

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

ROXANA SERBAN^{1,2*}, CRISTIANA FILIP^{1*}, LUMINITA MIHAELA RADULESCU^{2,3*},
MINERVA CODRUTA BADESCU⁴, MAGDA MARIANA BADESCU⁵, BOGDAN MIHAIL DIACONESCU⁶,
MIHAIL DAN COBZEANU³ and BOGDAN MIHAIL COBZEANU^{2,3}

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

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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 1 α (IL-1 α), 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-1 α 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-1 β . Keratinocytes produce mediators of inflammation such as IL-1 α and IL-1 β , 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-1 β 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-1 β receptor binding site, but platelets express the IL-1R1 receptor on their surface and respond to IL-1 β . IL-8 promotes procoagulant activity, initiating platelet activation. IL-1 β , 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-1 α , 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,

Correspondence to: Dr Roxana Serban, Department of Biochemistry, 'Grigore T. Popa' University of Medicine and Pharmacy, 16 Universitatii Street, 700115 Iasi, Romania
E-mail: roxana_serban10@yahoo.com

Dr Minerva Codruta Badescu, Department of Internal Medicine, 'Grigore T. Popa' University of Medicine and Pharmacy, 16 Universitatii Street, 700115 Iasi, Romania
E-mail: codruta.badescu@gmail.com

*Contributed equally

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Table I. Comparison of IL-1 α levels (pg/ml) between study groups.

Parameters	Group M	Group R	Group C	Group O
N	30	1121	28	
Mean	0.815	5.021 ^{a,c}	3.011 ^{a,d}	2.911 ^{a,b,e,f}
Median	0.796	5.024	3.230	2.974
Standard deviation	0.412	0.616	0.594	0.452
Variance	0.170	0.379	0.351	0.204
Skewness test	0.636	0.107	-1.038	-1.545
Standard error of skewness	0.427	0.661	0.501	0.441
Kurtosis	-0.533	-1.378	-0.354	1.510
Standard error of kurtosis	0.833	1.279	0.972	0.858
Minimum	0.270	4.080	1.910	1.810
Maximum	1.170	5.910	3.520	3.340
Percentile				
25	0.447	4.524	2.743	2.904
50	0.796	5.024	3.230	2.974
75	1.215	5.608	3.460	3.283

^aP<0.001, significantly different; ^bP>0.05, no significant difference. 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 groups R and M; ^eP<0.001 group O compared to groups R and M and ^fP>0.05 group O compared to group C. L, interleukin; CSOM, chronic suppurative otitis media.

21 with CSOM with cholesteatoma and 11 with cholesteatoma recidivism.

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. Group O included 12 individuals from rural areas and 16 from urban areas, 13 females and 15 males, aged between 16 and 59 years. Group C included 12 individuals from rural and 9 from urban areas, 10 males and 11 females aged between 9 and 54 years. Group R was comprised of 11 individuals, 7 of rural and 4 of urban origin, 5 males and 6 females, aged between 10 and 58 years. The control group comprised 30 clinically healthy individuals, aged between 6 and 54 years; 6 under 18 years and 24 over 18 years. Of these, 13 individuals were from rural and 17 from urban areas, 18 were female and 12 were male.

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-1 α was determined using the MaxDiscovery Human IL-1 α 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 \pm 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-1 α , IL-6 and IL-8 were homogenous.

IL-1 α values. The IL-1 α 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-1 α values were between 1.81 and 3.34 pg/ml. The mean values from the M, C and O groups were significantly

Table II. Mean IL-1 α values (pg/ml) compared by sex and study group.

Sex	Group M	Group R	Group C	Group O	F _{ANOVA} test ^a
Male	0.86 \pm 0.42	5.07 \pm 0.66	2.88 \pm 0.69	2.81 \pm 0.50	0.001
Female	0.79 \pm 0.42	4.97 \pm 0.63	3.13 \pm 0.49	3.03 \pm 0.37	0.001
Student's t-test	0.653	0.811	0.365	0.192	-

^aBonferroni 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.

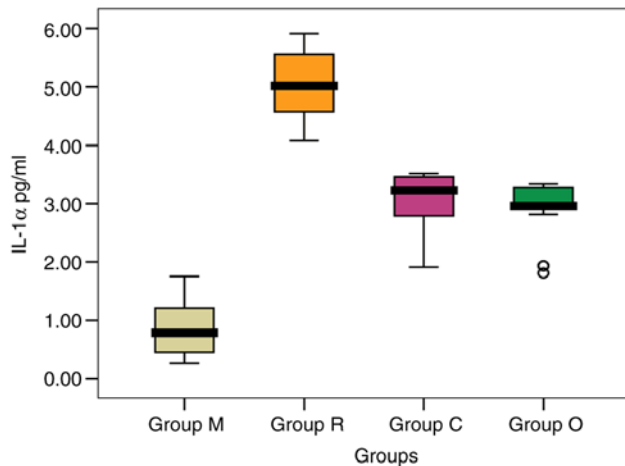


Figure 1. Comparison of IL-1 α mean values (pg/ml) between study groups. Group M included healthy individuals, group C included patients with CSOM with cholesteatoma, group R comprised patients with cholesteatoma recidivism, and group O included patients with simple CSOM. IL, interleukin; CSOM, chronic suppurative otitis media.

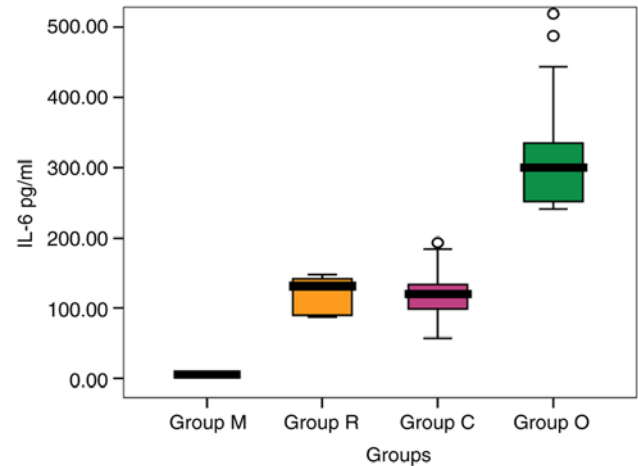


Figure 2. Comparison of IL-6 mean values (pg/ml) between study groups. Group M included healthy individuals, group C included patients with CSOM with cholesteatoma, group R comprised patients with cholesteatoma recidivism, and group O included patients with simple CSOM. IL, interleukin; CSOM, chronic suppurative otitis media.

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-1 α levels compared to patients with cholesteatoma recidivism.

In both males and females, the highest mean level of IL-1 α 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 57.23 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).

IL-8 values. Values obtained after the IL-8 dosing for the groups are: In group O the IL-8 level varied between 27.95 and 83.58 pg/ml, and in patients from group C the variation was in the range 31.72-77.39 pg/ml, with the lowest mean level being recorded in patients in group M, significantly lower compared to the levels recorded in groups C and O [34.31 vs. 53.91 and 54.20 pg/ml ($P=0.001$)]. The mean level in patients from group R was slightly lower than that in patients from groups O and C [53.74 vs. 54.20 and 53.91 pg/ml ($P=0.944$)], but significantly higher compared to the healthy subjects [53.74 vs. 34.31 pg/ml ($P=0.001$)] (Table V).

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).

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 ^{a,c}	121.23 ^{a,b,d,e}	316.59 ^{a,f}
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

^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 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 _{ANOVA} test ^a
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	-

^aBonferroni 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.

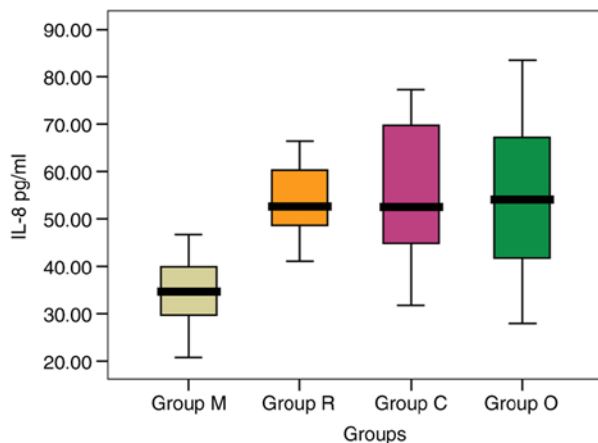


Figure 3. Comparison of IL-8 mean values (pg/ml) between study groups. Group M included healthy individuals, group C included patients with CSOM with cholesteatoma, group R comprised patients with cholesteatoma recidivism, and group O included patients with simple CSOM. IL, interleukin; CSOM, chronic suppurative otitis media.

Discussion

The study by Skotnicka and Hassmann (7) investigated the levels of IL-1 β , 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-1 β and IL-6 levels and also between IL-6 and IL-10 levels, no direct correlation could be established between IL-1 β 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-1 is synthesized by activated macrophages, where IL-1 production is stimulated by lipopolysaccharides and leukotrienes. IL-1 β is produced and released extracellularly by inflammatory cells, such as macrophages and monocytes. IL-1 α is located predominantly intracellularly or on the surface of these cells (3).

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 ^{a,c}	53.91 ^{a,b,d,e}	54.20 ^{a,b,f,g}
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				
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

^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.

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

Sex	Group M	Group R	Group C	Group O	F _{ANOVA} test ^a
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
Student's t-test	0.537	0.335	0.275	0.172	-

^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.

In the current study, the patients with CSOM with and without cholesteatoma had significantly lower IL-1 α 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-1 α 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-1 α 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-1 α in this category of patients (8).

Kuczkowski *et al* (9) found high levels of IL-1 α 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).

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 collagenases, 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-1 β reached peak values before IL-8, suggesting that it is produced by inflammatory cells accumulated by the influence of TNF- α and IL-1 β (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-1 β , IL-6 and TNF α 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-1 α , IL-6 and IL-8 were recorded in all otitis media groups compared to the healthy group. IL-1 α had the highest value in patients with cholesteatoma recidivism. IL-6 and IL-8 had the highest value in patients with CSOM.

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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.

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