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TITLE PAGE

Title: Multicenter prospective study for laboratory diagnosis of HHV8 infection in solid organ donors and transplant recipients and evaluation of the clinical impact after transplantation

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Author contributions

Angela Chiereghin participated in carrying out the research, in acquisition and analysis of data, as well as drafted the paper.

Patrizia Barozzi and Evangelia Petrisli participated in carrying out the research, in acquisition and analysis of data.

Giulia Piccirilli, Liliana Gabrielli, Giovanni Riva, Leonardo Potenza and Irene Libri participated in carrying out the research and contributed to the acquisition of the data.

Gianni Cappelli, Nicola De Ruvo, Umberto Maggiore, Maria Cristina Morelli, Luciano Potena, Antonio D. Pinna and Gaetano La Manna clinically managed the patients during the post-transplant period and provided the respective clinical information.

Manuel Labanti and Gabriela Sangiorgi managed the blood samples and information of the solid organ transplant donors.

Mario Luppi participated in research design and supervised the project.

Tiziana Lazzarotto participated in research design, supervised the project, as well as performed a critical revision of the article.

All authors discussed the results and commented on the manuscript at all stages. All of them provided final approval of the version to be submitted.

Conflict of Interest/Disclosures

The authors declare no conflicts of interest.

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ABBREVIATIONS PAGE

BL: border-line

D/R: donor/recipient

ELISA: enzyme-linked immunosorbent assay

ERR: Emilia Romagna Region

HHV8: human herpes virus 8

IFA: indirect immunofluorescent assay

KS: Kaposi's sarcoma

nd: not defined

PCR: polymerase chain reaction

SOT: solid organ transplant

WB: whole blood

Abstract:

Background. We performed a serological-virological screening in solid organ transplant (SOT) donors and recipients in north-central Italy and a surveillance program for human herpes virus 8 (HHV8) infection after transplant, aiming to establish a correct management of HHV8 infection in SOT recipients. **Methods.** For pre-transplant HHV8 screening in both donors and recipients, six serological, i.e. four indirect immunofluorescent assays (IFA) and two enzyme-linked immunosorbent assays (ELISA) - both HHV8 lytic and latent antigen-based - and two molecular assays were used. All transplant patients at risk to develop HHV8-related disease underwent virological post-transplant monitoring by quantitative real-time PCR assay. **Results.** HHV8 seroprevalence was 4% (10/249) in donors and 18% (93/517) in organ recipients. IFAs sensitivity values ranged from 98% (lytic IFA) to 37.8% (latent IFA) and lytic IFAs were more sensitive than ELISA (51.4%). HHV8-DNA was detected in 6.8% and 2.9% of HHV8-seropositive donor samples by in-house nested PCR and quantitative real-time PCR assays, respectively. After transplant, three out of 12 (25%) HHV8-mismatch patients (seropositive donor/seronegative recipient) developed a primary infection, one of these patients developed a lethal nonmalignant illness. Two out of 93 HHV8-seropositive recipients (1.2%) had a viral replication in post-transplant period, one of these developed Kaposi's sarcoma. **Conclusions.** Serological assays, in particular lytic IFAs, were the best methodological approach to identify SOT donors and recipients HHV8-infected. A very low incidence (1.9%) of post-transplant HHV8-related disease was observed.

INTRODUCTION

In solid organ transplant (SOT) recipients, human herpesvirus 8 (HHV8) has been associated with both neoplastic diseases, i.e. iatrogenic Kaposi's sarcoma (KS), primary effusion lymphoma, Castleman's disease and with non-neoplastic diseases including Castleman-like or otherwise called atypical HHV8-positive plasmacytic lymphoproliferations, bone marrow failure, peripheral cytopenias, associated or not associated with hemophagocytic syndromes and acute hepatitis syndromes. [1,2] Although the HHV8 transmission pathway seems to differ between populations and geographic areas,[3] international studies have provided evidence that HHV8 can be transmitted from the donor organ to transplant recipient.[4-6] Most of the post-transplant HHV8-associated diseases could be rapidly lethal if not timely recognized and adequately treated.[2] Moreover, it was observed that in SOT recipients KS risk is 400- to 500-fold higher than the general population.[7] Despite this, the serologic tests most frequently used internationally for donor and recipient screening prior to SOT do not include HHV8 serology. Screening for anti-HHV8 antibodies is included in the optional screening measures for viral infections probably due to the actual lack of a gold standard in serologic assays.[8-10] Despite the high prevalence of HHV8 infection in the southern regions of Italy, HHV8 serology testing is performed on a small percentage of SOT recipients and donors, 27.3% and 11.4%, respectively.[11]

From July 2008 to October 2010 we performed a multicenter prospective HHV8 screening program in SOT donors and recipients in the Emilia Romagna Region (ERR) in north-central Italy with the aim to establish a correct diagnostic screening and management of HHV8 infection in SOT recipients. Specifically, the primary end point was to establish a *gold standard* test for the diagnosis of HHV8 infection. Secondary outcomes included: *i*) identification of patients at risk of HHV8-related disease and thereby the implementation of a rigorous, at least two-year long post-transplantation monitoring; and *ii*) evaluation of the clinical impact of post-transplant HHV8 infection.

MATERIALS AND METHODS

STUDY DESIGN

HHV8 serological and virological screening in SOT donor/recipient (D/R) pairs at the time of organ donation and transplantation (baseline time) were performed. Serum and blood samples were collected and plasma samples were obtained by centrifugation of whole blood (WB). All donors procured in the ERR and recipients were tested using six different serological tests to detect serum antibodies against both HHV8 lytic and latent antigens. The study was launched after initial validation of these serological tests. The assays were validated in two well-defined populations consisting of 20 patients diagnosed with KS and 20 healthy adults. The 20 KS cases included 14 SOT recipients who developed KS after an average time of 9.2 (range, 3.5-16) months post-transplant and 6 elderly people who developed KS with cutaneous lesions. The 20 selected healthy adults were volunteer blood donors, born in low HHV8 prevalence countries and who never showed any prior HHV8-related clinical signs. Subsequently, the twenty true-positive and the twenty true-negative samples were tested with all the six assays in order to establish criteria for interpretation of serological results.

Virological screening was performed in all SOT D/R pairs by searching for HHV8-DNA using two molecular tests, i.e. quantitative real-time PCR and in-house nested PCR assays. In particular, all donors and recipients WB samples were tested by quantitative real-time PCR assay and plasma samples were tested using both the molecular assays. Based on serological-virological screening results obtained in D/R pairs, SOT recipients at higher risk to develop HHV8-related disease were identified and for these patients virological monitoring of HHV8 infection was performed on plasma samples by quantitative real-time PCR. Specifically, recipients in D+/R+, D-/R+ and D-/R not defined (nd) groups were monitored monthly until two years post-transplant; recipients in D+/R- and Dnd/R- groups were monitored every two weeks during the first 3 months and then monthly until two years post-transplant. Additional blood samples were processed if clinically

indicated. The 5% of patients (20/346) in the D-/R- group was also monitored for HHV8 infection. Finally, in order to identify HHV8 seroconversions, the recipients in the D+/R- and Dnd/R- groups were tested by serological assays monthly for the first 12 months, and then once every three months until 24 months post-transplant or until positive. HHV8 seroconversion was defined as the occurrence of at least two positive serological tests.

PARTICIPANTS

Between July 2008 and October 2010 all adult patients undergoing kidney, liver and heart transplantation in three transplant centers in the ERR (Bologna, Modena and Parma) were proposed to participate in the study, by providing their consent to pre-transplant screening and post-transplant monitoring for HHV8. The exclusion criteria were age <18 years and HIV infection at the time of transplantation. During the same period, deceased solid organ donors procured in the ERR were included.

The study was approved by the three transplant centers' Ethics Committee. All patients submitted written informed consent.

HHV8 SEROLOGICAL TESTS

Four indirect immunofluorescence (IFA) and two enzyme-linked immunosorbent (ELISA) tests made by three different companies, i.e. Advanced Biotechnologies Incorporated (ABI, Columbia-USA), Biotrin-Diasorin (Dublin, Ireland) and Scimedx Corporation (Denville, New Jersey-USA) were utilized. IFA and ELISA tests made by ABI are for research use only, IFA and ELISA tests made by Biotrin-Diasorin and IFA by Scimedx Corporation have In Vitro Diagnostic-European Conformity marking. **ABI IFA test.** The HHV8 IgG antibody IFA Kit uses a mixture of HHV8 infected/induced KS-1 cell line derived from a body-cavity-based lymphoma (No longer commercially available). **Biotrin-Diasorin IFA test.** The Human Herpes Virus 8 IgG IFA Kit is based on human lymphocytes that express lytic viral antigens. **Scimedx Corporation IFA tests.**

The Human Herpesvirus 8 Lytic and Latent IFA Kits use human lymphocytes expressing lytic and latent HHV8 antigens, respectively. **ABI ELISA test.** The HHV8 IgG antibody ELISA Kit is made from a whole virus extracted derived from sucrose gradient purified HHV8 virions isolated from the KS-1 cell line derived from a body-cavity-based lymphoma. **Biotrin-Diasorin EIA test.** The Human Herpes Virus 8 IgG ELISA Kit uses lytic peptide epitopes derived from different viral proteins (No longer commercially available).

All tests were performed following manufacturer's instruction.

MOLECULAR TESTS

Quantitative real-time PCR assay (HHV8 Q-PCR Alert Kit, ELITech Group, Italy). Blood samples were collected in ethylenediaminetetraacetic acid-anticoagulated tubes. DNA was extracted from 200 µL of plasma and 100 µL of WB using the NucliSens easyMAG System (bioMerieux, Marcy l'Etoile, France) according to the manufacturer's instructions and eluted in 50 µL and 25 µL, respectively. An aliquot of 5 µL of these extracted DNA samples was used for the quantitative real-time PCR on the ABI Prism 7300 real-time PCR System (PE Applied Biosystem, Foster City, Calif, United States). The PCR assay targets gene KS330 codifying the HHV8 capsid protein. The analytical sensitivity of the assay is 10 copies of target DNA per amplification reaction. The lower limit of quantification of the assay is 500 copies/mL plasma and WB. ***HHV8 in-house nested PCR assay*.** DNA was extracted from 500 µL of plasma using QIAamp DNA Mini Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions and eluted in 40 µL. For nested PCR, 10 µL of these extracted DNA samples was used. The first round PCR used primers from ORF26, which generated a 233-bp PCR product.[12] The primer sequences were: sense 5'AGCCGAAAGGATTCCACCAT3', and antisense 5'TCCGTGTTGTCTACGTCCAG3'. The second round PCR used inners primers, which generated a 172-bp PCR product.[13] An aliquot of 5 µL of products from the first round PCR was re-amplified in the second round PCR. The primer sequences were: sense 5'GTGCTCGAATCCAACGGATT3' and antisense

5'ATGACACATTGGTGGTATAT3'. The PCR program for both the first and second round were: 95°C for 10 minutes, followed by 44 cycles of 95°C for 30 seconds, 58°C for 1 minute, 72°C for 1 minute and 30 seconds, followed by a 7-minutes extension at 72°C. The analytical sensitivity of the assay is 5 copies of target DNA per amplification reaction.

STATISTICAL ANALYSIS

Continuous variables are expressed as means (range), categorical as numbers (percentages). Fisher's exact test was used to compare differences in HHV8-seroprevalence in donors and recipients according with gender, place of birth and age. We evaluated assay accuracy in terms of sensitivity and specificity. Based on preliminary experiments, we considered as "true HHV8-positive" those patients in whom at least two of the assays tested positive.

RESULTS

VALIDATION OF THE HHV8 SEROLOGICAL ASSAYS

All results obtained by each IFA assay were evaluated by two different investigators. The samples were considered HHV8-positive or HHV8-negative when there was consistency between the two evaluations otherwise the samples were considered HHV8-border-line (BL). All twenty samples collected from the healthy adults resulted negative in each serologic test, but not all tests detected anti-HHV8 serum antibodies in the 20 KS cases. Based on this variable performance of the different serological HHV8 tests observed (data not shown), patients were considered HHV8-seropositive when samples resulted positive in at least two HHV8-specific assays (IFA and/or ELISA). Patients were HHV8-seronegative when samples resulted negative in all tests. Patients were considered HHV8-not defined when samples resulted positive and/or BL in one serologic test.

ENROLLMENT

Two hundred forty-nine solid organ donors and 517 patients undergoing kidney (n = 250), liver (n = 230) or heart (n = 37) transplant were enrolled. Study population's characteristics at baseline time are reported in Table 1.

SEROLOGICAL SCREENING AT BASELINE TIME

The results obtained using the six serological assays in donors and recipients are shown in Table 2.

SOT donors. Ten out of 249 (4%) donors were HHV8-seropositive; 235/249 (94.4%) were HHV8-seronegative and 4/249 (1.6%) were HHV8-not defined.

SOT recipients. Ninety-three out of 517 (18%) recipients were HHV8-seropositive; 414/517 (80.1%) were HHV8-seronegative and 10/517 (1.9%) were HHV8-not defined. HHV8 serostatus according to the type of organ transplanted is reported in Table 3.

SOT donor/recipient pairs. The serological results are reported in Figure 1.

Performance of HHV8 serological assays

The specificity and sensitivity (95% confidence intervals) of each serological test were calculated taking into account all the 766 serum samples tested at baseline time (Table 4). Analysis was performed considering the 14 HHV8-not defined samples to be HHV8 false-positive samples, since no HHV8 primary infections and no viral reactivations occurred among the recipients in Dnd/R-group and in the HHV8-not defined recipients, respectively.

Correlates of HHV8 seropositivity

Gender, place of birth (northern/central/southern Italy or other countries) and age at donation/transplantation (<50 and ≥ 50 years) were evaluated (Table 5).

VIROLOGICAL SCREENING AT BASELINE TIME

The results obtained using the two molecular assays are shown in Table 6.

SOT donors. Three out of the 10 HHV8-seropositive donors resulted positive by virological screening. Specifically, 1 out of 3 donors was positive for HHV8-DNA by both the two molecular assays performed on plasma sample and negative by quantitative real-time PCR assay performed on WB sample. This donor transmitted HHV8 infection to two out of their own 3 HHV8-seronegative recipients. The remaining two donors were positive for HHV8-DNA by in-house nested PCR test and negative by quantitative real-time PCR assay; one of these donors transmitted HHV8 infection to 1 HHV8-seronegative recipient.

SOT recipients. Ninety-three out of 517 recipients were serologically positive at baseline time. Two out of 93 HHV8-seropositive recipients had a viral reactivation in the post-transplant. One out of the 2 patients was positive for HHV8-DNA by both the two molecular assays performed on plasma sample; WB sample resulted negative. The other one was among the 88 HHV8-seropositive recipients who resulted negative by both molecular tests.

HHV8 INFECTION FOLLOW-UP

The outcomes in according to HHV8 serology were reported in Figure 1.

HHV8 primary infection. The D+/R- group included 4 liver, 7 kidney (5 single and 2 dual kidney transplantations) and one heart transplant recipients. Three of these patients (25%) developed primary HHV8 infection in the post-transplant period (average time of onset 88 days, range 40-180) (Table 7).

Patient 1: Forty days post-transplant, the patient was found positive for HHV8-DNA on routine monitoring, with no clinical symptoms HHV8-related. The patient died ten days later from postoperative complications.

Patient 2: Forty-four days post-transplant the patient was positive for HHV8 viremia (DNA load < 500 copies/mL), during routine monitoring. The HHV8-DNA in the clinical course increased, ranging from 1,330 to 3,650 copies/mL and immunosuppressive therapy was reduced. At 5.4

months post-transplant, a blood viral load of 13,100 copies/mL was detected and the patient was treated with cidofovir (first dose; 5 mg/Kg). Six months after transplantation the patient developed dyspnea, malaise and pancytopenia; the X-rays showed pleural effusion and a high blood viral load was detected (98,900 copies/mL). The pleural fluid sample was positive for HHV8-DNA (150,400 copies/mL). A total of six doses of cidofovir (single doses of 5 mg/Kg; the dosage was modified in relation to creatinine clearance) between 5.4 and 8 months after transplantation were administrated. At 8 months post-transplant, although high viral loads were present in blood and pleural fluid, the antiviral therapy had been suspended due to the very critical clinical condition of the patient. At 9 months, the immunosuppressive treatment with tacrolimus was also suspended and at 10 months post-transplant, the patient died of multiorgan failure. **Patient 3:** The patient had a HHV8 seroconversion six months after transplantation without concomitant or preceding HHV8 positive viremia. During the entire HHV8 post-transplant monitoring, the viral load was undetectable in all plasma samples.

HHV8 reactivation. Among the 93 HHV8-seropositive recipients, two patients (2.1%; liver and single kidney transplants) in the D-/R+ group had a viral reactivation (Table 7).

Patient 4: Thirteen days post-transplant, the patient was positive for HHV8 viremia (HHV8-DNA equal to 1,960 copies/mL). The plasma sample collected at baseline time was DNA positive, whereas the WB sample was DNA negative (see paragraph "virological screening at baseline time"). **Patient 5:** The patient developed KS with cutaneous lesions and visceral involvement 10 months post-transplant without concomitant HHV8 positive viremia. KS was controlled with reduction of immunosuppression in association with administration of liposomal doxorubicin. During the virological-clinical monitoring conducted in the next 24 months post-transplant, there were another 2 relapsing of cutaneous lesions associated to HHV8-KS.

HHV8 infection in Dnd/R- and Rnd groups. No HHV8-not defined patient (4 kidney, 5 liver and 1 heart transplant recipients) and no HHV8-seronegative patients with a donor HHV8-not defined

(5 kidney, 2 liver and 1 heart transplant recipients) had positive HHV8 viremia and/or HHV8 related diseases during post-transplant follow-up.

DISCUSSION

In the post-transplant setting, both HHV8 primary infection and reactivation have been associated with sometimes life-threatening neoplastic and non-neoplastic diseases.[2] To our knowledge, this is the first study which performs a prospective both serological and virological screening program for HHV8 in organ donors and in kidney, liver and heart transplant recipients. Six different commercial serologic assays - both IFA and ELISA test - and two molecular assays were utilized. HHV8 seroprevalence assessed in SOT donors and recipients in the ERR was equal to 4% and to 18%, respectively. According to the type of organ transplanted, the higher HHV8 seroprevalence was observed in liver (23.5%), followed by kidney (14.4%) and heart (8.1%) transplant recipients. The discrepancies in seroprevalence among donors and recipients may be related to the different geographic area of origin of the two populations. In fact, unlike most other human herpesviruses widely distributed, HHV8 is not ubiquitous and its distribution shows remarkable geographic variations.[14] In Italy, the prevalence varies between less than 10% in the north to more than 20% in the south, with the highest rates observed on the islands of Sicily and Sardinia.[10] In agreement with these data, in the overall study population, higher HHV8 seroprevalence was found among individuals born in southern Italy than among those born in central and northern Italy and the differences were statistically significant ($P < 0.01$). The 11.7% of donors and 41.1% of recipients were born in southern Italy and this may explain the different seroprevalence assessed in the two populations. Similarly, the higher HHV8 seroprevalence observed among liver transplant recipients may be related to the presence of a higher percentage of individuals born in southern Italy in this group of patients (39.6%), than in kidney (31.6%) and heart (27%) transplant recipients. Furthermore, among the liver transplant recipients there were six patients who were born outside of Italy, in geographic areas with very high (Côte d'Ivoire) and moderate (Peru) prevalence rates of HHV8 infection, that resulted HHV8-seropositive. HHV8-seropositivity was also associated with

the age at the time of donation/transplantation: HHV8 seroprevalence was higher among persons over 50 years old (P = 0.01). Conversely, there was no association between gender and HHV8-seropositivity (P = 0.82).

The results obtained by serological screening showed different performance of the six HHV8 serological tests at individual level. With regard to IFAs, sensitivity values ranging from 98% to 37.8% and specificity values ranging from 99.8% to 98.3% were obtained. In agreement with others studies,[15,16] among the four IFAs, the latent was the one with the lowest sensitivity (37.8%) and the highest specificity (99.8%). The ABI IFA test was the most sensitive for detecting HHV8-specific antibodies (98%). The 1.8% of the samples processed by IFAs were HHV8-not determined. These samples were evenly distributed between the two populations and within the transplant recipient population; their presence reflected the investigator's subjectivity to the interpretation of the results. With regard to ELISAs, the sensitivity value of ABI ELISA test was equal to 51.4% and the specificity was equal to 99.4%; the Biotrin-Diasorin ELISA test did not detected HHV8-specific antibodies in any sera samples. The latter findings are not in agreement with data published by other authors who reported that these two ELISAs were shown to have high sensitivities but poor specificities.[17] However, the study were performed in higher risk populations, i.e. AIDS patients, AIDS-KS patients and KS patients, compared to our patients. Therefore, the IFA tests, except for latent IFA test, were found to be more sensitive than the ABI ELISA test. The different performance of the six serological assays might be related to differences in antigen preparation. In light of these findings, we believe that the use of latent IFA or ELISA assays is not recommended in a HHV8 screening program due to their low sensitivity. In order to have a high sensibility and specificity, it is generally recommended to use both lytic and latent antigen assays.[15,16] Nevertheless, on the basis of the data obtained in our study, we believe that it might be sufficient to test serum samples only by a lytic antigen-based IFA assay, in particular with ABI IFA test or Biotrin-Diasorin IFA test, that showed to have both high sensitivity (> 92%) and very good specificity (> 98%). Virological screening for HHV8 at baseline time was carried out using

quantitative real-time PCR assay both on plasma and WB samples and HHV8 in-house nested PCR assay only on plasma samples. False-positive results were not obtained: the 649 HHV8-seronegative samples and the 14 not determined samples were negative for both molecular tests. Among the seropositive plasma samples, HHV8-DNA was detected in 6.8% and in 2.9% of cases by in-house nested PCR assay and quantitative real-time PCR assay, respectively. Comparing the performance of the two molecular assay, we confirmed that in-house nested PCR assay was more sensitive than quantitative real-time PCR assay (5 versus 10 copies/amplification reaction). Nevertheless, a very low correlation between the seropositivity of our population and the detection of HHV8-DNA blood was observed; virological screening is not recommended to perform. Of note, among the three donors that resulted positive by virological screening, two (66.7%) donors transmitted HHV8 infection to their own HHV8-seronegative recipients and among the 5 patients resulted positive by virological screening, one (20%) patient had a viral reactivation in the post-transplant period. These data suggest that the positivity for HHV8-DNA in plasma samples detected at the time of organ donation and transplantation, could maybe be a risk factor for the developing of primary infection and viral reactivation, respectively. On the basis of the screening results, patients identified at risk of developing HHV8-related disease, were rigorously monitored for at least two years post-transplant, since the risk of KS (the most common HHV8-related post-transplant complication) peaks during the first two years post-transplant and then decreases.[5,18] In the study population, the rate of seroconversion observed was equal to 25% (3/12; D+/R-) and the seroconversion occurred within 6 months post-transplant. One patient - a liver transplant recipient - developed a HHV8 primary infection associated with a severe clinical course, in fact a HHV8-associated nonmalignant illness characterized by pleural effusions, ascites and renal failure was found. In agreement with other studies,[2,5,19] the high HHV8-DNA levels detected in the patient's peripheral blood and effusions, predicted the occurrence of the HHV8-related non neoplastic disease. The HHV8 infection was fatal for the patient leading to multiorgan failure and death. Regarding the remaining two patients with primary infection, for one patient there was a limited

number of virological measurements since the patient died in the early post-transplant period; whereas the other one resulted HHV8-DNA negative during the entire post-transplant follow-up. The episode of seroconversion without HHV8 positive viremia was also observed by other authors[20,21] and might be due to viral replication episodes of short duration and with very low HHV8-DNA levels (below those detected by molecular assay used). This patient did not develop any HHV8-related symptoms and is currently alive and well. The rate of viral reactivation observed was equal to 2.1% (2/93 R+). In one case, 10 months after kidney transplantation the development of KS was observed. Several studies reported that this neoplastic disease, in kidney transplant recipients, is more frequently associated with pre-transplant positive HHV8 serology than with *de novo* HHV8 infection.[4,22,23] The patient developed KS without preceding or concomitant HHV8 positive viremia and very low HHV8-DNA levels, below the lower limit of quantification of the PCR assay, were observed during the entire virological follow-up after KS diagnosis. These findings are in line with other authors reporting that in HHV8-seropositive kidney transplant recipients, virological monitoring by PCR assay showed a low sensitivity (23.8%) in predicting development of post-transplant KS[4]. Furthermore, virological follow-up was found useful with regard to disease progression, time from diagnosis and disease staging, only in a proportion (40%) of transplant recipients with post-transplant KS.[24]

Finally, none of the patients in D-/R- group showed HHV-8 related symptoms during the clinical follow-up, although some received blood transfusions during the post-transplant period (data not shown). Studies on this HHV8 transmission pathway have produced contradictory results, providing evidence of transfusion transmitted infection in high but not in low prevalence countries.[25]

In conclusion, our data showed that serological diagnosis performed by lytic antigen-based IFA assays was the best methodological approach to assess the prevalence of HHV8 infection and therefore the best way to identify patients at risk to develop post-transplant HHV8-related disease. Seroconversion was a frequent event during post-transplant period (25%), higher than viral

reactivation (1.2%). A very low incidence (1.9%) of HHV8-related disease was observed among the patients identified at risk. However, in one liver transplant recipient HHV8 infection had a fatal clinical course.

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Table 1: Baseline characteristics of the study population

	RECIPIENTS				DONORS
	TYPE OF ORGAN TRANSPLANTED			TOTAL (%)	
	KIDNEY **BO - MO - PR transplant centers**	**LIVER** **BO - MO transplant centers**	**HEART** **BO transplant center**		**ERR**
Number of patients (%)	250 (48.3)	230 (44.5)	37 (7.2)	517	249
Gender					
male	167	171	28	366 (70.8%)	140 (56.2%)
female	83	59	9	151 (29.2%)	109 (43.8 %)
Mean age in years (range)	51.4 (20-74)	54 (18-70)	53 (19-70)	52.8	60 (12-89)
Place of birth*					
northern Italy	81	70	18	169 (38.6)	196 (78.7)
central Italy	28	27	6	61 (13.9)	13 (5.2)
southern Italy	79	91	10	180 (41.1)	29 (11.7)
other country	10	15	3	28 (6.4)	11 (4.4)

BO: Bologna; MO: Modena; PR: Parma; ERR: Emilia Romagna Region.

* Information was available for 438 out of 517 (84.7%) solid organ transplant recipients and for all donors.

Table 2: Results obtained by performing serological HHV8 screening in solid organ donors and recipients

Number of sera/total (%)		ABI IFA	BIOTRIN-DIASORIN IFA	SCIMEDX CORP.		ABI ELISA	BIOTRIN-DIASORIN EIA
				LYTIC antigens	LATENT antigens		
Seropositive donors 10/249 (4%)	4/10	+	+	+	+	+	-
	3/10	+	+	+	-	-	-
	1/10	BL	+	+	+	-	-
	1/10	+	BL	+	-	-	-
	1/10	BL	+	+	-	-	-
Seronegative donors 235/249 (94.4%)	235	-	-	-	-	-	-
Not defined donors 4/249 (1.6%)	3/4	BL	-	-	-	BL	-
	1/4	BL	-	-	-	-	-
Seropositive recipients 93/517 (18%)	17/93	+	+	+	-	-	-
	2/93	+	-	+	+	-	-
	24/93	+	+	+	+	+	-
	5/93	+	+	+	+	-	-
	11/93	+	+	-	-	-	-
	1/93	+	+	-	+	+	-
	1/93	+	+	-	+	-	-
	17/93	+	+	+	-	+	-
	1/93	+	-	+	+	+	-
	5/93	+	+	-	-	+	-
	1/93	+	-	+	-	+	-
	5/93	+	+	-	-	BL	-
	3/93	+	-	+	-	-	-
Seronegative recipients 414/517 (80%)	414/414	-	-	-	-	-	-
Not defined recipients 10/517 (2%)	2/10	BL	BL	BL	-	-	-
	5/10	BL	-	-	-	-	-
	1/10	-	-	BL	BL	BL	-
	2/10	-	-	BL	-	-	-

+: positive; - : negative; BL: border-line.

Table 3: HHV8 serostatus according to the type of organ transplanted

TYPE OF ORGAN TRANSPLANTED	NUMBER OF PATIENTS	SEROPOSITIVE (%)	SERONEGATIVE (%)	NOT DEFINED (%)
Kidney	250	36 (14.4)	210 (84)	4 (1.6)
Liver	230	54 (23.5)	171 (74.3)	5 (2.2)
Heart	37	3 (8.1)	33 (89.2)	1 (2.7)

Table 4: Sensitivity and specificity of each serological tests

TEST	SENSITIVITY %	95% CI	SPECIFICITY %	95% CI
ABI IFA	98.0	93.1 – 99.7	98.3	97.0 – 99.1
BIOTRIN-DIASORIN IFA	92.2	85.3 – 96.6	99.7	98.9 – 99.9
SCIMEDX CORP. LYTIC IFA	77.6	68.4 – 85.3	99.2	98.2 – 99.7
SCIMEDX CORP. LATENT IFA	37.8	28.5 – 48.0	99.8	99.1 – 100
ABI ELISA	51.4	41.4 – 61.4	99.4	98.4 – 99.8
BIOTRIN-DIASORIN ELISA	nd	nd	nd	nd

95% CI: 95% confidence intervals; nd = not determinable, all samples resulted negative.

Table 5: Prevalence and correlates of HHV8-specific antibodies in solid organ transplant donors and recipients at baseline time

	Number of positive sera/total	**% of positive sera**	***P*** values*
Gender			
male	67/506	13.2	0.82
female	36/260	13.8	
Place of birth**			
northern Italy	32/365	8.7	< 0.01
central Italy	10/74	13.5	
southern Italy	43/209	20.5	
other country	8/39	20.5	
Age (in years)			
< 50	29/304	9.5	0.01
≥ 50	74/462	16	

* *P* values were computed with Fisher's exact test.
** Information was available for all 10 HHV8-seropositive donors and for 83 out of 93 (89.2%) HHV8-seropositive recipients.

Table 6: Results obtained by virological HHV8 screening performed in solid organ transplant donors and recipients

	HHV8 SEROSTATUS	NUMBER OF PATIENTS		QUANTITATIVE REAL-TIME PCR		IN-HOUSE NESTED PCR
				WHOLE BLOOD copies/mL	PLASMA copies/mL	PLASMA
DONORS n. 249	seropositive	10	1	-	Positive < 500	+
			2	-	-	+
			7	-	-	-
	not defined	4	4	-	-	-
	seronegative	235	235	-	-	-
RECIPIENTS n. 517	seropositive	93	1	Positive < 500	Positive < 500	-
			1	-	Positive < 500	+
			3	-	-	+
			88	-	-	-
	not defined	10	10	-	-	-
	seronegative	414	414	-	-	-

+: positive; - : negative.

Table 7: Patients who developed HHV8 infection during post-transplant period

PT	Age	Sex	HHV8 serostatus D/R	Organ transplanted	Etiology	Quantitative real-time PCR copies/mL plasma viral load peak	Onset of Infection Days post-TX	HHV8 related disease	Outcome
1	67	M	+/-	dual kidney	ESRD	positive < 500	40	none	death
2	59	M	+/-	liver	HCC	188,550	44	nonmalignant illness (pleural effusions, ascites, renal failure)	death
3	65	F	+/-	single kidney	renovascular disease	not detected	180	none	alive
4	60	F	-/+	liver	HCV-related liver cirrhosis	1,960	13	none	alive
5	62	F	-/+	single kidney	NAS	positive < 500	330	KS	alive

PT: patient; M: male; F: female; ESRD: end stage renal disease; HCC: hepatocellularcarcinoma; HCV: hepatitis C virus; NAS: nephroangiosclerosis; KS: Kaposi's sarcoma; +: positive; - : negative.

Figure 1: HHV8 serostatus in Donor/Recipient (D/R) pairs at baseline time and outcomes.

Patients were considered: *i)* HHV8-seropositive (+) when samples resulted positive in at least two HHV8-specific assays; *ii*) HHV8-seronegative (-) when samples resulted negative in all tests and *iii*) HHV8-not defined (nd) when samples resulted positive by one serologic test and/or border-line.