LABORATORY FINDINGS AND DIAGNOSTICS OF TBE

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SLOVENIA

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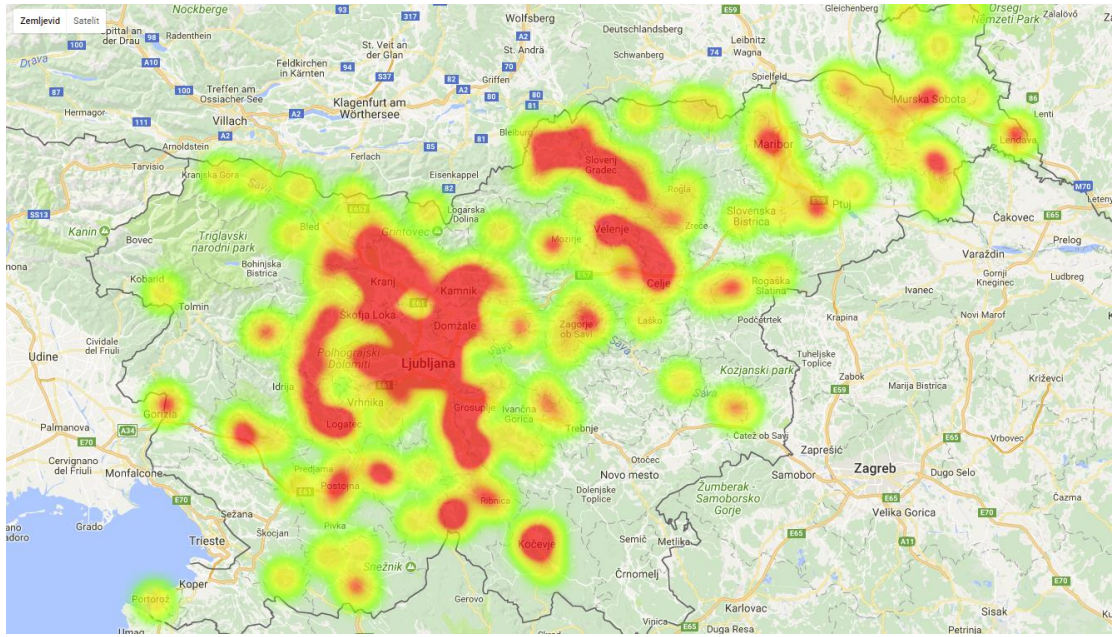
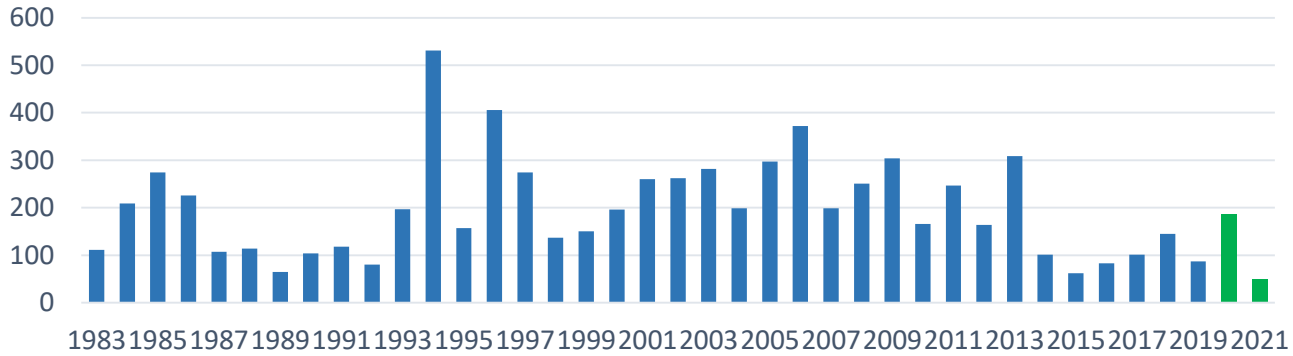
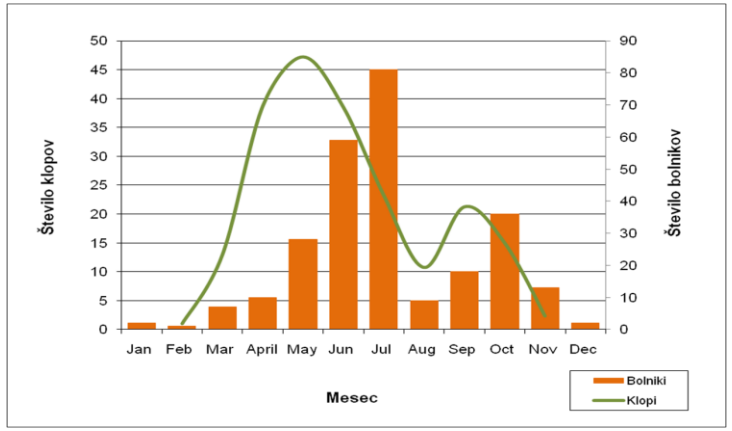
TBE IN SLOVENIA

- TBE was first diagnosed in 1953 and it became notifiable disease in 1977
- 200-300 cases/year; (14/100.000 population)

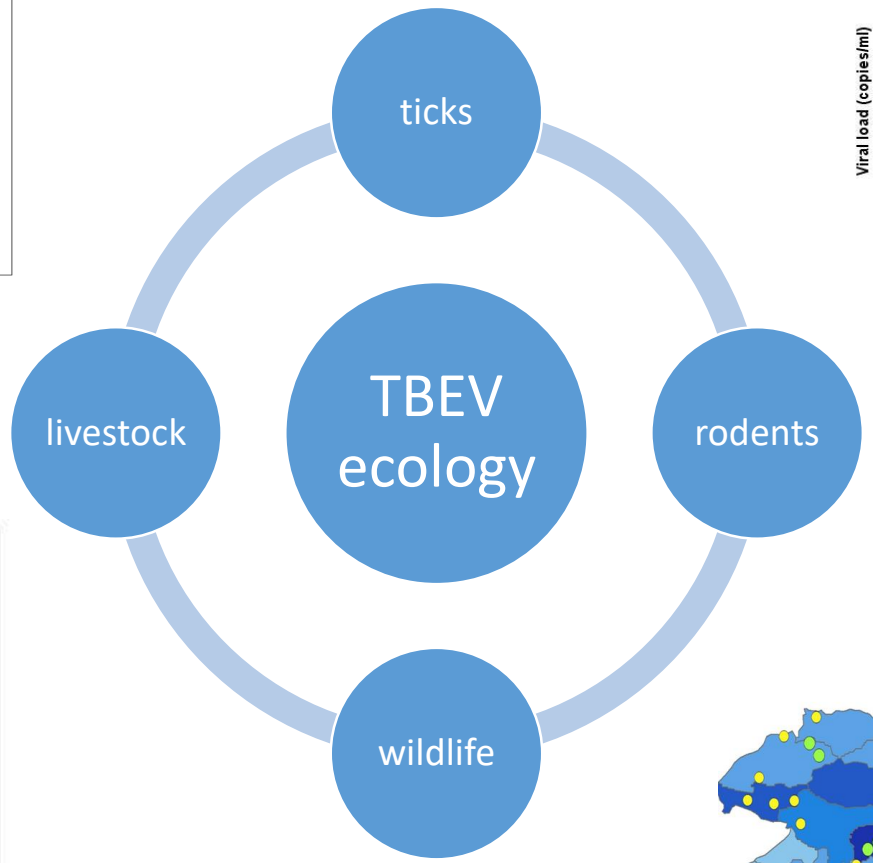


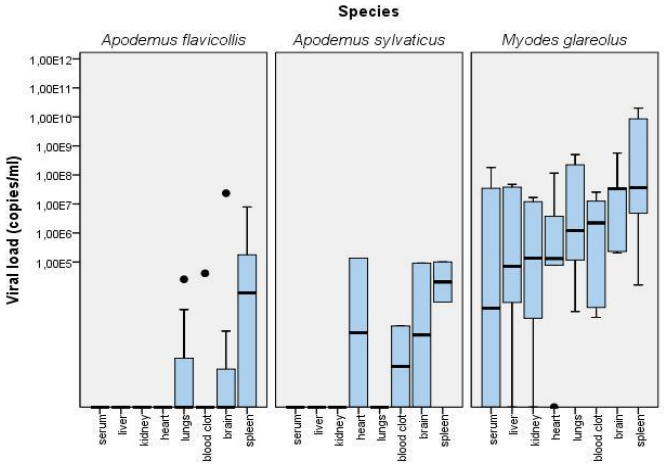




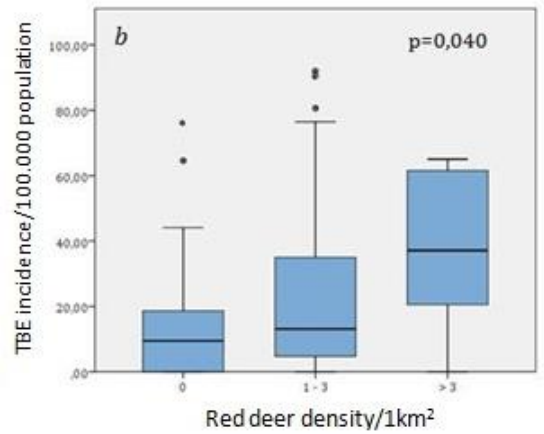
excretion of TBEV in milk!

- prevalence in ticks: 0.1-1.3 %
- bimodal seasonal dynamics

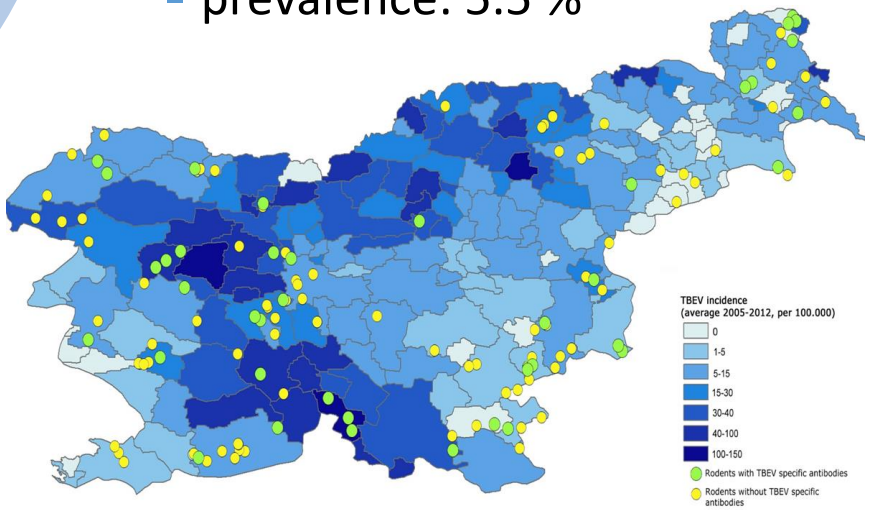




- viremic and non-viremic transmission
- prevalence: 5.5 %



- reproductive hosts for ticks
- red deer - direct influence on the incidence



Knap N. et all: numerous articles.

TBE CASE is DELINEATED by

EPIDEMIOLOGICAL CRITERIA

exposure to tick or consumption of unpasteurised dairy products

CLINICAL CRITERIA

symptoms/signs of inflammation of the CNS (e.g. meningitis, meningoencephalitis)

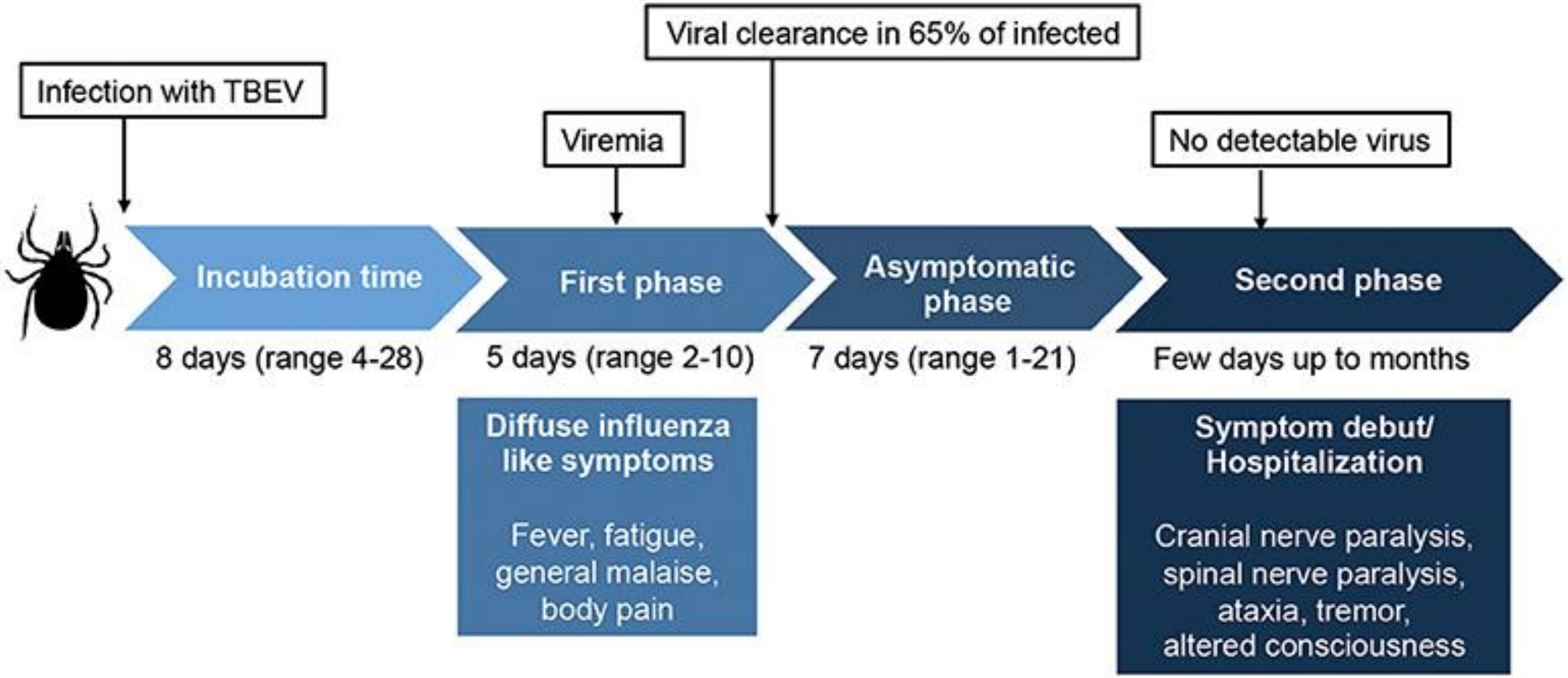
LABORATORY CRITERIA

an elevated cerebrospinal fluid cell count (> 5x 10^6 cells/L)

MICROBIOLOGICAL CRITERIA

TBE specific IgM AND IgG antibodies in blood or TBE specific IgM antibodies in CSF
Sero-conversion or 4-fold increase of specific antibodies in paired serum samples
Detection of TBE viral nucleic acid in a clinical specimen
Isolation of TBE virus from a clinical specimen

CLASSIC BIPHASIC DISEASE-PATTERN



Blom et al.: Front Immunol 2018.

FIRST PHASE

Non-specific symptoms – moderate fever, headache, body pain, fatigue, general malaise, nausea …

Meningeal signs absent

Leukopenia and/or **thrombocytopenia** (~70%)

Abnormal liver function tests (~20%)

Normal CSF findings !!

Last 2 – 7 days

Bogovič P et al.: numerous articles.

SECOND PHASE

CRP & blood leucocyte count normal/mildly elevated

biochemical examinations usually within normal range

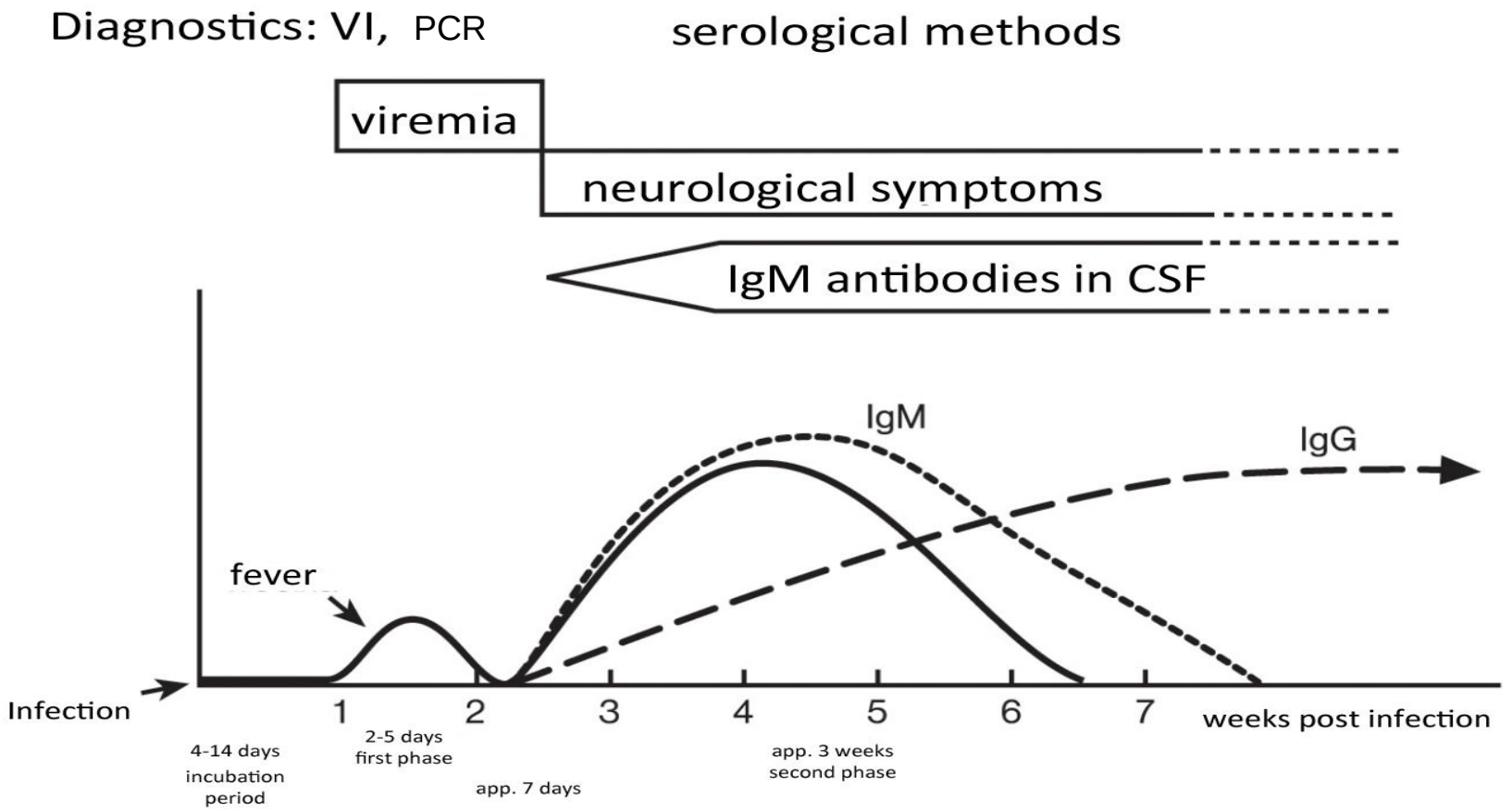
Pathological CSF findings !!

Elevated leucocytes (50–300 x 10^6/L, lymphocytic predominance)

Normal glucose concentration

Normal/moderately elevated protein concentration

TBE – MICROBIOLOGICAL DIAGNOSTICS



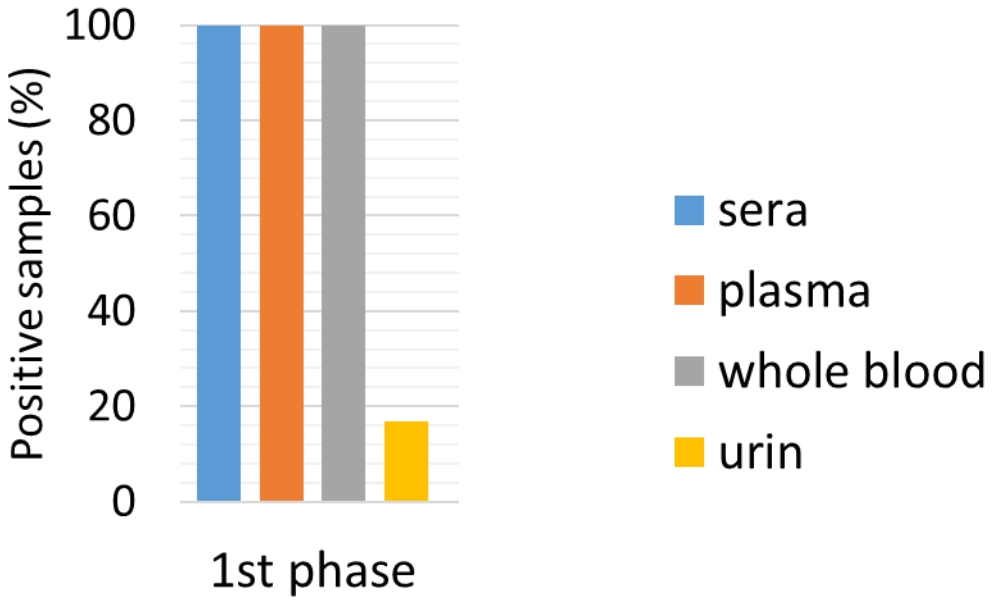
FIRST PHASE DIAGNOSTICS

TBE viremia – EDTA blood, serum, urin – **PCR** and virus isolation

100 % RNA detection in serum/plasma/whole blood before seroconversion;

only 16 % urine samples positive

No IgM or IgG antibodies in the initial phase of the disease!!!

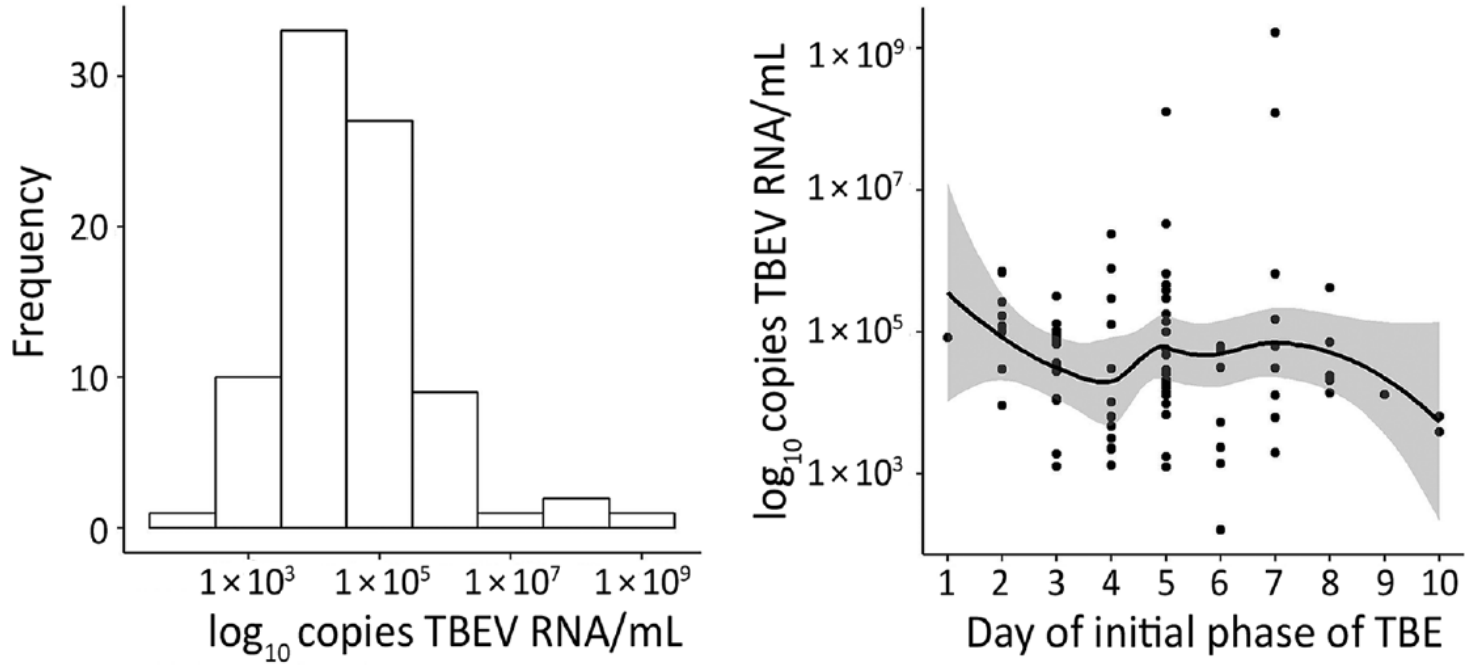


FIRST PHASE DIAGNOSTICS

TBEV RNA was detected in the time frame of 1–14 (median 5) days from the beginning of the initial phase of illness

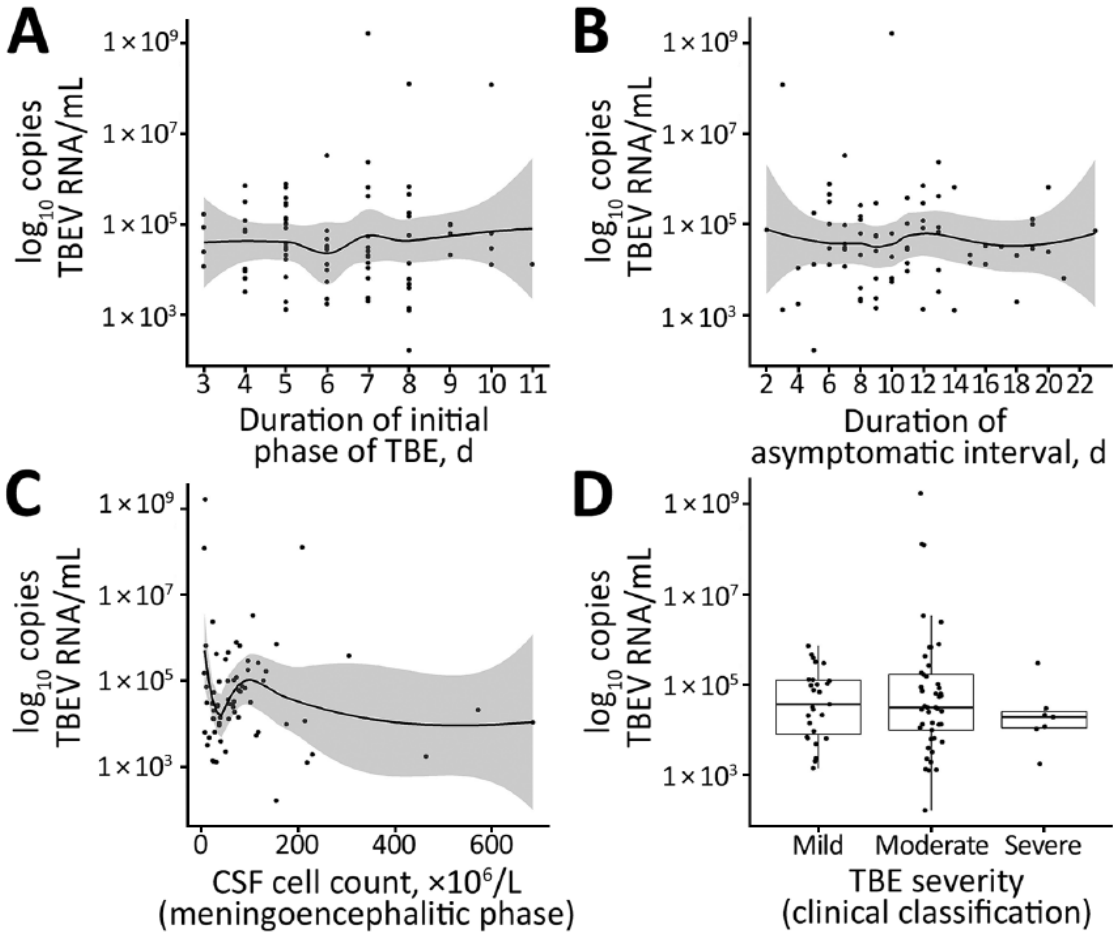
viral load ranged 3-6 $\log_{10}$ RNA/ml (median 4.65)

no significant VL dynamic in initial phase



Saksida A. et all. EID 2018.

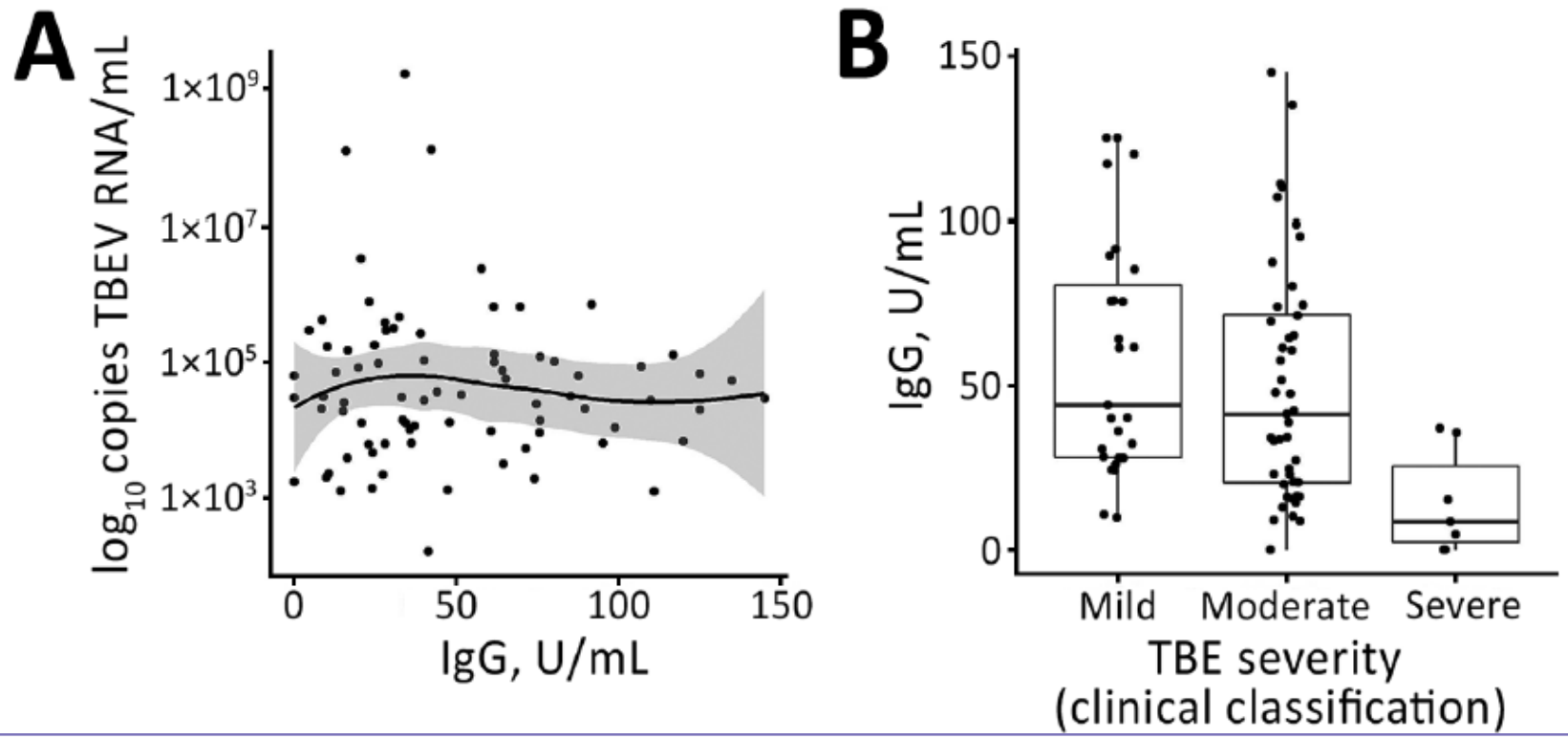
NO CORRELATION between viral load and duration of the first phase, duration of the asymptomatic interval, CSF cell count, leukocyte or platelet counts and clinical TBE severity



Saksida A. et all. EID 2018.

No direct association viral load and antibody response

But strong negative association between IgG and disease severity



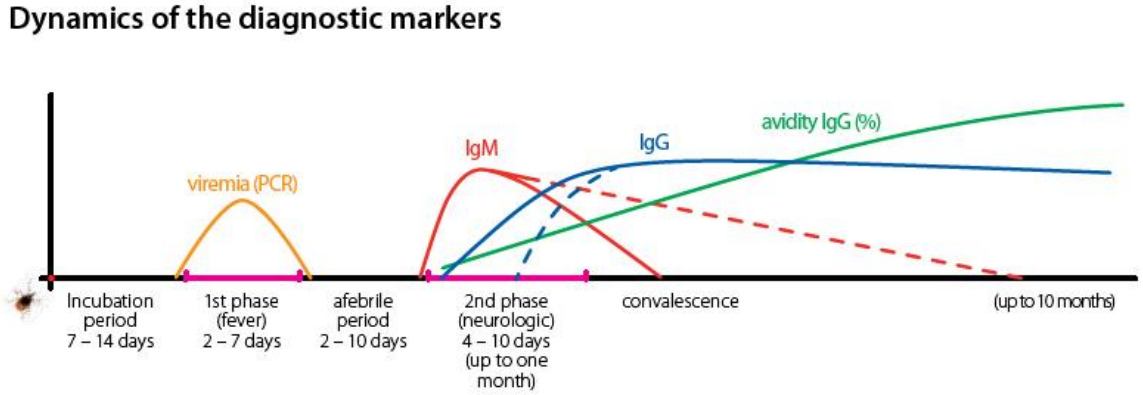
Failure of rapid viral clearance may be resulting in a more pronounced infection of neuronal cells and subsequently in a more severe clinical presentation.

Saksida A. et all. EID 2018.

SECOND PHASE DIAGNOSTICS

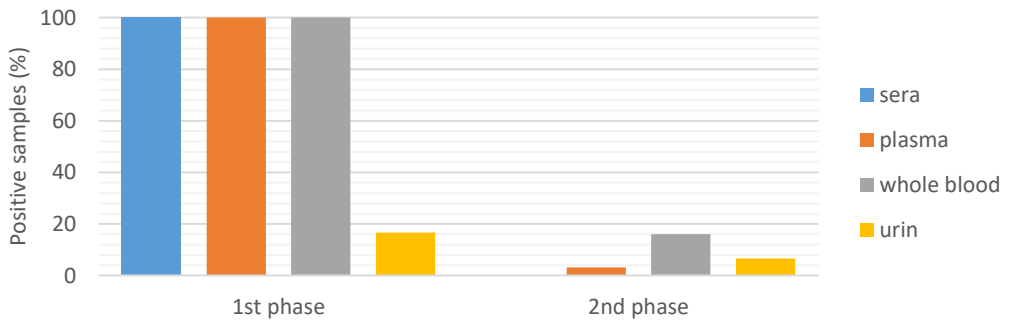
SEROLOGY:

- Specific IgM and IgG antibodies
- Detection of antibodies in sera and CSF
- Standardized method



MOLECULAR DETECTION -

- No virus in the blood after the apperance of IgG antibodies
- Only in 16 % low level RNA detection in blood after IgM antibody appearance and in 6.6 % of urine samples
- no RNA detected in CSF in neurological phase



SEROLOGICAL DIAGNOSTIC

Routine laboratory confirmation of TBEV infection is based on demonstration of:

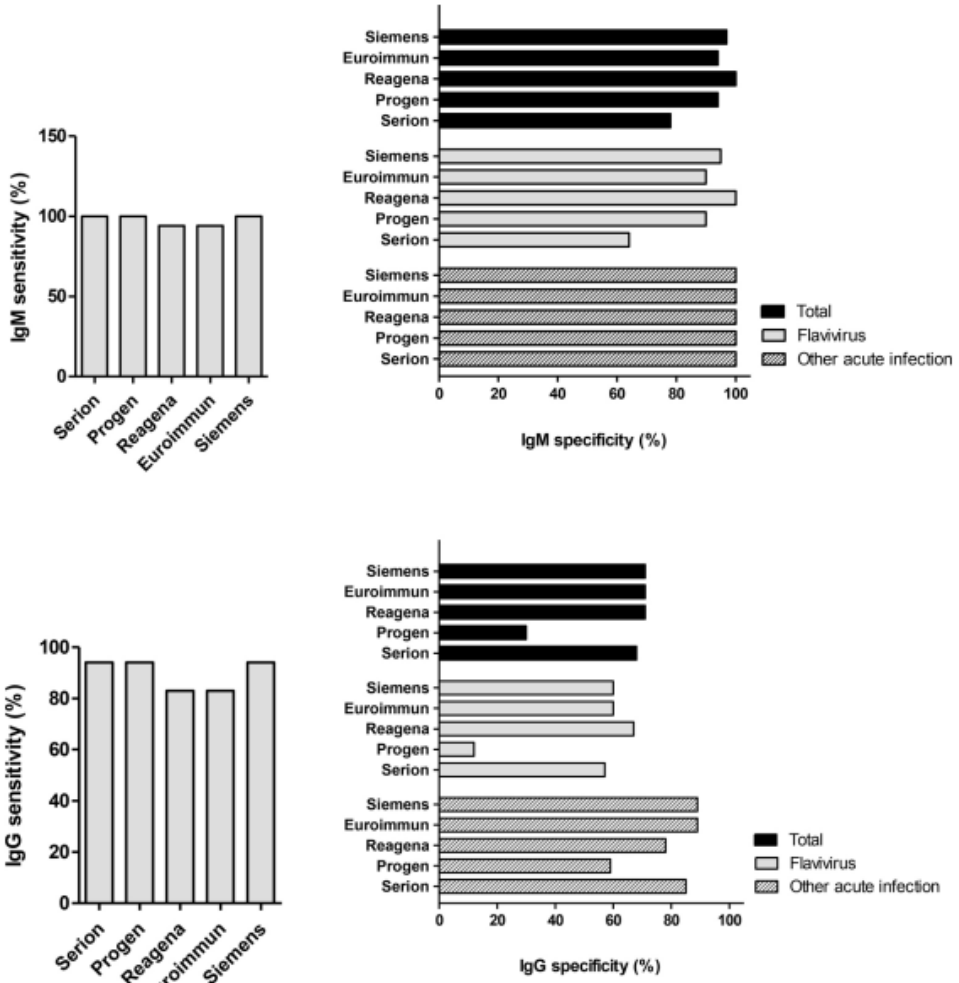
- specific IgM and IgG antibodies in serum and/or CSF
- 4-fold increase in specific antibody response in serum
- intrathecal TBE-specific antibody response in CSF
- Seroconversion

Demonstration of IgM ALONE is not sufficient for the diagnosis!

→ ELISA

- Standardized, automated method
- High sensitivity (IgM=98,8 %, IgG=99,5 %)
- High specificity (IgM=99,9 % (?), IgG =96,8%)
- Quantitative ELISA

Evaluation of serological methods ELISA

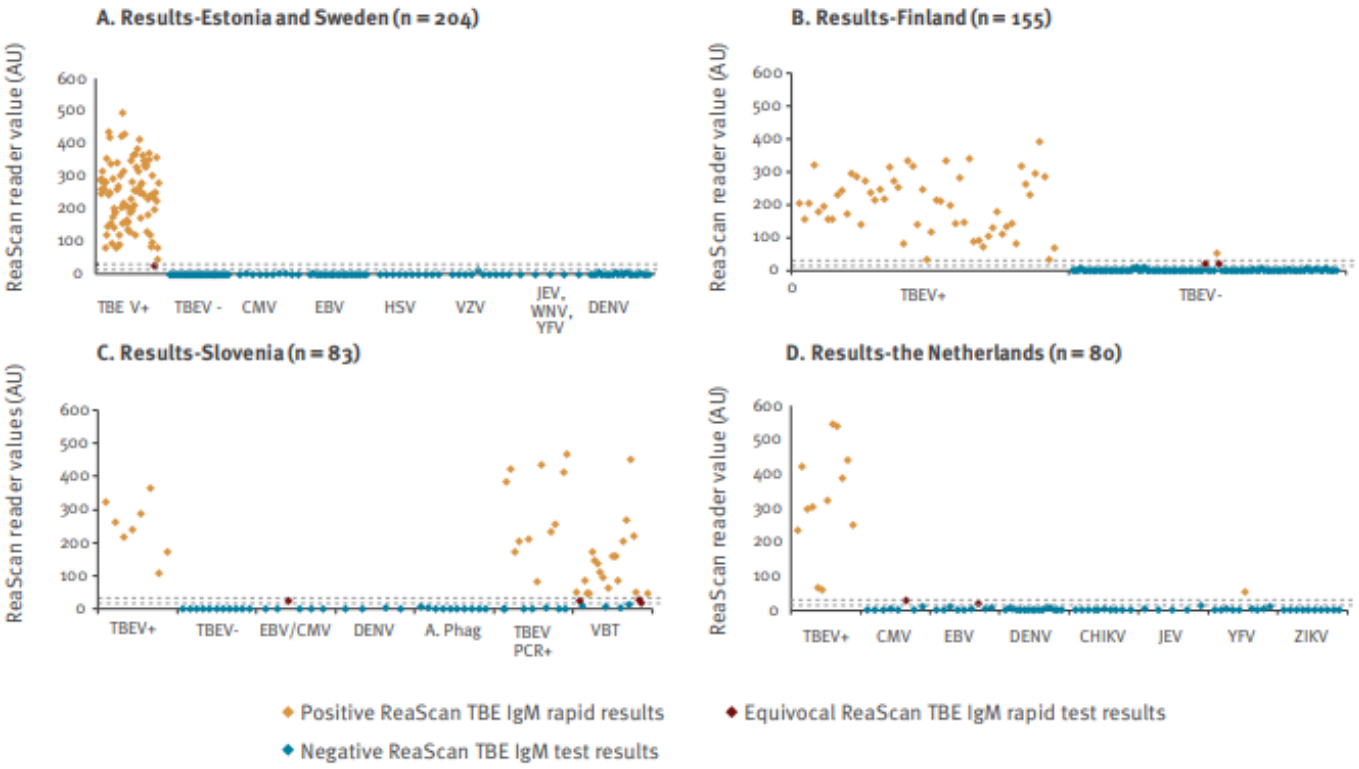


Sensitivity of the IgM TBEV ELISAs 94 – 100 %
Specificity of the IgM TBEV ELISAs 94 – 100%

Sensitivity of the IgG TBEV ELISAs 87 – 94%
Specificity of the IgG TBEV ELISAs 60 – 90%

Reusken C. et all.: J. Clin. Virol. 2019

ReaScan TBE IgM rapid test



Type of patients	ReaScan[a] result			Total	Sensitivity[b] (95% CI)	Specificity[c] (95% CI)
	TBE positive	TBE equivocal	TBE negative			
TBE patients	171	1	0	172	99.4% (96.8–100%)	97.9% (93.9–99.6%)
Non-TBE patients	1	2	138	141		

Type of samples	ReaScan[a] result			Total	Specificity (95% CI)
	TBE positive	TBE equivocal[b]	TBE negative		
Samples with potentially interfering agents (from patients with *A. phagocytophilum*, CMV, EBV, HSV and VZV infections)[c]	0	3	80	83	96.4% (89.8–99.3%)
Samples from patients with other flavivirus/togavirus (DENV, JEV, WNV, YFV, ZIKV, CHIKV) infections/vaccinations[d]	1*	0	81	82	98.8% (93.4–100%)

Albinsson B. et al.. Euro Surveill 2020

TICK BORNE ENCEPHALITIS – DIAGNOSTIC PITFALLS

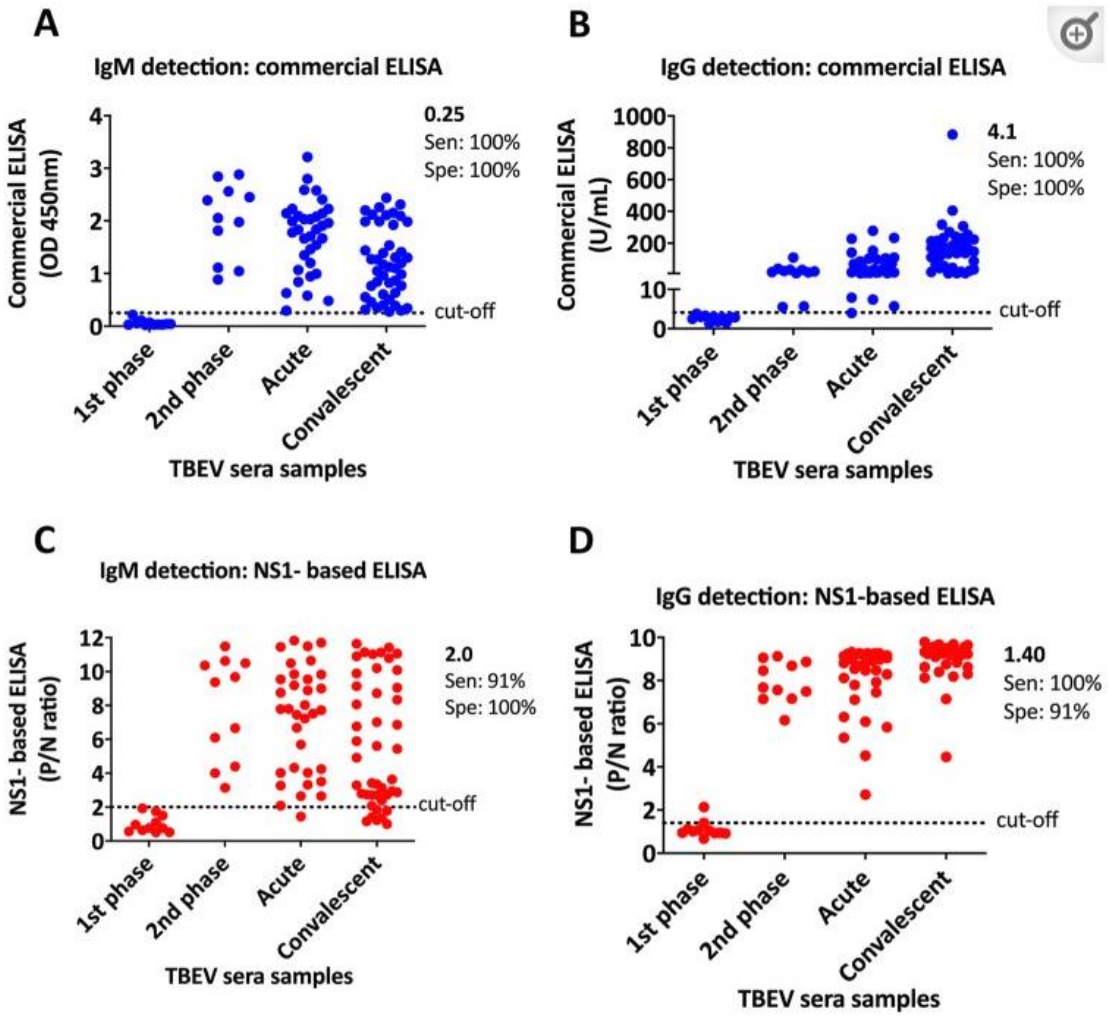
CROSS-REACTIVITY

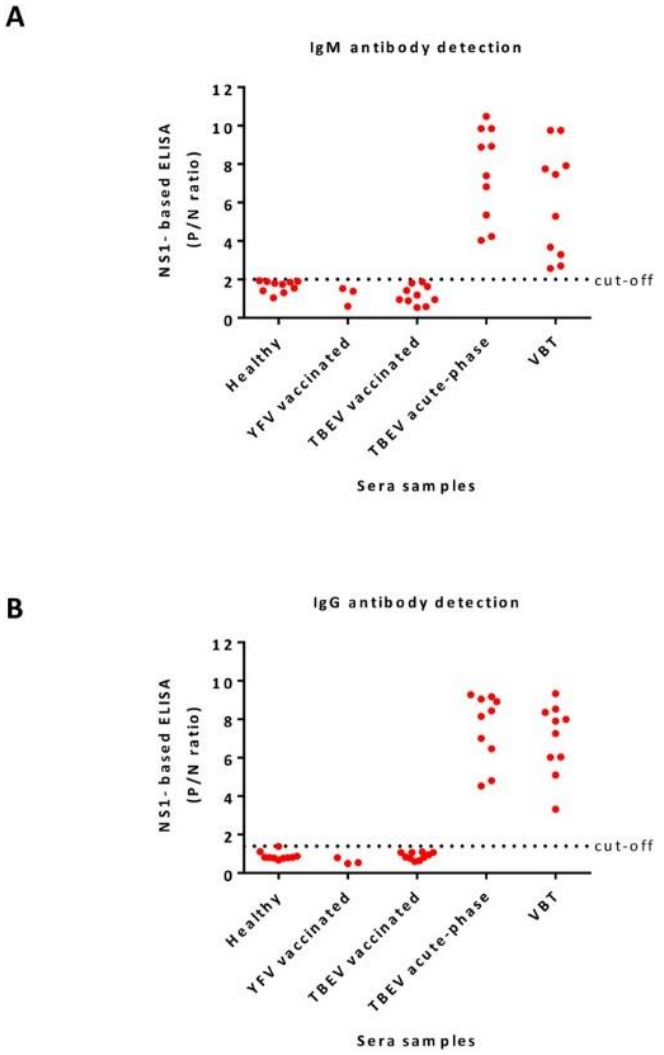
- exclusion of other viral exposure factors (vaccinations, travel to flavivirus endemic area)
- WNV, JEV, YFV, USUV, DENV, ZIKV
- neutralisation test, quantitative ELISA

VACCINATION BREAKTHROUGH

- Exclusion of other flavivirus infection
- Confirmation of CSF antibody formation
- Avidity testing for IgG antibodies
- TBE patients after vaccination have high avidity
- IgM antibodies are usually delayed!
- TBE NS1 specific antibodies only in infected, but not in vaccinated patients!
- Subsequent sera samples for confirmation of delayed IgM response, or an increase in specific IgG antibodies

NS1- based serological assays for the detection of TBEV immune response





Mora-Cardenas E. et all. PLoS NTD 2020

DIFFERENTIAL DIAGNOSIS

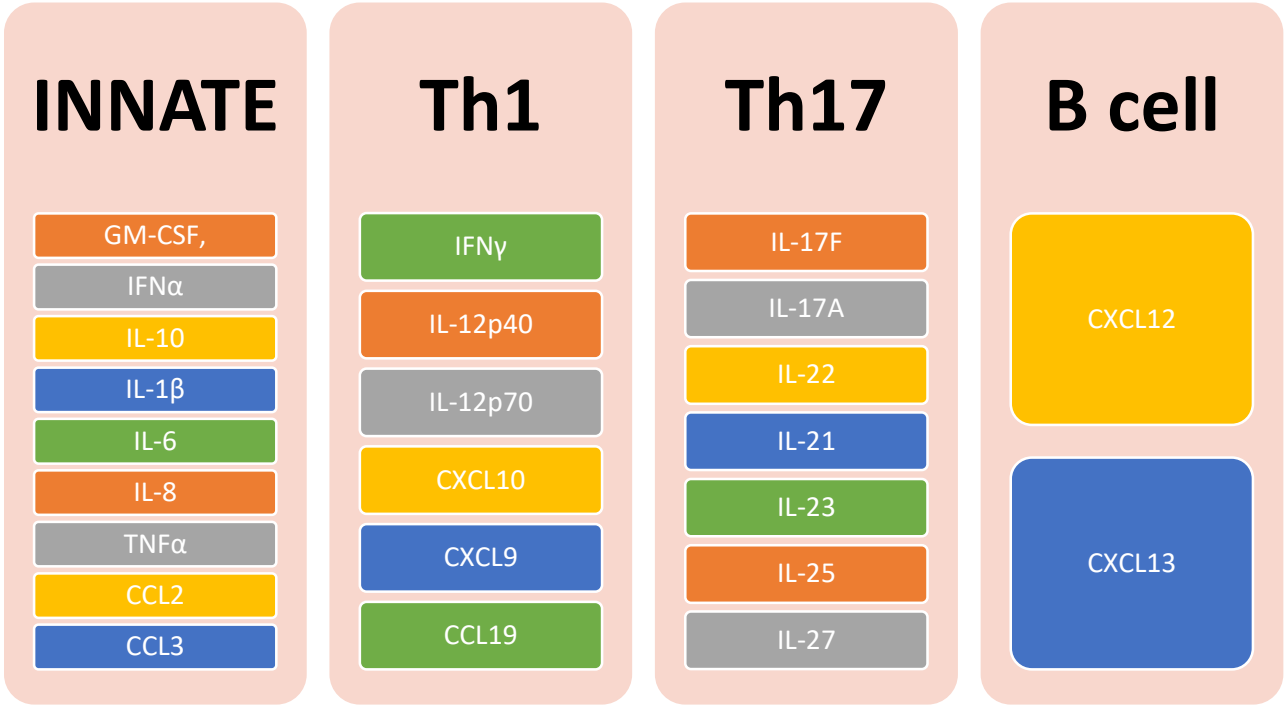
The differential diagnosis of TBE is extensive and includes a wide variety of CNS infections and non-infectious diseases.

- In initial phase anaplasmosis and also gastroenteritis is a possible explanation
- In neurological phase:
  - differentiation from other aseptic meningitis (WNV, JEV, HSV, adenovirus, enterovirus…)
  - other tick-borne diseases: Lyme borreliosis, babesiosis, HGA, tularemia, rickettsioses

- Co-infections!!!

CHARACTERIZATION OF CYTOKINES/CHEMOKINES

24 cytokines and chemokines were measured with Luminex bead assays



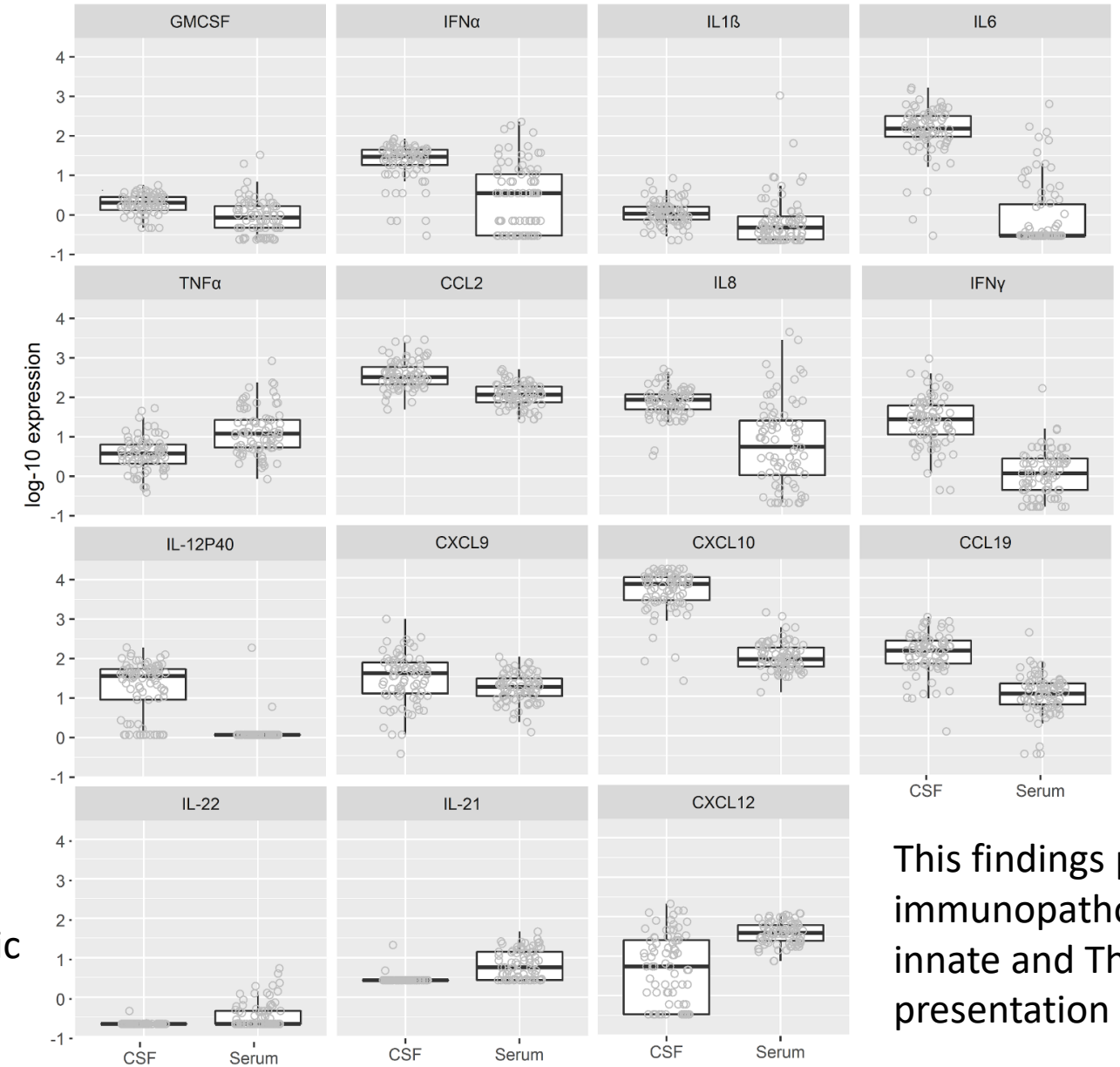
81 patients

Bogovič P. et all. J. Clin. Med. 2019

Cytokines differed substantially according to the compartment

Innate and **Th1** responses are occurring locally in CSF (markedly higher in CSF)

Th17 and **B cell** (generally higher in serum) responses may be triggered in systemic circulation



This findings provide new insights in immunopathogenesis of TBE and implicate innate and Th1 adaptive responses in clinical presentation and severity of acute illness.

Bogovič P. et all.: J. Clin. Med. 2019

DYNAMIC OF CYTOKINE RELEASE and association with the outcome of the diseases

Acute TBE: cytokines reached the highest levels on days 5–7 of the meningoencephalitic phase and decreased thereafter.

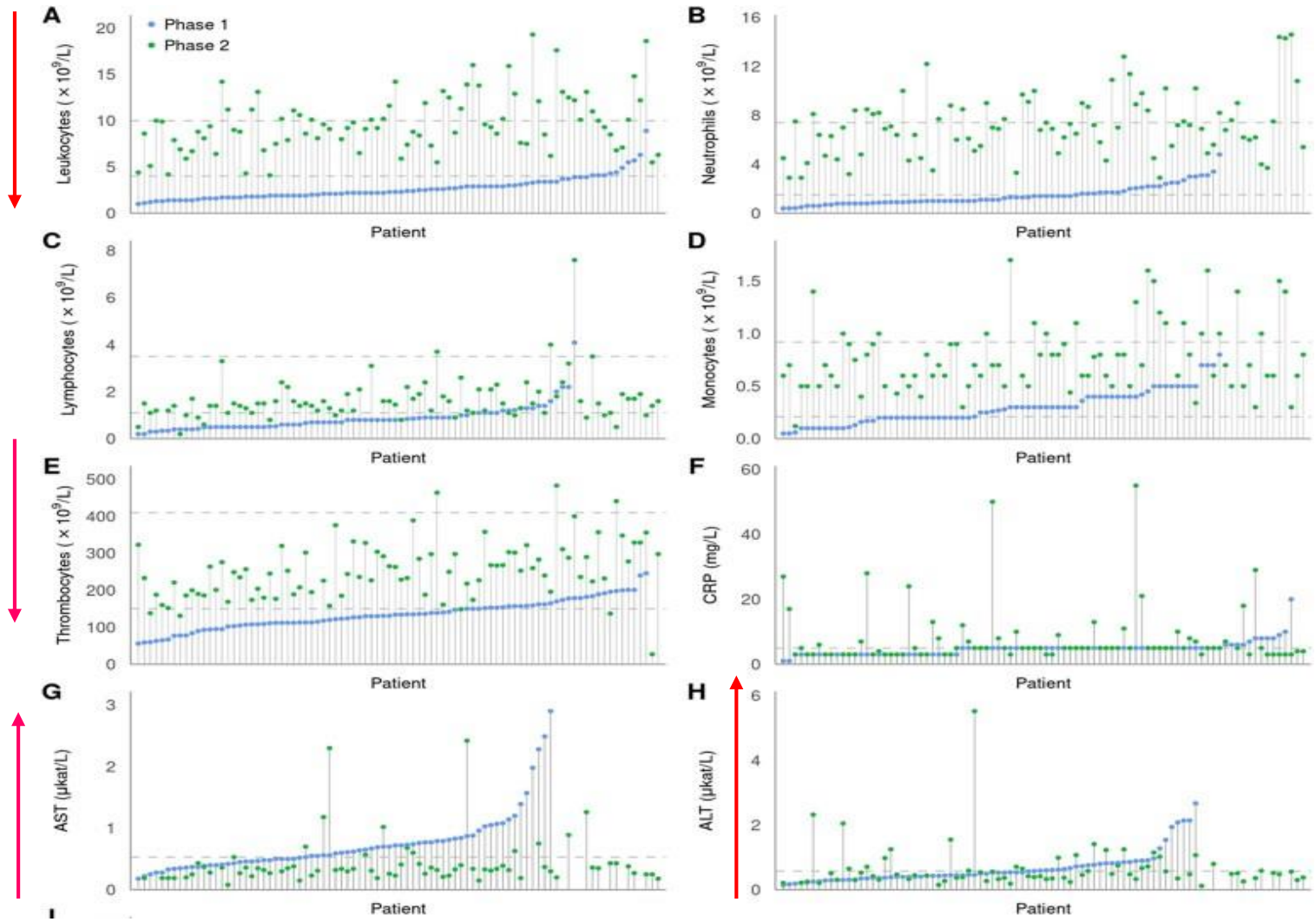
Duration of acute phase was correlated CXCL12 (B cell) and IL-27 (Th17).

Follow-up at 2 months: we found some differential expression of cytokines in serum, but none of the cytokines had a predictive value for early identification of patients who may develop PES

Follow-up at 2-7 years: interestingly, patients with PES had generally higher levels of innate cytokines and decreased levels of cytokines associated with Th1 and Th17 at the last visit

Bogovič P. et all. Microorganisms 2019

Laboratory characteristics of the initial and second phase of TBE



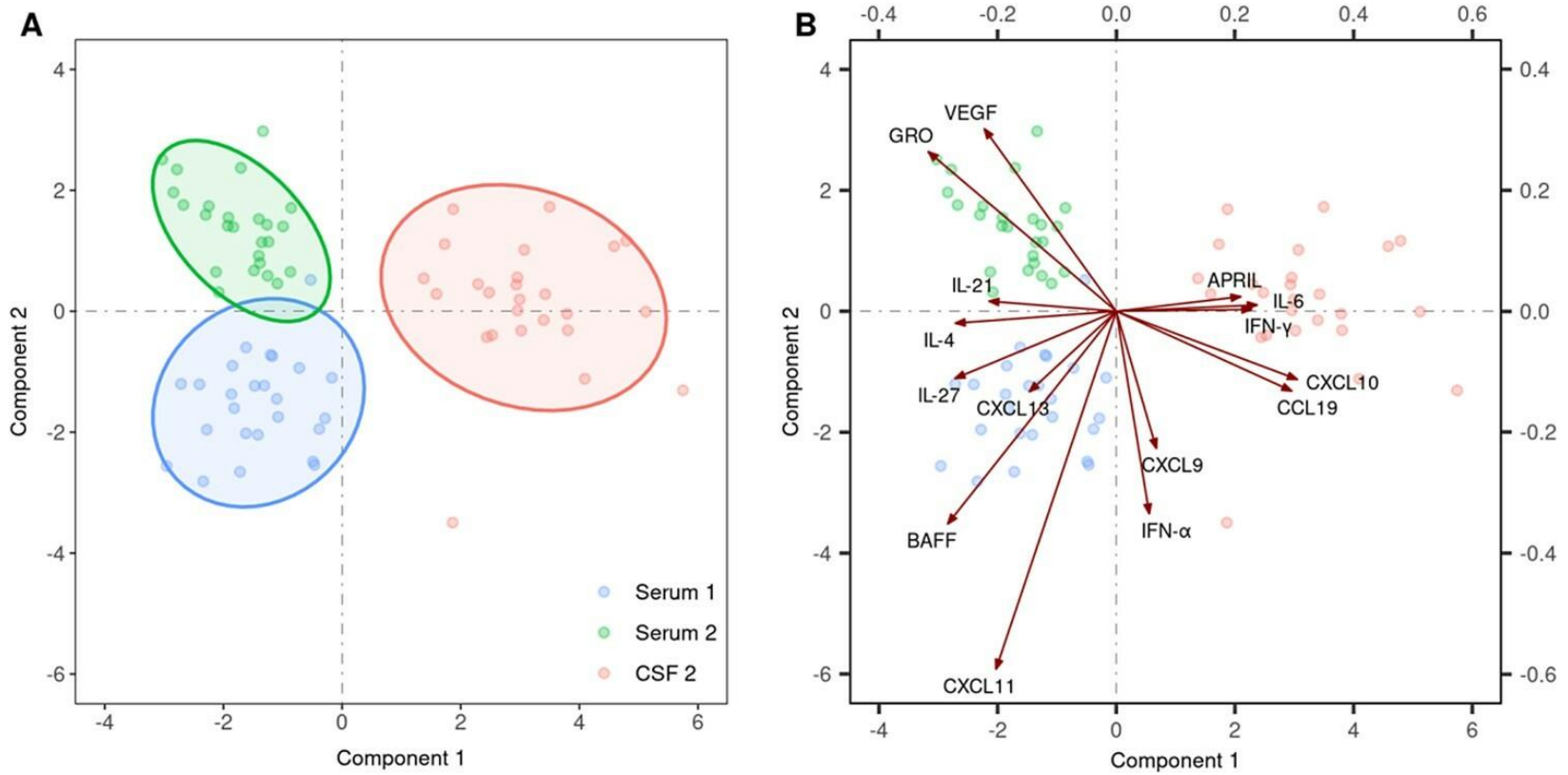
88 adult patients

- Phase 1
- Phase 2

Comparison of laboratory blood parameters in both phases of TBE revealed that laboratory abnormalities consisting of low leukocyte and platelet counts and increased liver enzymes level were associated with the first phase of TBE and resolved thereafter.

Bogovič P.et all.:Emerging Microbes & Infections 2022

Immune characteristics of the initial and second phase of TBE



25 adult patients

In the **initial phase** of TBE, the primary finding in serum was a rather heterogeneous immune response involving innate (CXCL11), B cell (CXCL13, BAFF), and T cell mediators (IL-27 and IL-4). During the **second phase** of TBE, growth factors associated with angiogenesis (GRO-α and VEGF-A) were the predominant characteristic in serum, whereas innate and Th1 mediators were the defining feature of immune responses in **CSF.** These findings imply that distinct immune processes play a role in the pathophysiology of different phases of TBE and in different compartments.

Bogovič P.et all.:Emerging Microbes & Infections 2022

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Klemen Strle

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