The neutrophil infiltration of the central nervous system in patients with tick-borne encephalitis

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Disclosures

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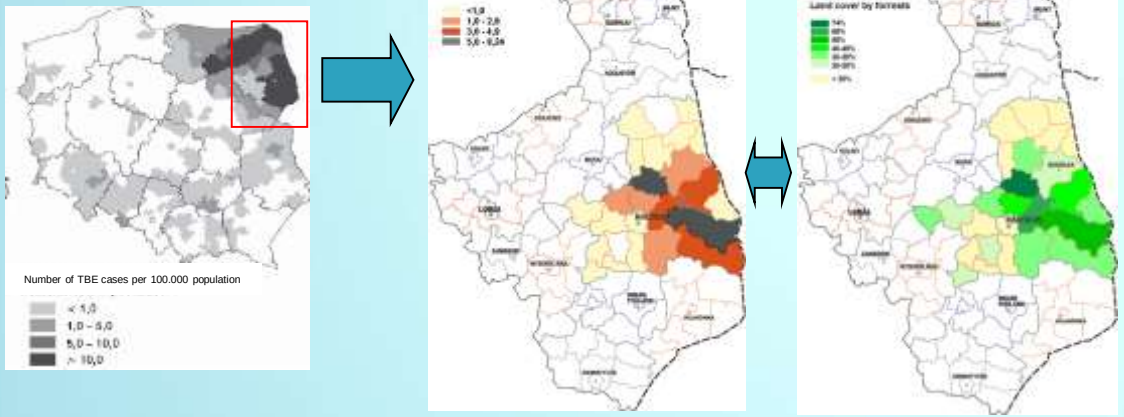
Tick-borne encephalitis (TBE)

Tick-borne encephalitis (TBE) is an anthropozoonotic disease caused by a TBE virus (TBEV, genus *Flavivirus,* family *Flaviviridae*) transmitted from wild animals to humans by *Ixodes sp.* ticks.

TBE – epidemiology



- TBE is endemic in the area stretching from Central Europe to Eastern Asia.
- Cases are concentrated in endemic areas, determined mostly by environmental factors.
- In Poland there were 283 cases reported in 2016, including 208 cases in the endemic region in the north-east.
- In the Department of the Infectious Diseases and Neuroinfections in Białystok – 1072 patients hospitalized from 1993 to 2014.

TBE – clinical presentation

I. **Peripheral phase** – viremia, flu-like infection

II. **Neurologic phase**

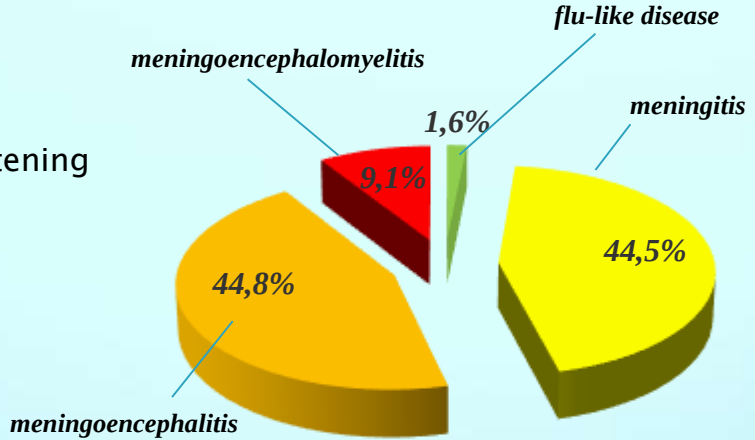
1) **meningitis**

2) **meningoencephalitis** – from mild to life-threatening

3) **meningoencephalomyelitis**

The disease limited to the peripheral phase is probably common, but rarely diagnosed.

The host factors determining the occurrence of the neuroinvasion, its clinical form and severity are not well known.



Clinical manifestations in 933 hospitalized TBE patients

TBE – pathogenesis

- The mode of TBEV entry into CNS is unclear and may depend on inflammatory response:

- blood-brain barrier (BBB) disruption by systemic inflammation ?
- migration with infected leukocytes?

(Wang et al., Nature Med. 2004; Arjona et al., J Clin Invest 2007; Bai et al., J Infect Dis 2010)

- The excessive intrathecal inflammatory/immune response to TBEV may contribute to the CNS pathology.

(eg. King et al. Immunol Cell Pathol 2007; Palus et al. J Neuroinflammation 2013; Růžek et al. Plos One 2011; Gelpi et al. J Neurovirol 2006)

TBE – neutrophils

- Neutrophils may constitute a majority of the CSF leukocyte population at the time of the diagnostic lumbar puncture in *Flavivirus* encephalitis, including TBE.

(Mickiené et al. Clin Infect Dis 2002; Kaiser and Holzmann, Infection 2000; Tyler et al. Arch Neurol 2004; Rawal et al. Diagn Cytopathol 2006; Srivastava et al. Int J Exp Path 1999)

- They might contribute both to the immunopathogenesis (blood-brain barrier disruption, excessive intrathecal inflammation) and to the protective anti-viral response.

- Infected neutrophils may carry *Flavivirus* into CNS and initiate/escalate neuroinfection (studied in animal WNV model so far).

(Bai et al., J Infect Dis 2010; Wang et al., PlosONE 2012)

Mediators of the neutrophilic inflammation…

- Th17-type cytokines: **IL-17A, IL-17F, IL-22** – act directly on tissue cells, promoting the inflammatory response dominated by neutrophils.

- Chemokines signaling through CXCR1 and CXCR2, acting preferentially on neutrophils: **CXCL1, CXCL2, CXCL5, CXCL8 (IL-8).**

  (Liang et al. J Immunol 2010; Stark et al. Immunity 2005; Gaffen Cytokine 2008; Eyerich et al. Trends Immunol 2010; Bachmann et al. PLoS Pathogens 2010; Zrioual et al. J Immunol 2009; Matusevicius et al. Multiple Sclerosis 1999)

- **IL-17 and IL-22 effect on blood-brain barrier endothelium:**
  - increased permeability,
  - secretion of chemokines,
  - facilitated transmigration by leukocytes.

  (Kebir et al., Nat Med. 2007)

…in *Flavivirus* encephalitis

Few studies so far, but…

- In WNV infection in mice **Il-22** is responsible for the neutrophil migration into CNS and for the BBB virus crossing a with infected neutrophils. Mediated by **Cxcl1** and **Cxcl5** secreted by BBB endothelium.

  (Wang et al., PlosONE 2012)

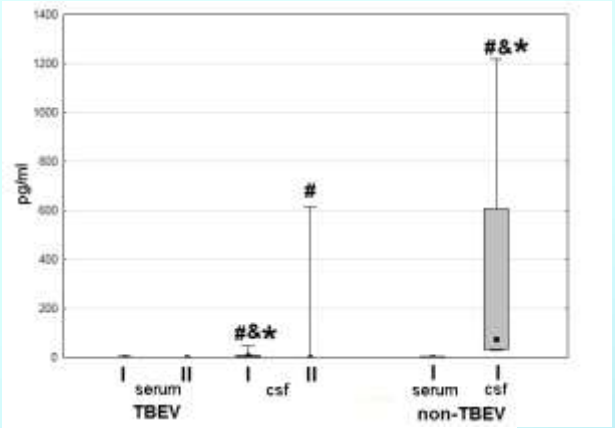
- In Japanese encephalitis in mice increased ratio of **Th17** to Treg cells is detrimental.

  (Kim et al. J Neuroinflammation 2016)

- **IL-8, IL-17** and **CXCL1** were detected in serum and/or CSF of TBE patients.

  (Palus et al., J med. Virol 2015; Pietikäinen et al. J Neuroinflammation 2016)

- We have recently detected an increased concentration of **IL-8** in CSF of TBE and non-TBE meningitis patients, with a significant concentration gradient towards CSF.



I – sample obtained on admission to hospital,
II – follow-up sample 12-16 days after admission;
* $p < 0.05$ for significant increase in comparison with control CSF
# $p < 0.05$ for comparison between csf and serum
& $p < 0.05$ for comparison between TBE and non-TBE groups

Study aim & design:

- We have attempted to gather more data on the role of neutrophils in the pathogenesis of TBE in humans and on the cytokines involved in their recruitment into CNS.

- We have analyzed associations between the CSF neutrophils count and other laboratory and clinical parameters retrospectively.

- We have studied concentrations of CXCR1/CXCR2-specific chemokines and of Th17-type cytokines in serum and CSF in a prospectively recruited TBE patient group.

Material/methods:

Retrospective study group

- 240 patients with serologically confirmed neurologic TBEV infection hospitalized from 2009 to 2014: meningitis (M) in 114, meningoencephalitis (ME) in 110, meningoencephalomyelitis (MEM) in 16
- Four patients with severe encephalitis, unconscious or comatose on admission, including one fatal case.
- Twelve surviving patients with permanent neurologic deficits.

Material

- Analysis of the medical records: the clinical presentation, the results of the diagnostic examinations.
- The severity of the disease and the mental status were scored with pre-defined point scales.
- The outcome was classified as: 0 – full recovery; 1 – persistent subjective complaints; 2 – objective neurologic deficits.

Statistical analysis

- We have searched for the association of the peripheral blood and csf neutrophil count with other laboratory parameters and with the clinical presentation.
- The chi-square test for correlations and Kruskal-Wallis ANOVA for comparisons between groups

Material/methods:

Prospective study group:

- 15 patients with TBE virus infection: meningitis (M) in 11, meningoencephalitis (ME) in 4: mild in 3, moderately severe in 1.

Material:

- Acute phase serum and cerebrospinal fluid (CSF) samples obtained early in the neurologic phase (usually on admission to hospital)
- Convalescent blood and csf samples 12-16 days later (IL-22, CXCL1)

Controls

- Meningitis: 6 patients with aseptic non-TBE meningitis hospitalized during Echovirus 30 meningitis outbreak (serum and CSF on admission, IL-17A, IL-17F, CXCL2).
- Healthy: 7 blood donors (serum) and 7 patients in whom cns infection was excluded (CSF).

Laboratory methods

- Concentrations of cytokines measured with commercial ELISA kits, following manufacturers' instructions. Sensitivity was 10 pg/ml for **CXCL1**, 5 pg/ml for **CXCL2**, 0.5 pg/ml for **IL-17A**, 0.5 pg/ml for **IL-17F**, 10 pg/ml for **IL-22**

Statistical analysis

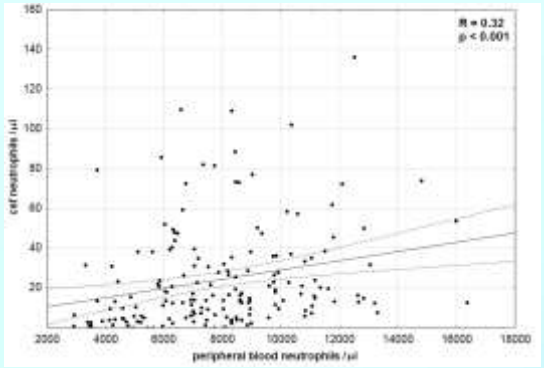
- Concentrations of cytokines were compared between groups, sub-groups of TBE patients defined by clinical manifestation, correlated with cerebrospinal fluid inflammatory parameters and stratified by clinical presentation.
- The data were analyzed with non-parametric tests (U Mann-Whitney test, Kruskal-Wallis ANOVA, Spearman test) and $p < 0.05$ was considered significant.

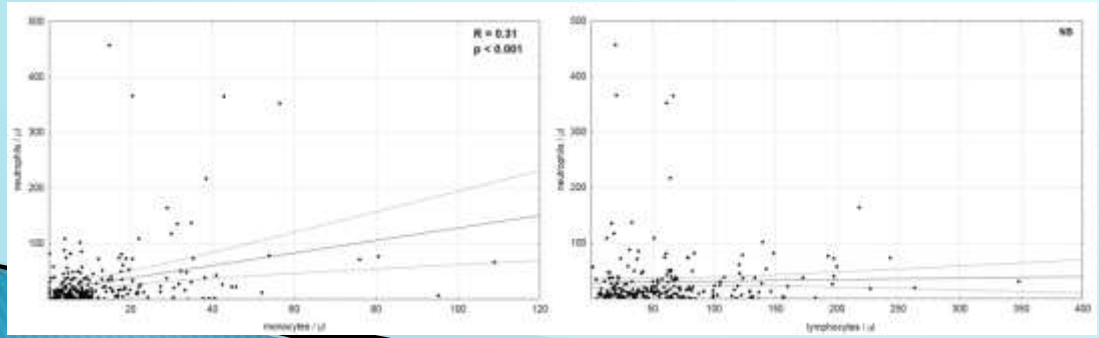
Results

- Retrospective study

CSF neutrophils

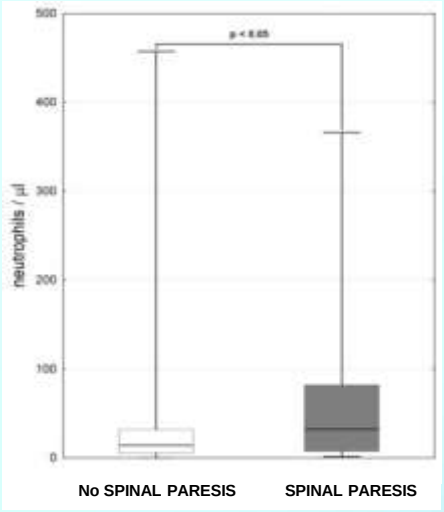
- Neutrophils constituted 25% of CSF pleocytosis on admission (median).
- They were present in a minority of follow-up samples, usually at << 10%.
- The CSF neutrophil count on admission correlated weakly with peripheral neutrophilia, but not with the concentration of C-reactive protein.
- The CSF neutrophil count on admission correlated weakly with csf monocyte count, but did not correlate with other CSF inflammatory parameters.

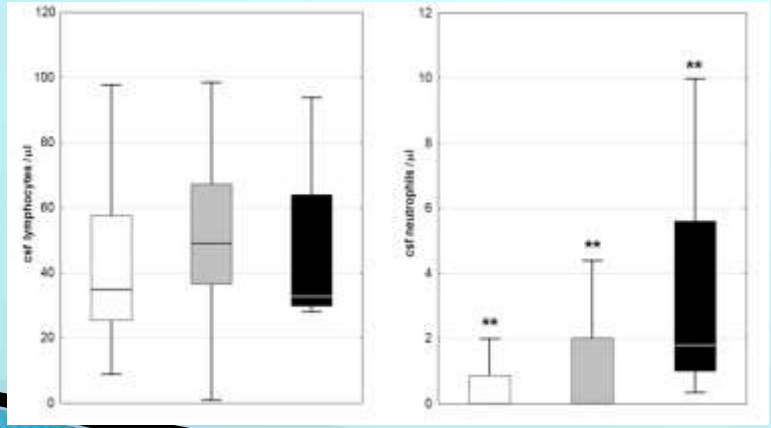




CSF neutrophils and clinical presentation

- CSF neutrophil count on admission was higher in the MEM group and in patients with spinal paresis than in the remaining TBE patients.
- There was no other significant correlation between admission neutrophil count and a clinical presentation or severity.
- The continued presence of neutrophils in the follow-up csf correlated with long-lasting neurologic sequelae.

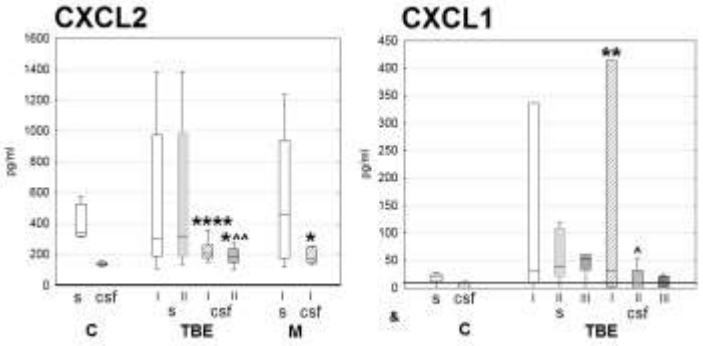


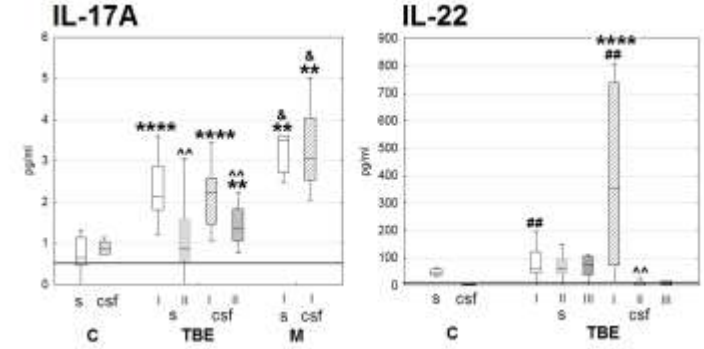


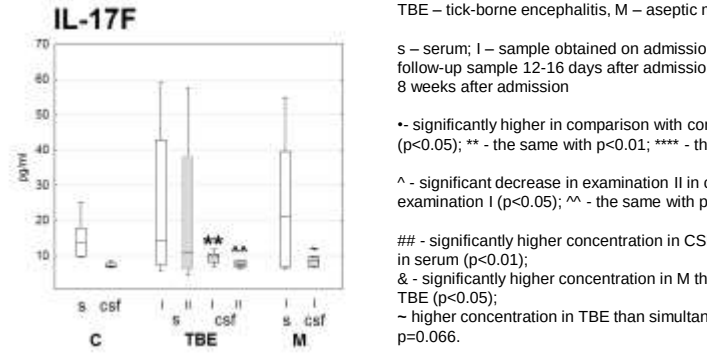
White – total recovery
Grey – subjctive complaints
Black – neurologic deficitis
** p < 0.01 for the trend

Results

- Prospective study







TBE – tick-borne encephalitis, M – aseptic meningitis, C – controls,

s – serum; I – sample obtained on admission to hospital, II – follow-up sample 12-16 days after admission; III – late follow-up 6-8 weeks after admission

•- significantly higher in comparison with control serum/CSF ($p<0.05$); ** - the same with $p<0.01$; **** - the same with $p<0.0001$;

^ - significant decrease in examination II in comparison with examination I ($p<0.05$); ^^ - the same with $p<0.01$;

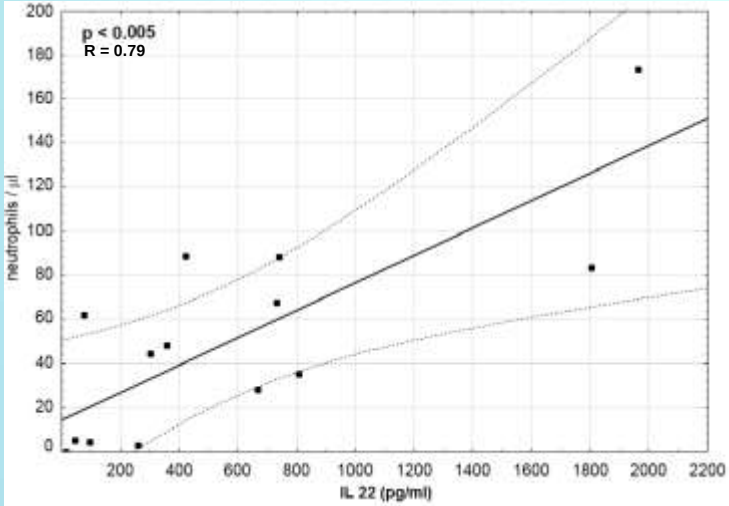
- significantly higher concentration in CSF than simultaneously in serum ($p<0.01$);
& - significantly higher concentration in M than simultaneously in TBE ($p<0.05$);
~ higher concentration in TBE than simultaneously in M with $p=0.066$.

Cytokine concentrations

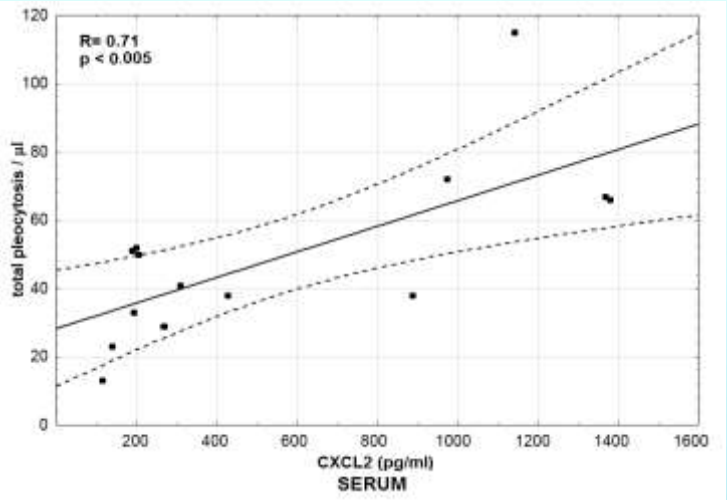
- **CXCL2** – up-regulated in admission and convalescent CSF, no difference between TBE and non-TBE groups.
- **IL-17A** – up-regulated in CSF and **serum** on admission, remained elevated in convalescent CSF, but lower concentration in TBE than in non-TBE meningitis.
- **IL-17F** – up-regulated in CSF on admission, higher concentration in TBE than in non-TBE meningitis.
- **CXCL1** – up-regulated in admission CSF only.
- **IL-22** – up-regulated in admission CSF, significantly higher than in serum.

Cytokine concentrations vs CSF cytosis

- **IL-17F** and **IL-22** concentration in CSF correlated with the neutrophil count, but not with other CSF parameters:



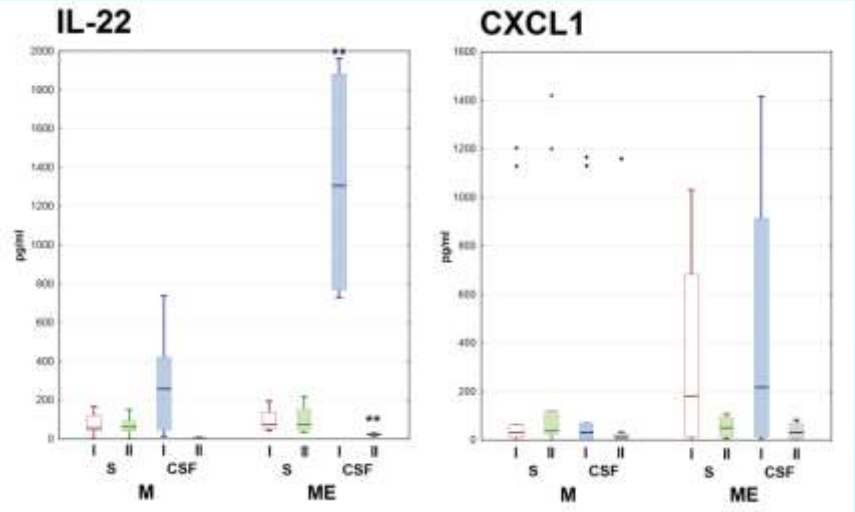
- **IL-17F** and **CXCL2** concentration in serum correlated with the neutrophil and lymphocyte count in CSF:



Cytokine concentrations vs clinical presentation

- Concentration of IL-22 in CSF on admission was higher in meningoencephalitis than in meningitis.
- It was the highest in a patient with the most severe presentation.
- Similar, non-significant trend for CXCL1, but not for other cytokines.
- IL-22 and CXCL1 concentrations in CSF correlated with one another.
- The association of IL-22 with the severity, neutrophil count and CXCL1 concentration is analogous to the animal model of WNV encephalitis, in which IL-22 was a factor driving CNS infiltration by WNV-infected neutrophils.

(Wang et al., PlosONE 2012)



M - meningitis (n=11)
ME - meningoencephalitis (n=4)
S – serum; CSF - cerebrospinal fluid; I – admission;II - follow-up;
** - significantly higher in ME than in M ($p < 0.01$)

Summary:

- The neutrophils migrate from the circulation into CSF of TBE patients early in the neurologic phase and independently of lymphocyte influx and BBB disruption.
- By analogy with animal models, they may be a vehicle of CNS invasion by TBEV.
- CNS neutrophil infiltration is associated with a spinal/radicular involvement and with persistent sequelae of encephalitis – involvement in CNS intrathecal pathology?

Summary:

- The Th17 cytokines and CXCR1/CXCR2-specific chemokines are up-regulated in TBE and involved in the neutrophilic response.
- Intrathecal IL-22 may be a factor determining the neutrophil influx into CNS, contributing to the neuroinvasion by TBEV and the occurrence of encephalitis.
- IL-22 concentration in CSF may be a marker of the CNS tissue involvement in TBE.

Future investigation:

- Is an increased expression of IL-22 in CSF specific for *Flavivirus* encephalitis?
- Could IL-22 concentration in CSF be used as a marker of the CNS tissue involvement and its severity?
- Are infected neutrophils a vehicle of CNS infection by TBEV in humans? Is it a main route of neuroinfection in TBE ?