

*Original Article***Human herpes virus 8 infection in kidney transplant patients in Belgium**

Julie Sheldon<sup>1</sup>, Stéphanie Henry<sup>2</sup>, Michel Mourad<sup>3</sup>, Monique Bodéus<sup>2</sup>, Jean-Paul Squifflet<sup>3</sup>, Thomas F. Schulz<sup>1</sup> and Patrick Goubau<sup>2</sup>

<sup>1</sup>Department of Medical Microbiology and Genitourinary Medicine, The University of Liverpool, Liverpool, UK,

<sup>2</sup>Department of Virology and <sup>3</sup>Department of Renal Transplantation, Cliniques Universitaires St Luc, Université Catholique de Louvain, Brussels, Belgium

**Abstract**

**Background.** Kaposi sarcoma (KS) may arise as a complication of kidney transplantation. In the Saint Luc Teaching Hospital in Brussels, patients of both Belgian and foreign origin are treated. The prevalence of human herpes virus 8 (HHV-8) infection differs in different geographical settings. We wanted to estimate the background infection rate and the risk of infection in our transplant population: a first step towards evaluating the necessity of HHV-8 screening.

**Methods.** Serum samples were taken from 210 organ donors over a period of 7 years (30 per year) and from 200 kidney recipients from whom two sera were tested, one pre-transplant and the second 6–12 months post-transplant. All sera were screened for HHV-8 by an enzyme-linked immunosorbent assay using recombinant ORF 65 and ORF 73 antigens and an immunofluorescence assay for the latent antigen. Reactive samples were confirmed by western blotting.

**Results.** Seven donors (3.3%) were positive for HHV-8 antibodies. Of 198 pre-transplant sera available for evaluation, 15 were positive (7.6%). Post-transplantation 18/199 (9%) were positive: four (2.1% of negatives) had a documented seroconversion and one lost the antibodies. No patients developed KS.

**Conclusions.** A substantial number of kidney transplant patients already had antibodies to HHV-8 at the time of transplantation. A further 2.1% of seronegative patients had seroconversion, which could have been acquired through the transplanted organ (3.3% of donors were positive) or through transfusion.

**Keywords:** Belgium; human herpes virus type 8; Kaposi sarcoma; kidney graft

rare cases of HIV-positive body cavity-based lymphoma and with multicentric Castleman disease [1–4]. The distribution of the virus in the population conforms to the pattern expected for a sexually transmitted disease and its prevalence corresponds to the risk for KS [5–7]. Some studies suggest that non-sexual modes of transmission may be important in highly endemic countries [8]. Before confirmation of the aetiological agent it had already been supposed on epidemiological grounds that a sexually transmitted infection might be the cause [9]. Many people infected with HHV-8 never develop disease [10,11], suggesting that some form of equilibrium usually exists between the virus and the immune system. Immuno-compromised individuals, such as people infected with HIV or transplant recipients may develop KS. The incidence of KS in renal transplant recipients varies widely from country to country; figures from 0.4–5.3% have been reported [12,13]. A report of two KS cases in patients who received kidneys from a single donor suggested that a graft may transmit the virus [14]. More recently in Switzerland HHV-8 was indeed transmitted through a graft with consequent risk of KS [15]. On the other hand post-transplant KS in transplant recipients from HHV-8 endemic areas appears to occur mainly in individuals who were already HHV-8 infected before transplantation [16,17]. As the risk of HHV-8 infection and KS may differ according to the ethnic background of patients, we thought it would be useful to evaluate the extent of the problem in a varied population of graft recipients. The patients treated at the St Luc University Hospital in Belgium are from various geographical origins.

**Materials and methods**

Sera (210) from potential donors were tested over a period of 7 years from 1992 to 1998 (30 successive sera each year). All of these donors died in Belgium and were mostly of Belgian origin. Samples had been stored at –20°C for up to 8 years. Samples from four kidney transplant-associated KS cases and from four post-transplant lymphomas were tested as controls. No pre-transplant sera from the eight control

**Introduction**

Human herpes virus 8 (HHV-8) has been associated with different forms of Kaposi sarcoma (KS), with

*Correspondence and offprint requests to:* Dr Patrick Goubau, Unité de Virologie, Ecole de Santé Publique 3055, Cliniques Universitaires St Luc, Clos Chapelle-aux-Champs, 30 B-1200 Bruxelles, Belgium. .

cases were available. A group of 198 renal transplant recipients was also tested serologically. For all patients at least two serum samples were available, one pre-transplant and one taken between 6 months and 1 year post-transplantation. For the purpose of the study, the recipient population was arbitrarily divided into two subgroups according to their origin at the time of transplantation: group B comprised 143 patients who had local residency at the time of transplantation; group M comprised 55 recipients who originated from a foreign state inside the European Community, mostly from Mediterranean countries.

Sera were tested for the presence of antibodies to HHV-8 in a dilution of 1:100 by an enzyme-linked immunosorbent assay (ELISA) using two HHV-8 antigens and a control antigen. The two HHV-8 antigens were a recombinant fragment (amino acids 86–170) of the HHV-8 capsid protein encoded by open reading frame (ORF) 65 [7] and a recombinant fragment (amino acids 951–1163) of the major latent nuclear antigen (LNA), encoded by ORF 73 [18]. The control antigen was the fusion protein employed to express these two HHV-8 antigens (dihydrofolate reductase) (7). Optical density values exceeded the average ( $+5$  standard deviations) seen in 10 HHV-8-negative UK blood donors [7,19]. In addition, sera were tested by immunofluorescence in a dilution of 1:50 for the presence of antibodies to the 'latency-associated nuclear antigen', as described previously [7]. Sera that were positive by ELISA, but negative by immunofluorescence, were retested by western blotting on the appropriate recombinant antigen.

Differences were analysed for statistical significance by the chi-squared test with Yates' correction using the Graphpad Prism 2.0 software (San Diego, CA).

## Results

In the donor group 7/210 were HHV-8 positive (3.3%). The four cases of post-transplant KS were seropositive and the four lymphoma cases were negative. Of 198 pre-transplant sera, 15 were positive (7.6%). Among these 15 positive cases, more group M patients (14.8% positive) were affected than others (4.9%); this difference is significant ( $P=0.02$ ). Results by gender are given in Table 1. Ten of 77 (12.9%) females were positive (six from the M group, four from the B group) and 5/123 (4%) men were positive (two from the M group, three from the B group). The difference of prevalence between sexes is significant ( $P=0.03$ ), but is largely due to the M group. Six months to 1 year post-transplantation, 18/198 (9%) were positive: four had documented seroconversion and one positive individual became negative. One seroconverter was from

group M, the others from group B. At present, each of the four seroconverters have a good functioning kidney and none has developed KS after 3–7 years.

## Discussion

KS is a relatively rare disease in our patient population. Between June 1963 and May 1999, 2807 renal transplantations were performed at our institution; only eight KS cases (0.3%) have been documented, which indicates that the risk is very low. Since the discovery of HHV-8 several serological assays have been developed both on the basis of lytic and latent antigens [5–7]. Comparative evaluation of assays has shown that the correlation is suboptimal and therefore individual test results of HHV-8 serology are difficult to interpret [20], but general population tendencies can be derived. The positive results in a few KS cases confirmed the validity of our tests. The lymphoma cases were negative. A low prevalence of HHV-8 antibodies (3.3%) was found among donors in our hospital. Only one positive donor gave a kidney and a liver to local recipients in 1993 (not in this study). Post-transplant samples were tested and the kidney recipient appears to be negative while the liver recipient is positive for HHV-8 antibodies. This indicates that infection is not an inescapable result of transplantation from a positive donor.

Pre-transplant prevalence of HHV-8 antibodies among graft recipients was 7.6%. Geographically, a gradient of prevalence can be seen between highly endemic areas like central Africa and areas of low endemicity such as northern Europe or the US [10,11]. Our findings confirmed this as 14.8% of recipients from group M were antibody-positive as compared with only 4.9% in group B. The prevalence is, however, not only defined geographically because within a given geographical area wide variations may exist between population groups. The higher prevalence in women (12.9%) than in men (4%) is curious but may be due to the small group of positive individuals. In other studies the rates of HHV-8 infection between men and women are generally similar, but KS is seen mainly in men. This suggests that risk factors other than HHV-8 are involved. The higher prevalence in women, if real, might be explained by a more efficient sexual transmission from men to women than *vice versa*, or by a

**Table 1.** HHV-8 serological results in geographical groups according to gender

|                       | M group* |         | B group** |        | Total     |
|-----------------------|----------|---------|-----------|--------|-----------|
|                       | Men      | Women   | Men       | Women  |           |
| Positive (% of group) | 1 (2.6%) | 6 (30%) | 4 (4.8%)  | 4 (7%) | 15 (7.6%) |
| Negative              | 37       | 14      | 79        | 53     | 183       |
| Total                 | 38       | 20      | 83        | 57     | 198       |

\*M group: individuals from Mediterranean countries. \*\*B group: individuals with residence in Belgium.

higher frequency of transfusions in women, as has been shown for other blood-borne viruses like HTLV-I.

When patients were retested 6–12 months post-transplantation, four were shown to have seroconverted. All four had received grafts from other centres of Eurotransplant. None of the seroconverted patients developed KS and all grafts are doing well.

We are unsure as to why one of the initially seropositive patients became negative: either the first result was falsely positive or antibody levels in the second sample had dropped below the detection level.

In a Swiss study 2/25 seroconverters developed KS [15], which is statistically no different from our results. The seroconversion rate in the Swiss study (12.1%) was much higher than the 2.1% found in our study. The magnitude of our figure is in accordance with transmission through graft, as it corresponds to the prevalence among our donors. In Italy, pre-transplant infection status seemed to be the main risk factor for KS [16]. A recent case-control study carried out in France on transplant patients from HHV-8 endemic and non-endemic countries concluded that being HHV-8 seropositive pre- or post-transplantation conferred a 28-fold increased risk of transplant-associated KS, and that the use of anti-lymphocyte T globulin should be avoided in HHV-8 seropositive patients [17]. This may be a factor, but among our patients who remained free of KS two-thirds of them received anti-lymphocyte T globulin.

Given the uncertainties related to serological assays [20] and the unknown risk of KS development in HHV-8-infected individuals, the establishment of a policy of systematic screening of donors seems to be premature at present.

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