes.[2]

Multicentric plasma cell variant is highly associated with infection by human herpesvirus 8 (HHV-8), increasing the risk for the development of other HHV-8 related neoplasms, including Kaposi's sarcoma and extranodal B-cell lymphoma, usually in immunosuppressed patients.[3] Other factors such as a dysregulated production of human IL-6 have recently been involved.[4]

The pathogenesis of the hyaline vascular variant of Castleman's disease is currently unknown. However, vascular and dendritic cell proliferations are common in this disorder as well as an increase of angiogenesis.[5]

On the other hand, Castleman's disease is exceedingly rare during pregnancy, with few cases reported in the medical literature. [6-8] Special immune conditions during pregnancy may play a role on its development.[9] We report a pregnant patient who develops a localized axillary hyaline-type giant lymph-node hyperplasia in which HHV-8 and EBV coinfection was demonstrated in the tissue sample.

### *Patient, material and methods*

#### *Case report*

A 31-year-old Caucasian woman was referred to our outpatient clinic because of a left axillary mass. The patient had been well until the eighth month of her second pregnancy, when she noted a painful left axillary nodule. During the first month of lactation, the nodule progressively enlarged, and she was referred to the hospital.

Her past medical history was unremarkable, and she took no medications. She had not undergone any vaccination in the left arm. On physical examination there was a mass 4 cm in diameter in the left axillary region, very tender on palpation. The remainder of the examination was normal.

Haematological tests and blood and urine chemical values were normal. Blood samples for estradiol, beta-HCG, and progesterone were within normal levels. Serologic tests for syphilis, herpes viruses, HIV and toxoplasma antibodies were negative. Serology and viral load for EBV and HHV-8 were not performed. A tuberculin skin test was also negative. Radiographs of the chest showed no abnormalities. A CT-scan disclosed a rounded mass 6.2 cm in diameter, with small calcifications, in the left axillary region, suggestive of hemangioma. Neither pleural effusion nor other lymphadenopathy was observed at the thoracic or abdominal regions. A contrast-enhanced thoracic MRI scan confirmed the CT findings (Figure 1).

A biopsy of the mass was performed (Figure 2, see below). Diagnosis of hyaline vascular variant of localized Castleman's disease was done, and complete surgical excision of the mass was performed two weeks later. A new histological examination confirmed the biopsy findings. Two years later the patient remains asymptomatic.

### *Material and methods*

#### *PCR analysis*

DNA from each tissue specimen was extracted in physically isolated laboratories from the laboratory where PCR analyses were performed. Samples were analysed in a blind fashion. Stringent laboratory conditions and appropriate negative controls were used to avoid cross-contamination and false positive results. All samples were tested for amplification using specific primers for beta-globin. We used a primer set amplifying a 700-bp fragment (sense and antisense, respectively) Kaposi sarcoma (KS) 4 (5'-AGC ACT CGC AGG GCA GTA CG-3') and KS5 (5'-GAC TCT TCG CTG ATG AAC TGG-3'). The amplification of the external region was of 2 minutes at 94°C for initial denaturation, 40 cycles at 95°C for 30 sec, 60°C for 30 sec, and 72°C for 30 sec and 72°C for 5 min as final extension. PCR product (2 μL) was added to an inner PCR reaction mixture and amplified for 40 additional cycles with an annealing temperature of 58°C with the use of the primers amplifying the 233-bp KS330233 region of the sequence associated with HHV-8: KS1 (5'AGC CGA AAG GAT TCC ACC AT-3') and KS2 (5'-TCC GTG TTG TCT ACG TCC AG-3'). Amplifications were performed in a Techne 500 Thermocycler. Amplification products were analysed for the presence or absence of the 233-bp expected band on a 2 percent agarose gel containing ethidium bromide. A case of cutaneous AIDS-Kaposi sarcoma was included as an internal positive control.

We also performed a PCR multiplex for human herpes viruses (herpes simplex type 1 and 2), varicella-zoster, cytomegalovirus, EBV and human herpes virus 6 (Herplex, PharmaGen. Madrid. Spain) according to the manufacturer's instructions. Briefly, after DNA extraction, we performed amplification with 40 cycles at 96°C for 1 min, 60°C for 1 min, and 72°C for 1 min. PCR product was analysed by microtiter plate hybridization (ELISA). An internal control (DNA polymerase gen) was included to assure the integrity of the sample and the process.

A seminested PCR was performed to detect heavy chain IgH rearrangement (Vitro-Imico. Granada. Spain)

according to manufacturer's instructions. Briefly, we amplified the Fr2/J segment that gives a 240-260 bp PCR product. After the amplification we performed a heteroduplex reaction in order to eliminate the possibility of unspecific amplification. We used as a DNA integrity internal control the exon 5 of p53 that is highly conserved in human genome. Amplification products were analysed for the presence or absence of the expected band on a 2 percent agarose gel containing ethidium bromide.

*Immunohistochemistry*

The following mouse monoclonal antibodies were used for immunohistochemical staining: LMP-1 (Dako. Glostrup. Denmark. Monoclonal, clone CS-1, 2, 3 and 4. Dilution 1/100), and monoclonal antibody against LNA-1 (ORF-73) of HHV8 (Advanced Biotechnologies. Columbia, Maryland. USA. Dilution 1/400) were performed using formalin-fixed and paraffin embedded tissue sections. Antigen retrieval was done only in the case of HHV8 antibody by boiling sections in 0,1mM EDTA pH8.0 buffer using a pressure cooker for 90 sec. The Dako EnVisionTM + kit was used as a visualization system according to the manufacters' instructions, in a Techmate 500 220 automatized immunostainer (Biotek, Santa Barbara, CA).

*Histopathological and genetics results.*

The biopsies showed a lymph node with irregular germinal centers with prominent hyalinised vascular proliferation. Radially penetrating capillaries surrounded by collagenous hyalinisation and concentrically layered mantle zones were patent. Some follicles had an atrophic appearance. Between the follicles, extensive capillary proliferation effaced the lymphoid sinuses (Figure 2). Morphological diagnosis was hyaline-vascular type Castleman's disease.

HHV-8 DNA sequences were detected in the lymph node lesions by nested PCR. Positive signal was observed also in the AIDS-KS specimen. The PCR multiplex showed a positivity for EBV with a 405nm absorbance of 0,931 (negative control: 0,100; positive control 3,485) (Figure 3). The remainder of herpes viruses analyzed were all negative.

VEB and HHV8 antibodies both gave positive results in cells of lymphoid proliferation (Figures 4 and 5). The IgH rearrangement showed a smearing band belonging to a polyclonal lymphoid population. No evidence of lymphoproliferative disorder was detected.

## *Discussion*

Castleman's disease is a rare disorder characterized by two distinct entities with similar histology but different time course and therapeutic response. Localized Castleman's disease is more frequent in mediastinal region, although abdominal and pelvic areas can be involved.[2] A few cases during pregnancy have been reported. Baser et al.[6] described an asymptomatic pelvic mass that was incidentally found by ultrasonography during follow up of a normal pregnancy. Ylinen *et al.*[7] described an adnexal mass of difficult localization in a non-pregnant woman. Once localized a year later, the mass was finally removed in the third trimester pregnancy period. Abramov *et al.*[8] noted a large abdominal mass associated with vaginal bleeding during the second trimester of pregnancy. As the patient we report, this cases were localized and of hyaline-vascular type, and thus successfully resolved after surgery. Nevertheless, not much light has been shed regarding the nature of Castleman's disease in pregnancy so far.

On the other hand, HHV-8, a recently discovered oncogenic human virus, has been associated with Kaposi's sarcoma, primary effusion lymphoma, and multicentric Castleman's disease. These disorders occur more frequently, but not exclusively, in immunodeficiency states such as in human immunodeficiency virus (HIV)-infected individuals or transplantation.[10] One of the most intriguing aspects of HHV-8-related disorders is the involvement of numerous growth factors in their pathogenesis. The virus contains cellular gene homologues that encode proteins involved in signal transduction, cell cycle regulation, inhibition of apoptosis, and immune modulation. Several HHV-8 gene products exhibit proinflammatory and proangiogenic activities or act as transcriptional activators of cellular cytokines that are involved in the pathogenesis of HHV-8-related disorders.[11,12]

Regarding EBV implication in localized Castleman's disease, Murray *et al.*[13] found the virus in 5 of 12 lymph node biopsies by in situ hybridization for the noncoding EBV early RNAs (EBERs). However, HHV-8 was not tested at this time.

Moreover, HHV-8 and EBV co-infection has been documented in the setting of Castleman's disease, typically associated with immunosupressed states.[14-16] The role of these viruses rests upon their ability to trigger changes at a cellular level that facilitate the occurrence of neoplasms, as well as their ability to produce proangiogenic factors such as IL-6.[11] In these cases the pattern of the disease is always multicentric. However, thus far only one case of unicentric Castleman's disease has been reported to be associated with HHV-8, suggesting that it may be a different disease than multicentric variant.[17] Our results, provide the first evidence of the presence of HHV-8 and EBV sequences in localized Castleman's disease, suggesting a possible role of the association of these herpes virus in the pathogenesis of this type of disorder. Further studies are needed to confirm this hypothesis.

Interestingly, in our patient Castleman's disease has been diagnosed during pregnancy. This represents a period in which angiogenesis is clearly increased, with detection of high serum vascular endothelial growth factor and angiopoietins levels.[18] Changes in hormone levels associated with normal pregnancy modify immune mechanisms. More precisely, adjustments in cytokine production trigger a dominant humoral response, whereas cellular immunity activity diminishes.[19] Thus, pregnant women are more vulnerable to intracellular and viral infections due to a normally increased (TH2/ TH1) cell share.[20]

It is tempting to speculate that several factors may be playing a role in the current case, including a proangyogenic state associated to pregnancy, cellular changes due to a concomitant viral infection, angiogenesis induced by the viral infection, and the physiological pregnancy changes in lymphocyte T cells subtypes' balance. Future approaches may test whether those conditions may justify the finding of a localized Castleman's disease in pregnancy, as their immune system is partially able to respond to viruses. This case highlights that searching for HHV-8 and EBV sequences in cases of localized Castleman's disease is strongly advised.

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**Figure 4.** LMP-1 immunostaining. Note the positive nuclei (LMP-1. Original magnification 100x).

**Figure 5.** HHV8 immunostaining. Note the positive nuclei (LNA-1. Original magnification 100x).

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## References

1. Castleman B, Towne VW. Case records of Massachusetts General Hospital, Case 40001. N Eng J Med 1954;250:26-30.
2. Herrada J, Cabanillas F, Rice L, et al. The clinical behavior of localized and multicentric Castleman disease. Ann Intern Med 1998;128:657-62.
3. Hengge UR, Ruzicka T, Tyring SK, Stuschke M, Roggendorf M, Schwartz RA, et al. Update on Kaposi's sarcoma and other HHV8 associated diseases. Part 2: pathogenesis, Castleman's disease, and pleural effusion lymphoma. Lancet Infect Dis 2002;2:344-52.
4. Yoshizaki K, Matsuda T, Nishimoto N, et al. Pathogenic significance of interleukin-6 (IL-6/BSF-2) in Castleman's disease. Blood. 1989;74:1360-7.
5. Keller, AR, Hochholzer, L, Castleman, B. Hyaline-vascular and plasma-cell types of giant lymph node hyperplasia of the mediastinum and other locations. Cancer 1972; 29:670.
6. Baser I, Dilek S, Pabuccu R, Ulgenalp I, Mermut S, Finci R. Unexpected nature of a pelvic mass in pregnancy: Castleman's disease. Gynecol Obstet Invest. 1989; 28: 53-5.
7. Ylinen C, Sarlomo-Rikala M, Laatikainen T. Pelvis Castleman disease mimicking an adnexal tumor. Obstet Gynecol 1995; 85: 894-7.
8. Abramov Y, Nadjari M, Abramov A, Constantin R, Anteby SO. Castleman disease in pregnancy. Obstet Gynecol 1997; 90: 653-4.
9. Luppi P. How immune mechanisms are affected by pregnancy. Vaccine 2003; 21: 3352-3357.
10. Aoki Y, Tosato G. Pathogenesis and manifestations of human herpesvirus-8-associated disorders. Semin Hematol 2003;40:143-53.
11. Wang L, Wakisaka N, Tomlinson C, et al. The Kaposi's Sarcoma Associated Herpesvirus (KSHV/ HHV-8) K1 protein induces expression of angiogenic and invasion factors. Cancer Research 2004; 64: 2774-81.
12. Menke DM, Chadbum A, Cesarman E, et al. Analysis of the human herpesvirus 8 (HHV-8) genome and HHV-8 vIL-6 expression in archival cases of Castleman's disease at low risk for HIV infection. Am J Clin Pathol 2002; 117: 168-75.
13. Murray PG, Deacon E, Young LS, et al. Localization of Epstein-Barr virus in Castleman's disease by in situ hybridization and immunohistochemistry. Hematol Pathol. 1995;9:17-26.
14. Larroche G, Agbalika F, Poirel H, et al. Epstein-Barr virus and human herpes virus 8 coinfection and concomitant extranodal nasal-type NK/T cell-lymphoma and Castleman disease. CID 2003; 36: 107-10.
15. Theate I, Michaux L, Squifflet J-P, Martin A, Raphael M. Human herpesvirus 8 and Epstein-Barr virus related monotopic large B-cell lymphoproliferative disorder coexisting with mixed variant of Castleman's disease in a lymph node of a renal transplant recipient. Clin Transplant 2003; 17: 451-4.
16. Weisenburger DD, Nathwani BN, Winberg Castleman's disease, et al. Simultaneous occurrence of Epstein-Barr virus associated Hodgkin's disease and HHV-8 related multicentric Castleman's disease: a fortuitous event? J Clin Pathol 2001;54:790–1.
17. Kikuta H, Itakura O, Taneichi K, Kohno M. Tropism of human herpesvirus 8 for peripheral blood lymphocytes in patients with Castleman's disease.Br J Haematol 1997;99:790-3.
18. Kaufmann P, Mayhew TM, Charnock-Jones DS. Aspects of human fetoplacental vasculogenesis and angiogenesis. II. Changes during normal pregnancy. Placenta 2004; 25: 114-26.
19. van Niewnhoven ALV, Bouman A, Moes H, et al. Cytokine production in natural killer cells and lymphocytes in pregnant women compared with women in the follicular phase of the ovarian cycle. Fertility and Sterility 2002; 77:1032-37.
20. Piccinni M-P, Scaletti C, Maggi E, Romagnani S. Role of hormonal-controlled Th1 and Th2 type cytokines in successful pregnancy. J Neuroinmunol 2000; 109: 30-33.