Santa Rasa

PERSISTENT VIRAL INFECTIONS (HHV-6, HHV-7 & B19) IN MYALGIC ENCEPHALOMYELITIS/CHRONIC FATIGUE SYNDROME

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Euromene
European Network on Myalgic Encephalomyelitis/ Chronic Fatigue Syndrome

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Infectious agents

Viruses

- Herpes simplex viruses 1 and 2, varicella zoster, Epstein-Barr, Cytomegalovirus, HHV-6, HHV-7, HHV-8
- Parvovirus B19
- Enteroviruses
- Polyomaviruses JC and BK
- Adenovirus
- Rubella virus
- Hepatitis C virus
- XMRV

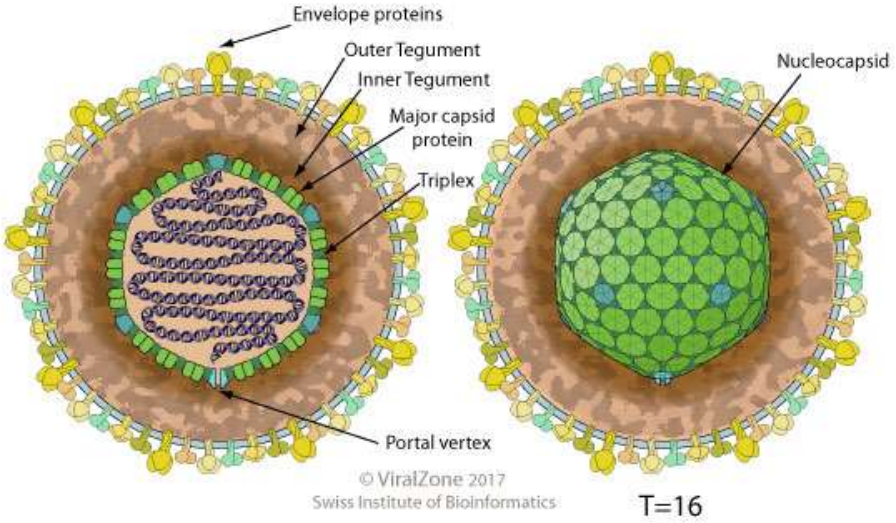
Bacteria

- *Coxiella burnetii*
- *Borrelia*
- *Chlamydophila pneumoniae*
- *Mycoplasma*

Parasite

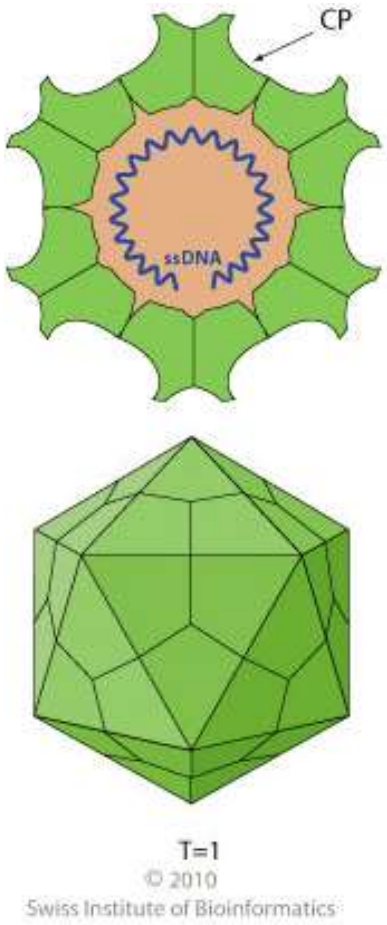
- *Giardia lamblia (Giardia intestinalis)*

Viruses



HHV-6 and HHV-7 (*Herpesviridae* family, *Beta-herpesvirinae* subfamily, *Roseolovirus* genus)

(viralzone.expasy.org/16)



B19V (*Parvoviridae* family, *Parvovirinae* subfamily, *Erythrovirus* genus

(viralzone.expasy.org/103?outline=all_by_species)

Aim of the study

To determine the involvement of human herpesvirus-6, human herpesvirus-7 and parvovirus B19 in etiopathogenesis of myalgic encephalomyelitis/chronic fatigue syndrome

Objectives of the study

1. To estimate the frequency of HHV-6 and HHV-7 specific antibodies and genomic sequences, infection activity phase, viral load, as well as HHV-6 type and antigen expression in patients with ME/CFS

2. To detect the frequency of B19V specific antibodies and genomic sequences, infection activity phase, viral load, genotype and period of time from B19V infection appearance in ME/CFS patients

3. To determine the expression level of cytokines (IL-6, TNF-α, IL-12, IL-4 and IL-10) in patients with persistent infection/co-infection in latent and active phase

4. To analyse the association of HHV-6, HHV-7 and B19V infection/co-infection with ME/CFS clinical symptoms

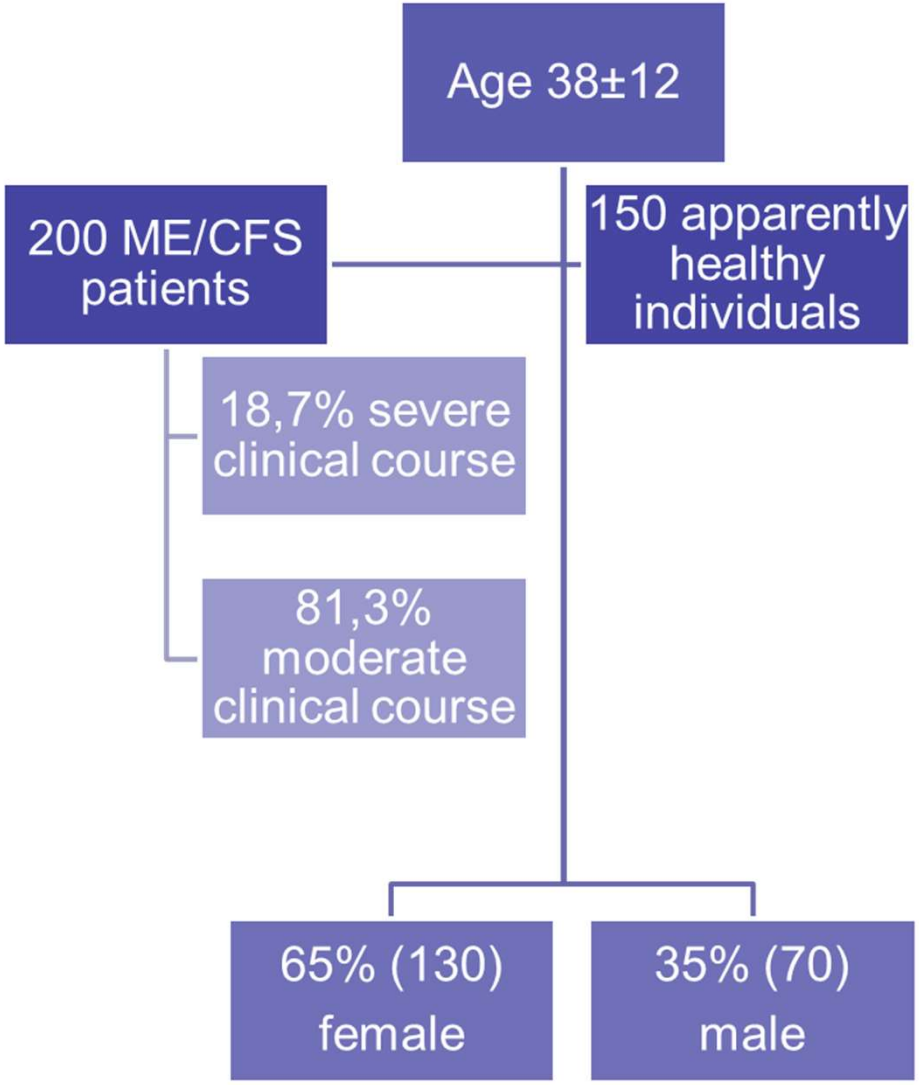
5. To estimate the influence of infection activity on severity of ME/CFS clinical course

Hypothesis of the study

- Persistent viral infections, like beta-herpesviruses HHV-6 and HHV-7, and parvovirus B19V infections, are ME/CFS trigger factors and are associated with the development of ME/CFS
- The activity phase of virus infection is of the greatest importance because – an active infection causes much deeper immunological disturbances and is associated with a more severe ME/CFS clinical course

MATERIAL AND METHODS

- Clinically diagnosed ME/CFS corresponding to 1994 Fukuda Centers for Disease Control and Prevention (CDC) criteria
- According to International Statistical Classification of Diseases and Related Health Problems (ICD-10), G93.3 – postviral fatigue syndrome (benign myalgic encephalomyelitis)



MATERIAL AND METHODS

Criteria for ME/CFS patients to be **included** in the study were the following**:**

1. Fatigue lasting at least for six months.

2. At least 4/8 following criteria:

- post-exertional malaise
- impaired memory and concentration
- un-refreshing sleep
- muscle pain
- multi-joint pain
- tender lymph nodes
- sore throat
- headache

MATERIAL AND METHODS

Exclusion criteria:

- Anaemia (Fe, B12 deficiency)
- 2. Cancer in the past, radiation therapy, chemotherapy
- 3. Radiation exposure
- 4. Pregnancy and postpartum period within 1st year
- 5. Endocrine disorders, including, diabetes mellitus, thyroid and adrenal diseases
- 6. Orthostatic hypotension
- 7. Cardiac disorders (congestive heart failure, endocarditis, arrhythmias)
- 8. Renal disorders (uraemia, electrolyte disturbance)
- 9. Hepatic disorders (hepatitis, cirrhosis)
- 10. Connective tissue diseases
- 11. Myopathy, myositis, peripheral neuropathies
- 12. CNS diseases with motor, sensory, cognitive and mental impairment (stroke,
- multiple sclerosis, traumatic brain injury, moto-neuron diseases, etc.)
- 13. Infectious diseases (Lyme disease, HIV)
- 14. Trauma
- 15. Toxic substance influence (including alcohol, drugs)
- 16. Psycho-organic diseases (depression, affective and neurotic conditions).

MATERIAL AND METHODS

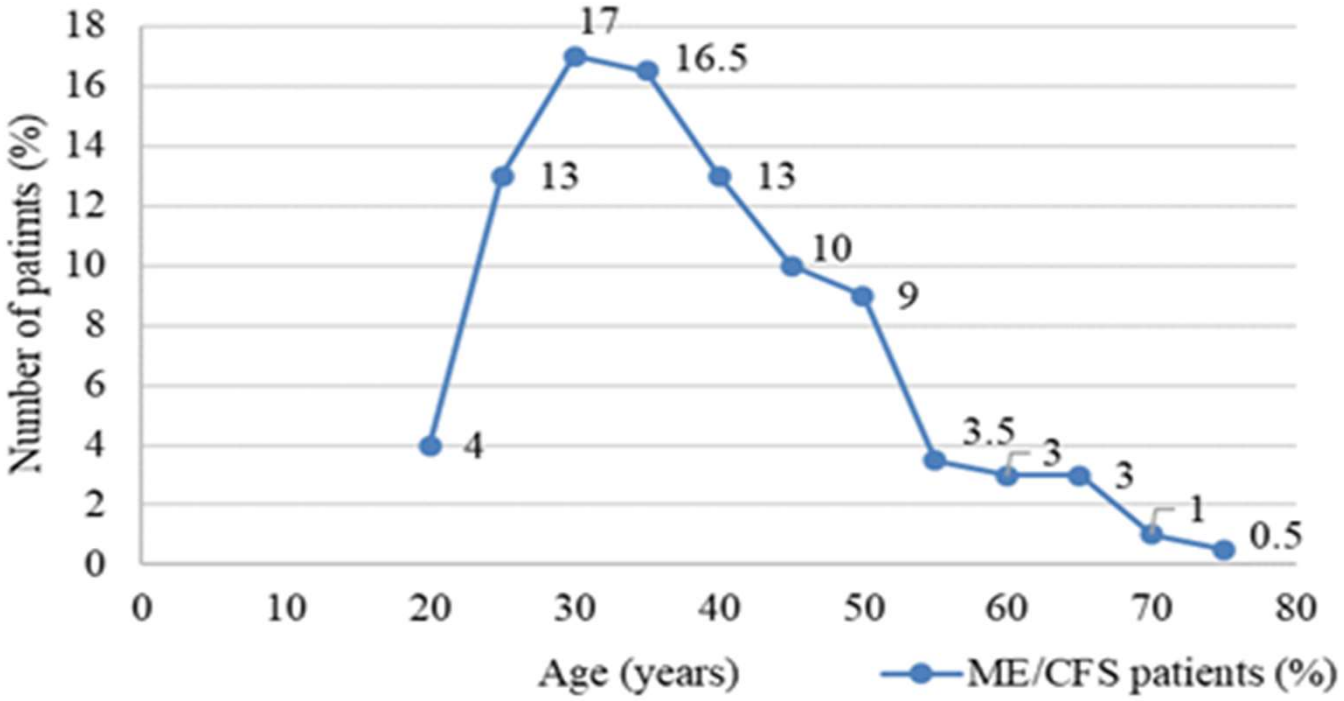
- **Molecular methods**
  - DNA isolated from peripheral blood and plasma samples
  - RNA extracted from PBMCs
  - Spectrophotometrically measured concentration of the extracted DNA and RNA
  - cDNA was synthesized with reverse transcription
  - β-globin PCR – to assure the quality of cDNA and DNA
  - nPCR – to detect viral genomic sequences
  - HHV-6A and HHV-6B differentiated with nPCR and HindIII restriction endonuclease
  - PCR – to amplify virus specific DNA sequences in cDNA samples
  - Electrophoretic analysis – to separate and identify DNA fragments amplified by PCR
  - HHV-6, HHV-7 and B19V load was estimated using real-time PCR

MATERIAL AND METHODS

Immunological methods

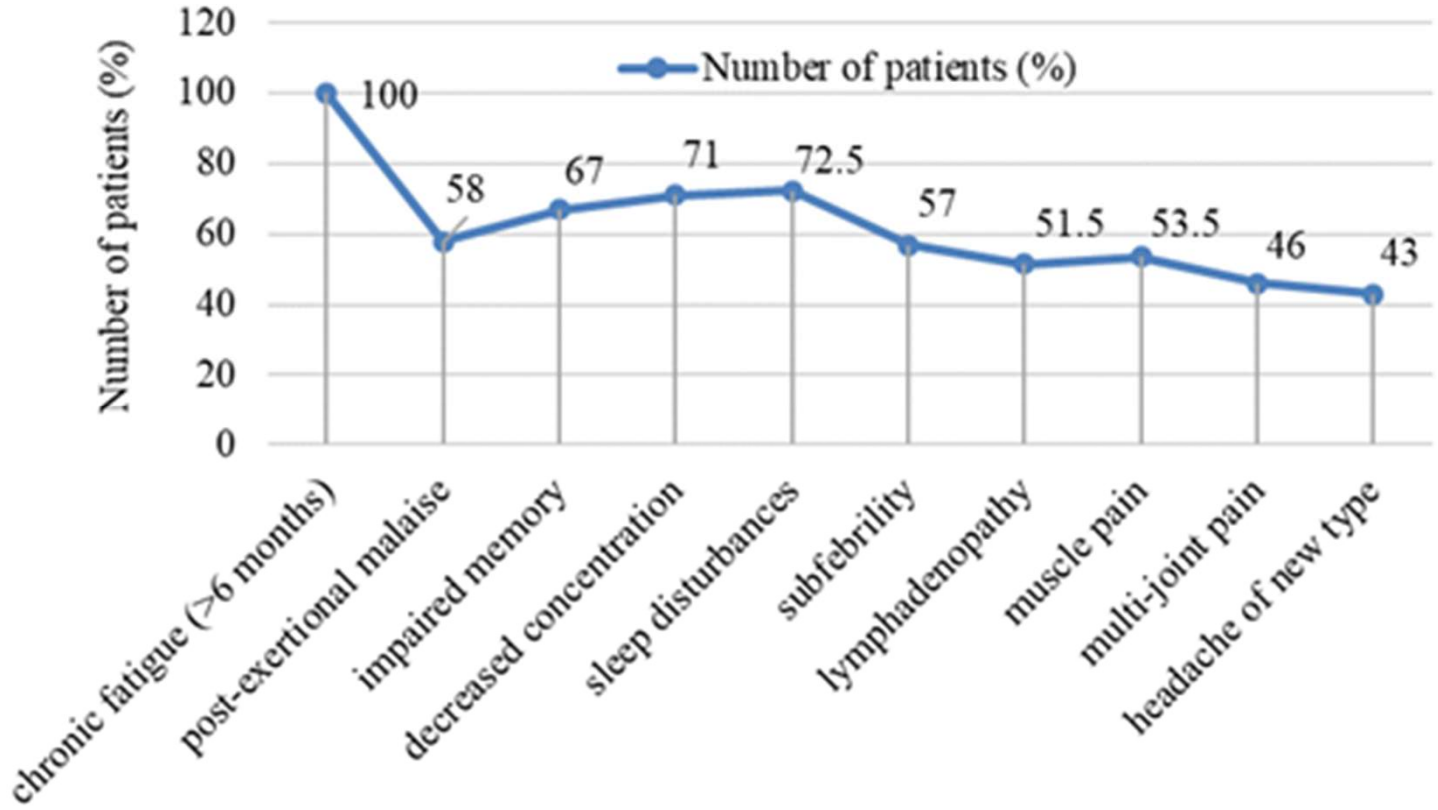
- Immunoassays
  - IgM and IgG class antibody detection
  - Determination of cytokine level
  - » Indirect immunofluorescence
    - HHV-6 antigen expression detection
- Phylogenetic analysis
- Statistical analysis

Results



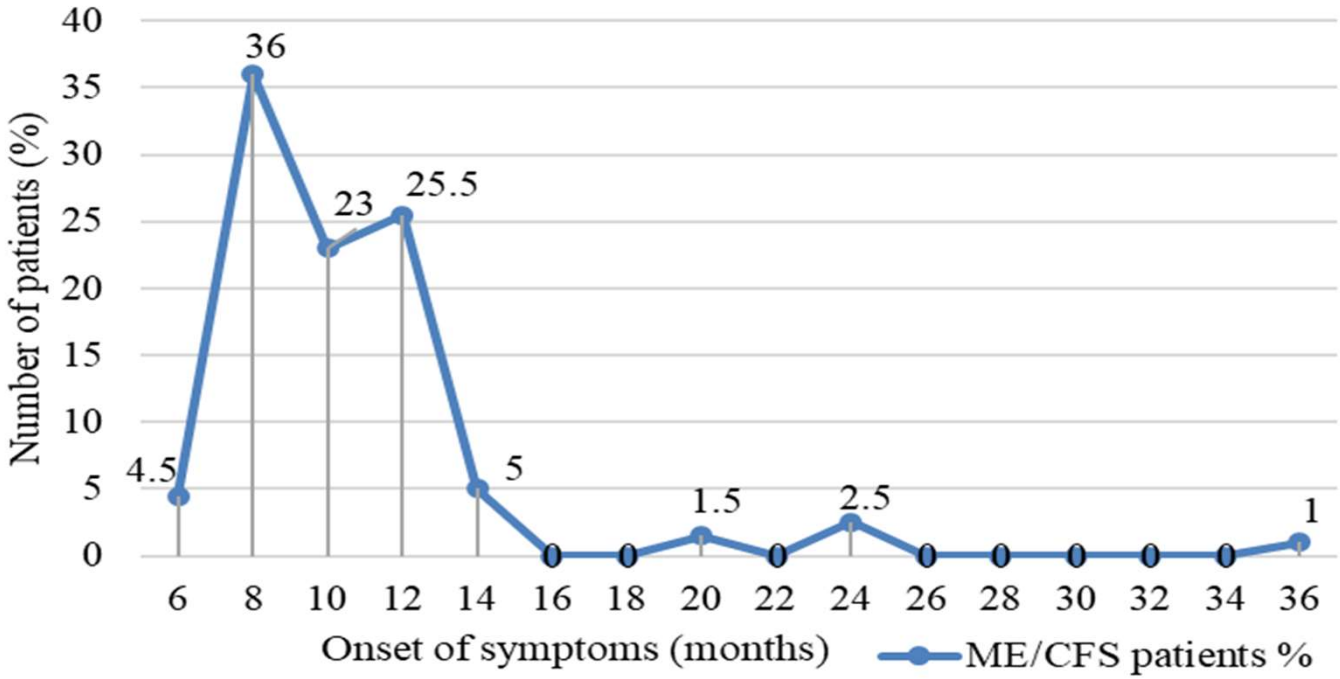
- 65% (130/200) were female and 35% (70/200) - male ($p < 0.0001$)
- Mean (± SD) age was 38 ± 12 years
- 79% of patients were between age of 25–50 years

Results



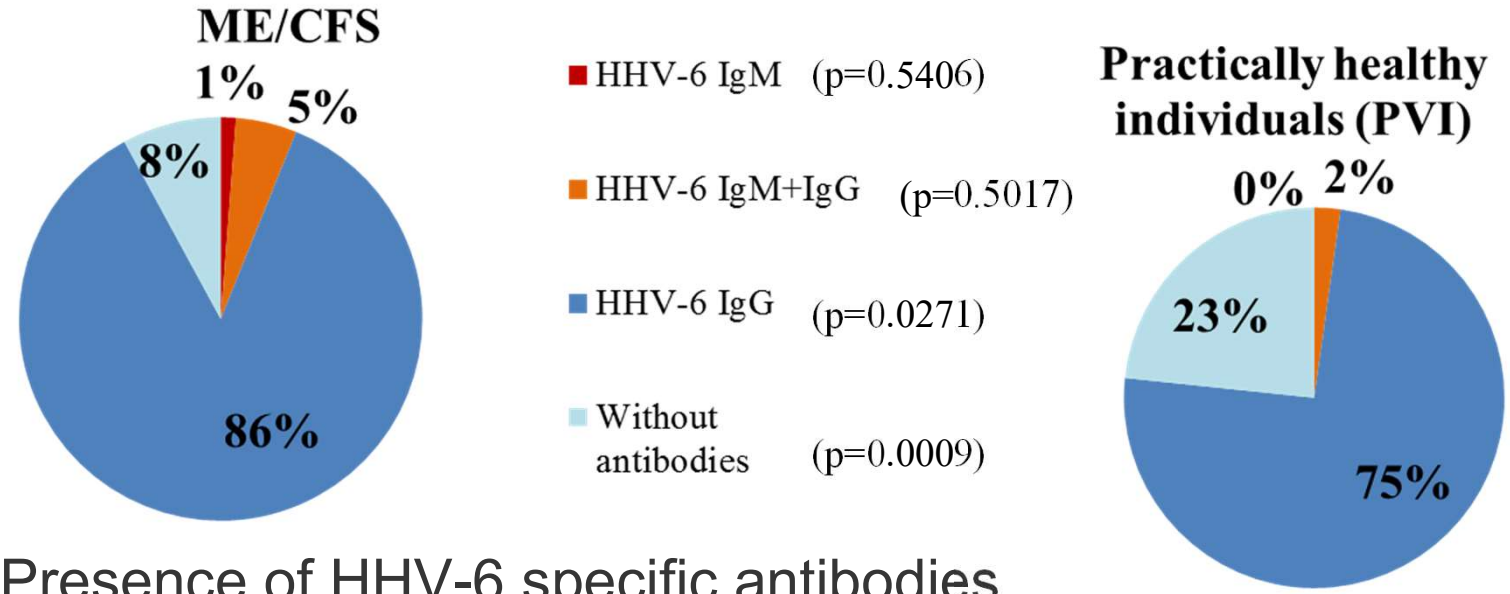
- Most frequently observed symptoms - impaired memory, decreased concentration and sleep disturbances

Results



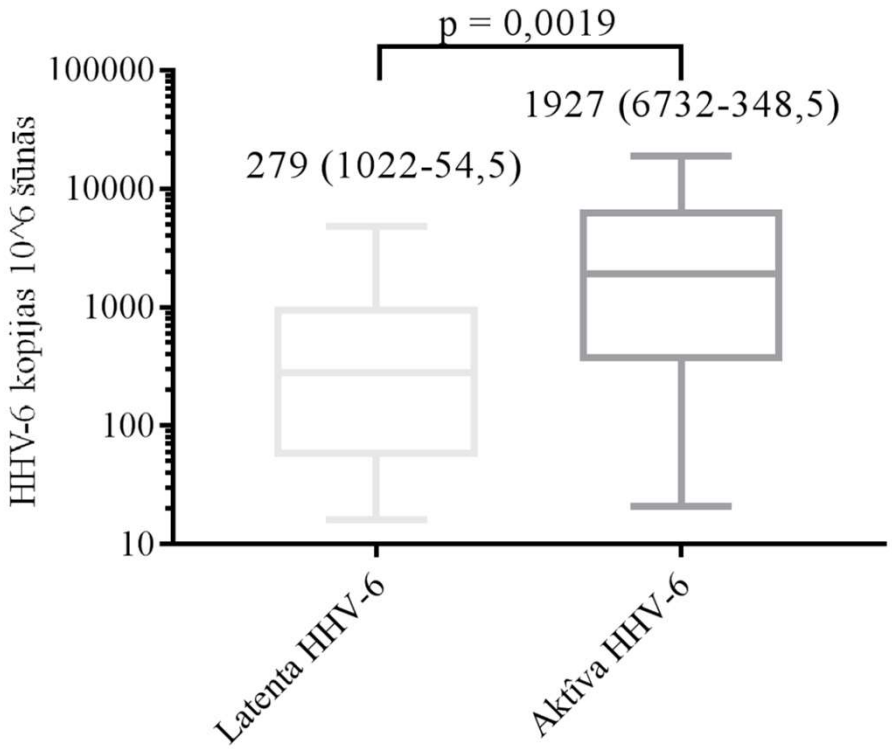
- Onset of ME/CFS symptoms occurred 6–36 months before inclusion in this study, 10.2 ± 4.2 months

Involvement of HHV-6 in development of ME/CFS



- Presence of HHV-6 specific antibodies
  - » 92.1% (151/164) ME/CFS vs 76.7% (69/90) PVI (p = 0.0009)
- Presence of HHV-6 genomic sequences:
  - » DNA from PBL (marker of latent phase) – 42% (84/200) ME/CFS and 28,7% (43/150) PVI (p = 0.0133)
  - » DNA from PBL and plasma (marker of active phase) – 11% (22/200) ME/CFS and none of PVI (p < 0.0001)

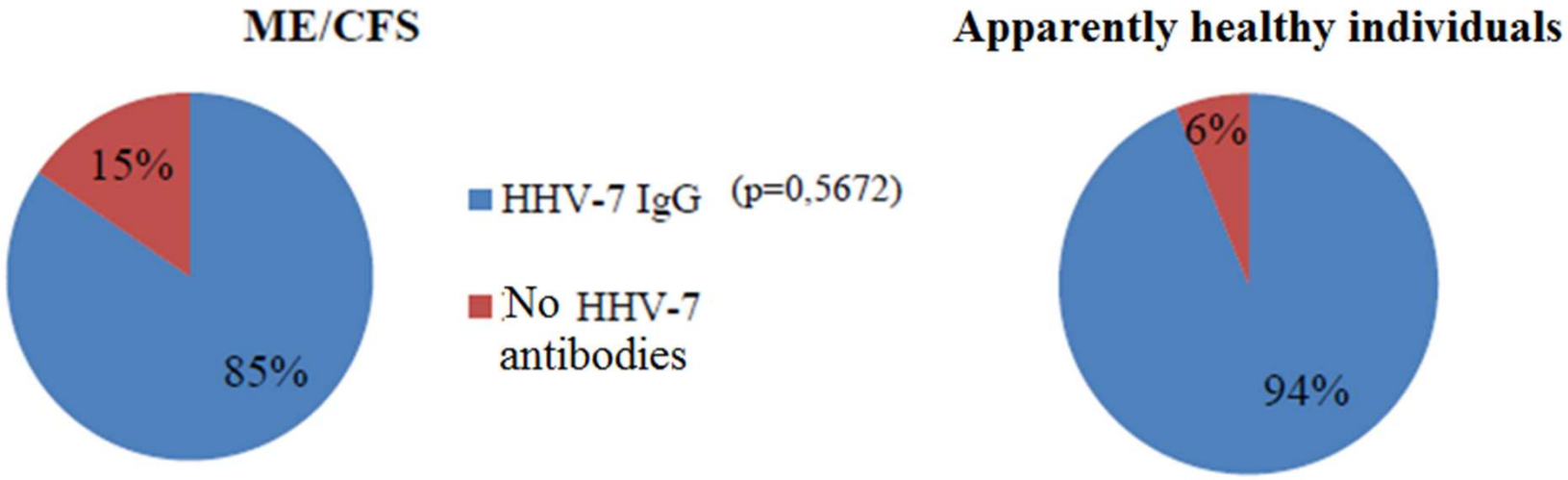
Involvement of HHV-6 in development of ME/CFS



- HHV-6 load
  - Elevated HHV-6 load (> 10 copies/10^6 cells) 66% (66/100) ME/CFS and 2/10 PVI (p = 0.0064)
    - 56.4% (44/78) latent phase
    - 100% (22/22) active phase (p < 0.0001)
  - Six patients had $1,21x10^6$ (1.45 – 0.81) copies/10^6 cells

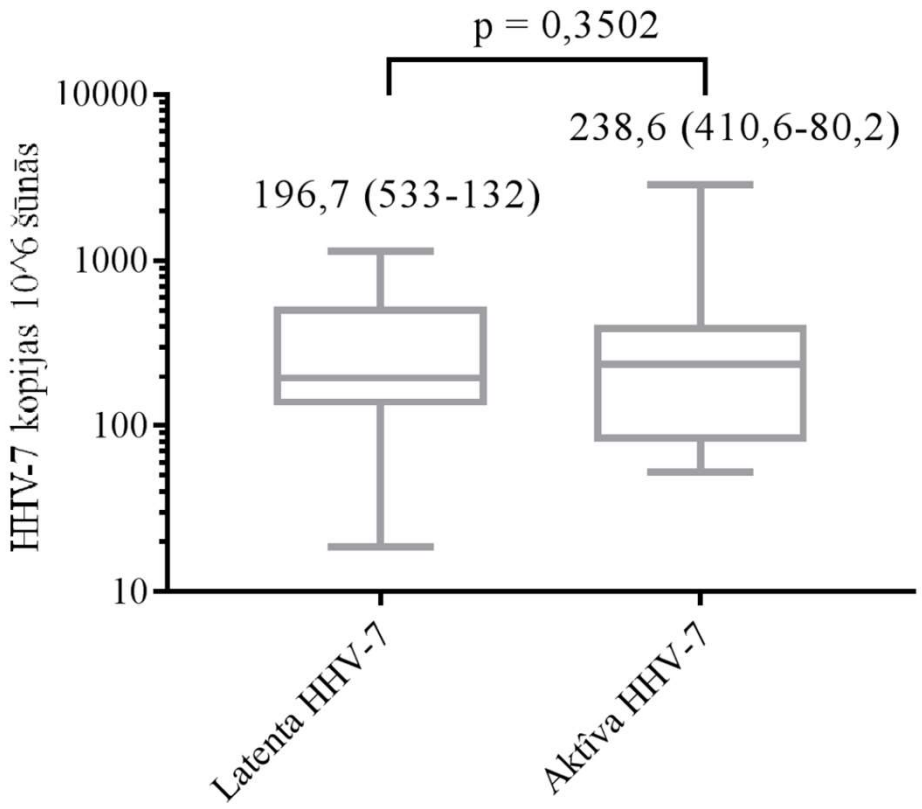
Involvement of HHV-7 in development of ME/CFS

- Presence of HHV-7 specific antibodies



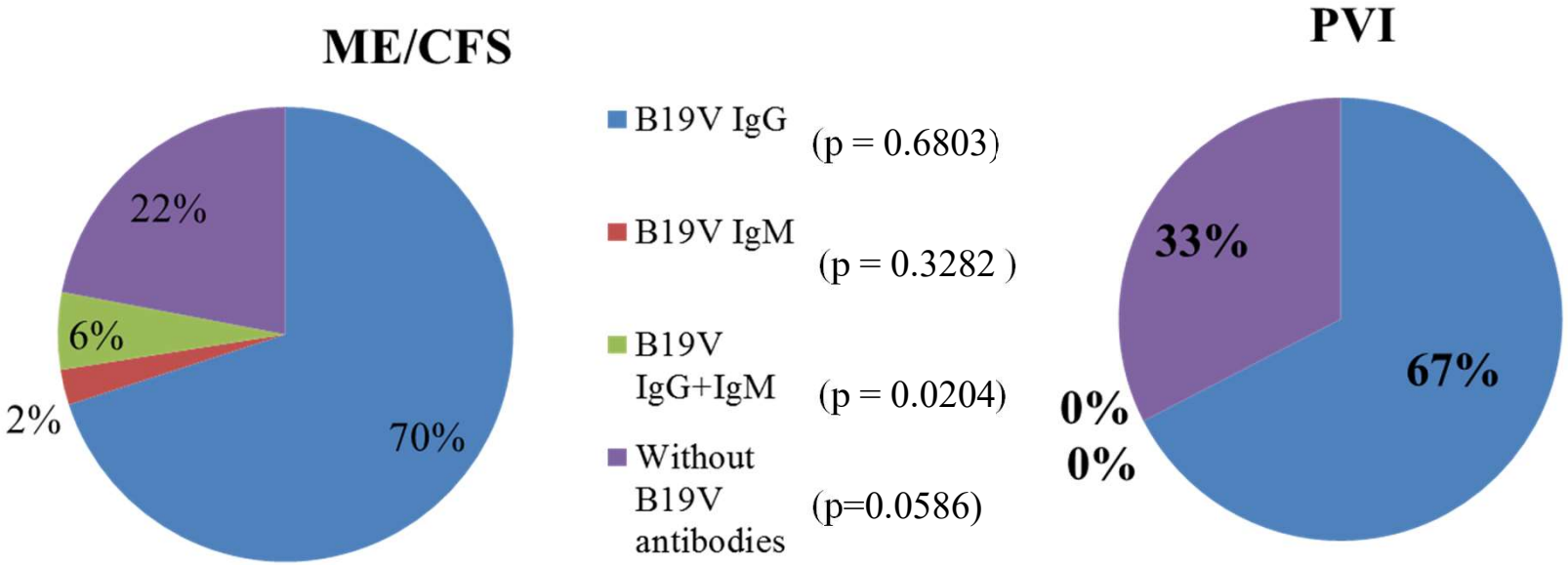
- Presence of HHV-7 genomic sequences:
  - » Markers of latent phase – 58% (116/200) ME/CFS patients and 67.3% (101/150) PVI (p = 0.0766)
  - » Markers of active phase – 34% (68/200) patients and 8% (12/150) from PVI (p < 0.0001)

Involvement of HHV-7 in development of ME/CFS



- HHV-7 load
  - Elevated HHV-7 load (> 10 copies/10^6 cells) 67.3% (113/168) ME/CFS and 31.4% (16/51) PVI ($p < 0.0001$)
    - 62.9% (66/105) latent phase
    - 74.6% (47/63) active phase ($p = 0.1292$)
  - One patient had HHV-7 load in blood and in hair follicle DNA > 1 x10^6 copies/10^6 cells. Patients mother hair follicle > 2 x10^6 copies/10^6 cells (Prusty et al., 2017).

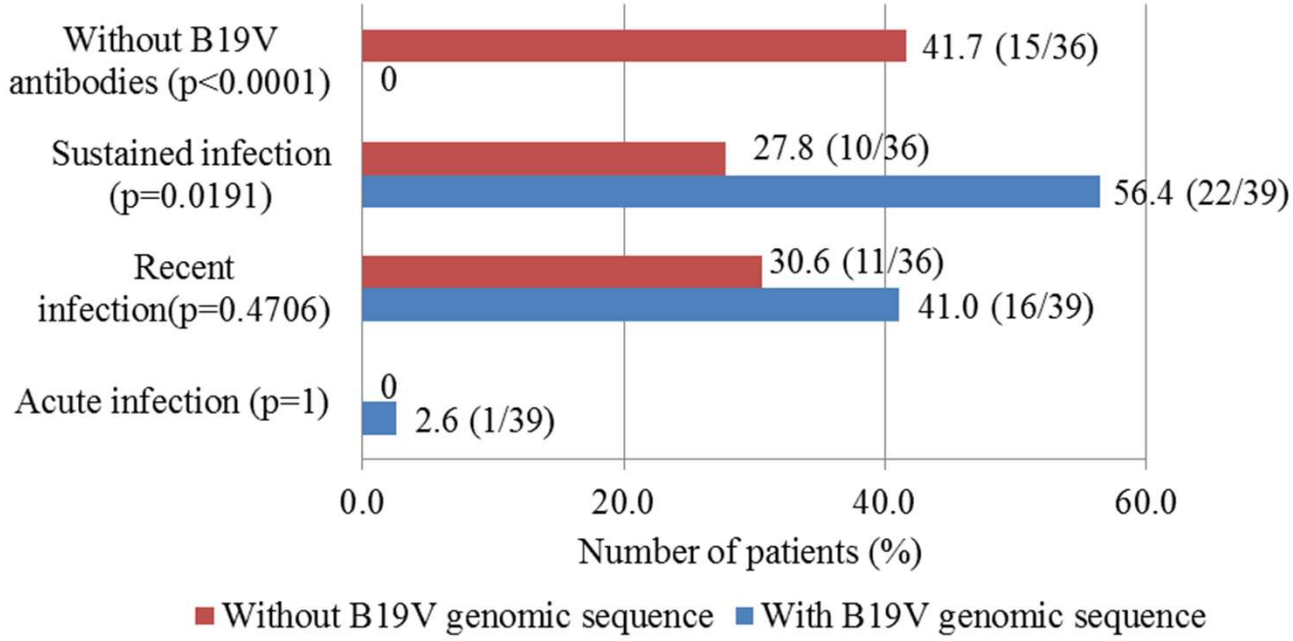
Involvement of B19V in development of ME/CFS



- Presence of B19V specific antibodies
  - » 78% (156/200) ME/CFS and 67.4% (60/89) PVI (p = 0.0586)

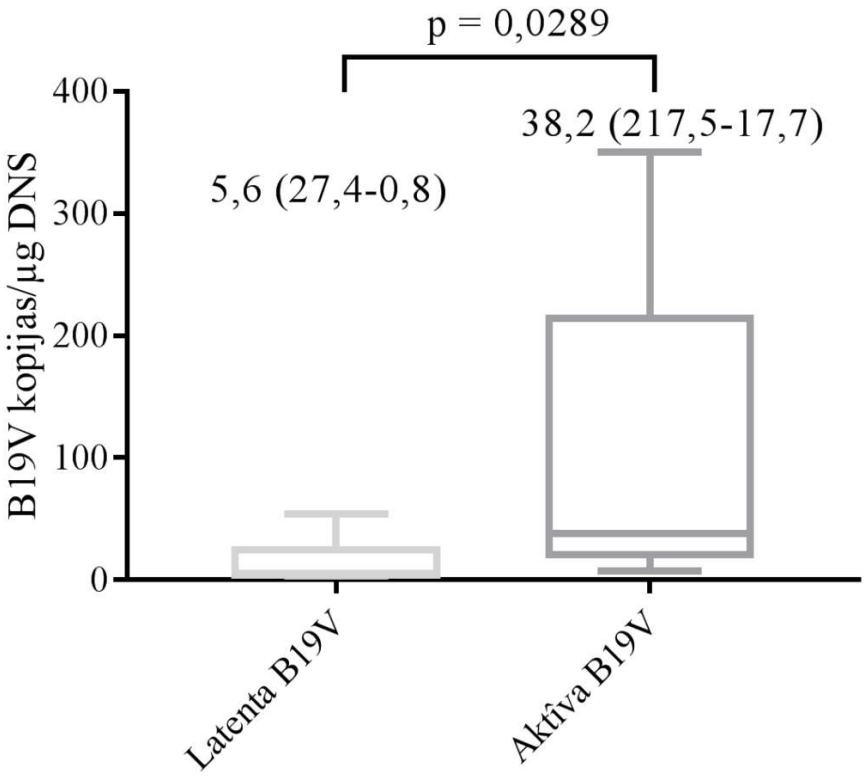
Involvement of B19V in development of ME/CFS

- Various reaction patterns of antibodies against 6 antigens of B19V (Vp-2p; VP-N; VP-1S; VP-2r; VP-C; NS-1)
- Association of time period after B19V infection with onset of ME/CFS



- Onset of ME/CFS 10,2 ± 4,2 months before the study
- 85% of ME/CFS patients symptoms started 8 – 12 months ago

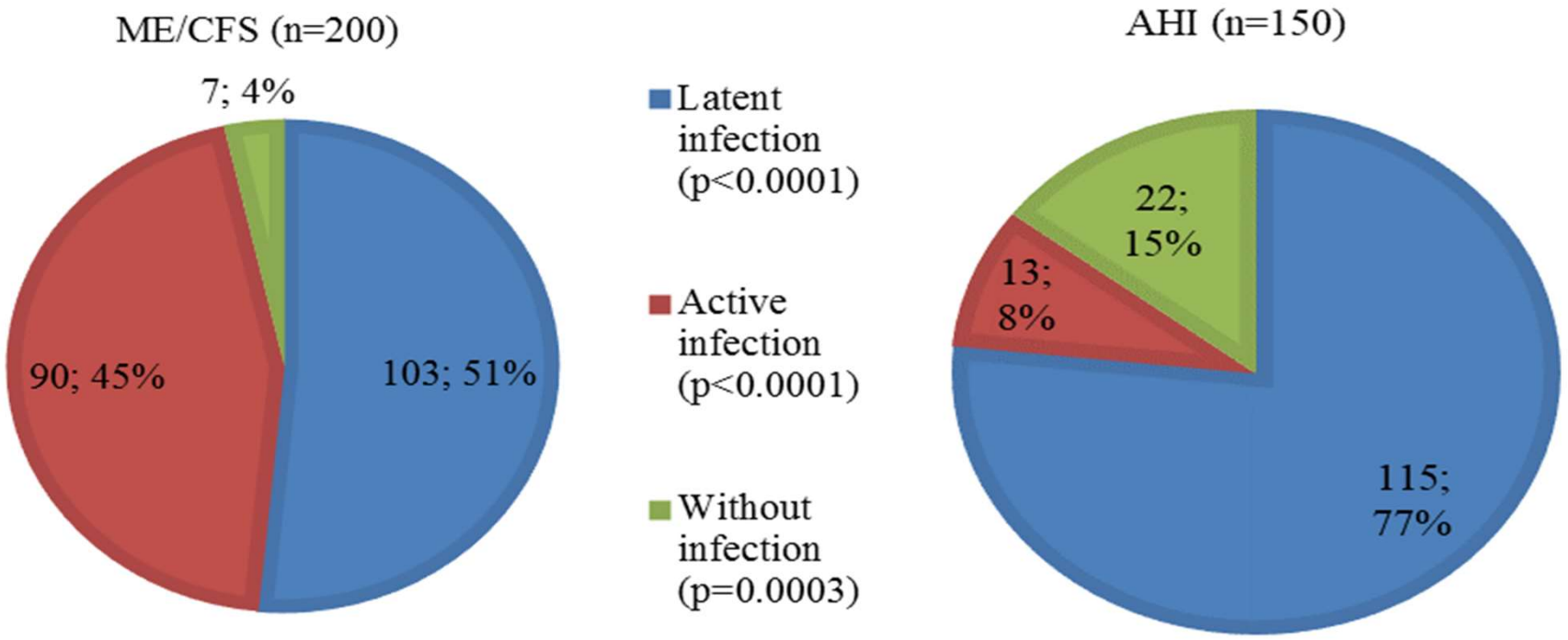
Involvement of B19V in development of ME/CFS



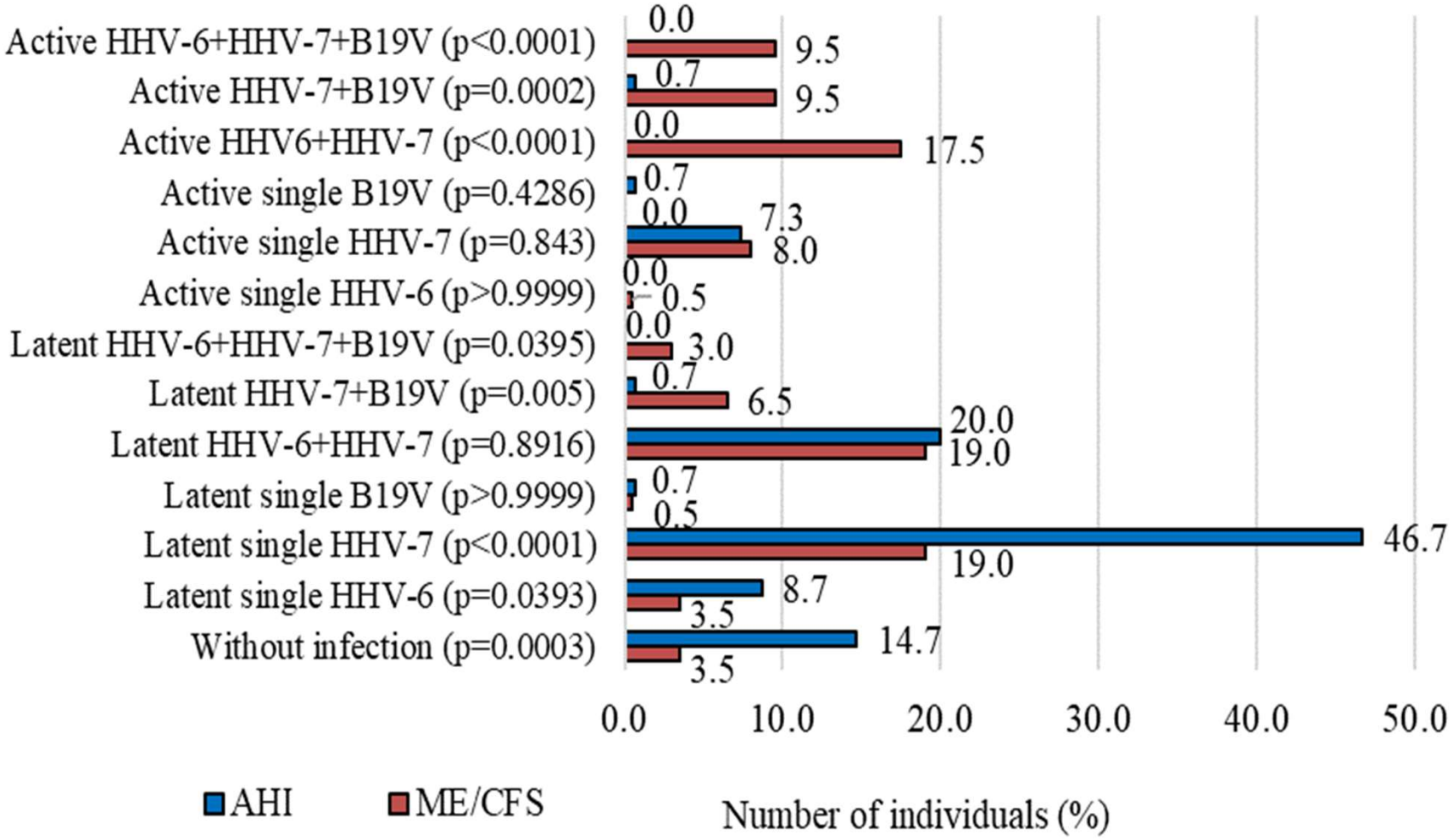
- Presence of B19V genomic sequences:
  - » Markers of latent phase – 12% (24/200) ME/CFS and 1.9% (2/104) PVI (p = 0.002)
  - » Markers of active phase – 17% (34/200) ME/CFS and 1.9% (2/104) from PVI (p < 0.0001)
- B19V load
  - » Elevated B19V load (> 0.2 copies/µg DNA) – 10% (20/200) ME/CFS and 0/104 PVI (p = 0.0003)
    - 9/24 latent phase
    - 11/34 active phase

Involvement of HHV-6, HHV-7 and B19V infection/co-infection in development of ME/CFS

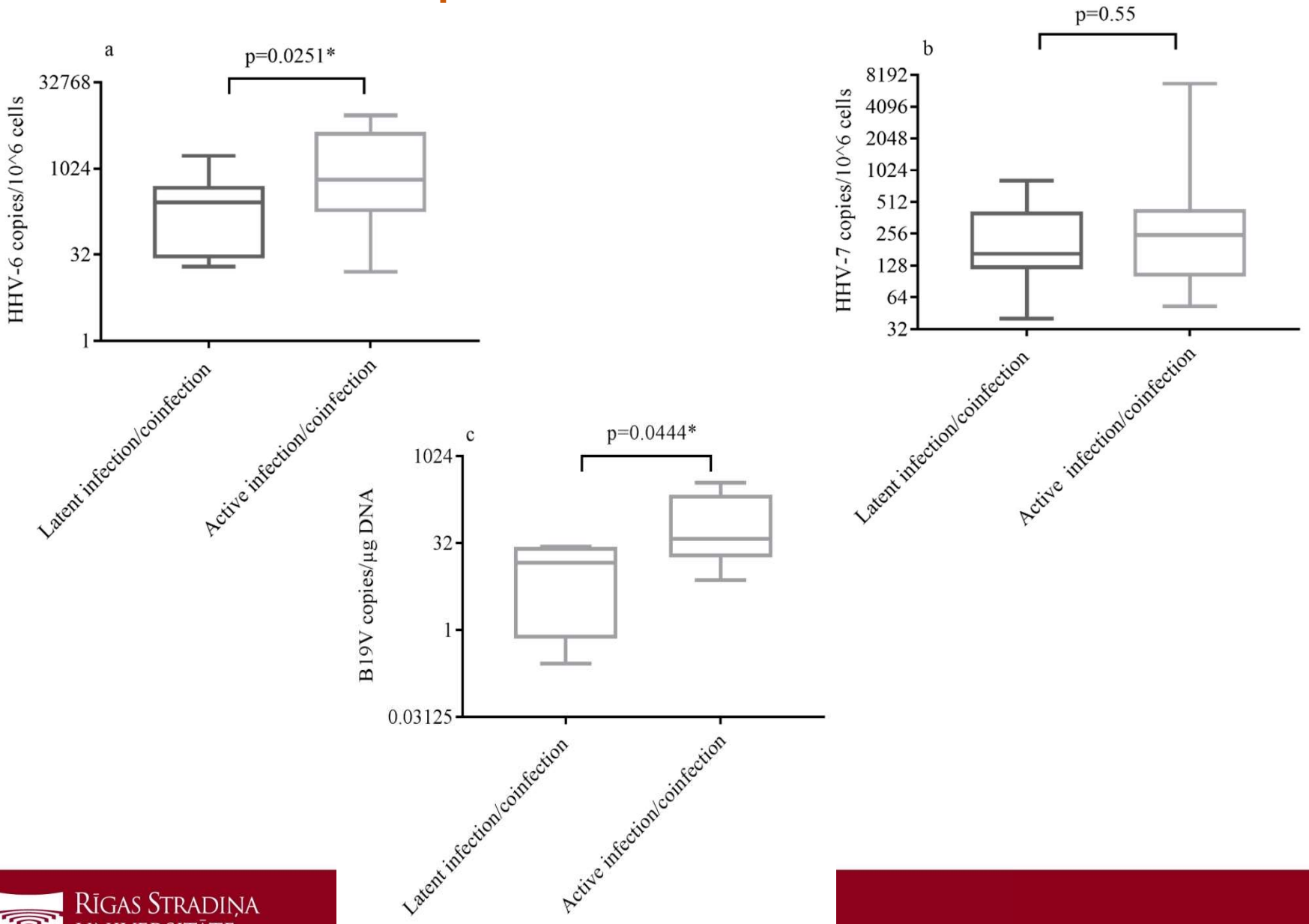
» Persistent viral infection/co-infection – 96.5% (193/200) of patients with ME/CFS and in 85.3% (128/150) of apparently healthy individuals (p = 0.0003)



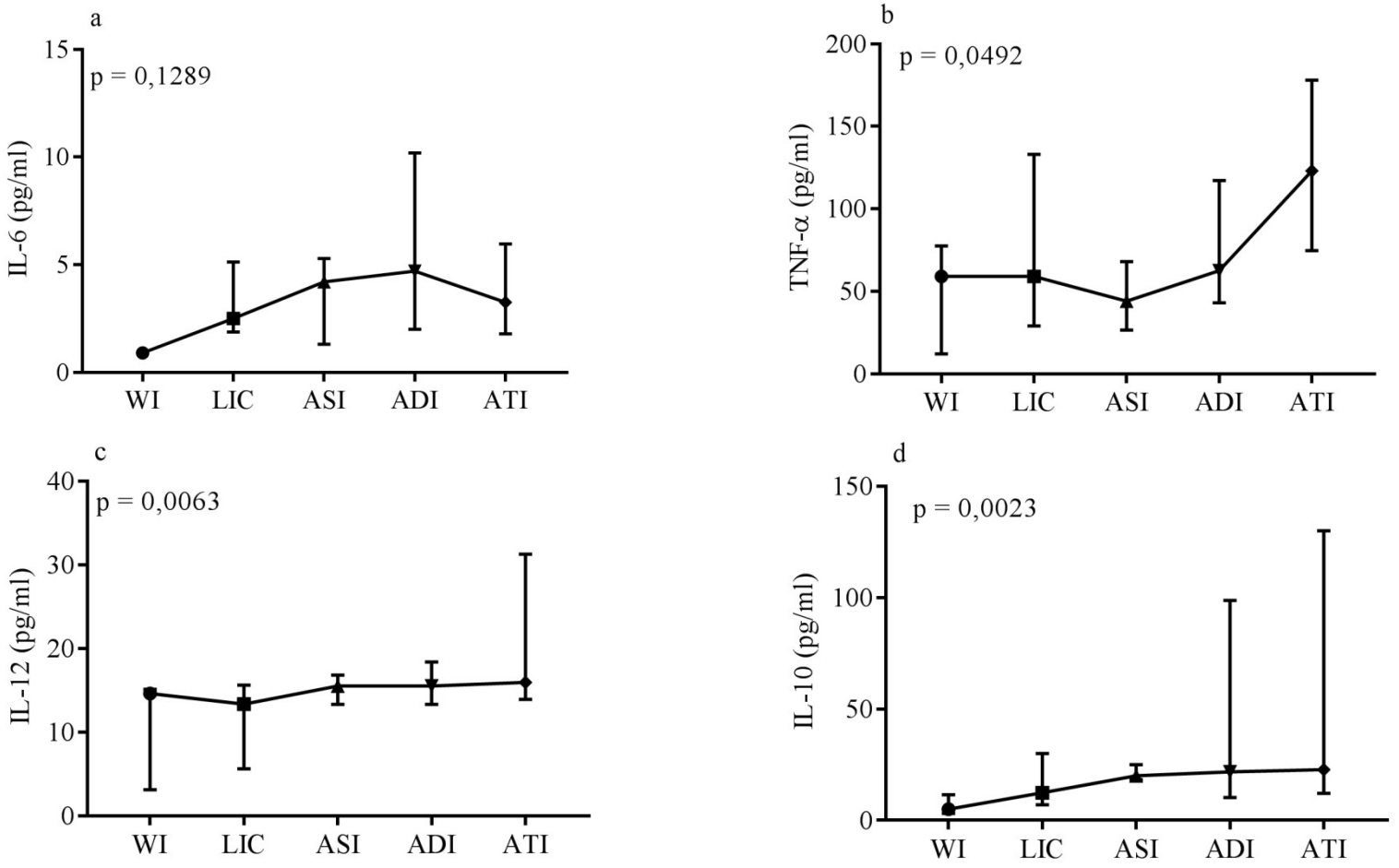
Frequency of persistent HHV-6, HHV-7 and B19V infection/co-infection (%) in latent or active phase



Viral load in patients with co-infection

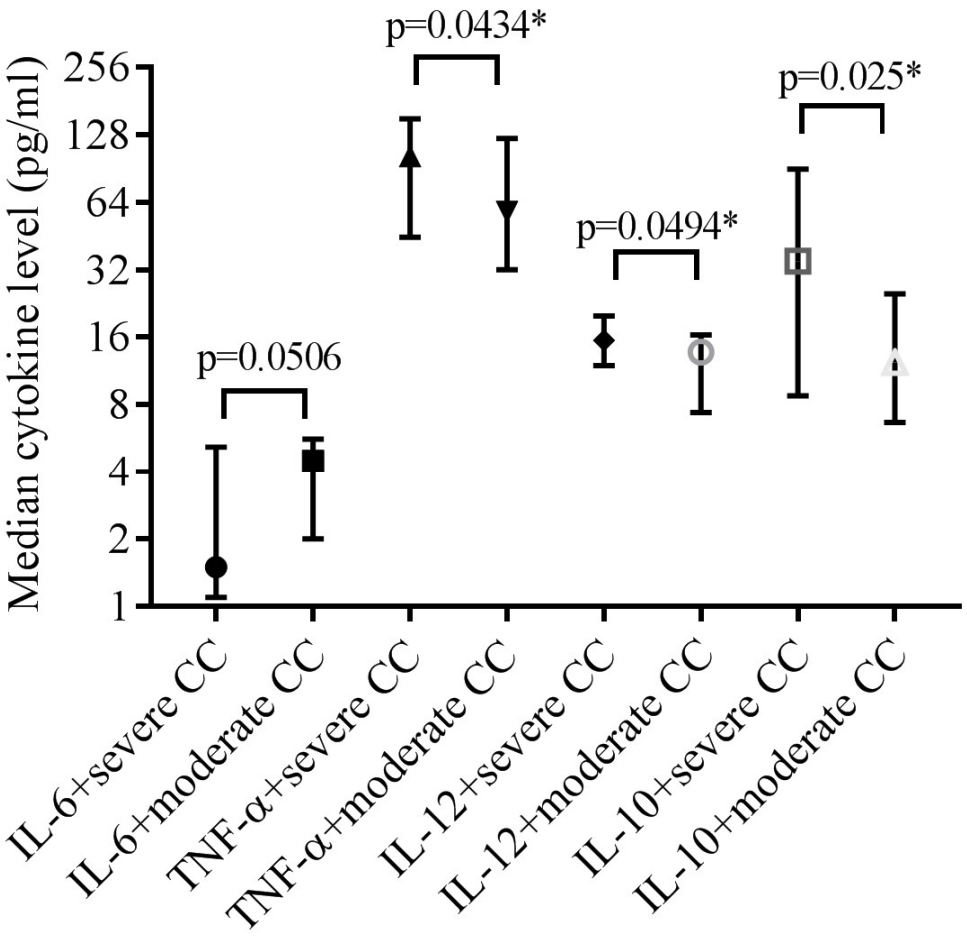


Cytokine level in ME/CFS patients with viral infection/co-infection



WI-without infection, LIC – latent infection/co-infection, ASI – active single infection, ADI –active double infection, ATI – active triple infection

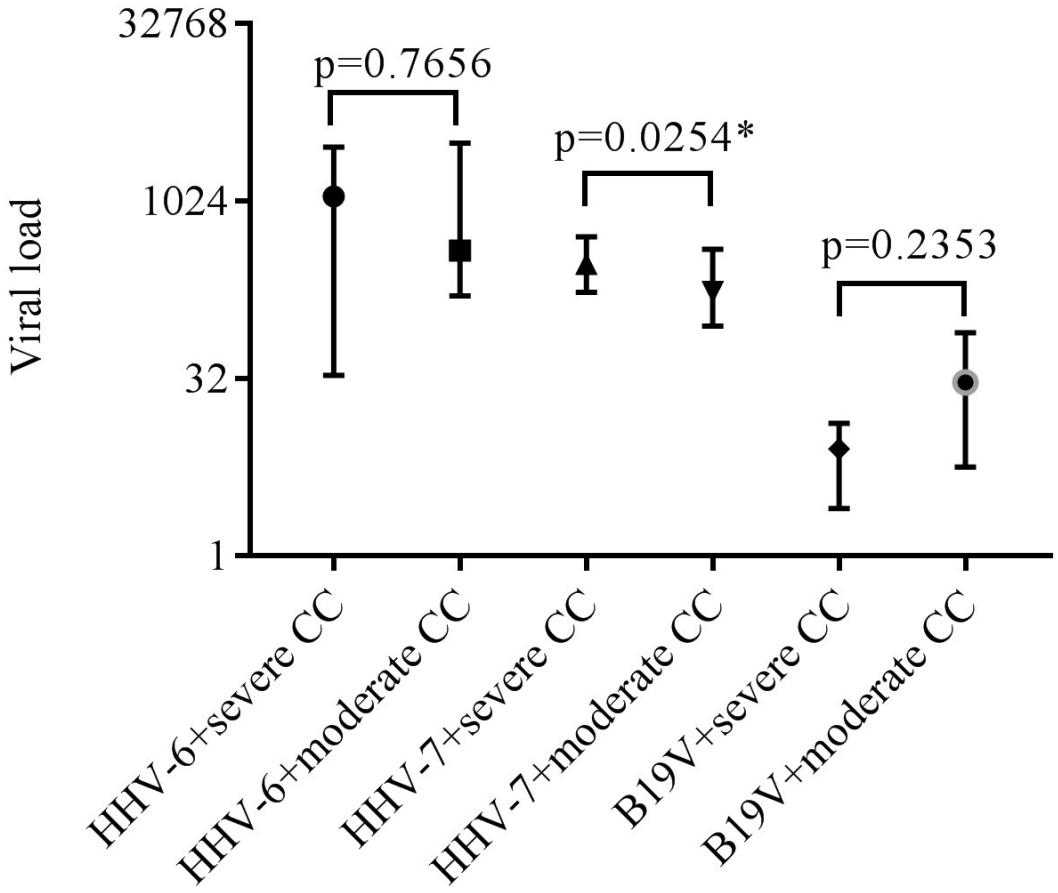
Association of level of cytokines with ME/CFS clinical course



» Median (IQR) IL-6, TNF-α, IL-10 and IL-12 level in ME/CFS patients with severe and moderate course of the disease

CC – clinical course

Association of viral infection with ME/CFS clinical course



» Median (IQR) HHV-6, HHV-7 (copies/106 cells) and B19V (copies/µg DNA) load in ME/CFS patients with severe and moderate course of the disease

CC – clinical course

Conclusions I

- Persistent HHV-6 and HHV-7 infection in an active phase is presented significantly more frequently and with a higher viral load among patients with ME/CFS than apparently healthy individuals, and HHV-6B is prevalent in Latvian ME/CFS patients.
- A more common finding of B19V (genotype 1) active infection with a higher B19V load in ME/CFS patients than in apparently healthy individuals and the coincidence of the infection time with the onset of the disease symptoms point to B19V as a possible trigger factor in ME/CFS development.
- HHV-6, HHV-7 and B19V persistent co-infection in an active phase is significantly more widespread among patients with ME/CFS compared to healthy donors and is characterized by a higher a viral load and level of cytokines in comparison to the latent phase of infection. Therefore, markers of HHV-6, HHV-7 and B19V infection could be used as one of biomarkers in ME/CFS diagnostics.

Conclusions II

- The level of cytokines is elevated in patients with ME/CFS indicating immune response to inflammation that could be caused by a viral infection. Also persistent HHV-6, HHV-7 and B19V co-infection in an active phase might significantly influence elevation of pro-inflammatory and anti-inflammatory cytokine levels, which can lead to immune disturbances and the development of ME/CFS symptoms.
- A higher HHV-6 and HHV-7 load and a significantly elevated level of pro-inflammatory cytokines TNF-α, IL-12 and anti-inflammatory cytokine IL-10 in patients with a more severe ME/CFS clinical course advocate on the involvement of these viral infections in ME/CFS development.

Review

- Retroviruses
- Enteroviruses
- B19V

- ~ 8 – 9 p

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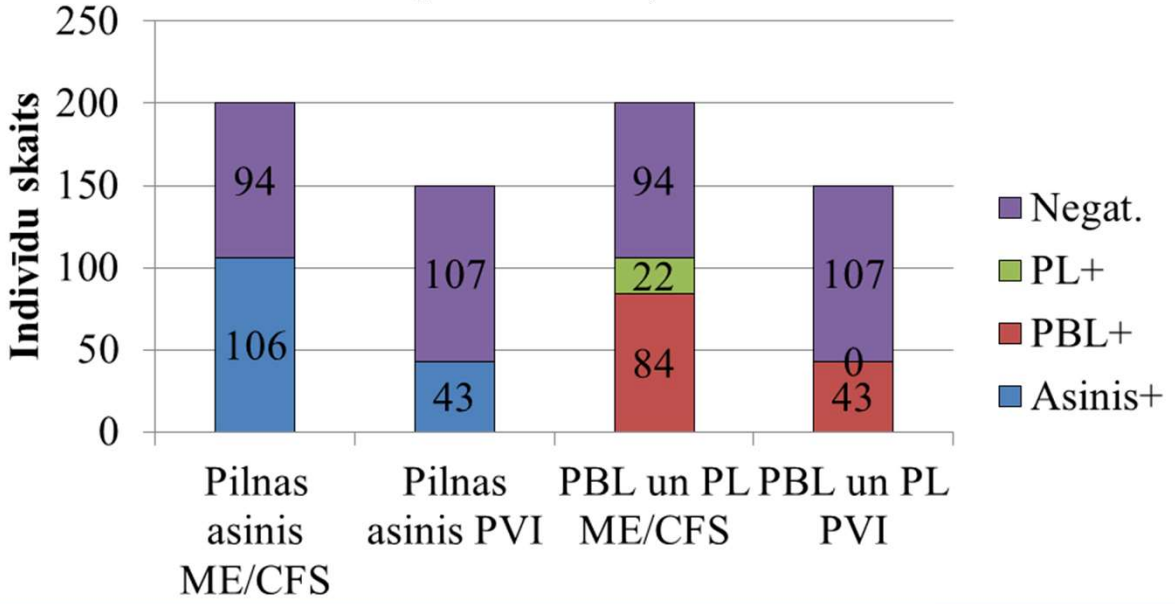
HHV-6 iesaiste ME/CFS attīstībā

- HHV-6 genoma secību biežums
  - » Persistenta HHV-6 - 53% (106/200) no pacientiem ar ME/CFS un 28,7% (43/150) no praktiski veseliem indivīdiem (p < 0,0001)
    - Latentā fāzē - 42% (84/200) ME/CFS un 28,7% (43/150) praktiski veselo indivīdu (p = 0,0133)
    - Aktīvā fāzē – 11% (22/200) ME/CFS un nevienam no praktiski veseliem indivīdiem (p < 0,0001)

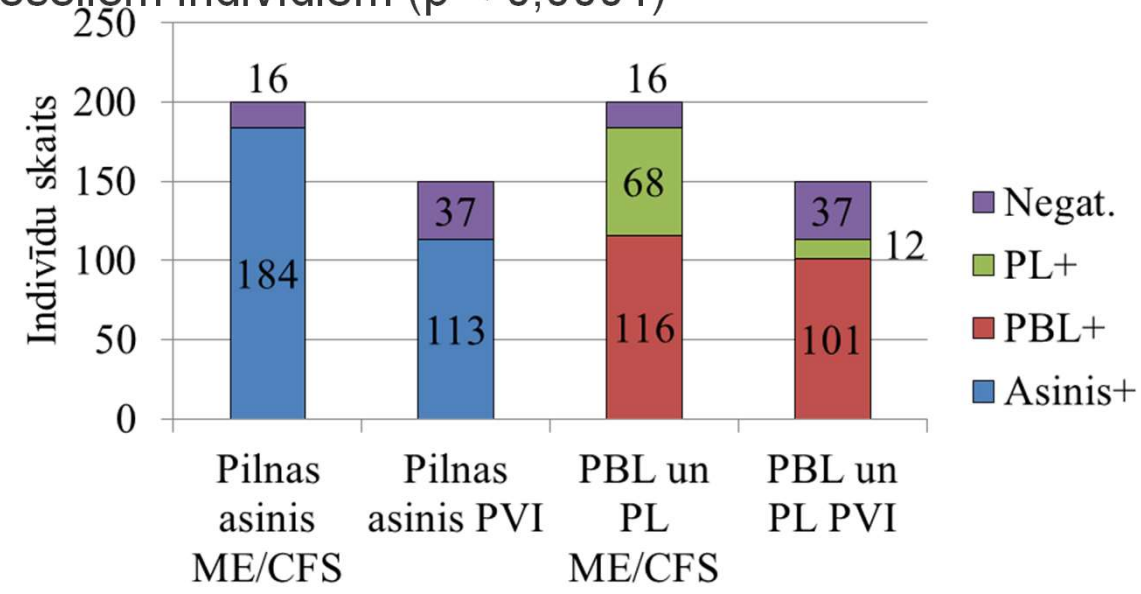


HHV-6 iesaiste ME/CFS attīstībā

- HHV-6A konstatēja vienam un HHV-6B pārējiem no analizētajiem pacientiem ar ME/CFS ($p < 0,0001$)
- Lietojot RT-PĶR, HHV-6 U89/90 gēna ekspresiju PBMC atrada 78% (57/73) no pacientiem ar ME/CFS
- HHV-6 antigēnu noteikšana
  - 36 pacientu PBMC ar netiešo imūnfluorescenci noteikta:
    - p41 ekspresija (6)
    - gp100 ekspresija (15)
    - gp116 ekspresija (7)

HHV-7 iesaiste ME/CFS attīstībā

- HHV-7 genoma secību biežums
  - » Persistenta HHV-7 - 92% (184/200) no pacientiem ar ME/CFS un 75,3% (113/150) no praktiski veseliem indivīdiem (p<0,0001)
    - Latentā fāzē - 58% (116/200) ME/CFS pacientu un 67,3% (101/150) praktiski veselo indivīdu (p = 0,0766)
    - Aktīvā fāzē – 34% (68/200) no pacientiem un 8% (12/150) no praktiski veseliem indivīdiem (p < 0,0001)

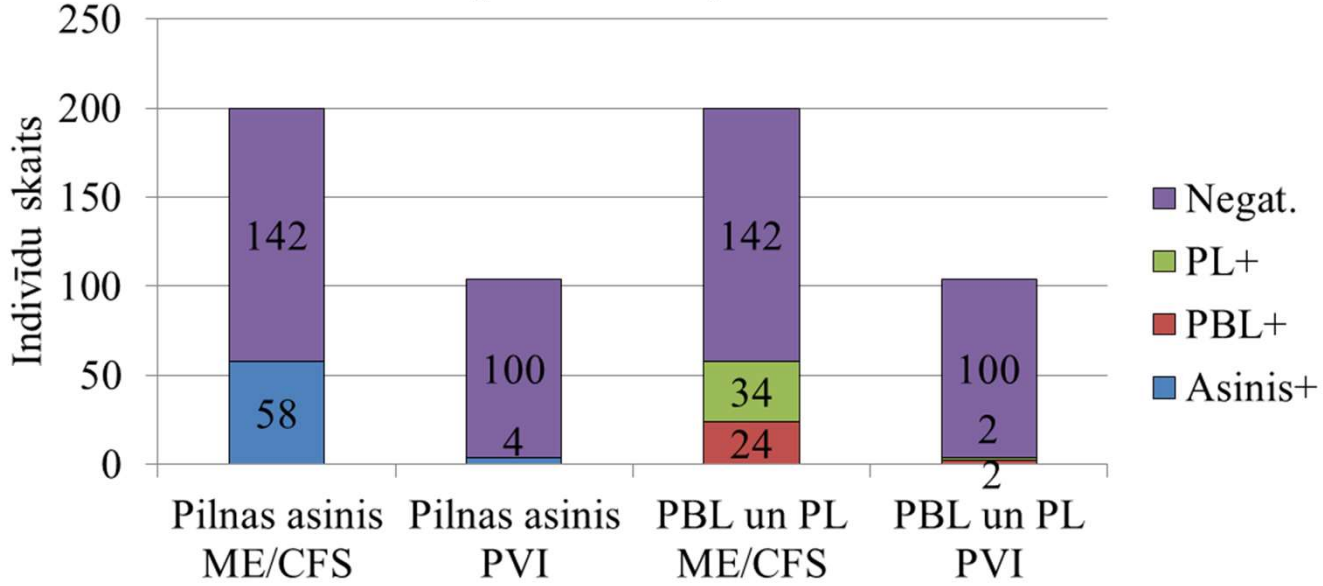


HHV-7 iesaiste ME/CFS attīstībā

- HHV-7 U57 gēna ekspresiju PBMC atrada 45,7% (58/127) ME/CFS pacientiem ar RT-PĶR

B19V iesaiste ME/CFS attīstībā

- B19V genoma secību biežums
  - » Persistenta B19V - 29% (58/200) ME/CFS pacientu un 3.8% (4/104) praktiski veselo indivīdu ($p < 0,0001$)
    - Latenta/persistenta - 12% (24/200) ME/CFS un 1,9% (2/104) no praktiski veseliem indivīdiem ($p = 0,002$)
    - Aktīvā fāzē – 17% (34/200) ME/CFS un 1,9% (2/104) no praktiski veseliem indivīdiem ($p < 0,0001$)



B19V iesaiste ME/CFS attīstībā

» ME/CFS tipisko klīnisko simptomu procentuālais sadalījums pacientiem ar un bez detektējamām NS1 antivielām