Failure of multiple antivirals to affect high HHV-6 DNAaemia resulting from viral chromosomal integration in case of severe aplastic anaemia

We report a fifty-year-old woman presenting with severe aplastic anaemia (SAA) and prolonged high *Human Herpesvirus 6 (HHV6)* variant A DNAeamia detected by quantitative PCR. Multiple antiviral treatments failed to affect the HHV6 DNAemia and subsequent immunosuppressive treatment reached only partial improvement as judged by bone marrow examinations. The patient remained dependent on thrombocyte transfusions and G-CSF treatment. After one year of steady high *HHV6* DNA load in blood, viral chromosomal integration was proved by demonstrating the viral DNA in hair follicles. This condition appeared to be unconnected with, and to have no effect, on the original SAA

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We report a fifty-year-old woman who was admitted to hospital after one month of fatigue, easy bruising and loss of weight with fever and acute tonsilitis at the end of August 2005. Her blood counts showed pancytopaenia - 1,600 leucocytes/microL (89% of lymphocytes), 55,000 thrombocytes/microL, 94 g/L haemoglobin.

Bone marrow aspiration and trephine biopsy showed hypocellular bone marrow with 14.4% of granulopoiesis, almost no megakaryocyte precursor detectable, low counts of erythropoiesis cell precursors, lipophages, erythrophagocytosis and 78% of lymphoid cells. Most of the lymphoid cells were T lymphocytes, of which 16.3% were HLA DR positive. Based on the above findings, severe aplastic anaemia (SAA) was diagnosed. An immediate treatment was commenced with antibiotics and granulocyte colony stimulating factor (G-CSF), however no reaction to G-CSF was observed in the peripheral blood.

There was nothing remarkable in the family, pharmacological or social history and no evidence of exposure to harmful chemical substances, drugs or radiation and no signs of autoimmunity or paroxysmal nocturnal haemoglobinuria.

On testing whole blood for viral infections by quantitative real-time PCR, there was no evidence of *Human Cytomegalovirus and Parvovirus B19*, while *Epstein-Barr virus* was detected at a minute viral load. However, we detected high *Human Herpesvirus 6 (HHV6)* DNA load of 150,000 copies normalized to 100,000 human genome equivalents which were obtained by quantification of albumin gene.[1,2] We also detected 4,200 viral copies of HHV6 per mL of plasma. The virus was subsequently identified to be the variant A using a nested PCR system.

Because of high *HHV6 DNA* load (Figure 1) and the reported suppressive effect of *HHV6* on haematopoiesis,[3-5] we considered that the *HHV6* infection was a possible explanation of the patient's low blood count. Antiviral treatment (Figure 1) was started with foscarnet 120 mg/kg per day. After two weeks of treatment without any decrease in the level of *HHV6* DNA, we changed the therapy to cidofovir 5 mg/kg once a week. Four weeks later because the *HHV6* DNA level remained high, we altered the therapy to ganciclovir 2.5 mg/kg every 12 hours. Virostatic treatment failed to reduce viral load documented by the mean of 150,000 normalized copies of *HHV6* (range 89,000–200,000) during the treatment. During these two months, the patient was kept on an antibiotic and antimycotic prophylaxis and immunoglobulin supplementation.

Since the antiviral treatment did not result in any decrease in viral load and no improvement in the patient's clinical status had been observed two month later, we started immunosuppressive treatment for SAA. The treatment consisted of four doses of rabbit antithymocyte globulin (ATG Fresenius) at a dose of 40 mg/kg, 1.5 mg/kg per day of corticosteroids and cyclosporine A adjusted to maintain a serum level of 100 to 200 ng/mL and complemented by G-CSF. During the immunosuppressive treatment, the *HHV6* levels remained constantly high with a mean of 160,000 normalized copies (range 37,000–1 300,000) (Figure 1).

After two month of immunosuppressive treatment, there were no signs of improvement in haematopoiesis in control trephine biopsy of bone marrow and because there was no sibling donor available, we searched for unrelated haematopoietic stem cell donor but without success. However, after four months of treatment (January 2006), there was a temporary improvement in peripheral blood and the white blood cell count reached 4,000 leucocytes/microL with granulocytes above 1,500/ microL, although patient remained dependent on thrombocyte transfusions.

Two months later, the blood count worsened again. Therefore we started administration of pegfilgrastim 6 mg every three weeks, keeping the granulocytes above 3,000 leucocytes/microL. In June 2006, thrice weekly haemodialysis was required for progressively worsening renal function. The patient did not suffer from severe anemia although she was remaining dependent on thrombocyte transfusions and random G-CSF treatment. She died due to peracute sepsis caused by *Staphylococcus aureus* in January 2007.

Considering the contribution of *HHV6* infection to our patient's illness, extensive antiviral therapy failed to induce any improvement in stem cell activity or a fall in viral DNA levels and lengthy immunosuppressive treatment did not cause a rise in viral DNA levels both of which findings indicate that active viral infection was not responsible for the primary disease in our patient. Moreover the patient's virus was variant A, which is not connected with any known disease but is commonly found in chromosomal integration of *HHV6* (CHHV6); indeed we have recently reported a case of CHHV6 with variant A (Hubacek *et al.*, in press).

CHHV6 is relatively common phenomenon being

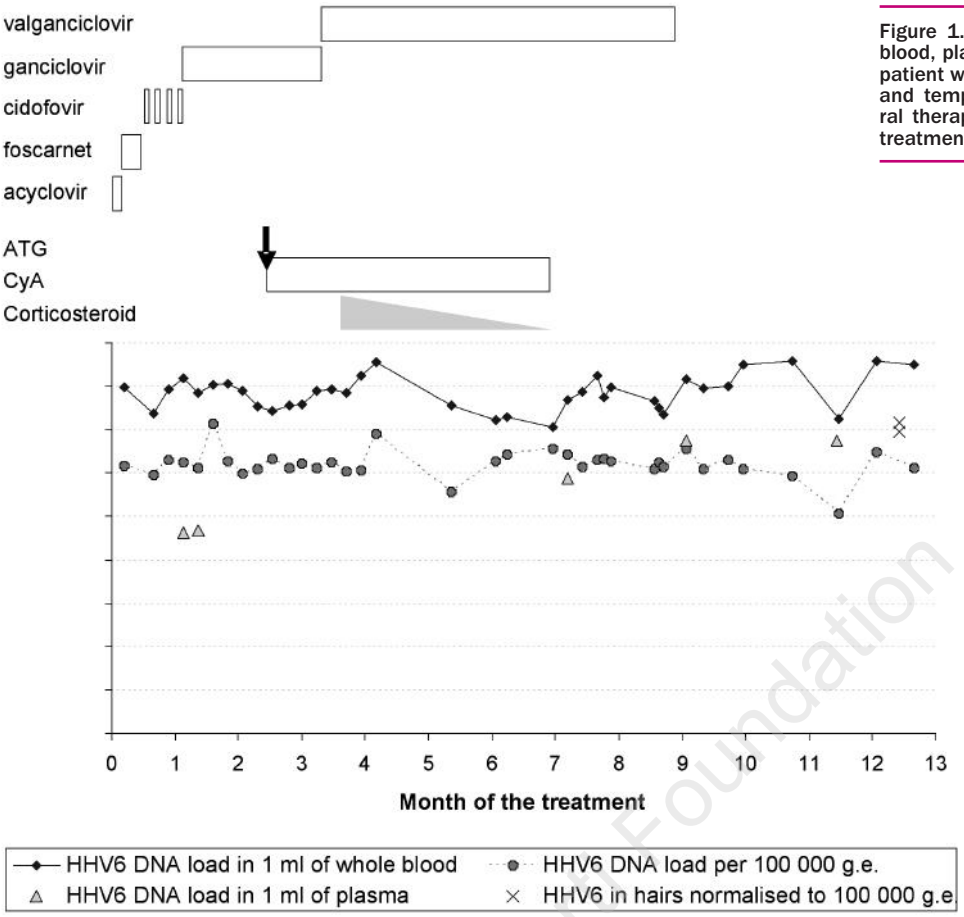

Figure 1. HHV6 DNA load in whole blood, plasma and hair follicles of a patient with severe aplastic anaemia and temporal relationship to antiviral therapy and immunosuppressive treatment.

found between 0.2 and 2.9% of the human population[6,7] and was first described by Luppi et al. in 1993[8] in patients without any clinical manifestation of *HHV6* disease. Daibata *et al.*[9] demonstrated the inheritance of *HHV6* DNA through two generations. Indeed it seems that *HHV6* may be found in any cell in the body since it has been found not only in the blood but also in the hair follicles of individuals with CHHV6 and in both these sites there is at least one copy of *HHV6* DNA per cell.[10] Thus, very high, persistent levels of *HHV6* DNAaemia at about one copy/cell in our patient were highly suggestive of CHHV6 rather than active infection. We therefore tested for *HHV6* DNA in the hair follicles of our patient and found it at a high level comparable with that in the patient's blood (Figure 1) thus confirming chromosomal integration.

This case reminded us the necessity of critical review of the results obtained by molecular biological techniques. We were initially misled by the detection of high level of *HHV6* DNA in both whole blood and plasma and concluded that this implied active virus infection was whereas in fact the *HHV6* DNA found in plasma was derived from cellular chromosomal DNA containing *HHV6*.[10] Detection of *HHV6* DNA in the hair roots proved the genome integration into the cells. Testing for the presence of *HHV6* DNA in the hair follicles proved to be an easy way to discriminate between active infection and integration of *HHV6* DNA into human genome among the suspected patients according to the published data.[10] In agreement with our experience, there was not published evidence of viral reactivation from chromosomally integrated *HHV6* genome. Therefore in similar case we would not lose any precious time with the virostatic drugs and treat the primary disease as soon as *HHV6* chromosomal integration is confirmed.

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