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Immune profiling in solid organ transplant recipients with HHV-8 infection: Identification of immunological biomarkers for KICS and Kaposi's sarcoma

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ABSTRACT

Human Herpesvirus 8 (HHV-8) poses a significant risk in solid organ transplant recipients (SOTRs). HHV-8 is implicated in both neoplastic and non-neoplastic conditions. This study investigates immune dysregulation in HHV-8-infected SOTRs by analysing cytokine profiles and virus-specific T cell responses across different clinical manifestations. Our findings reveal a progressive decline in HHV-8-specific T cell responses correlating with disease severity, alongside a distinct cytokine signature. KICS patients exhibit heightened inflammation with elevated IL-6, IL-10, IFNα, TNFα, IL-1β, IL-17 A, IDO, sCD14, and immune exhaustion markers (PD-1, LAG-3), whereas KS is associated with angiogenic and macrophage activation factors (HGF, CD163). Given these insights, monitoring HHV-8 DNAemia, inflammatory cytokines, and T cell functionality is crucial for early detection and risk stratification. This study underscores the importance of immune monitoring in transplant recipients, paving the way for targeted interventions to mitigate HHV-8-associated complications.

1. Introduction

Human Herpesvirus 8 (HHV-8) infection in immunocompetent individuals often remains asymptomatic throughout life. However, in immunocompromised patients, particularly solid organ transplant recipients (SOTRs), undergoing long-term immunosuppressive therapy, HHV-8 can pose a significant risk. HHV-8, also known as Kaposi's Sarcoma-associated Herpesvirus (KSHV), is the etiological agent of both neoplastic and non-neoplastic disease. Kaposi's sarcoma (KS) is a malignant vascular tumour that predominantly affects immunocompromised individuals, including transplant recipients receiving chronic immunosuppression [1,2]. Less common neoplastic diseases associated with HHV-8 include lymphoproliferative diseases, such as primary effusion lymphomas and multicentric Castleman disease, which primarily occur in immunodeficiency virus-infected subjects [3]. Among non-neoplastic diseases, the clinical manifestations of HHV-8 infection in transplant patients range from asymptomatic viremia (DNAemia) to severe and life-threatening conditions such as Kaposi's Sarcoma Inflammatory Cytokine Syndrome (KICS) and KS [4]. KICS is an emerging and potentially fatal disorder that closely mimics cytokine release syndrome (CRS). This cytokine storm exacerbates pre-existing complications in transplant recipients, significantly increasing morbidity and mortality while complicating clinical management [5,6]. Given its severe impact, early identification and targeted intervention are crucial. In

Abbreviations: CD, cluster of differentiation; CNI, calcineurin inhibitor; D, donor; HHV-8, human herpesvirus-8; HIV, human immunodeficiency virus; IL, interleukin; ISMETT, Mediterranean Institute for Transplantation and Advanced Specialized Therapy; KICS, Kaposi sarcoma herpesvirus-associated inflammatory cytokine syndrome; IFN, interferon; KS, Kaposi sarcoma; mTOR, mammalian target of rapamycin; R, recipient; SOT, solid organ transplant..

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contrast, KS is a neoplastic disorder driven by HHV-8-mediated endothelial cell proliferation and angiogenesis [7,8]. The disease is characterized by the development of multiple violaceous skin lesions, mucosal involvement, and visceral organ dissemination, with a more aggressive clinical course in immunocompromised hosts. In transplant recipients, KS progression is influenced by the degree of immunosuppression and the specific immunosuppressive regimen used. In addition, the switch of immunosuppressive regimen from Calcineurin inhibitors (CNIs) to mTOR inhibitors (mainly sirolimus) has been reported to induce dramatic KS regressions [2,9,10], together with the recovery of HHV-8-specific T cells [11]. Due to the potentially severe clinical consequences of HHV-8-related complications, early identification of primary HHV-8 infection, whether donor- or non-donor-derived, is essential. This can be achieved through monitoring of viral DNA load (PCR for HHV-8 DNAemia) and assessment of inflammatory markers, such as interleukin-6 (IL-6) and interleukin-10 (IL-10), enabling timely and appropriate intervention [6,12]. Management strategies often involve modifying immunosuppressive therapy, such as switching from CNIs to mTOR, as well as administering antiviral agents like cidofovir or foscarnet. Additionally, targeted immunotherapies, including rituximab (anti-CD20 monoclonal antibody) and IL-6 inhibitors (*e.g.*, tocilizumab), have shown promise in treating KICS and other HHV-8-associated conditions [5,6]. Expanding upon our previous findings on HHV-8 incidence and clinical outcomes [6], this study aimed to identify diagnostic and predictive biomarkers associated with HHV-8-related conditions by evaluating cytokine profiles and specific T cell responses across clinical groups (asymptomatic, KICS, and KS). Our results revealed a progressive decline in HHV-8-specific T cell responses, indicative of immune dysfunction, along with a strong inflammatory signature in KICS that closely resembled cytokine storms observed in other severe infections [13,14]. Furthermore, KS patients exhibited significant T cell exhaustion and tumour-associated macrophages (TAMs) activation, further emphasizing the role of immune dysregulation in HHV-8-associated diseases. These findings underscore the importance of immune monitoring in HHV-8-related conditions and suggest potential biomarkers and therapeutic targets to improve clinical management in transplant recipients.

2. Materials and methods

2.1. Subjects studied

This retrospective study was conducted at IRCCS-ISMETT using biobanked samples from 14 transplant recipients who developed post-transplant HHV-8 DNAemia. A total of 16 episodes were included in the analysis, as two patients experienced both KICS and KS at different time points and were therefore counted separately for each clinical manifestation. Among them, 10 patients acquired the infection from the donor (pre-transplant serological mismatch, D+/R-), 2 patients developed a non-donor-derived primary infection (D−/R-), and 2 experienced viral reactivations (R+). The clinical characteristics, management, and outcomes of this population have already been described in a recently published study by our group [6]. All study participants were confirmed to be HIV-negative at the time of inclusion. Patients with detectable HHV-8 DNAemia were categorized into three groups based on their clinical manifestations (16): six patients showed detectable DNAemia but remained asymptomatic (D), six developed KICS (K), and four were diagnosed with Kaposi's Sarcoma (KS), as detailed in Table 1. Notably, two patients experienced both KICS and KS at different time points, though not simultaneously. In our cohort, KICS was defined according to the working case definition of KICS proposed by Polizzotto et al. [5].The criteria required for the diagnosis of KICS were: 1) an elevated HHV-8 plasma viral load (> 1000 cp/mL), 2) increased C-reactive protein level (higher than 3 g/dL), 3) no evidence of multicentric Castleman disease (MCD) upon histologic examination of lymphadenopathy if present, 4) and at least two clinical abnormalities from three categories (clinical symptoms, laboratory abnormalities, radiographic abnormalities: lymphadenopathy, hepatomegaly, splenomegaly). All patients with KICS included in our cohort met the criteria for KICS diagnosis, and MCD was ruled out by biopsy whenever lymphadenopathy was present. The mean age of group D (asymptomatic) was 58.2 years (range: 49–64 years), and all of them had an HHV-8 serological mismatch (D+/R-) after liver transplantation, and no one died for HHV-8 attributable causes. The KICS group showed a mean age of 57.2 years (range: 41–65 years); 5/6 of them developed HHV-8 infection after liver transplant and 1/6 after lung transplant. Within this group, 4 had an HHV-8 serological mismatch (D+/R-), while 2 liver transplant recipients developed a non-donor-derived primary infection (D−/R-). One lung transplant recipient with mismatch (D+/R-), first developed a disseminated KS and later experienced KICS, and died of progressive KS. Finally, the KS group showed a mean age of 53 years (range: 41–63 years). This group includes 2 R+ heart transplant recipients with viral reactivation that died due to KS, 1 lung transplant recipient with mismatch that had KS and KICS with fatal outcome as described above, and the remaining patient was a liver transplant recipients who developed a primary non-donor-derived infection that progressed to KICS and subsequently KS with survival.

The study was approved by our Institutional Review Board (IRRB/37/19) and conducted following the guidelines of the Declaration of

Table 1
Classification of patients based on clinical manifestations.

	Patient ID	Age	Gender	Tx	D/R matching	Switch to mTor inhibitor	Ohter therapies	Attributable death
Asymptomatic	KSHV_157	64	M	Liver	D+/R-	Yes	NA	No
	KSHV_62	56	M	Liver	D+/R-	Yes	NA	No
	KSHV_179	49	M	Liver	D+/R-	Yes	NA	No
	KSHV_176	60	M	Liver	D+/R-	Yes	NA	No
	KSHV_100	64	M	Liver	D+/R-	Yes	NA	No
	KSHV_195	56	F	Liver	D+/R-	Yes	NA	No
KICS	KSHV_92	57	F	Liver	D+/R-	Yes	Rituximab + Foscarnet	No
	KSHV_114	62	F	Liver	D+/R-	Yes	Rituximab + Foscarnet	No
	KSHV_177	41[#]	M	Lung	D+/R-	Yes	Rituximab + Foscarnet	Yes
	KSHV_190	51*	M	Liver	D-/R-	Yes	Rituximab + Foscarnet	No
	KSHV_196	65	M	Liver	D-/R-	Yes	Rituximab + Foscarnet	No
	KSHV_204	65	M	Liver	D+/R-	Yes	Rituximab + Foscarnet	No
KS	KSHV_177	41[†#]	M	Lung	D+/R-	Yes	CHT	Yes
	KSHV_190	51*	M	Liver	D-/R-	Yes	CHT	No
	KSHV_146	63[†]	F	Heart	R+	Yes	CHT	Yes
	KSHV_61	55[†]	M	Heart	R+	No	CHT	Yes

This table presents detailed information on patient age, sex, type of transplant, pattern of viral transmission, immunosuppressive and/or other therapies, and HHV-8–attributable mortality. The patient marked with # developed Kaposi Sarcoma (KS) first, followed by KSHV Inflammatory Cytokine Syndrome (KICS), whereas the patient marked with * experienced KICS prior to KS (in this case, KS resulted from HHV-8 reactivation). The † symbol denotes a deceased patient. [D = donor; R = recipient; CHT = chemotherapy].

Helsinki. All patients provided written informed consent for the use of their anonymized data for research purposes.

2.2. Cytokine assay

Our primary objective was to characterize immunological differences among D, K, and KS SOTRs. We retrospectively analysed a panel of 59 cytokines, chemokines, and immune checkpoint markers in plasma samples of 16 cases of transplant recipients with detectable HHV-8 DNAemia. The selected time points were the first detected DNAemia, the peak of DNAemia, and the declining phase or resolution. Luminex™ magnetic bead technology was used with the ProcartaPlex Human Magnetic Luminex Kits (Affymetrix, Wien, Austria). The panels contained a total of 59 analytes: IFNα, IFNγ, IL-1β, IL-1RA, IL-2, IL-4, IL-6, IL-8 (CXCL8), IL-9, IL-10, IL-17 A (CTLA-8), IP-10 (CXCL10), PTX3, TNFα, Arginase-1, B7-H6, BTLA, CD134 (OX40), CD137 (4-1BB), CD14, CD152 (CTLA4), CD163, CD27, CD276 (B7-H3), CD28, CD47 (IAP), CD48 (BLAST-1), CD73 (NT5E), CD80, CD96 (Tactile), E-cadherin, Fractalkine (CX3CL1), GITR, HGF, HVEM, ICOS ligand (B7-H2), IDO, LAG-3, MICA, MICB, MR-proADM, nectin-2, PD-1, PD-L1, PD-L2, perforin, PVR, S100A8/A9 (calprotectin), SAA, siglec-7, siglec-9, TIM-3, TIMD-4, TREM-1, uPAR (CD87), ULBP-1, ULBP-3, ULBP-4, and VISTA (B7-H5). The assays were conducted according to the manufacturer's instructions and analysed using Luminex™ xMAP™ INTELLIFLEX System (Luminex Corporation, Austin, TX, USA). Data were expressed as mean fluorescence intensity (MFI), rather than concentration to increase statistical power as previously described [15].

2.3. ELISpot assay

We analysed HHV-8-specific T cell response in SOTRs using ELISpot assays. Responses to four major HHV-8 antigens (LANA, K8, K12, and gB) were assessed at the peak of DNAemia, comparing each other's D, K and KS patients. Peripheral blood mononuclear cells (PBMCs) were isolated from blood samples collected in BD Vacutainer® CPT™ tubes using density gradient centrifugation within four hours of venipuncture. Following isolation, PBMCs were counted using the Sysmex XN-2000™ Hematology System to assess cell number and viability both pre-freezing and post-thaw. The cellular immune response was evaluated through an interferon-γ (IFNγ) enzyme-linked immunospot (ELISpot) assay to quantify antigen-specific T cells, as previously described [16]. ELISpot assays were conducted using thawed PBMCs, resuspended in RPMI-1640 medium supplemented with 20 % fetal bovine serum (FBS) and 1 % L-glutamine at a concentration of $2.5 \pm 0.5 \times 10^6$ cells/mL. A 96-well ELISpot strip plate pre-coated with monoclonal IFNγ antibodies (Human IFNγ ELISpot PLUS Kit ALP, Mabtech, Stockholm, Sweden) was used, and 100 μL of each PBMC sample was added to duplicate wells. Cells were stimulated overnight under various conditions: complete medium as a negative control, complete medium containing 1 μg/mL anti-human CD3 monoclonal antibody as a positive control (included in the Mabtech kit), complete medium containing 1 μg/mL of a CEFX PepMix (a pool of 176 known peptides from various infectious agents, JPT Peptide Technologies, Germany), and complete medium containing 1 μg/mL of peptide pools specific for HHV-8 antigens, including latent (LANA, 106 peptides; K12, 13 peptides) and lytic (K8.1, 60 peptides; gB, 236 peptides) proteins (See Supplementary Table 1). The assay was performed in duplicate, stimulating $2.5 \times 10^5 \pm 0.5 \times 10^5$ PBMCs/mL for 20–22 h at 37 °C in a 5 % CO_2 humidified atmosphere, with 1 μg/mL overlapping peptides spanning the HHV-8 antigens (15-mers with 11 amino acid overlaps, purity >90 %, JPT Peptide Technologies, Germany). Following stimulation, IFNγ-secreting T cells were quantified using an ELISpot Reader (Autoimmun Diagnostika GmbH, Straßberg, Germany) and analysed with ELISpot 7.0 Software (AID). Results were expressed as the number of IFNγ spot-forming cells (SFC), normalized to 10^6 PBMCs after background subtraction, and averaged from duplicate wells. A positive response cut-off was determined by calculating the mean response of unstimulated wells plus two standard deviations (SDs), resulting in a threshold of 50 SFC/10^6 PBMCs. These methods allowed for the serial monitoring of HHV-8-specific T cell responses, providing insights into the immune response to both lytic and latent viral antigens.

2.4. Statistical analysis

Microsoft Office Excel (version 2305) was used for data collection. Statistical analyses were performed with R (version 4.2.3) and Graph Pad Prism (version 9.0). Depending on the type of samples being compared, the Wilcox test, the Mann-Whitney test, or Kruskal-Wallis test with Dunn's multiple comparisons were used ($p < 0.05$ was considered significant). Correlations were evaluated using Pearson's rank correlation coefficient. Statistical significance levels were defined as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$. The cytokines heatmap was generated using the R package pheatmap (version 1.0.12), with cytokine values standardized through z-score transformation, expressed as MFI, and hierarchical clustering applied to classify both patients and cytokines.

3. Results

3.1. Cytokine and immune marker profiling in HHV8-positive SOTRs

Our comprehensive analysis aimed to define the distinct immune profiles associated with each condition, providing insights into immune activation, tumour progression, and viral persistence. The heatmap in Fig. 1A presents a hierarchical clustering of the analysed cytokines in our HHV-8-infected SOTRs cohorts. Patients are categorized based on clinical manifestation into three groups: asymptomatic with active DNAemia (D), KICS (K), and KS. Additionally, the heatmap includes annotations for DNAemia levels, distinguishing between low ($<10^3$ HHV8-DNA copies/mL), intermediate (10^3–10^5 HHV8-DNA copies/mL), and high ($>10^5$ HHV8-DNA copies/mL) viral loads. Cytokine clustering primarily follows DNAemia levels rather than clinical manifestation, with higher viremia ($>10^5$) predominantly observed in K patients, while distinct cytokine subgroups are associated with specific disease states, suggesting their potential as biomarkers for disease progression. High-DNAemia patients, especially those with KICS and KS, show increased pro-inflammatory cytokines (IL-6, TNFα, IL-1β, IFNγ). KS is linked to angiogenic factors (VEGF, HGF, IL-8) and monocyte/macrophage activation (sCD163), while K shows elevated IL-10, IL-1RA, IDO, and PD-1, indicating immune suppression and chronic inflammation. On the other hand, low-DNAemia patients show relatively lower levels of inflammatory cytokines, indicating controlled immune responses. Overall, DNAemia drives cytokine patterns, with distinct inflammatory, angiogenic, and immunosuppressive signatures defining K and KS. The box plot analysis (Fig. 1B) shows the statistically significant differences observed across the groups during the peak of DNAemia. K group exhibited significantly ($p < 0.05$) elevated levels of IL-10, IL-6, IFNα, LAG-3, PD-1, CD48, TNFα, IL-1β, IL-17 A, IDO, sCD14, MIC-B, ULBP-4, and VISTA compared to D group, indicating a state of pronounced immune activation. Additionally, when compared to KS, K patients showed significantly ($p < 0.05$) higher levels of IL-10, IL-6, IFNα, LAG-3, PD-1, and CD48, further supporting the notion that KICS is characterized by heightened immune activation and T cell exhaustion, in stark contrast to the tumour-promoting environment of KS. Conversely, KS patients exhibited a more immunosuppressive profile compared to both K and D. Specifically, KS was associated with significantly higher levels of CD163 ($p < 0.05$) and HGF ($p < 0.05$), two markers linked to macrophage activation, tissue remodelling, and angiogenesis, key processes driving KS pathogenesis. The elevated levels of these factors in KS underscore their role in tumour progression rather than in immune activation. At last, we detected the correlations between all analysed cytokines and viremia levels at all-time points (first DNAemia, peak of DNAemia, and

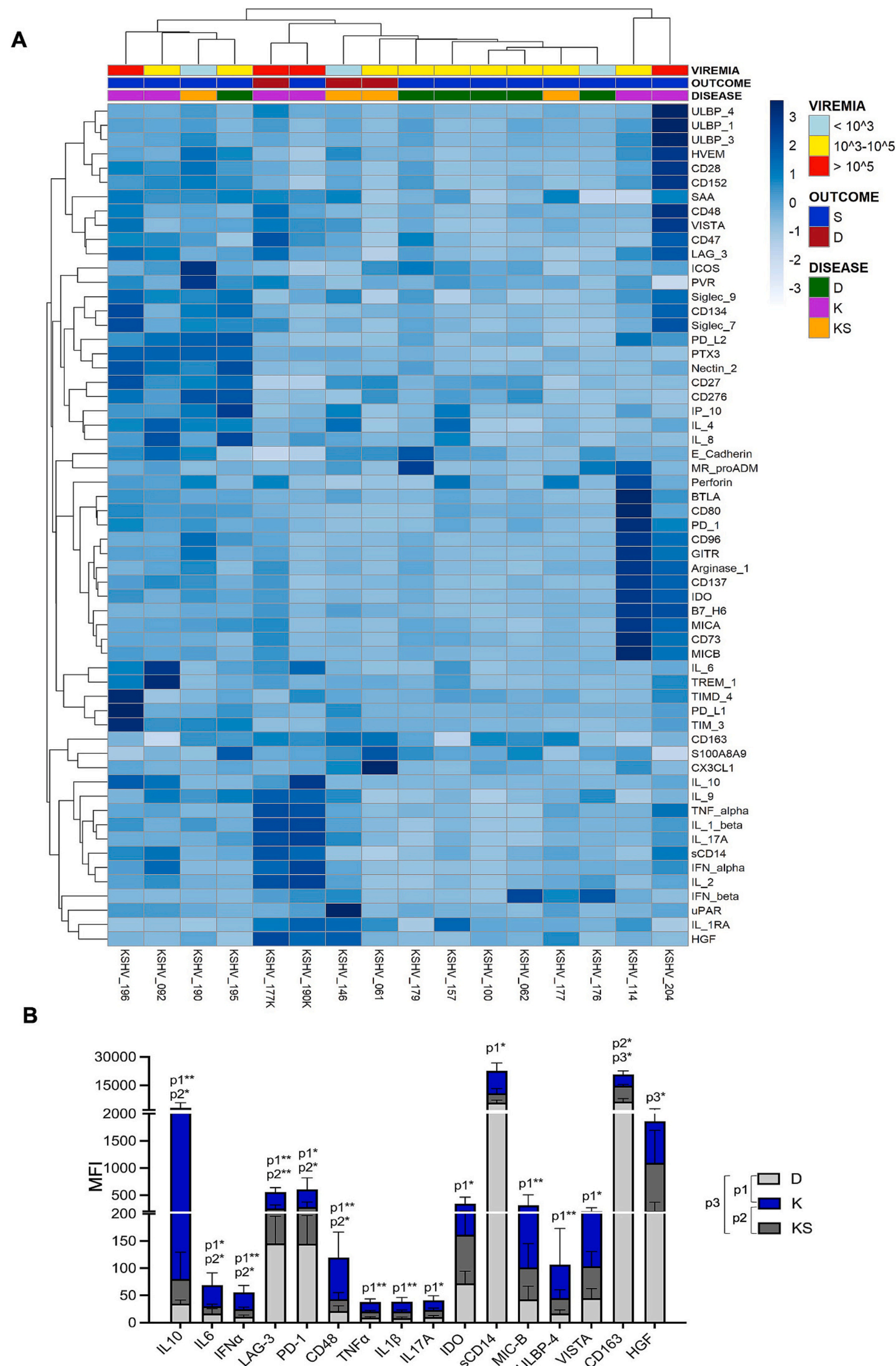
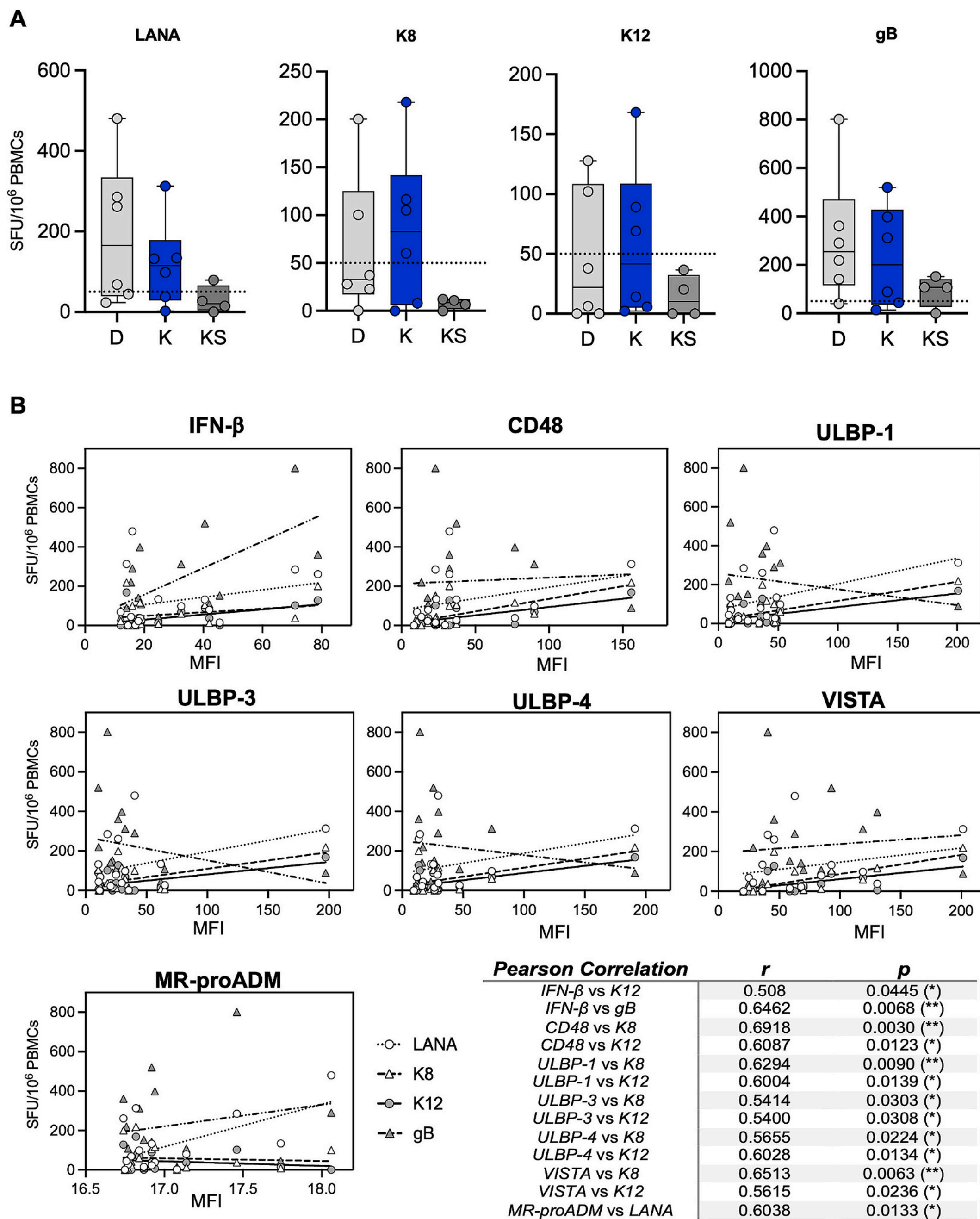


Fig. 1. (A) The Heatmap represents a hierarchical analysis of 59 cytokines in HHV8-infected SOTRs. Rows represent different cytokines; each column represents one patient; hierarchical clustering highlights similar expression patterns among patients and cytokines. Shades of blue indicate cytokine expression levels, ranging from low to high values. (B) Plasma levels of only significant cytokines during higher DNAemia in patients asymptomatic (D), with KICS (K), and with Kaposi sarcoma (KS). p1 = K *vs* D; p2 = *K vs KS*; p3 = D *vs* KS. * $p < 0.05$, ** $p < 0.01$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the declining phase) across all patients. The results revealed significant associations between viral load and immune responses (Supplementary Table 2). Particularly, we observed strong correlations ($r > 0.5$, $p < 0.05$) between DNAemia and IFNα, IL-1β, IL-2, IL-6, IL-10, IL-17 A, TNFα, PD-L1, TIMD-4, and sCD14, supporting the notion that the immune response to HHV-8 infection is closely tied to viral replication. Additionally, moderate correlations ($r < 0.5$, $p < 0.05$) were found with IL-9, PTX3, CD47, LAG-3, and VISTA, suggesting the involvement of further immune regulatory mechanisms, potentially resembling those seen in EBV infections, where immune evasion plays a key role in viral persistence [17].

3.2. ELISpot assay analysis of T-cell responses to HHV-8 antigens

To further explore immune control mechanisms, we analysed HHV-8-specific T cell responses in our studied cohorts using ELISpot assays. The plot in Fig. 2A illustrates responses to four major HHV-8 antigens (LANA, K8, K12, and gB) during the peak DNAemia, comparing D, K,



Pearson Correlation	r	p
IFN-β vs K12	0.508	0.0445 (*)
IFN-β vs gB	0.6462	0.0068 (**)
CD48 vs K8	0.6918	0.0030 (**)
CD48 vs K12	0.6087	0.0123 (*)
ULBP-1 vs K8	0.6294	0.0090 (**)
ULBP-1 vs K12	0.6004	0.0139 (*)
ULBP-3 vs K8	0.5414	0.0303 (*)
ULBP-3 vs K12	0.5400	0.0308 (*)
ULBP-4 vs K8	0.5655	0.0224 (*)
ULBP-4 vs K12	0.6028	0.0134 (*)
VISTA vs K8	0.6513	0.0063 (**)
VISTA vs K12	0.5615	0.0236 (*)
MR-proADM vs LANA	0.6038	0.0133 (*)

Fig. 2. (A) ELISpot assay analysis of HHV-8-specific T-cell responses in SOTRs, categorized by clinical outcome, during peak DNAemia. The patient groups include D (asymptomatic), K (KICS), and KS (Kaposi's sarcoma). T-cell responses were evaluated against four major HHV-8 antigens (LANA, K8, K12, and gB). Each graph depicts the magnitude of the immune response in individual patients, measured by the number of spot-forming cells, which reflects the frequency of antigen-specific T cells. The dashed horizontal line represents the cutoff for a positive response. A stronger T-cell response suggests more effective immune control, while weaker responses may indicate immune dysfunction, exhaustion, or suppression associated with disease progression. (B) Correlations between HHV-8-specific T-cell responses and cytokine levels in HHV-8 infected SOTRs during peak of DNAemia. Scatter plots display correlations between T-cell responses to HHV-8 antigens (LANA, K8, K12, gB) and cytokine levels across all patient groups (asymptomatic viremia, KICS, and KS). Each point represents an individual patient. Statistically significant correlations are highlighted, with cytokine expression levels plotted on the x-axis and T-cell responses on the y-axis.

and KS groups of patients. The strongest responses were observed in the D group, those without clinical manifestation, with a majority of individuals mounting an immune reaction above the cut-off, suggesting effective immune control that prevents disease progression despite persistent viral replication. In contrast, the K group exhibited a weaker response, with fewer individuals reaching the threshold for a significant T cell reaction, reflecting partial immune dysfunction, which, combined with excessive cytokine production, may contribute to the inflammatory nature of the syndrome. At last, KS group displayed the most impaired responses, with most individuals failing to exceed the cut-off, indicative of profound immune suppression or exhaustion, further supporting the notion that inadequate T cell immunity plays a crucial role in tumour development. These findings reinforce the importance of HHV-8-specific cellular immunity in modulating disease outcomes, with a progressive decline in T cell responsiveness correlating with increasing clinical severity. Although statistical significance was not reached, likely due to the small sample size and inter-individual variability (immunosuppressive therapy, genetic diversity, and variations in viral load), the

observed trends suggest biologically relevant differences. To gain a broader immunological perspective, we investigated correlations between HHV-8-specific T cell responses and cytokine levels across the three patient groups during the peak of DNAemia, focusing exclusively on statistically significant associations (Fig. 2B). This analysis aimed to identify key immune modulators influencing antiviral immunity, immune activation, and immune evasion. Significant correlations revealed that higher IFNβ levels were associated with stronger HHV-8-specific T cell responses, suggesting that type I interferon signalling may enhance antiviral immunity. CD48 expression correlated with K8/K12-specific T-cell responses, indicating its role in immune regulation and the prevention of excessive exhaustion. Similarly, NKG2D ligands (ULBP-1, ULBP-3, and ULBP-4) showed positive correlations with T cell responses, highlighting their involvement in HHV-8 immune surveillance. In contrast, elevated VISTA expression was linked to a weaker T cell response, supporting its function as an immune checkpoint inhibitor that may contribute to viral persistence. Additionally, MR-proADM levels correlated with LANA-specific T cell responses, suggesting a potential link between immune regulation and endothelial dysfunction.

3.3. Longitudinal dynamics of IFNγ-producing T cells and viremia in HHV-8-associated KICS and Kaposi's sarcoma

To explore how immune responses evolve over time in HHV-8-associated diseases, we analysed IFNγ-producing T cells and viral load in three transplant recipients with different clinical outcomes. This exemplificative approach aimed to highlight the link between T cell dynamics, viral control, and disease progression. Longitudinal profiling of IFNγ-producing T cells, alongside viral load, reveals distinct immunological trajectories in HHV-8-infected transplant recipients with KICS and/or Kaposi sarcoma. In the non-fatal case (Fig. 3A), the patient developed KICS with a peak in viremia at T1 (grey area), accompanied by gB- and LANA-specific T cell responses, reflecting active recognition of both lytic and latent antigens. The administration of rituximab at this point led to rapid clearance of viremia and a contraction of all antigen-specific T cells, consistent with B cell depletion impairing antigen presentation. Everolimus, introduced early, may have helped preserve T cell function during the initial immune activation. In a fatal case (Fig. 3B), a biphasic viremia pattern emerged, first at T2 (Kaposi diagnosis), then at T5 (KICS), each associated with marked expansions of gB- and K8-specific T cells, indicative of strong responses to lytic viral replication. However, sequential treatment with doxorubicin, foscarnet, and rituximab resulted in a collapse of all T cell responses. LANA-specific T cells remained modest throughout, and K12 responses were minimal, suggesting limited immune engagement with latent antigens. This transient activation, followed by deep immune suppression, paralleled the patient's clinical deterioration. In the third case (Fig. 3C), KICS occurred first, with a gB-specific T cell peak and high viremia at T0. Subsequent viral control preceded KS onset at T2, at which point LANA-specific T cells gradually expanded, suggesting delayed development of tumour-specific immunity. This suggests a potential protective role of latent antigen-directed responses. Across all cases, gB-specific T cell dynamics closely track lytic activity, while LANA responses may mark effective immune surveillance. These findings underscore the utility of longitudinal IFNγ+ T cell monitoring to assess antiviral and antitumor immunity and to guide immunomodulatory strategies in HHV-8-associated disease post-transplant.

4. Discussion

The observed cytokine profiles suggest a progression of HHV-8-related diseases, transitioning from immune activation in KICS-affected patients to immune exhaustion in KS ones. High-viremia cases are characterized by a pro-inflammatory cytokine milieu, while low-DNAemia patients show relatively lower levels of inflammatory cytokines, indicating controlled immune responses. This suggests that asymptomatic individuals maintain a balance between immune surveillance and viral control, reinforcing the idea that viral reactivation alone is not sufficient to trigger severe disease manifestations without concurrent immune dysregulation. On the other hand, K patients experience a breakdown in immune regulation, resulting in a state of hyperinflammatory activation characterized by elevated levels of IL-6, IL-10, TNFα, IDO, sCD14, and IFNα. This cytokine storm closely resembles CRS observed in severe SARS-CoV-2 infections, where excessive immune activation leads to systemic inflammation and multi-organ dysfunction [13,14]. However, despite this heightened immune response, K patients exhibit features of immune exhaustion, as indicated by the upregulation of PD-1 and LAG-3, molecules known to impair T cell function and promote viral persistence [17]. HHV-8 appears to exploit immune evasion mechanisms similar to those of Epstein-Barr virus (EBV), further sustaining viral replication [17]. The involvement of CD48 and NKG2D ligands in immune activation underscores the crucial role of NK and T cell responses in HHV-8 surveillance [18–21], suggesting that disruption of these pathways could contribute to disease progression. Functional analyses showed a decline in HHV-8-specific T cell responses, strongest in asymptomatic patients and weakest in K and KS ones, reflecting immune exhaustion. This supports the role of cellular immunity in disease progression, with T cell impairment correlating with severity. Although statistical significance was limited by sample size and interindividual variability, the observed trends suggest biologically relevant differences. IFNβ enhanced HHV-8-specific T cell responses, indicating a potential therapeutic role for type I interferons [22–24]. Elevated PD-1, LAG-3, and VISTA expression in K patients further suggests immune exhaustion and HHV-8 immune evasion [22–24]. Additionally, MR-proADM links HHV-8 immunity to systemic inflammation and endothelial dysfunction, supporting its use as a severity biomarker [25]. Building on these insights, longitudinal analysis of IFNγ-producing T cells and viremia reveals how the timing and strength of antiviral responses influence disease course. Coordinated T cell activity was associated with viral control and better outcomes, while transient or weak responses preceded clinical deterioration. These observations support the use of T cell monitoring to guide risk stratification and therapy in transplant recipients. Beyond these findings, we also uncovered significant insights within the KS group. Although KS is typically associated with viral reactivation [2,6,26], it has been shown that in lung transplant recipients, primary HHV-8 infection can also lead to KS. Studies on donor-derived HHV-8 infection found lung recipients particularly vulnerable, with a high incidence of KS [4,27]. Among our four KS patients, three developed KS from reactivation, while one, a lung transplant recipient with a D+/R- mismatch, developed KS from primary infection, in line with literature findings. Our findings indicate a tumour-promoting environment in KS patients, characterized by elevated levels of CD163 and HGF, which are associated with macrophage activation, tissue remodelling, and angiogenesis [28–31]. CD163, expressed on M2 macrophages, contributes to an immunosuppressive microenvironment, while HGF promotes proliferation, invasion, and metastasis. From a therapeutic perspective, targeting key inflammatory pathways in KICS-affected patients, such as IL-6 blockade or immune checkpoint inhibition, *via* PD-1 and LAG-3, alongside the already utilized rituximab [6], may help restore immune function and mitigate excessive inflammation. In contrast, interventions in KS patients should focus on modifying the tumour microenvironment, including inhibition of the HGF/c-Met axis or targeting CD163-positive TAMs [32].

Despite its significance, this study has some limitations associated precisely with the scarce clinical cases available. We are aware that the small sample size (16 cases in 14 SOTRs) may limit the generalizability of the findings, and interindividual variability in immunosuppressive therapy and viral load could introduce confounding factors. Additionally, the retrospective design limits causal inferences between cytokine profiles and disease progression. While ELISpot assays provided valuable insights into HHV-8-specific T cell responses, further analyses, such as single-cell RNA sequencing or flow cytometry, are needed to fully

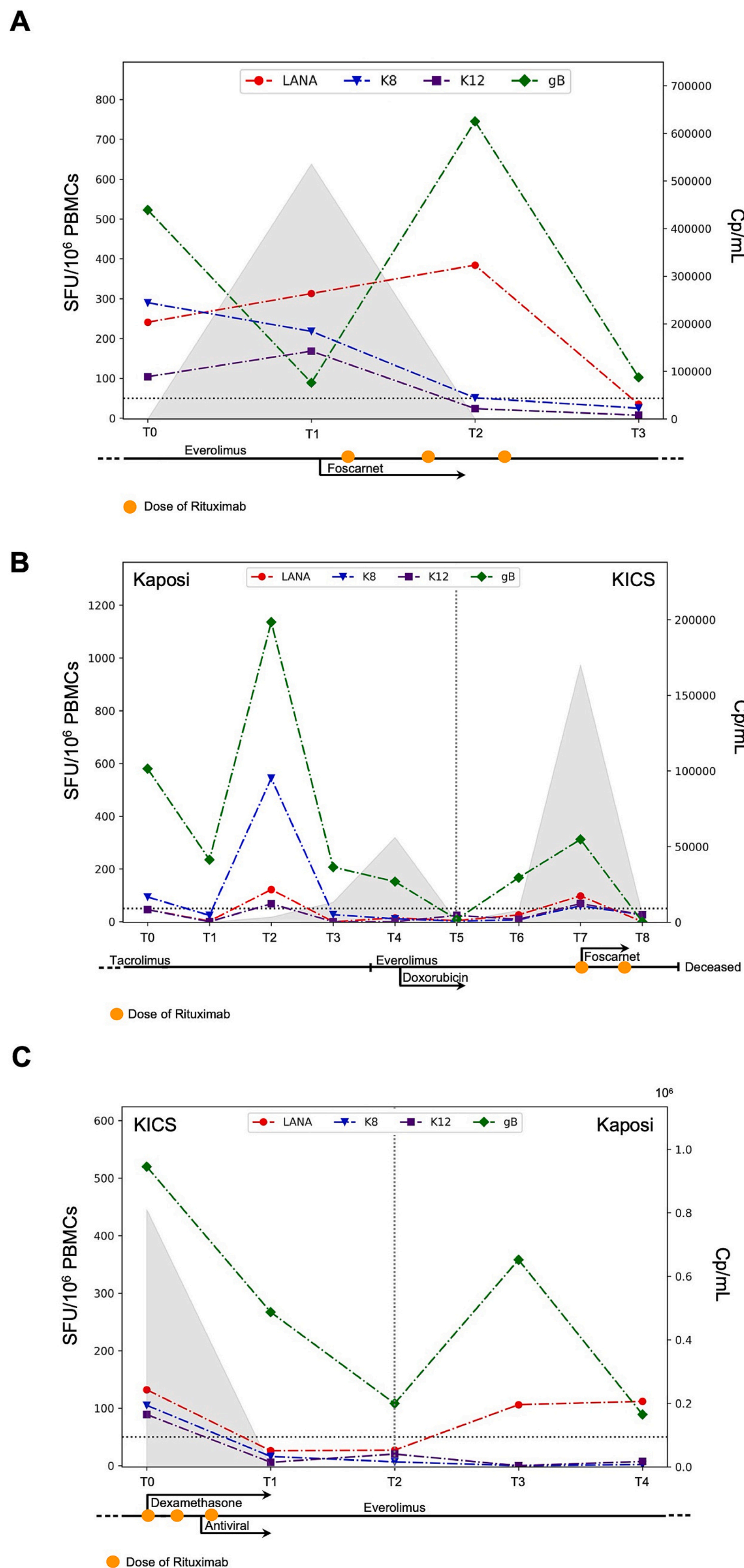


Fig. 3. Longitudinal IFNγ^{+} T cell responses and viremia in a non-fatal KICS case (KSHV_204) (**A**), a fatal case with sequential KS and KICS showing transient immune activation followed by collapse (KSHV_177) (**B**), and a case with early KICS followed by KS marked by progressive T cell recovery and viral control (KSHV_190) (**C**). Grey area, viremia levels. Red line and circle, LANA levels. Blue line and triangle, K8 levels. Purple line and square, K12 levels. Green line and diamond, gB levels. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

characterize immune exhaustion. Nonetheless, this study offers key strengths: ELISpot assays enabled a detailed assessment of HHV-8-specific immunity, while cytokine profiling provided critical insights into immune dysregulation.

To address these limitations and expand our understanding of HHV-8 immunity, we have recently established a monitoring protocol at IRCCS-ISMETT to prospectively collect biological samples from transplant recipients with an HHV-8 donor-recipient mismatch, the highest-risk group for complications.

Our findings underscore the key role of immune dysregulation in HHV-8-related diseases, particularly in differentiating asymptomatic individuals from those with KICS. The decline in HHV-8-specific T cell responses with disease severity highlights the importance of cellular immunity. While asymptomatic individuals maintain immune balance, KICS is marked by excessive inflammation and immune exhaustion, driven by chronic activation. Integrating cytokine profiling and HHV-8-specific T cell responses enhances disease classification, progression monitoring, and biomarker identification. These insights refine our understanding of HHV-8 conditions, supporting improved classification and tailored therapies for immunosuppressed patients. Furthermore, our findings in SOTRs align with those in HIV-immunocompromised individuals. While most studies focus exclusively on HIV [3,33,34], our data underscore similar immune dysregulation in SOTRs, highlighting the novelty of our study. Although HIV- and transplant-related immunosuppression share common features, they also exhibit distinct immune dysfunction patterns that may influence disease progression differently. The identification of similar cytokine profiles and T cell responses in SOTRs suggests that immune mechanisms, described in HIV patients, may extend to other forms of acquired immunosuppression, broadening the clinical implications of our findings.

CRediT authorship contribution statement

Rosalia Busà: Writing – review & editing, Writing – original draft, Visualization, Supervision, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Francesca Timoneri:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Monica Miele:** Formal analysis, Data curation. **Mariangela Di Bella:** Formal analysis, Data curation. **Andrea Cona:** Writing – review & editing, Investigation, Data curation, Conceptualization. **Salvatore Castelbuono:** Writing – review & editing, Visualization, Validation, Software, Methodology, Formal analysis, Data curation. **Mattia Emanuela Ligotti:** Writing – review & editing, Formal analysis, Data curation. **Alessia Gallo:** Writing – review & editing, Formal analysis, Data curation. **Francesca Pecoraro:** Data curation. **Giuseppe Randazzo:** Methodology. **Caterina Amato:** Methodology. **Clara Pipia:** Methodology, Formal analysis, Data curation. **Giandomenico Amico:** Methodology. **Valentina Agnese:** Supervision. **Pier Giulio Conaldi:** Supervision. **Mario Luppi:** Supervision. **Alessandra Mularoni:** Supervision, Conceptualization. **Matteo Bulati:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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Declaration of competing interest

The authors of this manuscript have no conflicts of interest to disclose as described by the American Journal of Transplantation.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clim.2025.110562.

Data availability statement

The data presented in this study will be available from the corresponding author upon reasonable request.

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