IEGULDĪJUMS TAVĀ NĀKOTNĒ

EIROPAS SAVIENĪBA

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# Frequency of human herpesvirus-6 and -7 infections activation in patients with autologous and allogeneic peripheral blood stem cell transplantation

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# History

- Stem cell transplantation was pioneered using bone-marrow-derived stem cells by a team at the Fred Hutchinson Cancer Research Center from the 1950s through the 1970s led by E. Donnall Thomas, whose work later recognized with a Nobel Prize in Medicine. Thomas, work showed that bone marrow cells infused intravenously cold repopulate the bone marrow and produce new blood cells.
- The first physician to perform a successful human bone marrow transplant was Robert Good at the University of Minnesota in 1968.
- Successful stem cell transplantation in Latvia, using bone-marrow-derived stem cells, was carried out in 2001.

# INDICATIONS FOR STEM CELL TRANSPLANTATION

- Candidates for haematopoietic stem cell transplantation (HSCT) are a patients with:
- Leukaemia ( acute myeloid leukaemia, acute lymphopblastic leukaemia, chronic myeloid leukaemia, myelodysplastic syndrome, chronic lymphatic leukaemia)
- Lymphomas,
- Multiple myeloma,
- Severe aplastic anaemia, Fanconi anaemia, Thalasaemia,
- Solid tumors ( neuroblastoma, germinal tumors, Ewing sarcoma) and others,

- who would not benefit from prolonged treatment with, or are already resistant to, polychemotherapy.

# GRAFT TYPES

- Autologous HSCT

  - requires the extraction (apheresis) of haematopoietic stem cell from the patients and then returned to his/her body. Auto-SCT is used to treat Hodkin's disease, non-Hodkin's lymphoma, myeloma

- Allogeneic HSCT

  - involved the donor of HSC and the recipient. Allogeneic HSC donors must have a tissue (HLA) type that matches the recipient and may be related, singeneic or unrelated.

  - Allo-HSCT is used to treat acute leukaemia, chronic myeloleukaemia, aplastic anaemia

# SOURCE of HAEMATOPOIETIC STEM CELL

- - Bone marrow,
- - Peripheral blood stem cells,
- - Umbilical cord blood
- Peripheral blood stem cells (PBSC) are now the most common source of stem cells.
- Granulocyte-colony stimulating factor (G-CSF) serving to mobilize stem cells from bone marrow into the peripheral circulation.

# CHEMOTHERAPY

- Autologous SCT:
- BEAM- carmustin, etoposide, cytosar, melphalan
- High dose melphalan

- Allogeneic SCT:
- BuCY- busulphan, cyclophosphamide
- Fludarabine, cyclophosphamide, ATG, and other.

- T- cell depletion – Campath in bag or ATG
- GVHD prophylaxis – MTX, CyA

# COMPLICATIONS AND SIDE EFFECTS

- HSCT is associated with a high treatment-related mortality in the recipients (2.8%- autoSCT and 25-30% aloSCT), limits its use to conditions that are themselves life-threating.
- Major complications are:
- Early complications (haemorrhagic cystitis, early complications of vascular origin – sinusoidal obstruction syndrome of the liver, capillary leak syndrome, engraftment syndrome, diffuse alveolar haemorrhage, thrombotic microangiopathy, idiopatic pneumonia syndrome, multiple-organ dysfunction syndrome),
- Infection ( bacterial, fungal, viral, especially HSV),
- Acute (occurs in the first 3 months after transplantation) and chronic graft-versus-host disease (GVHD),and other.

- Despite to successful HSCT, the viral infections remains one of the causes of post-transplant morbidity and mortality.

- Human herpesvirus -6 and -7 (HHV-6, HHV-7) are ubiquitous (seroprevalence rate 50-90%) T-lymphotropic immunomodulating viruses that after primary infection establish a life-time latent/persistent infection and can be reactivate in immunosuppressed hosts, that may potentially cause immune dysregulation.
- CD34+ cells which are a major source of haematopoietic progenitor cells for transplantation can be infected by HHV-6 and HHV-7 that may cause myelosuppression in patients.
- Two variants of HHV-6 (A and B) have been described. The specific pathogenicity of each variant remains poorly understood.
- The pathogenic role of HHV-7 still remain unclear as well as interaction between HHV-7 and HHV-6 by concurrent infection.
- The application of different antiviral specific drugs for the prevention and treatment of these viruses infection activation remain contradictory.

- Aim of this study was to evaluate the incidence of latent/persistent HHV-6 and HHV-7 infection and frequency of these viruses infection activation in early period after auto- and related allo-PBSCT; the potential interaction with post-transplant complication development; the potential interactions between both viruses.

## Patients and grafts donors

- In this retrospective study were enrolled
- - 44 patients (27 females, 17 males; mean age 34.1±10.7) underwent auto-PBSCT (group I) with Hodgkin's disease - 29, non-Hodgkin's lymphoma - 5, with myeloma - 10 patients.
- - 12 patients (6 females, 6 males; mean age 35.7±7.2) underwent related allo-PBSCT (group II) with acute myeloid leukaemia -7 patients, acute lymphoblastic leukaemia – 3, with Hodgkin's disease – 2 patients.
- All patients was transplanted in the Chemotherapy and Haematology Clinic, Riga, Latvia.
- The stem cells for auto- and allo- grafts were harvested from peripheral blood

- Pre-transplant myeloablative therapy for lymphoma patients was carried out by BEAM, for myeloma patients - with melphalan and for allo-PBST patients conditioning therapy by BuCy scheme (busulfan, cyclophosphamide) were used. To limit the risks of GVHD allo-PBSCT recipients received immunosuppressive therapies (cyclosporine + MTX or medrole). For prophylaxis against herpesviruses infection activation and bacterial infections valacyclovir and trimethoprinum after transplantation to all patients were administered.
- EDTA blood samples were collected from the grafts donors and patients before transplantation as baseline and during 3 months after transplantation.

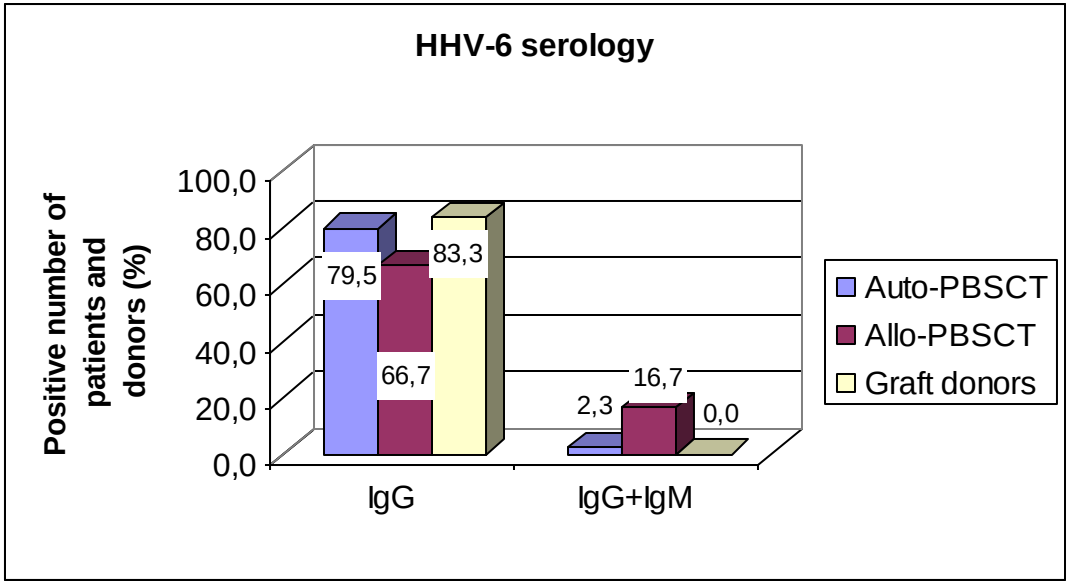
# Methods

- - ELISA kits were used to the detection of HHV-6 specific IgM and IgG class antibodies.
- - Nested polymerase chain reaction (nPCR) and Real-Time PCR were used for the detection of HHV-6 and HHV-7 genomic sequences in DNA isolated from peripheral blood leukocytes (PBL) and cell free plasma (markers of latent/persistent and active infection, respectively) and HHV-6 viral load in PBL DNS samples.
- - Restriction endonuclease analysis was carried out using enzyme HindIII for the detection HHV-6A and HHV-6B virus variants.
- - ELISA and CLIA tests were used for the cytokine levels in serum/plasma samples.
- - Statistical difference in the prevalence of latent/persistent and active HHV-6 and HHV-7 infection was assessed by Fisher's exact test. SPSS software was used to assess the continuous variable values of cytokines levels with a value of $p<0.05$ considered as significant.

## Results

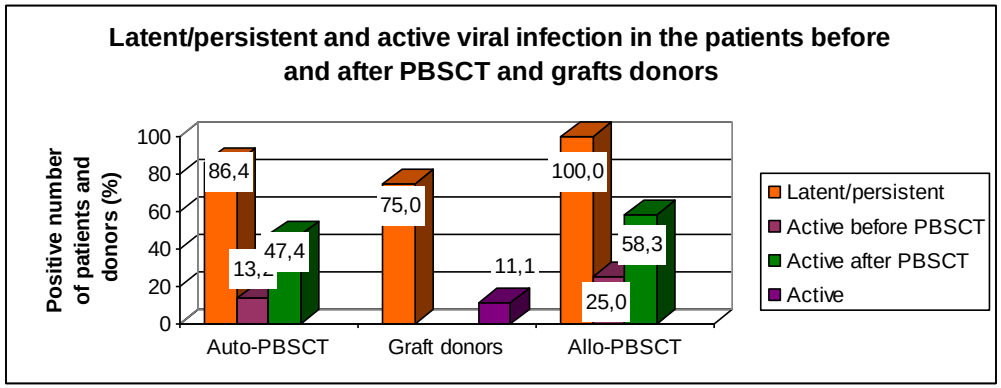
### HHV-6 serology. (Fig. 1)

Significant difference in the prevalence of anti-HHV-6 specific IgG class antibodies was not detected between auto- and allo-PBSCT patients before transplantation (35/44, 79.2% and 8/12, 66.7%, respectively) and grafts donors (Fig. 1) Simultaneous presence of IgM and IgG class antibodies was found in 2.3% (1/44) of I group patients and in 16.7% (2/12, p=0.11) of II group patients.



# Latent/persistent and active viral infection in the patients before and after PBSCT and grafts donors (Fig. 2)

Significant difference in the frequency of latent/persistent and active HHV-6 and/or HHV-7 infection between I and II group of the pre-transplant patients and grafts donors was not found.




**Latent/persistent infection was revealed in:**
**86.4% (38/44) I group patients;**
**100% samples (12/12) II group patients;**
**75.0% (9/12) grafts donors. (Fig. 2).**

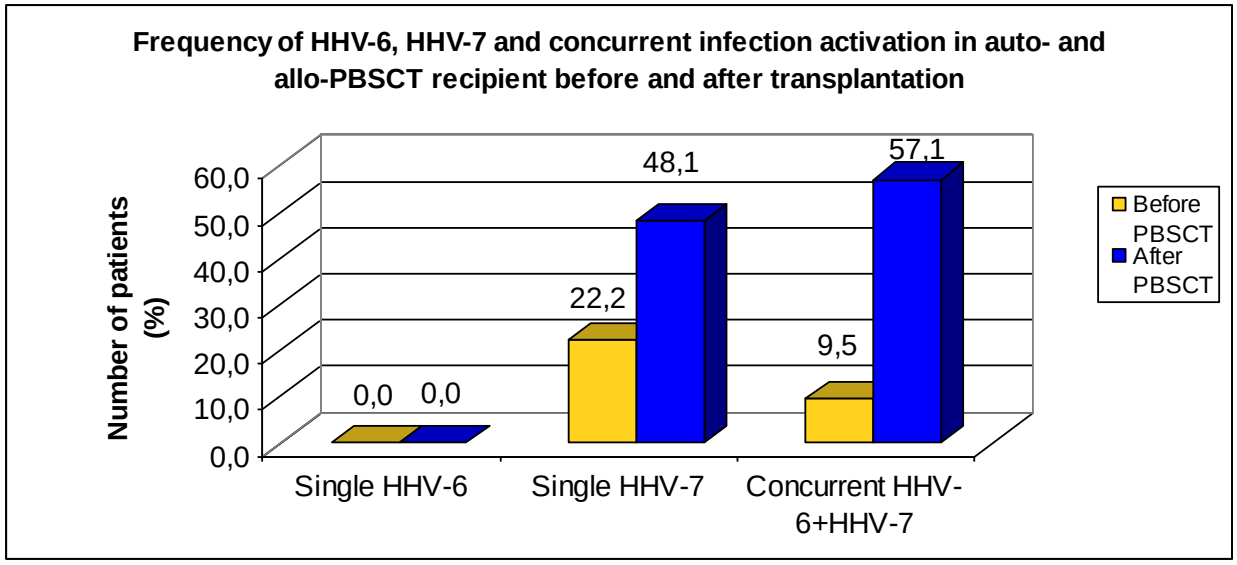
**Active viral infection (plasma viremia) before transplantation was detected in:**
**13.2% (5/38) I group patients);**
**25.0% (3/12) II group patients);**
**11.1% (1/9) grafts donors (Fig. 2). (p=0.113)**

**The frequency of viral infection activation was significantly higher after auto-PBSCT and two time higher after allo-PBSCT in comparison with the frequency before transplantation.**
**47.4% (18/38) I group patients (p=0.0003); 58.3% (7/12) II group patients (Fig. 2).**

It was of interest to compare frequency of **each infection activation before and after transplantation in the patients (n=21) with dual HHV-6+HHV-7 infection**.

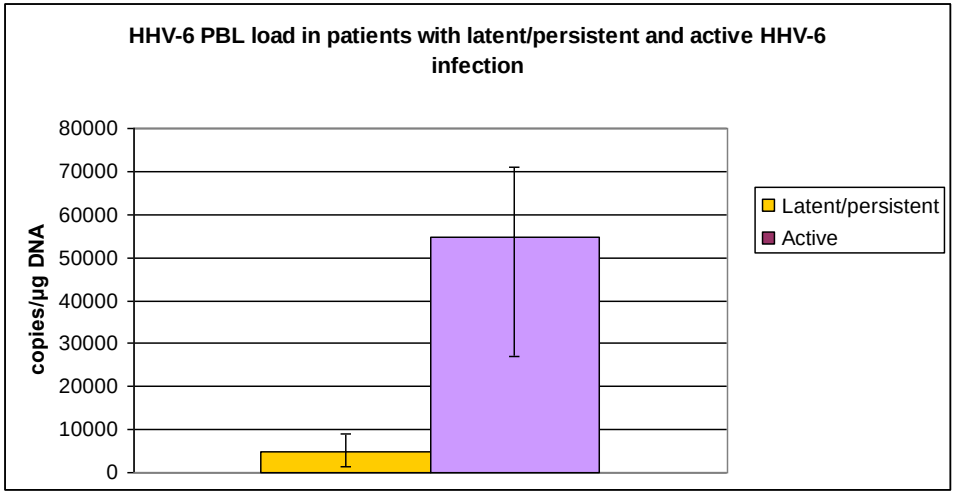
The analysis of data showed that:

- activation of single HHV-6 infection was not detected before and after PBSCT.
- At the same time the frequency of single HHV-7 infection activation after transplantation was four time (22.2% before and 48.1% after) then before transplantation.
- The frequency of concurrent (HHV-6+HHV-7) infection activation was significantly higher after then before PBSCT (9.5% and 57.1%, respectively (Fig. 3).



# HHV-6 PBL load in patients with latent/persistent and active HHV-6 infection

**Fig. 4.**
**The significant increase of mean HHV-6 load in DNA of peripheral blood leukocytes was detected in the period active faze of viral infection 54676 (range: 26867 - 71163) copies/µg DNA then in the period of latent/persistent faze of infection 4781 (range:1548 – 8963) copies/µg DNA.**




**HHV-6B genomic sequence only was found in all peripheral blood leukocytes and plasma DNA samples from the donors and recipients.**

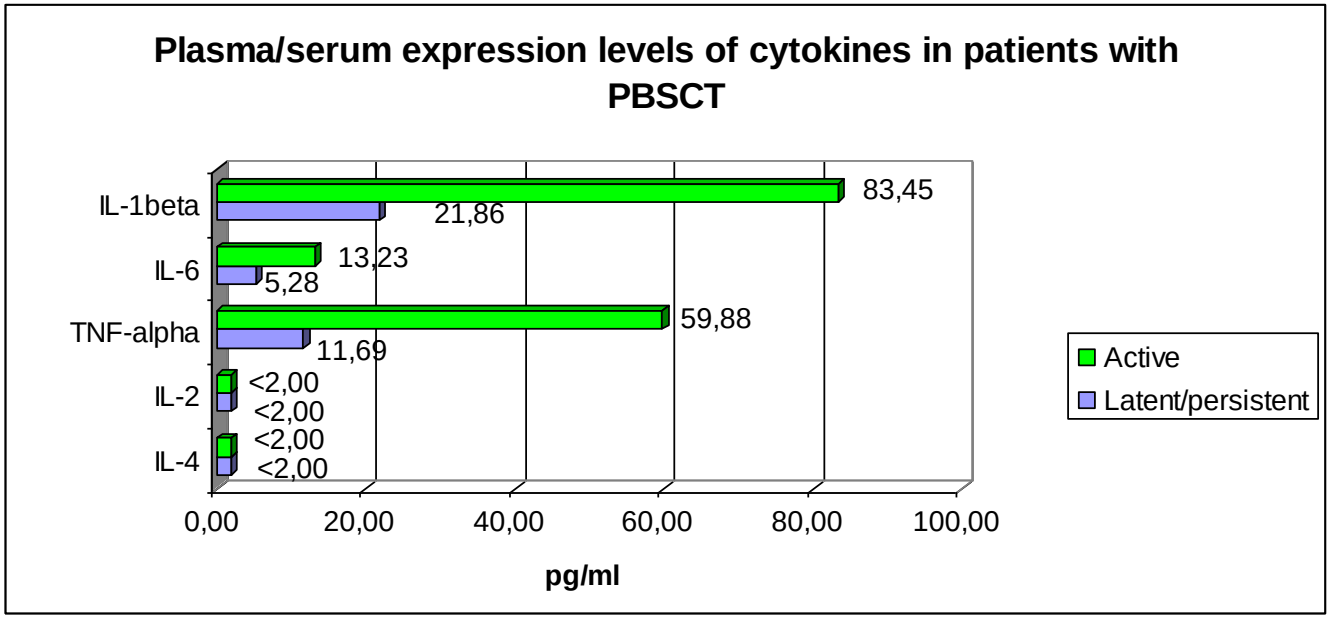
**The mean number of days before detecting HHV-7 and HHV-6 reactivation was 11 days (range: 8 -14 days) and 27 days (range: 12-34 days), respectively.**

## Plasma/serum expression levels of cytokines in patients with PBSCT

**Fig. 5**

**Significant increase in mean expression levels of pro-inflammatory cytokines TNF-α, IL-6, IL-1β was detected in the plasma/serum samples from the auto- and allo-PBSCT recipients with active viral infection in comparison to the expression levels in patients with latent infection.**

**None of the transplant patients had detectable value of IL-4 and IL-2 in the serums/plasma samples.**



**Relationship between active beta-herpesviruses infection and the clinical complications development in early period after PBSCT**

| Complication | Auto-PBSCT (n=12) | | Allo-PBSCT (n=5) | |
|---|---|---|---|---|
| | Active viral infection | | | |
| | HHV-7 | HHV- 6 + HHV-7 | HHV-7 | HHV-6 +HHV-7 |
| Febrile syndrome (FS) | 3 | 1 | 4 | |
| FS + pneumonitis | | 3 | | |
| Gastrointeritis | 1 | | | |
| Pneumonitis | | 2 | | |
| Gastroenteritis+pneumonitis | | 1 | | |
| FS+ Gastrointeritis+pneumonitis | | 1 | | |
| FS+cutaneous rash+GVHD | | | | 1 |

Different clinical complications after transplantation, not connected with basic disease, were detected in 12 out of 44 auto-PBSC transplant recipients and in 5 out of 12 allo-PBSC transplant recipients. From them reactivation of HHV-7 (8/17) or both viruses (9/17) was found. Febrile syndrome was often diagnosed (3-14days) after transplantation and frequently preceded or combined with gastroenteritis and/or pneumonitis (4/8 patients) development. The complications after allo-PBSCT, that may be associated with viral infection activation, was diagnosed in 5 patients: febrile syndrome - in 4 and febrile syndrome, cutaneous rash and acute GVHD - in one patient.

## Conclusion

- High frequency of HHV-7 and concurrent (HHV-6+HHV-7) infection activation with simultaneous increase of pro-inflammatory cytokines, serum/plasma expression levels suggest that both viruses are involved in the complications development in the early period after auto- and allo-PBSCT via their immunomodulatory ability.

- The kinetics of the viruses, reactivation reflects the potential role of HHV-7 as co-factor of HHV-6 reactivation.

- Our data suggest that valacyclovir used in experience for antiviral prophylaxis and therapy is not sufficiently effective to prevent of HHV-6 and HHV-7 infection activation after PBSC. However, stem cell transplantation is a recommendation to use for patients after aloPBSCT and autoPBSCT, prophylaxis with acyclovir or valaciclovir to decrease the risk of reactivation during the early phase of transplant.

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