IL-1α, IL-6 and IL-8 serum values in patients with chronic suppurative otitis media

ROXANA SERBAN1,2*, CRISTIANA FILIP1*, LUMINITA MIHAELA RADULESCU2,3*, MINERVA CODRUTA BADESCU4, MAGDA MARIANA BADESCU5, BOGDAN MIHAIL DIACONESCU6, MIHAIL DAN COBZEANU3 and BOGDAN MIHAIL COBZEANU2,3

1Department of Biochemistry, ‘Grigore T. Popa’ University of Medicine and Pharmacy, 700115 Iasi; 2Clinical Rehabilitation Hospital, 700656 Iasi; Departments of 3Otorhinolaryngology, 4Internal Medicine, 5Physiopathology and 6Occupational Medicine, ‘Grigore T. Popa’ University of Medicine and Pharmacy, 700115 Iasi, Romania

Received April 27, 2021; Accepted May 27, 2021

DOI: 10.3892/etm.2021.10660

Abstract. Inflammatory mediators play an important role in the pathogenesis of otitis media by initiating and maintaining an inflammatory response to infection. The presence of inflammatory mediators may be one of the reasons, in some patients, for acute otitis media transforming into chronic otitis media. The present study included 60 patients admitted to the Clinical Rehabilitation Hospital, Iasi, Romania, for surgery. The control group comprised 30 healthy individuals. Serum levels of interleukin 1α (IL-1α), interleukin 6 (IL-6) and interleukin 8 (IL-8) were measured prior to surgery and were compared among patients with chronic suppurative otitis media (CSOM), cholesteatoma and cholesteatoma recidivism and the control group. High serum levels of interleukins were recorded in all the groups compared to the healthy control group. IL-6 and IL-8 had the highest value in patients with CSOM and IL-1α had the highest value in patients with cholesteatoma recidivism. Thus, we can consider that inflammatory mediators play a central role in the pathogenesis of CSOM and cholesteatoma by maintaining a systemic and local inflammatory response.

Introduction

Cytokines are proteins that regulate the nature, intensity and duration of the immune response, binding to specific receptors in target cells (1,2). Bacterial otitis media is caused by the migration of pathogens from the nasopharynx to the middle ear, considering that the endotoxin in the bacterial cell wall component causes the initiation of inflammation in the middle ear. The endotoxin is a modulator of the immune response, stimulates macrophages to produce tumor necrosis factor α (TNF-α) and IL-1β. Keratinocytes produce mediators of inflammation such as IL-1α and IL-1β, IL-6 and IL-8 (3). Recent findings suggest a link between processes that occur in the middle ear during otitis and cytokine levels (4).

IL-6 is involved in regulating the activity of the tissue factor that leads to the initiation of the coagulation process. IL-1β regulates thrombomodulin by causing defects in anticoagulant proteins, thus affecting the activation of protein C, which is a step in the anticoagulant process that occurs in healthy individuals. Erythrocytes do not have an IL-1β receptor binding site, but platelets express the IL-1R1 receptor on their surface and respond to IL-1β. IL-8 promotes procoagulant activity, initiating platelet activation. IL-1β, IL-6 and IL-8 produce changes in the coagulation process by binding to platelets. IL-6 can have the most effects on erythrocytes, due to the presence of a binding site on them (5,6).

In the present study, IL-1α, IL-6 and IL-8 serum levels were measured to determine which inflammatory mediators play a key role in the pathogenesis of chronic suppurative otitis media (CSOM) and cholesteatoma.

Patients and methods

Patients. The prospective study was performed on a group of 60 patients aged between 9 and 58 years who were diagnosed with CSOM with and without cholesteatoma, and were hospitalized for surgery at the Clinical Rehabilitation Hospital, Iasi, Romania. Of these, 28 patients presented with simple CSOM,
IL-1α, IL-6, IL-8 Serum Values in CSOM

The study was approved by the Ethics Committee of the ‘Grigore T. Popa’ University of Medicine and Pharmacy (Iasi, Romania) on 15.07.2017, according to the law of medical research, no. 206 from 27.05.2004. Informed consent was obtained from all the patients included in the study.

The inclusion criteria were: Imaging and clinical diagnosis of CSOM. The exclusion criteria were: Acute or chronic diseases other than those of the ear, alcohol consumer or smoker, under any medical treatment, including vitamins.

Patients enrolled were divided into 4 groups: Group M included healthy individuals, group C included patients with CSOM with cholesteatoma, group R comprised patients with cholesteatoma recidivism, and group O included patients with simple CSOM.

Methods. Blood samples for analysis were taken after 12 h of fasting and collected into heparin-free tubes and stored on ice at 4°C. Subsequently, the serum was separated from the cells by centrifugation at 3,000 x g for 10 min at 4°C. Serum samples were stored at -20°C until they were used.

IL-1α was determined using the MaxDiscovery Human IL-1α ELISA test kit produced by Bioo Scientific Corporation (cat. no. 2109). The ELISA technique used specific antibodies and antigens and different dilutions. IL-6 was determined using the Human IL-6 ELISA Test Kit produced by Bioo Scientific Corporation (cat. no. 2107). IL-8 was determined using the Human IL-8 ELISA Test Kit produced by Bioo Scientific Corporation (cat. no. 2134).

Statistical analysis. Data obtained were processed using SPSS database version 18.0 (SPSS, Inc.) and processed with specific statistical functions. Data are represented as mean ± standard deviation. The tests of normality in frequency statistics, Skewness and Kurtosis (-2 < P < 2), were used to evaluate the distribution of continuous variables. The following indicators were used in data processing: Mean, median, variance, standard deviation, minimum and maximum values. The Student's t-test, a parametric test that compares the average values recorded in two groups with normal distributions was used. The F test (ANOVA) was also used to compare three or more groups with a normal distribution. Post hoc analysis was made with Bonferroni test.

Results

The Skewness and Kurtosis tests (-2 < P < 2) suggested that the value series of IL-1α, IL-6 and IL-8 were homogenous.

IL-1α values. The IL-1α values in group C ranged between 1.91 and 3.52 pg/ml with a mean value of 3.011 pg/ml (Table I). In group O, IL-1α values were between 1.81 and 3.34 pg/ml. The mean values from the M, C and O groups were significantly
lower compared to that recorded in group R. [0.815, 3.011 and 2.911 pg/ml (P=0.964) vs. 5.021 pg/ml (P=0.001)].

The mean level recorded in group M was significantly lower compared to all the other groups analyzed (0.815 pg/ml; P=0.001) (Fig. 1). The highest mean value was recorded in patients with cholesteatoma recidivism (group R, 5.021 pg/ml).

Analyzing the mean values obtained, the patients with CSOM with cholesteatoma and CSOM without cholesteatoma had significantly lower IL-1α levels compared to patients with cholesteatoma recidivism.

In both males and females, the highest mean level of IL-1α was found in patients with recurrence, and the lowest in controls. Within the group, no significant differences between sexes were recorded (P>0.05) (Table II).

IL-6 serum values. IL-6 serum values recorded the highest mean level in patients from group O ranging from 5.723 to 193.33 pg/ml, followed by that recorded in patients from group C with variations in the range from 240.62 to 519.63 pg/ml. The values were significantly higher compared to group M [316.59 and 121.23 pg/ml (P=0.001)] vs. 5.81 pg/ml (P=0.001)] (Table III).

The mean level in group R was slightly lower than in patients from group C. The serum level obtained was significantly higher compared to group M [117.77 and 121.23 pg/ml (P=0.551) vs. 5.81 pg/ml (P=0.001)] (Fig. 2). The highest mean value was recorded in group O (316.59 pg/ml).

In both males and females, the highest mean level of IL-6 was found in patients from group O and the lowest in group M. Within the group, only in patients from group O was there a significantly higher mean value in males than females (P=0.001) (Table IV).

In males, the highest mean level of IL-8 was found in patients from group O, while in females, the highest mean level of IL-8 was found in patients from group C, and in both sexes the lowest level average IL-8 was observed in group M (Fig. 3). Within the group, no significant differences between sexes were registered (P>0.05) (Table VI).
The study by Skotnicka and Hassmann (7) investigated the levels of IL-1β, IL-8 and IL-10 in the middle ear effusions of 38 patients with otitis media. While there was a strong statistical correlation between IL-1β and IL-6 levels and also between IL-6 and IL-10 levels, no direct correlation could be established between IL-1β and IL-10 levels. It was also not possible to establish a correlation between age, hearing loss, number of episodes of acute otitis media and cytokine levels (7).

IL-1 stimulates the release of other cytokines, arachidonic acid metabolism, cyclooxygenase and lipoxygenase pathways. IL-1β is produced and released extracellularly by inflammatory cells, such as macrophages and monocytes. IL-1α is located predominantly intracellularly or on the surface of these cells (3).

Table III. Comparison of IL-6 levels (pg/ml) between study groups.

| Parameters            | Group M | Group R | Group C | Group O |
|-----------------------|---------|---------|---------|---------|
| N                     | 30      | 11      | 21      | 28      |
| Mean                  | 5.81    | 117.77<sup>ac</sup> | 121.23<sup>b,de</sup> | 316.59<sup>f</sup> |
| Median                | 6.20    | 131.93  | 121.11  | 300.63  |
| Standard deviation    | 1.63    | 26.01   | 40.25   | 75.84   |
| Variance              | 2.64    | 22.09   | 33.20   | 23.96   |
| Skewness test         | -0.304  | -0.168  | 0.320   | 1.255   |
| Standard error of skewness | 0.427 | 0.661  | 0.501   | 0.441   |
| Kurtosis              | -1.100  | -2.265  | -0.415  | 1.064   |
| Standard error of kurtosis | 0.833 | 1.279  | 0.972   | 0.858   |
| Minimum               | 3.09    | 87.71   | 57.23   | 240.62  |
| Maximum               | 8.06    | 147.38  | 193.33  | 519.63  |
| Percentiles           |         |         |         |         |
| 25                    | 4.32    | 89.40   | 97.01   | 251.71  |
| 50                    | 6.20    | 131.93  | 121.11  | 300.63  |
| 75                    | 7.17    | 142.18  | 144.66  | 335.46  |

<sup>a</sup>P<0.001, statistically significant; <sup>b</sup>P>0.05, not significant. Group M included healthy individuals, group C included patients with CSOM with cholesteatoma, group R comprised patients with cholesteatoma recidivism, and group O included patients with simple CSOM. <sup>c</sup>P<0.001 group R compared to group M; <sup>d</sup>P<0.001 group C compared to group M and <sup>e</sup>P>0.05 group C compared to group R; <sup>f</sup>P<0.001 group O compared to groups M, R and C. IL, interleukin; CSOM, chronic suppurative otitis media.

Table IV. Mean IL-6 values (pg/ml) compared by sex and study group.

| Sex         | Group M | Group R | Group C | Group O | F<sub>ANOVA</sub> test<sup>a</sup> |
|-------------|---------|---------|---------|---------|-------------------------------|
| Male        | 5.89±1.72 | 123.86±27.91 | 107.43±43.19 | 356.38±80.94 | 0.001 |
| Female      | 5.75±1.61 | 110.47±24.40 | 133.78±34.61 | 270.69±32.16 | 0.001 |
| Student’s t-test | 0.817 | 0.424 | 0.138 | 0.001 | - |

<sup>a</sup>Bonferroni post hoc test was used. Group M included healthy individuals, group C included patients with CSOM with cholesteatoma, group R comprised patients with cholesteatoma recidivism, and group O included patients with simple CSOM. IL, interleukin; CSOM, chronic suppurative otitis media.

Figure 3. Comparison of IL-8 mean values (pg/ml) between study groups. Group M included healthy individuals, group C included patients with CSOM with cholesteatoma, group R comprised patients with cholesteatoma recidivism, and group O included patients with simple CSOM. IL, interleukin; CSOM, chronic suppurative otitis media.
In the current study, the patients with CSOM with and without cholesteatoma had significantly lower IL-1α levels compared to patients with cholesteatoma recidivism. However, the lowest value of this cytokine were found in the healthy group. In both males and females, the highest mean level of IL-1α was found in patients with cholesteatoma recidivism, and the lowest in the healthy group. It can be stated that a higher systemic inflammatory status is maintained with the chronicity of the disease, particularly in the group of patients with cholesteatoma recidivism.

Recent findings demonstrated the association between TNF-α and IL-1α and the degree of bone destruction in individuals diagnosed with CSOM with cholesteatoma, suggesting the necessity of complementary therapy to reduce TNF-α and IL-1α in this category of patients (8).

Kuczkowski et al (9) found high levels of IL-1α and IL-6 in cholesteatoma compared to granulation tissue, the highest values being recorded in cases with significant lesions of the ossicles in the middle ear, thus justifying the aggressiveness of cholesteatoma (9). IL-1 stimulates bone resorption by increasing the number of osteoclast precursor cells (10) and the proliferation of keratinocytes (11), which are part of the cholesteatoma, causing both an increase in size and an increased rate of relapse.

IL-6 is an acute phase cytokine and can induce CRP production. It is also frequently used as a marker of bacterial infection (12). IL-6 appears to play an important role in inflammation in otitis media. Elevated concentrations of IL-1, IL-6 and TNF-α have been correlated with otitis media in children (13). Kerschner et al stated that IL-6 regulates mucin secretion in the epithelial cells of the middle ear, a process involved in the pathogenesis of serous and mucous otitis media (14).

IL-6 induces osteoclast formation. High concentrations of IL-6 have been correlated with bone chain erosion and the presence of large amounts of granular tissue intraoperatively (10).

### Table V. Comparison of IL-8 levels (pg/ml) between study groups.

| Parameters     | Group M | Group R | Group C | Group O |
|----------------|---------|---------|---------|---------|
| N              | 30      | 11      | 21      | 28      |
| Mean           | 34.31   | 53.74<sup>ac</sup> | 53.91<sup>abde</sup> | 54.20<sup>bdeg</sup> |
| Median         | 34.79   | 52.72   | 52.65   | 54.17   |
| Standard deviation | 7.28    | 8.60    | 14.64   | 16.99   |
| Variance       | 21.21   | 16.00   | 27.16   | 31.37   |
| Skewness test  | -0.171  | 0.154   | 0.203   | 0.117   |
| Standard error of skewness | 0.427 | 0.661 | 0.501 | 0.441 |
| Kurtosis       | -0.882  | -1.030  | -1.123  | -1.058  |
| Standard error of kurtosis | 0.833 | 1.279 | 0.972 | 0.858 |
| Minimum        | 20.76   | 41.10   | 31.72   | 27.95   |
| Maximum        | 46.68   | 66.45   | 77.39   | 83.58   |

| Percentiles | Group M | Group R | Group C | Group O |
|-------------|---------|---------|---------|---------|
| 25          | 29.44   | 47.74   | 42.19   | 41.75   |
| 50          | 34.79   | 52.72   | 52.65   | 54.17   |
| 75          | 40.28   | 60.84   | 70.04   | 67.63   |

<sup>aP<0.001, statistically significant; bP>0.05, not significant. Group M included healthy individuals, group C included patients with CSOM with cholesteatoma, group R comprised patients with cholesteatoma recidivism, and group O included patients with simple CSOM. cP<0.001 group R compared to group M; dP<0.001 group C compared to group M and eP>0.05 group C compared to group R; fP<0.001 group O compared to group M and gP>0.05 group O compared to groups R and C. IL, interleukin; CSOM, chronic suppurative otitis media; CSOM, chronic suppurative otitis media. </sup>

### Table VI. Mean IL-8 values (pg/ml) compared by sex and study group.

| Sex           | Group M  | Group R   | Group C    | Group O    | F<sub>ANOVA test</sub><sup>a</sup> |
|---------------|----------|-----------|------------|------------|-----------------------------------|
| Male          | 35.34±7.80 | 56.15±11.33 | 50.18±13.45 | 58.33±19.45 | 0.002                              |
| Female        | 33.62±7.06 | 50.85±2.32  | 57.31±15.46 | 49.44±12.76 | 0.001                              |

<sup>aBonferroni post hoc test used. Group M included healthy individuals, group C included patients with CSOM with cholesteatoma, group R comprised patients with cholesteatoma recidivism, and group O included patients with simple CSOM. IL, interleukin; CSOM, chronic suppurative otitis media. </sup>
IL-6 serum values obtained in the present study were statistically significant higher compared to the control group, the highest being in patients with CSOM without cholesteatoma. The mean level recorded in patients with recurrence was slightly lower compared to that recorded in patients with CSOM with cholesteatoma, without statistical significance, but significantly higher compared to the control. In both males and females, the highest mean level of IL-6 was found in patients with CSOM, and the lowest in individuals without otic impairment.

Similar results with elevated serum IL-6 levels were obtained in a recent study showing that IL-6, metalloproteinase-2 and metalloproteinase-9 are related to the degree of destruction in the middle ear and the severity of the disease (15).

The results were similar to the study conducted by Nofal and Kwatly (16) which obtained a higher serum level of IL-6 in acute otitis media and CSOM, compared to a control group of healthy individuals. Authors of that study argue that IL-6 and CRP may be factors in predicting streptococcal otitis media (16).

Liu et al (17) observed an association between IL-6 and p-STAT3 expression: Increased expression of p-STAT3 in the cholesteatoma epithelium causes an increase in IL-6, leading to activation of IL-6/JAK/STAT3 signaling in cholesteatoma epithelial hyperplasia (17).

Certain authors consider IL-8 responsible for the formation of serous otitis media by: Accumulation of leukocytes in the middle ear, and in situ activation of leukocytes followed by tissue damage. IL-8 promotes the production of collage- nases, proteins involved in the process of bone lysis (10). It is a secondary cytokine that intervenes in inflammation in the middle ear, being a chemotactic agent for neutrophils (3). IL-8 also increases the production of adhesion molecules for the attachment and migration of neutrophils that occur during the acute inflammatory response. As IL-8 can cause the release of lysosomal enzymes, it can initiate tissue damage leading to chronic inflammation in the ear (18-20).

In the present study, the mean level of IL-8 in patients with cholesteatoma recurrence was slightly lower than that in patients with CSOM with or without cholesteatoma, but significantly higher compared to the control group.

In males, the highest mean level of IL-8 was found in patients with CSOM, while in females, the highest mean level of IL-8 was found in patients with CSOM with cholesteatoma, in both cases there are higher values compared to the control.

Elevated levels of IL-8 have also been identified in middle ear fluid in otitis serosa, with higher values in children than in adults (21). In experiments performed in guinea pigs, TNF-α and IL-1β reached peak values before IL-8, suggesting that it is produced by inflammatory cells accumulated by the influence of TNF-α and IL-1β (3,22).

The most recent study found a reduction in IL-8 and A20 (protein that is encoded by the TNFAIP3 gene) expression and an almost complete decrease in IL-1β, IL-6 and TNFα expression in cholesteatoma stem cells exposed to both lipopolysaccharides and the antagonist lipopolysaccharide toll-like receptor 4 (TLR4) from R. sphaeroides, compared to those treated with lipopolysaccharides only and untreated subjects. This could become a specific local treatment strategy that acts on TLR4-mediated signaling in cholesteatoma stem cells. It is therefore suggested that local administration of post-surgical drugs could reduce the cholesteatoma recidivism (23).

The current study has some limitations. Firstly, the study enrolled a relatively small number of patients, making it difficult to generalize the results. Secondly, children and adults were included together in the analysis and we consider that in the future differentiated studies on pediatric and adult populations are needed, which would allow a more accurate quantification of the inflammation parameters.

In conclusion, high serum levels of IL-1α, IL-6 and IL-8 were recorded in all otitis media groups compared to the healthy group. IL-1α had the highest value in patients with cholesteatoma recidivism. IL-6 and IL-8 had the highest value in patients with CSOM.

Acknowledgements

Not applicable.

Funding

The present study was financially supported by the ‘Grigore T. Popa’ University of Medicine and Pharmacy.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors’ contributions

RS, CF and LMR acquired experimental data and created the database. MCB, MMB and BMD analyzed the data and drafted the manuscript. BMC and MDC designed the study. RS, CF, MDC and LMR supervised data analysis. RS, CF and LMR checked and approved the authenticity of the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of the ‘Grigore T. Popa’ University of Medicine and Pharmacy (Iasi, Romania) on 09.06.2015, according to the law of medical research, no. 206 from 27.05.2004. Informed consent was obtained from all the patients included in the study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Dinarello CA: Historical insights into cytokines. Eur J Immunol 37 (Suppl 1): S34-S45, 2007.
2. Turner MD, Nedjai B, Hurst T and Pennington DJ: Cytokines and chemokines: At the crossroads of cell signalling and inflammatory disease. Biochim Biophys Acta 1843: 2563-2582, 2014.
3. Juhn SK, Jung MK, Hoffman MD, Drew BR, Preciado DA, Sausen NJ, Jung TT, Kim BH, Park SY, Lin J, et al: The role of inflammatory mediators in the pathogenesis of otitis media and sequelae. Clin Exp Otorhinolaryngol 1: 117-138, 2008.

4. Skovbjerg S, Roos K, Nowrouzian F, Lindh M, Holm SE, Adlerberth I, Olofsson S and Wold AE: High cytokine levels in perforated acute otitis media exudates containing live bacteria. Clin Biol Infect 16: 1382-1388, 2010.

5. Regnault V, de Maisire E, Carteaux JP, Gruel Y, Nguyen P, Tardy B and Lecompte T: Platelet activation induced by human antibodies to interleukin-8. Blood 101: 1419-1421, 2003.

6. Bester J and Pretorious E: Effects of IL-1β, IL-6 and IL-8 on erythrocytes, platelets and clot viscoelasticity. Sci Rep 6: 32188, 2016.

7. Skotnicka B and Hassmann E: Proinflammatory and immunoregulatory cytokines in the middle ear effusions. Int J Pediatr Otorhinolaryngol 72: 13-17, 2008.

8. Artono Surarto B, Purnami N, Hutahaen F and Mahardhika MR: The association of IL-1α level and TNF α expressions on bone destruction in chronic suppurative otitis media and cholesteatoma. Indian J Otolaryngol Head Neck Surg 72: 1-7, 2020.

9. Kuczkowski J, Sakowicz-Burkiewicz M, Iżycka-Świeszewska E, Mikaszewski B and Pawelczyk T: Expression of tumor necrosis factor-α, interleukin-1α, interleukin-6 and interleukin-10 in chronic otitis media with bone osteolysis. ORL J Otorhinolaryngol Relat Spec 73: 93-9, 2011.

10. Alves AL and Ribeiro FAQ: The role of cytokines in acquired middle ear cholesteatoma: Literature review. Rev Bras Otorrinol 70: 813-818, 2004.

11. Didierjean L, Salomon D, Méro Y, Siegenthaler G, Shaw A, Dayer JM and Saurat JH: Localization and characterization of the interleukin 1 immunoreactive pool (IL-1 alpha and beta forms) in normal human epidermis. J Invest Dermatol 92: 809-816, 1989.

12. Kishimoto T: The biology of interleukin-6. Blood 74: 1-10, 1989.

13. Yellon RF, Doyle WJ, Whiteside TL, Diven WF, March AR and Fireman P: Cytokines, immunoglobulins, and bacterial pathogens in middle ear effusions. Arch Otolaryngol Head Neck Surg 121: 865-869, 1995.

14. Kerschner JE, Meyer TK, Yang C and Burrows A: Middle ear epithelial mucin production in response to interleukin-6 exposure in vitro. Cytokine 26: 30-36, 2004.

15. Wu Y, Tang X, Shao W and Lu Y: Effect of CT manifestations of cholesteatoma on MMP-2, MMP-9 and IL-6 in the serum of patients. Exp Ther Med 17: 4441-4446, 2019.

16. Nofal K and Kwatly K: Serum interleukin-6 and C-reactive protein in bacterial otitis media patients in Damascus city. Int J Chem Farm 7: 403-408, 2015.

17. Liu W, Xie S, Chen X, Rao X, Ren H, Hu B, Yin T, Xiang Y and Ren J: Activation of the IL-6/JAK/STAT3 signaling pathway in human middle ear cholesteatoma epithelium. Int J Clin Exp Pathol 7: 709-715, 2014.

18. Schröder JM: The neutrophil-activating peptide 1/interleukin 8, a novel neutrophil chemoattract cytokine. Arch Immunol Ther Exp (Warsz) 40: 23-31, 1992.

19. Takeuchi K, Maesako K, Yuta A, and Sakakura Y: Interleukin-8 gene expression in middle ear effusions. Ann Otol Rhinol Laryngol 103: 404-407, 1994.

20. Butnaru C, Serban R, Martu C, Lungu A, Doroftei EA, Cobzeanu B and Cozma S: Otitis media complications. In: Proceeding of National ENT Head and Neck Surgery Conference. Berteșteanu SVG and Grigore R (eds). Filodiritto Publ., Arad, pp102-106, 2018.

21. Hotomi M, Samukawa T and Yamanaka N: Interleukin-8 in otitis media with effusion. Acta Otolaryngol 114: 406-409, 1994.

22. Schürmann M, Griene JFW, Volland-Thurn V, Oppel F, Kalschmidt C, Sudhoff H and Kaltschmidt B: Stem cell-induced inflammation in cholesteatoma is inhibited by the TLR4 antagonist LPS-RS. Cells 9: 199, 2020.

This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.