

## EKSPERIMENTINIAI TYRIMAI

### Tympanic membrane changes in experimental acute otitis media and myringotomy

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**Key words:** acute otitis media; myringotomy; myringosclerosis; animal model.

**Summary.** *Objective.* The present experimental study explored pathomorphological changes and calcium depositions in the tympanic membrane during experimental acute otitis media caused by nontypeable *Haemophilus influenzae* in myringotomized and nonmyringotomized ears.

*Material and methods.* A rat model of experimental acute otitis media caused by nontypeable *Haemophilus influenzae* was employed. Sixteen Sprague-Dawley rats were used. Four days following middle ear inoculation, a bilateral myringotomy was performed in six randomly selected animals. Another group of 10 animals was inoculated only. On days 4, 7, 14, and 28 after inoculation, two animals from each group were sacrificed. The temporal bones were removed and the tympanic membranes were dissected, followed by paraffin embedding. Adjacent sections were stained with PAS-alcian blue for basic histopathological observations and by von Kossa method for determination of calcium phosphate depositions.

*Results.* Particularly intense invasion of polymorphonuclear neutrophil leukocytes was seen on day 4 after inoculation. The highest infiltration of macrophages was observed on day 7. The peak number of lymphocytes was seen on day 14. No difference occurred in the number of polymorphonuclear leukocytes in myringotomized and nonmyringotomized tympanic membranes. The infiltration with lymphocytes and activated macrophages in all parts of the myringotomized tympanic membranes was statistically significantly higher than in the nonmyringotomized animals. The total amount of interstitial calcium phosphate depositions during days 7, 14, and 28 of study was statistically higher in the sections of *pars tensa* from myringotomized membranes compared to the nonmyringotomized membranes.

*Conclusion.* Nontypeable *Haemophilus influenzae*-induced acute otitis media and myringotomy provoke more extensive inflammatory reaction with microcalcification in the tympanic membranes.

#### Introduction

Myringotomy is an incision of the tympanic membrane. Until the 18th century, myringotomy was used for the treatment of deafness (1). Later myringotomy was applied in the treatment of acute otitis media (AOM) and secretory otitis media (SOM). However, there are no evident data that myringotomy during uncomplicated AOM alone or together with antibiotic treatment is more effective than antibiotic treatment alone (2, 3). However, myringotomy is still recommended in case of complicated AOM.

It has been shown in experimental myringotomy that early inflammatory reaction develops in the tym-

panic membrane (TM). In the *pars flaccida*, the inflammatory reaction starts earlier than in the *pars tensa* (4). Experimental myringotomy in rats has been shown to heal within 9–11 days. Interestingly, myringotomy performed during *Streptococcus pneumoniae*-induced AOM was shown to yield a slower resolution of the AOM compared nonmyringotomized ears (5). Later, another study on the rats concluded that myringotomy in AOM provoked a delayed recovery from the inflammatory process within the TM (6).

Otherwise, a study where the healing of the myringotomized TMs was compared in the infected and noninfected ears showed interesting findings. The

occurrence of myringosclerosis in infected specimens was less evident than in the noninfected ears. It seems possible that infection-induced inflammation of the connective tissue layer interferes with the process of myringosclerosis formation (7). Furthermore, the development of myringosclerosis in noninfected myringotomized TMs has been associated with exposure to a hyperoxic environment (8).

The present experimental study was performed with the aim to explore pathomorphological changes and calcium depositions in the TM of laboratory rat during experimental AOM caused by nontypeable *Haemophilus influenzae* (NTHi) in myringotomized and nonmyringotomized ears.

### Materials and methods

A well-established rat model of experimental AOM caused by NTHi was employed (9–11).

### Experimental animals

Sixteen healthy Sprague-Dawley male rats were used. The research project and animal housing conditions were approved by the Ethical Committee for Animal Studies (approval M18-04, Lund University, Lund, Sweden).

### Bacteria

The animals were challenged with suspension containing viable, nontypeable (nonencapsulated) *Haemophilus influenzae* (NTHi) strain 3655, biotype II.

### Operative procedures and experimental design

The rats were anesthetized by intraperitoneal injection of 36 mg/100 g body weight chloralhydrate (Apoteket Produktion & Laboratorier, Malmö, Sweden). A normal TM status was ensured by otomicroscopy before operation. A ventral midline incision was made in the neck, and the right tympanic bulla was exposed. The right middle ear of all animals used was inoculated with 0.05 mL of a suspension of viable, nontypeable *Haemophilus influenzae* strain 3655, biotype II, by a fine needle through the bony bulla wall. The core of the suspension contained  $1.0 \times 10^6$  colony forming units/mL.

Four days following middle ear inoculation, under otomicroscopical guidance, a bilateral myringotomy was performed in the posterior, inferior quadrant of the TM in six randomly selected animals. Another group of 10 animals was inoculated only. Later, eight contralateral noninoculated ears without otomicroscopical signs of inflammation from the nonmyringotomized animal group were used as controls. Nonin-

fecting myringotomized left TMs of six animals were not used in this study.

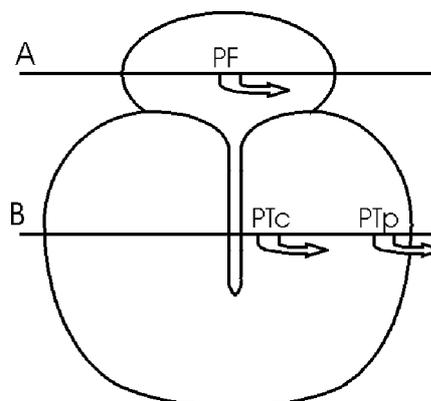
On day 4, middle ear effusion was sampled for bacteriological analysis from four randomly selected rats. On days 4, 7, 14, and 28 after inoculation, two animals from each group were sacrificed with an overdose of chloralhydrate intraperitoneally and simultaneous perfusion fixation with paraformaldehyde, through the left cardiac ventricle. The rats were decapitated, and the head stored in paraformaldehyde for at least one week. The temporal bone was removed and the tympanic membrane was dissected, followed by paraffin embedding.

### Tissue staining

Paraffin-embedded tympanic membranes were serially sectioned transversely on a microtome in 5–10- $\mu$ m sections (Fig. 1). Adjacent sections were stained with PAS-alcian blue for basic histopathological observations (five sections from the each eardrum) or by von Kossa method for determination of calcium phosphate deposition (five sections from the each eardrum). Number of sections according to the day of study is shown in Table 1.

### Histological evaluation

The sections were studied under a light Olympus CX41 microscope at  $\times 400$  magnification. All histological signs were evaluated in one field of vision at three parts of the each section: *pars flaccida* (PF), peripheral part of *pars tensa* (PTp), and central part of *pars tensa* (PTc) (Fig. 1). For measurement of the TM thickness, calibrated ocular Olympus WH10X-H/22 was used. Thickness of the drum was measured only



**Fig. 1. Schematic view of the rat tympanic membrane**

The horizontal lines (A, B) indicate direction of sections. Arrows indicate places of histological evaluation. PF – *pars flaccida*; PTp – peripheral part of *pars tensa*; PTc – central part of *pars tensa*.

**Table 1. Number of sections (N) from *pars flaccida* and *pars tensa* membranae tympani**

Day	Number of animals	Method of staining	Infected		Controls (N)	Total number of sections (N)
			myringotomized (N)	nonmyringotomized (N)		
0	16	–	–	–	–	–
4	16	PAS-alcian blue Von Kossa	–	4×5=20 4×5=20	2×5=10 2×5=10	30 30
7	12	PAS-alcian blue Von Kossa	2×5=10 2×5=10	2×5=10 2×5=10	2×5=10 2×5=10	30 30
14	8	PAS-alcian blue Von Kossa	2×5=10 2×5=10	2×5=10 2×5=10	2×5=10 2×5=10	30 30
28	4	PAS-alcian blue Von Kossa	2×5=10 2×5=10	2×5=10 2×5=10	2×5=10 2×5=10	30 30

in PAS-alcian blue-stained specimens. For each specimen, four measurements in each above-mentioned part of the drum were performed, and the average thickness was calculated.

The absolute number of cells was counted in one field of vision. A general number of inflammatory cells (polymorphonuclear neutrophil leukocytes (PMN), macrophages, lymphocytes) and fibrocytes were counted in PAS-alcian blue-stained specimens. Calcium phosphate depositions were evaluated in the von Kossa-stained specimens.

For evaluation of the expression of specific calcium phosphate staining in the interstitium of the *lamina propria* of examined TM, sign intensity level was described as follows: “0” – no signs of specific staining or only sporadic or isolated staining, “1” – staining in up to approximately 25% of relevant components of examined tissue, “2” – staining in up to approximately 50% of relevant components of examined tissue, and “3” – staining in more than 50% of relevant components of examined tissue.

#### **Statistical analysis**

Statistical data analysis was performed using the standard software Statistica 6.0 and MS Excel 2000. The following statistical estimates were calculated: mean ( $\bar{x}$ ), standard error (SE), and 95% confidence interval (CI). Mainly nonparametric Mann-Whitney test was used for evaluations of the differences between means. For comparison of distributions and variances, the Kruskal-Wallis and F tests were applied. The significance of differences was accepted, when error probability P is less than significance level  $\alpha=0.05$ , *i.e.* when  $P<0.05$ .

#### **Results**

On day 4 after inoculation, all ears infected with NTHi had typical signs of AOM. Apart from the otitis media, the animals appeared clinically healthy throughout the study. The same strain of bacteria as inoculated was grown on day 4 from the samples of middle ear effusion from four randomly selected rats. The tympanic membranes were without visible sclerotic deposits when examined in the otomicroscope.

#### **Investigation of the course of the AOM**

For evaluation of AOM course, thickness of TM and the infiltration of inflammatory cells were assessed.

Thickness of the TMs changed during AOM. The most prominent changes of thickness were observed in *pars flaccida* membranae tympani. The thickest TMs were observed on day 4 after NTHi inoculation in the middle ear cavity. The average thickness of the PF, PTp, and PTc membranae tympani was more than two-fold greater as compared to controls. The thinnest membranes were observed on day 28 after inoculation.

The average thickness of the peripheral part of the PT was greater than that of the central part. These differences in the thickness between PTp and PTc were statistically significant during all days of investigation in infected and control TMs.

During the course of AOM, the total average thickness of the TMs was gradually decreasing from day 4 and onwards. The differences in the total average thickness of the all parts of TM were statistically significant between day 7, day 14, and day 28. The difference in the total average thickness was statistically significant between TMs on day 28 and control

**Table 2. Comparison of the total thickness of the all parts of TM sections (PF, PTp, and PTc) and the total thickness from the sections of all parts of myringotomized and nonmyringotomized TMs during investigation (mean ± standard error)**

Day	Total thickness ( $\bar{x} \pm SE$ ), $\mu\text{m}$	Day of comparison	Mann-Whitney test			Thickness ( $\bar{x} \pm SE$ ), $\mu\text{m}$		Mann-Whitney test		
			U	Z	P	Nonmyringotomized (N=30)	Myringotomized (N=30)	U	Z	P
4	32.45±2.43	7 (N=20)	1496.50	1.59	0.111170	–	–	–	–	–
7	26.28±1.37	14 (N=20)	877.00	4.84	<b>0.000001</b>	26.36±1.84	27.19±2.05	301.00	-2.20	<b>0.0276</b>
14	20.36±1.32	28 (N=20)	1095.50	3.70	<b>0.000218</b>	19.07±1.82	21.66±1.95	238.50	-3.13	<b>0.0018</b>
28	17.71±1.37	Control (N=40)	512.50	3.32	<b>0.000911</b>	16.50±1.96	18.92±1.91	208.50	-3.57	<b>0.0004</b>
Control	14.88±1.85	–	–	–	–	–	–	–	–	–

TM – tympanic membrane; PF – *pars flaccida*; PTp – peripheral part of *pars tensa*; PTc – central part of *pars tensa*; SE – standard error.

TMs as well (Table 2).

At the beginning of experiment, prominent inflammatory reaction in PT and PF membranae tympani with infiltration of numerous inflammatory cells was observed. Particularly intense perivascular invasion of PMNs was seen in the stroma of PF on day 4 after NTHi inoculation. Later, the number of PMNs gradually decreased, and on day 28, only a few PMNs were observed in the stroma of PF and PT. Control TMs did not show any PMNs cells (Table 3). Infiltration with PMNs was not the same in the PTp and PTc parts of the eardrums. On days 4 and 7, more PMNs were observed in the central part of PT compared to the peripheral part, and this difference was

statistically significant. On day 14 and 28, the difference was not statistically significant.

Four days after induction of AOM, all parts of TM were invaded by macrophages, under the epithelial layer. The highest infiltration of macrophages was observed on day 7, deep in the stroma and especially in the central part of PT. Later in the study, the number of macrophages gradually decreased in the all parts of the eardrum, and on day 28, only a few macrophages were seen. In the control TMs, sporadic appearance of macrophages was observed in the subepithelial layer (Table 3). On day 4 and 7 after NTHi-induced AOM, more numerous macrophages were observed in the central part of PT compare to the peripheral

**Table 3. Number of PMN, macrophages, and lymphocytes in the sections of PF, PTp, and PTc (mean ± standard error)**

Day	PMN ( $\bar{x} \pm SE$ )/field			Macrophages ( $\bar{x} \pm SE$ )/field			Lymphocytes ( $\bar{x} \pm SE$ )/field		
	PF	PTp	PTc	PF	PTp	PTc	PF	PTp	PTc
4 (N=20)	<b>45.90</b> ±1.7	<b>19.85</b> ±0.67	<b>27.85</b> ±0.39	19.10 ±0.42	5.75 ±0.22	12.10 ±0.37	27.2 ±0.48	8.20 ±0.34	13.9 ±0.64
7 (N=20)	22.60 ±0.37	11.55 ±0.36	17.45 ±0.30	<b>21.10</b> ±0.29	<b>6.90</b> ±0.30	<b>18.05</b> ±0.22	27.50 ±0.42	9.95 ±0.42	16.80 ±0.39
14 (N=20)	6.85 ±0.35	3.90 ±0.22	3.95 ±0.21	12.10 ±0.33	3.85 ±0.15	4.90 ±0.24	<b>31.80</b> ±0.55	<b>15.70</b> ±0.33	<b>21.60</b> ±0.63
28 (N=20)	1.05 ±0.15	0.50 ±0.11	0.35 ±0.11	8.35 ±0.49	2.60 ±0.20	1.45 ±0.11	21.45 ±0.41	10.15 ±0.48	14.651 ±0.61
Control (N=40)	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	2.70 ±0.33	1.40 ±0.16	0.90 ±0.10	1.50 ±0.22	0.00 ±0.00	0.00 ±0.00

PMN – polymorphonuclear neutrophil leukocyte; PF – *pars flaccida*; PTp – peripheral part of *pars tensa*; PTc – central part of *pars tensa*; SE – standard error.

part of PT of the drum, and these differences were statistically significant. On day 14 after NTHi inoculation, the difference between PTP and PTC infiltration with macrophages was not statistically significant, whereas on day 28, statistically significantly higher infiltration of macrophages was observed in PTP compared to PTC part.

On day 4, equal infiltration with lymphocytes was observed in the stroma of all parts of the TMs. Later, the number of lymphocytes gradually increased in all parts of TMs with the peak on day 14. On day 28, the number of lymphocytes decreased. In the PF of TM from controls, only sparse lymphocytes were found, whereas lymphocytes were absent in PT (Table 3). During the whole study (days 4, 7, 14, and 28), the central part of PT membranae tympani was more heavily invaded by lymphocytes than the peripheral part, and this difference was statistically significant.

**Investigation of microcalcification in the TM**

Sections of the drums were divided into groups according to the presence or absence of interstitial deposition of calcium phosphate (Table 4). Sections with interstitial calcium accumulation (on days 14 and 28) were assigned to the drums with the late microcalcification. The differences in the average thickness in PF, PTP, and PTC parts of TM between drums with and without microcalcification were not statistically significant.

**Influence of myringotomy on the inflammatory changes and calcification in the TM**

The total average thickness of the TMs in the groups of myringotomized and nonmyringotomized drums following AOM decreased gradually. However, the average thickness of the sections from myringotomized drums was greater than that of nonmyringotomized ones, and this difference was statistically significant on days 7, 14, and 28 after NTHi inoculation

(Table 2). The average thickness of the different parts (PF, PTP, and PTC) of myringotomized drums was also greater than in nonmyringotomized drums, and this difference was statistically significant as well.

No difference occurred in the number of PMNs in myringotomized and nonmyringotomized TMs.

The lymphocytic infiltration in the all parts of myringotomized TMs (PF, PTP, and PTC) was higher than in nonmyringotomized membranes, and the difference was statistically significant (Fig. 2).

The number of fibrocytes in the drum stroma changed minutely during the study. However, fibrocytes in the sections from myringotomized TMs were more numerous in comparison to the nonmyringotomized drums, and this difference was statistically significant on days 7, 14, and 28 after induction of NTHi acute otitis media (Fig. 3).

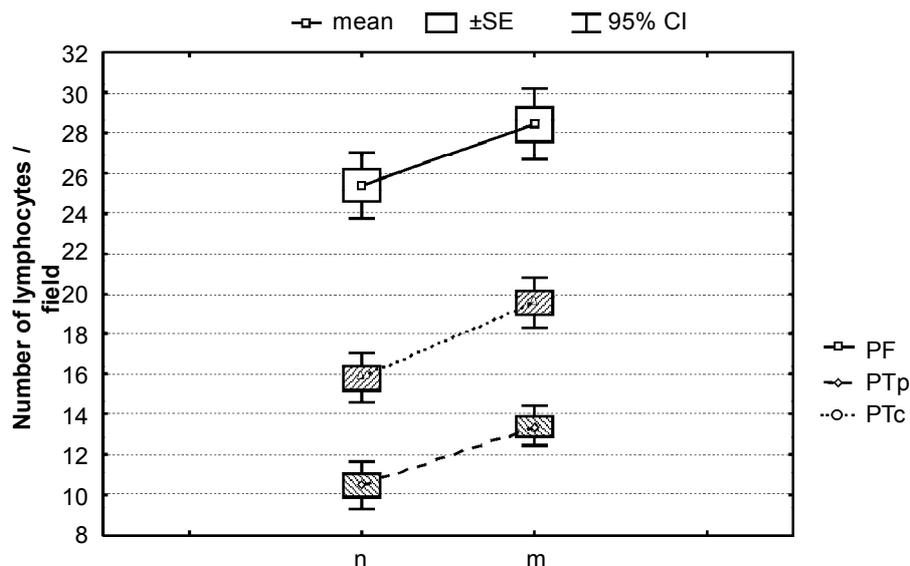
The infiltration with activated macrophages in the all parts of myringotomized TMs (PF, PTP, and PTC) was higher compared to the nonmyringotomized membranes, and the difference was statistically significant (Fig. 4). Meanwhile, more numerous macrophages with phagocytosed calcium in the cell cytoplasm were observed only in the sections from PF of myringotomized drums during days 7, 14, and 28 after inoculation compared to the nonmyringotomized ears, and the difference was statistically significant. However, no macrophages with cytoplasmic calcium phosphate were observed in PT on days 14 and 28.

The total amount of interstitial calcium phosphate depositions during days 7, 14, and 28 of study was higher in the sections of PT from myringotomized membranes as compared to the nonmyringotomized membranes, and this difference was statistically significant. This difference did not occur in PF (Table 5).

The most intensive calcium phosphate depositions (during days 14 and 28 after NTHi inoculation) were found in the lamina propria of PT of myringotomized TMs.

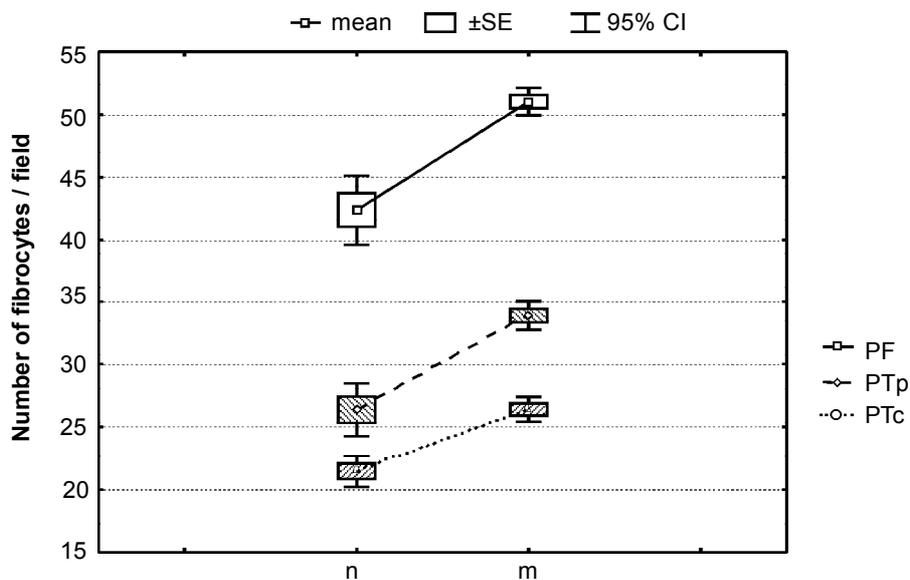
**Table 4. Number of sections with interstitial microcalcification (Ca+) and without microcalcification (Ca-)**

Microcalcification		Day			
		4	7	14	28
Ca+	Myringotomized	–	2/10	5/10	5/10
	Nonmyringotomized	5/20	4/10	3/10	2/10
	Controls	0/10	0/10	0/10	0/10
Ca–	Myringotomized	–	8/10	5/10	5/10
	Non-myringotomized	15/20	6/10	7/10	8/10
	Controls	10/10	10/10	10/10	10/10



**Fig. 2.** Comparison of the total average number of lymphocytes in the sections of different parts of myringotomized (m) (N=30) and nonmyringotomized (n) (N=30) TMs during investigation (days 7, 14, and 28)

TM – tympanic membrane; PF – *pars flaccida*; PTp – peripheral part of *pars tensa*; PTc – central part of *pars tensa*; SE – standard error; CI – confidence interval.



**Fig. 3.** Comparison of the total average number of fibrocytes in the sections of different parts of myringotomized (m) (N=30) and nonmyringotomized (n) (N=30) TMs during investigation (days 7, 14, and 28)

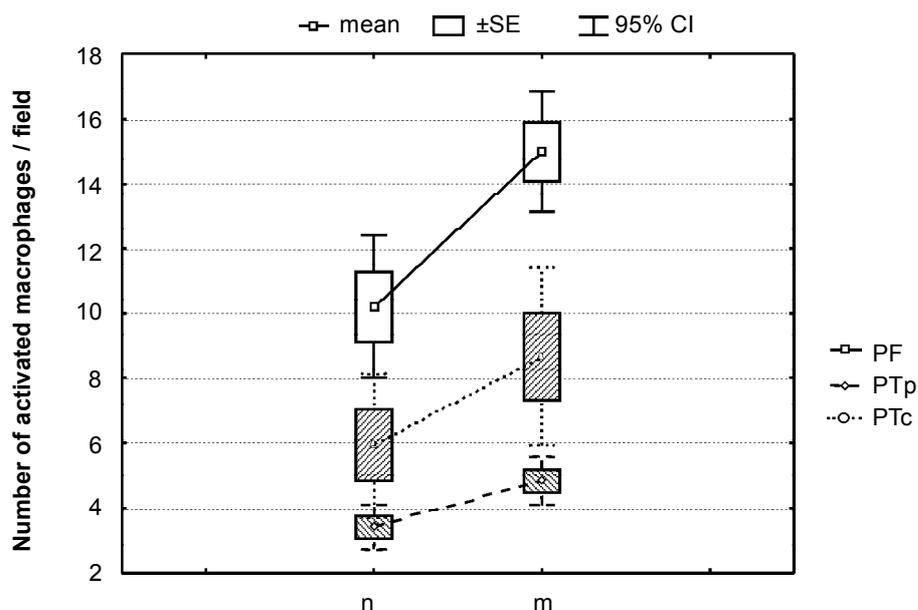
TM – tympanic membrane; PF – *pars flaccida*; PTp – peripheral part of *pars tensa*; PTc – central part of *pars tensa*; SE – standard error; CI – confidence interval.

### Discussion

The clinical course of AOM caused by NTHi in an animal model, as well as the increased thickness of the TM, is in accordance with earlier studies (10, 12). The most prominent changes of thickness in this

study were seen in the *pars flaccida* membranae tympani, and this is in agreement with the earlier observation that *pars flaccida* is the first and most extensively reacting site of the TM (13).

Previously it has been shown in otomicroscopical



**Fig. 4.** Comparison of the total average number of activated macrophages in the sections of different parts of myringotomized (m) (N=30) and nonmyringotomized (n) (N=30) TMs during investigation (days 7, 14, and 28)

TM – tympanic membrane; PF – *pars flaccida*; PTp – peripheral part of *pars tensa*; PTc – central part of *pars tensa*; SE – standard error; CI – confidence interval.

**Table 5.** Comparison of the total intensity level of interstitial calcium depositions (during days 7, 14, and 28 after inoculation) in the myringotomized and nonmyringotomized TMs

Part of TM	Intensity level of interstitial calcium depositions ( $\bar{x}\pm SE$ )		Kruskal-Wallis test		F test	
	nonmyringotomized (N=30)	myringotomized (N=30)	KW-H	P	F	P
PF	0.29±0.13	0.33±0.22	0.18	0.6750	0.04	0.8492
PTp	0.64±0.13	1.50±0.15	10.88	<b>0.0010</b>	18.33	<b>0.0003</b>
PTc	0.64±0.13	1.50±0.15	10.88	<b>0.0010</b>	18.33	<b>0.0003</b>

TM – tympanic membrane; PF – *pars flaccida*; PTp – peripheral part of *pars tensa*; PTc – central part of *pars tensa*; SE – standard error.

observation of NTHi-induced AOM that the fluid in the middle ear cavity was observed longer and TMs seemed to be more affected, e.g. thicker and with larger vessels, in myringotomized ears compared to nonmyringotomized ears (14). These otomicroscopical findings of the TM now are shown to be in accordance with this histological study. The TMs of the myringotomized animals were significantly thicker than nonmyringotomized and showed abundant infiltration with inflammatory cells (macrophages and lymphocytes). It has been shown earlier in a study of myringotomy performed during pneumococci-induced AOM that the infection resolved more slowly in myringoto-

mized ears compared to nonmyringotomized ears (5). Another study concluded that myringotomy in AOM provoked a delayed recovery from the inflammatory process within the TM (6).

Furthermore, there was a tendency toward more extensive microcalcification in ears with a relatively mild inflammatory reaction (e.g. pathological signs of lower intensity and shorter duration) following inoculation. Previous studies on the healing of myringotomized TMs in infected ears have reported some interesting findings. The infected TMs regenerated faster and closed their perforations at an earlier stage compared to uninfected TMs.

It thus seems possible that the inflammation of the connective tissue layer induced by the infection affects the process of myringosclerosis formation (7).

It is also interesting to note that macrophages within the connective tissues engulf and accumulate some of the calcium phosphate particles. The macrophage may be central in the process and pathogenesis of myringosclerosis. Calcified microspherules constitute the microscopic subunits of the sclerotic plaques and are broadly recognized as calcified remnants or particles of previous inflammation (15). The present finding of initial accumulation of calcium phosphate particles within the cytoplasm of macrophages during the acute phase of inflammation and subsequent disappearance of these particles suggests that the macrophage is a scavenger of calcium phosphate particles.

A myringotomy induces a wound in the tympanic membrane. The wound healing involves an inflammatory reaction, which leads to sclerosis (4, 16, 17). In case of preexisting inflammation at myringotomy, as seen during AOM, a high number of calcium-scavenging macrophages is already present, and this may explain why myringosclerosis is less extensive in case of a concurrent inflammation in an experimental setting (18). Thus, the balance between calcium deposition and scavenging is by conception decisive for the development of permanent sclerosis, and this balance tips towards sufficient calcium removal when activated macrophages are already locally present when the myringotomy is performed, which is the case during AOM. The calcium-scavenging macrophages migrate to the myringotomy site in the process of wound healing. Thus, an increased number of calcium-removing macrophages occur at the site of myringotomy, and this may explain why clinically observed myringosclerosis often is more extensive at sites other than

the myringotomy. However, the pathogenesis of myringosclerosis formation is complex, and the increased tissue oxygen tension and release of free oxygen radicals following myringotomy is without doubt an essential issue (8, 19, 20). Activated macrophages are one source of free oxygen radicals and may thus stimulate sclerosis formation, which opposes the above-proposed calcium-scavenging potential of this cell.

In this model of only microscopic myringosclerosis, some apparent differences have been found between myringotomized and nonmyringotomized ears, although late *lamina propria* calcification of the *pars tensa* was especially evident in myringotomized cases.

A more detailed histological study of these TMs is very relevant in order to elucidate early pathomorphological changes, which might be very important for the development of sequela after AOM – myringosclerosis.

Further studies on an AOM model of clinically evident sclerosis are needed in order to explore further possible differences between myringotomized and nonmyringotomized ears and to determine tympanosclerotic plaque lesions.

### Conclusions

We conclude that more extensive inflammatory reaction with microscopic calcification in the tympanic membrane was seen in myringotomized ears than in nonmyringotomized ears during nontypeable *Haemophilus influenzae*-induced acute otitis media.

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## Būgnelio pokyčiai eksperimentinio ūminio vidurinės ausies uždegimo metu atlikus miringotomiją

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**Raktažodžiai:** ūminis vidurinės ausies uždegimas, miringotomija, miringosklerozė, eksperimentinis gyvūnų modelis.

**Santrauka.** *Tyrimo tikslas.* Ištirti būgnelių patomorfologinius pokyčius ir kalcio sankaupą, atsirandančius dėl netipuojamo *Haemophilus influenzae* sukulto eksperimentinio ūminio vidurinės ausies uždegimo, taikant miringotomiją ir be jos.

*Tyrimo medžiaga ir metodai.* Tyrimui pasirinktas eksperimentinis laboratorinės žiurkės ūminio vidurinės

ausies uždegimo, sukkelto netipuojamo *Haemophilus influenzae*, tyrimo metodus. Tyrimui panaudota 16 *Sprague-Dawley* veislės žiurkių. Ketvirtąją parą po bakterijų įterpimo į vidurinę ausį šešioms atsitiktinės atrankos būdu atrinktiems gyvūnams atliktos abiejų būgnelių miringotomijos. Likusios 10 žiurkių buvo tik inokuliuotos. Praėjus 4, 7, 14 ir 28 dienoms po bakterijų įterpimo, po dvi iš kiekvienos grupės žiurkės buvo panaudotos būgnelių histologiniam tyrimui. Išpreparavus smilkinkaulius, išimti būgneliai įlieti į parafiną. Serijiniai būgnelių pjūviai dažyti PAS-alciano mėliu histopatologiniam įvertinimui ir von Kossa metodu – kalcio fosfato sandaigoms nustatyti.

**Rezultatai.** Ypač ryški polimorfinių branduolinių leukocitų infiltracija nustatyta ketvirtąją dieną po inokuliacijos. Žymesnė makrofagocitų infiltracija buvo septintąją tyrimo dieną. Gausiausia limfocitų infiltracija buvo 14 dieną po bakterijų įterpimo. Statistiškai reikšmingo skirtumo tarp polimorfinių branduolinių leukocitų kiekio miringotomuotuose ir nemiringotomuotuose būgneliuose nenustatyta. Pastebėta reikšmingai gausesnė infiltracija limfocitais ir aktyvintais makrofagocitais visose miringotomuotų būgnelių dalyse. Statistiškai reikšmingai didesnis bendrasis kalcio fosfato sandaigų tarpląstelinėje medžiagoje kiekis 7, 14 ir 28 tyrimo dieną buvo miringotomuotų būgnelių *pars tensa* dalyje palyginus su nemiringotomuotais.

**Išvados.** Netipuojamo *Haemophilus influenzae* sukeltas eksperimentinis ūminis vidurinės ausies uždegimas sukelia ryškesnę uždegiminę būgnelių reakciją ir mikrokalcifikaciją.

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