Summary

Despite significant progress in the prevention and treatment of tuberculosis (TB), this disease still remains a global public health problem, leading to nearly million of deaths annually. Among the main reasons for that, the incomplete protective effect of commonly used BCG (Bacillus Calmette-Guérin) vaccine and the often not strict conforming to treatment regimen are mentioned. In many countries, the percentage of infections caused by *Mycobacterium tuberculosis* (Mtb) resistant to numerous antibiotics is increasing, in result of insufficient treatment control. Migrations facilitate the spread of multi-resistant strains. Diagnosis of tuberculosis is based mainly on microbiological and radiological methods. There are no specific markers of infection that would enable effective and prompt diagnosis at the early disease stage and limiting its spread. One untreated person with active TB may give the disease to a dozen people within a year, while during pharmacotherapy, the risk of transmission decreases markedly after a few weeks.

Various mechanisms of innate immunity are crucial in the first-line antimicrobial defence. The pattern recognition receptors (PRRs) group consists of numerous transmembrane and soluble factors, which recognize pathogen-associated molecular patterns (PAMPs). In the initial phase of infection, effective immune mechanisms commonly (in 90-95% of cases) prevent host from developement of active disease. The interaction of *Mycobacterium tuberculosis* cells with various receptors and triggering the process of phagocytosis play a key role during infection. The effect of pathogen-host interactions often depends on the type of receptor involved in the recognition of bacterial antigens. The aim of this study was to investigate the significance of selected innate immunity factors in pulmonary tuberculosis, caused by *M. tuberculosis* infection. Adults patients diagnosed with active disease were recruited from the Institute of Tuberculosis and Lung Diseases in Warsaw, The Voivodeship Hospital of Lung Diseases in Jaroszowiec, Masovian Center of Lung Diseases and Tuberculosis Treatment in Otwock, Subcarpathian Center of Lung Diseases in Rzeszów and the Kuyavian-Pomeranian Pulmonology Center in Bydgoszcz. The diagnosis was confirmed by positive Mtb culture and chest radiography. Blood samples (for DNA and serum isolation) were collected immediately after the diagnosis. In minority of cases, serum samples were collected twice, before and after few weeks of anti-tuberculosis treatment, to estimate possible changes in the serum concentration of selected immune

factors. Healthy adults, who did not report a history of severe/recurrent infections, autoimmune or malignant diseases were qualified to the control group.

To achieve the aim of the study, serum concentrations of collectins [mannose binding lectin (MBL) and pulmonary surfactant protein D (SP-D)], activity of MBL complexes with associated serine proteases (MASP-1 and MASP-2) as well as levels of ficolins (ficolin-1, -2 and -3) were determined. Selected polymorphisms of genes encoding for the above-mentioned factors, surfactant protein A (SP-A) as well as genes for selected Toll-like receptors (TLR-1, -2, -4, -6) and the adaptor protein TIRAP, involved in signal transduction from activated TLRs, were also studied. The differences in median concentrations/activities of the afore-mentioned factors between the groups, the incidence of low and high values among patients and controls as well frequencies of polymorphic variants of corresponding genes and their impact on the serum levels of tested proteins were investigated.

It was shown, that the median serum concentration of MBL and the activity of its complexes with MASP-1 and MASP-2 as well as the median concentrations of SP-D and ficolin-1 were significantly higher in the group of patients than in the reference one (those differences were gender- and age-independent) In addition, within TB group, serum levels of the mentioned factors (and activities of MBL-MASP complexes) were found significantly higher in males than in females.

However, the frequency of the *MBL2* genotypes associated with the highest serum levels of fully active MBL (YA/YA) was lower (though insignificantly) among tuberculosis patients in comparison with healthy controls. Within TB group, the *FCN1* genotypes related to low serum concentrations of ficolin-1 (G/G at position -542; C/C at position -144) were in turn significantly more common than in reference one. This may indicate significance of other (not studied here) polymorphisms of these genes, or an impact of epigenetic regulation on their expression. MBL or ficolin-1 levels may also increase in response to active infection.

The median serum concentration of ficolin-2 in the TB group was significantly lower than in the C group (again, independently age and sex), while median ficolin-3 level did not differ between the groups. Furthermore, investigation of selected polymorphisms of the *FCN2* (ficolin-2) gene revealed significantly lower frequency of heterozygosity for -4 A>G polymorphism among patients compared with controls.

Interestingly, during anti-tuberculosis treatment, median serum MBL level increased significantly, while no difference was observed when concentrations of SP-D or ficolins in samples taken before and several weeks after starting therapy were compared.

The ROC analysis showed that ficolin-1 and ficolin-2 have a high potential to differentiate between individuals with active pulmonary tuberculosis and those qualified to the control group, therefore it is worth considering them as potential biomarkers in the early diagnosis of this disease. It was also demonstrated that two-factor analysis, using concentrations of both proteins offers a higher sensitivity and specificity of differentiation.

The differences in frequency of polymorphic variants of the *SFTPA2* gene at positions +26 (C>A) and +667 (C>A) (A/A genotypes were noted significantly less commonly in TB than in C group) seem to confirm the involvement of SP-A in defence against Mtb and may indicate an impact of the mentioned polymorphisms on the host's susceptibility to tuberculosis. In contrast, investigated SNPs of the following genes: *MASP-2, SFTPA1, SFTPD, FCN2, FCN3, TLR1, TLR2, TLR4, TLR6* and *TIRAP* have rather no significant influence on the risk of developing active infection.

The presented work expanded knowledge concerning the associations of selected innate immunity factors with susceptibility to *Mycobacterium tuberculosis* infections and the development of active pulmonary tuberculosis. The demonstrated and discussed data suggest continuation and extension of the study (including other polymorphisms of investigated genes, other factors and carrying out multifactorial analyzes) to be advisable.