Summary

Cholesteatoma is described as a cystic lesion consisting of keratinizing squamous cell epithelium (matrix), filled with keratin debris, surrounded by inflammatory fibrous tissue (perimatrix). Cholesteatoma gradually expands in the middle ear and causes the destruction of surrounding bone structures. This process can lead to the damage of the ossicular chain, consecutive hearing loss, vestibular dysfunction, and facial nerve paralysis. It also poses a risk for intratemporal and intracranial complications. Despite the development of molecular research and the confirmation of the impact of numerous proteolytic enzymes, growth factors, like cytokines potentially involved in the pathogenesis of cholesteatoma, this process is not fully understood. Surgery remains the treatment of choice. In the development of cholesteatoma, the role of metalloproteinase-9 (MMP-9) seems to be particularly significant. As an enzyme that digests extracellular matrix proteins, including type IV collagen, it thereby influences the process of apoptosis and hyperproliferation of keratinocytes, angiogenesis, as well as bone resorption.

The aim of the study was to: 1. determine the localization and intensity of inflammatory reaction in cholesteatoma tissues; 2. determine expression of MMP-9 and TIMP-1 in cholesteatoma tissues compared to healthy skin specimens obtained from the retroauricular area (perimatrix and matrix); 3. assess the concentration of MMP-9 and TIMP-1 in serum and plasma obtained from the blood of patients with cholesteatoma compared to a control group; 4. evaluate the dependence between the concentration of MMP-9 and TIMP-1in serum and plasma in the groups.

The study included 25 patients with chronic otitis media with cholesteatoma, who were selected for surgical treatment. During the same surgical procedure, cholesteatoma and a 3x2mm of a healthy skin specimen were taken. Before the surgical procedure, venous blood was collected from the same patients (study group: serum, plasma) as well as from 25 patients selected for septoplasty due to impaired nasal patency (control group: serum, plasma). The expression of MMP-9 and TIMP-1 in the collected tissues was assessed using immunohistochemical methods. The concentrations of MMP-9 and TIMP-1 in the serum and plasma of the study and control group were determined using the enzyme-linked immunosorbent assay (ELISA) method.

The statistical analysis of the obtained results was performed using the STATISTICA 13 software, Dell Inc 2016. Not all data had a normal distribution, therefore the nonparametric Mann-Whitney U test was used to compare the concentrations of MMP-9, TIMP-1 between groups, Spearman's rank correlation coefficient was used to assess the strength of

interdependence between these parameters, and the Chi-square test was used to evaluate the expression in tissues. A p-value of less than 0.05 was considered statistically significant.
In each cholesteatoma tissue sample, the localization and the intensity of inflammation was evaluated with haematoxylins and eosin staining. Inflammatory reactions were observed mainly within perimatrix of cholestetoma with the intensity of 60% of specimens as strong and 25% as moderate. Inflammation was not observed within the layers of healthy retroauricular skin specimens. A significantly higher MMP-9 expression was observed in the cholesteatoma perimatrix compared to the perimatrix of healthy skin ($p<0.010$) and to the cholesteatoma matrix ($p<0.013$). I demonstrated significantly higher TIMP-1 expression in the cholesteatoma matrix compared to the cholesteatoma perimatrix ($p<0.043$), and significantly higher TIMP-1 expression in the perimatrix of healthy skin compared to the cholesteatoma perimatrix ($p<0.001$). In the cholesteatoma perimatrix, there was significantly higher MMP-9 expression compared to TIMP-1 ($p<0.001$). I did not observe such a difference in the cholesteatoma matrix or in any layer of the healthy skin specimen.The concentration of MMP-9 in serum ($p<0.69$) and TIMP-1 in plasma ($p<0.323$) was higher in the study group compared to the control group, as well as lower concentrations of MMP-9 in plasma ($p<0.962$) and TIMP-1 in serum ($p<0.194$) in the study group compared to the control group. The results were not significant. In the assessment of relationships, I demonstrated a positive correlation between MMP-9 and TIMP-1 in the plasma of the control group ($p<0.48$), I did not demonstrate a relationship between MMP-9 and TIMP-1 in the experimental group.

Based on the obtained results the following conclusions were formulated:
1. Inflammatory infiltration limited to the layer of cholesteatoma perimatrix may promote its development.

2. The assessment of MMP-9 and TIMP-1 expression in cholesteatoma is useful in determining the severity of the inflammatory process.

3. Due to the lack of differences in the concentration of MMP-9 and TIMP-1 in the blood of patients with chronic otitis media with cholesteatoma, it is suggested that the inflammation in these patients is not systemic, but only local.

4. The assessment of MMP-9 and TIMP-1 concentrations in serum and plasma obtained from the blood of patients with chronic otitis with cholesteatoma has little clinical usefulness in the diagnosis of this disease.

Based on the obtained research results, I confirmed the first hypothesis. The second hypothesis was confirmed regarding the expression of MMP-9, but not regarding the expression of TIMP-1 in cholesteatoma tissues and samples of healthy periauricular skin. I did not confirm the third and fourth hypotheses.

In conclusion, the results of my research indicate the involvement of the cholesteatoma perimatrix and the usefulness of evaluating MMP-9 and TIMP-1 expression in the development of this disease. Further studies can contribute to elucidating new pathomechanisms and identifying novel areas for potential therapeutic targets in chronic middle ear inflammation with cholesteatoma.