# Summary

**Introduction**

Tick-borne diseases, both viral and bacterial, are still a serious epidemiological
and clinical problem. Pathogenesis, clinical manifestation, coexistence of various tick-borne diseases and their co-infections are not fully understood. The influence of individual pathogens on each other is also unknown. Only few studies aimed to the effect
of co-infection on the course of the inflammatory process and the participation of chemokines in the pathomechanism of these diseases. Various cytokines, e.g. IL-4, IL-10, IL-12; interferons, e.g. INF-β, INF-γ and chemokines, e.g. CCL-3, CXCL-10, CXCL-11, have been shown to be involved in the pathomechanism of tick-borne encephalitis (TBE)
and anaplasmosis (*Human granulocytic anaplasmosis*, HGA). Still, the role of many chemokines, including CCL-4 (MIP-1b), CCL-17 (TARC), CCL-20 (LARC, MIP3a, exodus1), CXCL-8 (IL-8) in the pathogenesis of tick-borne diseases is not fully understood. Also, their participation in co-infected patients is still unknown. Determination of the role
of these chemokines in inflammatory processes can be used to develop new diagnostic
and therapeutic methods in cases of monoinfections, as well as potentially co-infection after tick bite.

**The aim of the study**

1. Evaluation of serum CCL-4, CCL-17, CCL-20 and IL-8 concentrations in patients
with anaplasmosis.
2. Evaluation of serum CCL-4, CCL-17, CCL-20 and IL-8 concentrations in patients
with tick-borne encephalitis.
3. Evaluation of serum CCL-4, CCL-17, CCL-20 and IL-8 concentrations in patients
with bacterium *Anaplasma phagocytophilum* and tick-borne encephalitis virus co-infection.
4. Analysis of the differences in CCL-4, CCL-17, CCL-20 and IL-8 concentrations
in patients with monoinfection compared to patients with *Anaplasma phagocytophilum* and tick-borne encephalitis virus co-infection.
5. Analysis of correlation of CCL-4, CCL-17, CCL-20 and IL-8 concentrations with severity of clinical course in patients with tick-borne encephalitis, anaplasmosis and with co-infection with *Anaplasma phagocytophilum* and tick-borne encephalitis virus.
6. Evaluation of the usefulness of CCL-4, CCL-17, CCL-20 and IL-8 concentrations
in the diagnosis, monitoring the effectiveness of treatment monoinfections
and co-infections after tick bite.

**Methods**

Eighty-seven patients were included to the study: 30 women and 57 men, aged
from 18 to 77 years old, treated in the Clinic of Infectious Diseases and Neuroinfections
of the Medical University of Bialystok in 2011-2014 because of anaplasmosis (*human granulocytic anaplasmosis,* HGA) and tick-borne encephalitis (TBE). The research was carried out in three groups of patients: Group I – 20 patients diagnosed with HGA; group II
– 49 people diagnosed with TBE; group III – 18 patients diagnosed with *A. phagocytophilum* and TBE virus co-infection. The control group (CG) consisted of 20 healthy people.
The diagnosis was based on the history of a recent tick bite or exposition to ticks in TBE endemic areas, physical examination and the results of laboratory tests. TBE was confirmed by ELISA by detection of antibodies anti-TBE (Enzygnost Anti-TBE IgG / IgM, Germany)
in serum and cerebrospinal fluid. HGA was confirmed by the presence of *A. phagocytophilum* DNA in the blood by PCR (DNA amplification by nested end-point PCR, 16S rDNA gene
*A. phagocytophilum* – Anaplasma, BLIRT-DNA Gdańsk, Poland).

Concentrations of cytokines were measured in serum with following ELISA method:

* CCL-4 (Human CCL4 / MIP-1β ELISA Kit (hCCL4-ELISA; ScienCell)
* CCL-17 (ab100644 - TARC Human ELISA Kit; Abcam)
* CCL-20 (ab100599 Macrophage Inflammatory Protein 3 alpha Human ELISA Kit; Abcam)
* IL-8 (ab100575 - IL-8 (Interleukin-8) Human ELISA Kit; Abcam)

**Results**

1. Significantly higher concentrations of IL-8 and CCL-20 before treatment were observed
in HGA patients compared to CG.
2. Significantly higher concentrations of CCL-4 and CCL-20 before treatment were observed in TBE patients compared to CG.
3. Significantly higher concentrations of IL-8, CCL-4 and CCL-20 were observed in patients with *A. phagocytophilum* and TBE virus co-infection before treatment in comparison
with CG.
4. Significant reduction of IL-8, CCL-4 and CCL-20 concentrations in the group of patients with TBE and IL-8 in patients co-infected with *A. phagocytophilum* and TBE virus
after treatment was demonstrated.
5. A higher concentration of CCL-4 was demonstrated in the group of patients with *A. phagocytophilum* and TBE virus co-infection in comparison to patients with TBE monoinfection after treatment.

**Conclusions**

1. IL-8, CCL-4 and CCL-20 are involved in the pathogenesis of anaplasmosis and tick-borne encephalitis.
2. Analysis of IL-8, CCL-4 and CCL-20 concentrations can be used to monitor the course
of tick-borne encephalitis and anaplasmosis as well as the effectiveness of treatment for tick-borne encephalitis.
3. Tick-borne encephalitis virus and *Anaplasma phagocytophilum* co-infection does not increase the production of IL-8, CCL-4, CCL-17 and CCL-20 compared
to monoinfections.
4. Serum CCL-4 concentration can be used to monitor the course of tick-borne encephalitis virus and *Anaplasma phagocytophilum* co-infection*.*