Development of myringosclerosis during acute otitis media caused by *Streptococcus pneumoniae* and non-typeable *Haemophilus influenzae*: a clinical otomicroscopical study using the rat model

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

**Summary.** Objective. The present study was performed in order to study development of myringosclerosis during acute otitis media caused by different bacteria in myringotomized and non-myringotomized ears.

Material and methods. A rat model of acute otitis media caused by *Streptococcus pneumoniae* type 3 and non-typeable *Haemophilus influenzae* was used. A sample consisted of 42 animals. Four days following middle ear inoculation, a myringotomy was performed in 10 animals from the *Streptococcus pneumoniae* group and 6 from the non-typeable *Haemophilus influenzae* group. Another group of 24 animals was inoculated only. On day 4, 7, 14 and 28 after inoculation the status of the drum was inspected under the otomicroscope for vascular reaction, effusion, perforation, myringosclerosis and scarring.

Results. On day 4 after inoculation all infected ears had typical signs of acute otitis media. Tympanic membranes healed with scar formation in most cases of myringotomized *Streptococcus pneumoniae* type 3 infection and deposition of sclerotic plaques was observed by day 14. Otomicroscopically visible myringosclerosis was not found after non-typeable *Haemophilus influenzae* induced acute otitis media neither in myringotomized nor in non-myringotomized animals.

We conclude that *Streptococcus pneumoniae* type 3 provokes a severe clinical course of acute otitis media that healed with scarring and myringosclerosis formation in the tympanic membrane. Clinically visible myringosclerosis develops after middle ear infection caused by *Streptococcus pneumoniae* type 3, but not in cases caused by non-typeable *Haemophilus influenzae*.

**Introduction**

Acute otitis media (AOM) is one of the most frequent and painful diseases in childhood. The incidence is highest in the first year of life, peaking during the second half of the first year of life and decreasing gradually thereafter. Some studies have shown that 19–62% of children have had at least one episode of AOM within their first year of life and by the third year, 50 to 84% had experienced AOM at least once (1–5).

Nowadays, the most frequent pathogens found in AOM are *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis* (6, 7). Of these, *Streptococcus pneumoniae* and non-typeable *Haemophilus influenzae* have been isolated as major causative agents, growing respectively in 18–46% and in 23–30% cases of children with AOM (8). The third common pathogen causing AOM is *Moraxella catarrhalis*, which has been isolated in 10–23% of cases (9).

Treatment of AOM usually starts with empiric antimicrobial treatment. However, in some children with AOM myringotomy (tymanocentesis) is indicated, especially when patients fail to improve or worsen while receiving antibiotics (10). Myringotomy is recommended for the reduction of severe persistent otalgia, fever or both, to avoid serious suppurative complications (e.g. mastoiditis) or a concurrent infection (e.g. meningitis) (10, 11). Whether myringotomy is effective in the treatment of uncomplicated AOM in children, or whether it changes the clinical course and prognosis of AOM are still questions of controversy.

Myringosclerosis is a sequela seen after otitis me-
dia with effusion and chronic otitis media and sometimes after multiple acute episodes of AOM (12). It is also related to the treatment with ventilation tubes or myringotomy (13, 14). Myringosclerosis is described as a pathological condition affecting the lamina propria of the tympanic membrane (TM), characterized by a hyalinization and calcification of the connective tissue layer. Clinically, myringosclerotic lesions are seen as whitish, sclerotic plaques in the TM (Fig.). According to some epidemiological investigations on non-selected population, prevalence of the sclerotic plaques in the eardrum varies from 1.7% to 22.4% (15, 16). Myringosclerosis is not known to affect significantly the hearing threshold. Audiological investigations have shown that hearing impairment caused by myringosclerosis is less than 0.5 dB (17, 18). However, when sclerosis affects the middle ear mucosa (tympanosclerosis), about 60% of patients have a conductive hearing loss due to ossicular chain fixation (19, 20). But still it is not clear whether the myringosclerosis and tympanosclerosis are diseases of the same pathophysiology.

AOM can be induced experimentally in the rat middle ear by inoculating Streptococcus pneumoniae (21) or Haemophilus influenzae (21, 22). The TM of the rat shares similar architectural features with the TM in humans (23). The natural course of the infection in the rat is similar to humans. In general, infection resolves spontaneously within 10 days, without any clinically visible sequela although structural changes in the middle ear mucosa can persist for several months (24).

The aim of the present study was to investigate development of myringosclerosis in a well-established rat model of AOM caused by viable Streptococcus pneumoniae type 3 (PnC type 3) or non-typeable Haemophilus influenzae (NTHi). In this paper, we report otomicroscopical findings and try to determine whether development of myringosclerosis during AOM differs due to bacterial species in myringomized and non-myringomized ears.

Materials and methods
Experimental animals

Forty-two male healthy Sprague-Dawley 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). The animals weighed about 250 g at the beginning of the study, were maintained under normal laboratory conditions with free access to water and were fed a standard pellet diet.

Operating procedures and experimental design

The animals were anesthetized with chloralhydrate (36 mg/ml, ApoteketProduktion & Laboratorier, Malmö, Sweden) intraperitoneally (1 ml/100 g body weight). All animals were inspected under an Olympus operating otomicroscope before operation and inoculation and had normal status of the eardrums.

A ventral midline incision was made in the neck and the right tympanic bulla of the rat was exposed. By a fine needle through the bony bulla wall the middle ear of 26 rats was inoculated with 0.05 ml of suspension of PnC type 3 and the middle ear of 16 rats was inoculated with 0.05 ml of suspension of NTHi. The left bulla was untouched and used as control.

Under otomicroscopical guidance, a tympanocentesis was performed in the posterior, inferior quadrant of the TM in 10 randomly selected animals from the PnC type 3 group and 6 from the NTHi group, four days following middle ear inoculation. In order to keep perforations open for a period of one week after inoculation, re-myringotomy was performed on day 7 if perforation in drum has been closed. On day 4, 7, 14 and 28 after inoculation, the status of TM was inspected under the otomicroscope. On day 4, middle ear effusion was sampled with a cotton swab for bacteriological analysis (culture) from 4 randomly selected rats of each group. Experimental design is shown in Table 1.

Bacteria

The bacteria used were cultured at the Department of Microbiology, University of Lund. The concentration of bacteria was determined as colony forming units per milliliter (CFU/ml). The animals were chal-

*Fig. Myringosclerotic plaques in the pars tensa of human tympanic membrane*
Table 1. Experimental design of the study

<table>
<thead>
<tr>
<th>Day</th>
<th>Viable Streptococcus pneumoniae type 3</th>
<th>Viable non-typeable Haemophilus influenzae</th>
<th>Evaluation (number of animals)</th>
<th>Excluded from study (number of animals)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>26</td>
<td>16</td>
<td>Otomicroscopy 26+16</td>
<td>Died 2</td>
</tr>
<tr>
<td>4</td>
<td>24</td>
<td>16</td>
<td>Otomicroscopy 24+16, tympanocentesis 10+6, middle ear culture 4+4</td>
<td>Histology 4+4</td>
</tr>
<tr>
<td>7</td>
<td>20</td>
<td>12</td>
<td>Otomicroscopy 20+12, re-myringotomy, if necessary</td>
<td>Histology 4+4</td>
</tr>
<tr>
<td>14</td>
<td>16</td>
<td>8</td>
<td>Otomicroscopy 16+8</td>
<td>Histology 4+4</td>
</tr>
<tr>
<td>28</td>
<td>12</td>
<td>4</td>
<td>Otomicroscopy 12+4</td>
<td>Histology 12+4</td>
</tr>
</tbody>
</table>

lenged with suspension containing viable PnC type 3 (2.5×10⁶ CFU/ml) or NTHi strain 3655, biotype II (1.0×10⁶ CFU/ml).

**Otomicroscopical examination**

The status of the TM was assessed for vascular reaction, quality and quantity of the effusion behind the eardrum, perforation, bulging, retraction, myringosclerosis and scarring. Using otomicroscopy the intensity of each pathological sign was graded and recorded for each pathology. Vascular reaction was described as follows: −, no changes; +, vessels dilated along the malleus; ++, vessels dilated along the malleus and spread out along the malleus ligament; ++++, vessels dilated and spread and covering *pars flaccida*. Quantity of the effusion was described as follows: −, none; +, *pars flaccida* or inferior to the malleus; ++, 50% of the middle ear cavity; +++, the whole middle ear cavity filled.

Retractions were evaluated according to their localization in *pars tensa* and/or *pars flaccida*. Scarring in the TM was described as changes consisting of thin, transparent, dark areas. Myringosclerosis was defined as whitish, thickened areas in the TM with loss of normal transparency as well as chalky plaques in the TM. Otomicroscopic evaluation of the sclerotic lesions of *pars tensa* was classified as −, no visible sclerotic deposits; +, occasional lesions covering less than 25% of the TM; ++, moderate sclerosis covering up to 50%; ++++, grave sclerosis covering more than 50% of the TM.

**Results**

Apart from the otitis media, the animals appeared clinically healthy throughout the study in the NTHi group. Two animals inoculated with PnC type 3 died from generalized infection within 3 days.

The same strains of bacteria as inoculated were grown on day 4 from the samples of middle ear effusion from 4 randomly selected rats of each group.

**Otomicroscopical observations in PnC type 3 inoculated ears**

On day 4 after inoculation AOM was otomicroscopically verified in the all 24 ears challenged with PnC type 3. All showed a bulging TM with diluted blood vessels and a purulent effusion in the middle ear. Extensive vascular reaction was mostly described as ++++. The quantity of the purulent effusion was mostly ++/++. A fluid filled external canal of the 4 infected ears observed on day 4 after inoculation referred to spontaneous perforations of TMs. On day 7, diluted blood vessels (mostly ++) and purulent effusion (mostly +) were seen in both myringotomized and non-myringotomized ears. Perforations were found open in 7 of 10 myringotomized animals and re-myringotomy was performed in the 3 closed TMs. On day 14, a small amount of the purulent effusion and a small perforation were still persisted in 1 myringotomized ear. Mild (mostly not more intensive than +) vascular reaction was observed in 7 myringotomized and 5 non-myringotomized TMs. Slight retraction of *pars flaccida* was found in 2 myringotomized and 1 non-myringotomized TM, and bulging in 1 myringotomized and 2 non-myringotomized TMs. On day 14, scarring of the TM appeared in 6 out of 8 myringotomized TMs and 2 out of 8 non-myringotomized TMs.

On day 14, the appearance of myringosclerotic plaques was seen in 1 myringotomized and 3 non-
myringotomized ears inoculated with PnC type 3. These changes still persisted on day 28.

In the otomicroscope, a horseshoe shaped whitish lesion was noted in the anterior, non-injured part of the *pars tensa* of the myringotomized animals. Myringosclerotic plaques did not develop at the place of the myringotomy, but they developed in the remaining part of the *pars tensa*. The sclerotic lesions in the *pars tensa* of the non-myringotomized ears were less extensive. Myringosclerosis was not seen in *pars flaccida*.

Fluid was observed in 3 contra-lateral control ears on day 4, in 6 contra-lateral ears on day 7, and in 1 on day 14. No fluid was present in any animal on day 28.

The otomicroscopical findings of PnC type 3 inoculated animals are summarized in Table 2.

**Otomicroscopical observations in NTHi inoculated ears**

On day 4 after inoculation all ears infected with NTHi had typical signs of AOM, as indicated by bulging TM with dilated blood vessels. Accumulation of purulent effusion behind the TM from a small amount (+) to a full tympanic cavity (++++) was found in the majority of inoculated ears. Spontaneous TM perforation and pus in the external ear canal were seen in one ear only. Fluid was observed in 2 contra-lateral control ears on day 4. On day 7, a purulent or mixed effusion with vascular reaction of the TM was still seen in all infected ears. Perforations in the TMs were still open in all myringotomized ears. There were no signs of reactive inflammation in the contralateral ear on day 7, or later. In the myringotomized group, 2 TMs still showed signs of inflammation with mild vascular reaction and small amount of clear effusion on day 14. There were no signs of inflammation in non-myringotomized TM on day 14, or later. Under the otomicroscope, the appearance of scarring was found in 1 myringotomized TM and 1 non-myringotomized TM on day 14. On day 28, there were no signs of inflammation and all TMs seemed to be normal otomicroscopically. The animals were completely free from visible sclerotic deposits when examined in the otomicroscope. The otomicroscopical findings of NTHi inoculated animals are summarized in Table 3.

**Discussion**

The present study outlines the clinical course of AOM caused by PnC type 3 and NTHi in an animal model, as well as the development of myringosclerosis following the middle ear infection.

Initially, our study showed that experimental NTHi induced AOM caused no systemic infection, whereas animals challenged with PnC type 3 showed systemic reactions, and approximately 8% died within 3 days,

**Table 2. Otomicroscopical signs of PnC type 3 inoculated animals**

<table>
<thead>
<tr>
<th>Day</th>
<th>Vasa dilatation</th>
<th>Effusion</th>
<th>Perforation</th>
<th>Scarring</th>
<th>Visible myringosclerosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Myr</td>
<td>Non Myr</td>
<td>Myr</td>
<td>Non Myr</td>
<td>Myr</td>
</tr>
<tr>
<td>Day 4</td>
<td>+/4</td>
<td></td>
<td>+/4</td>
<td></td>
<td>4/24</td>
</tr>
<tr>
<td>(n=24)</td>
<td>++/18</td>
<td></td>
<td>++/11</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+++/2</td>
<td></td>
<td>+++/9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 7</td>
<td>++/10</td>
<td></td>
<td>+/1</td>
<td></td>
<td>7/10</td>
</tr>
<tr>
<td>(n=20)</td>
<td>–/1</td>
<td></td>
<td>++/6</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>++/2</td>
<td></td>
<td>++/3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+++/1</td>
<td></td>
<td>+++/4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 14</td>
<td>–/1</td>
<td></td>
<td>–/7</td>
<td></td>
<td>1/8</td>
</tr>
<tr>
<td>(n=16)</td>
<td>+/6</td>
<td></td>
<td>++/1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+++/1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 28</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>6/6</td>
</tr>
<tr>
<td>(n=12)</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td></td>
</tr>
</tbody>
</table>

Myr – myringotomized animals; Non Myr – non-myringotomized animals; n – number of rats; –, +, ++, +++/x – represent different levels of sign intensity, expressed in the number of inoculated ears. For additional explanation see text.
Table 3. Otomicroscopical signs of NTHi inoculated animals

<table>
<thead>
<tr>
<th>Day</th>
<th>Vasa dilatation</th>
<th>Effusion</th>
<th>Perforation</th>
<th>Scarring</th>
<th>Visible myringosclerosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Myr</td>
<td>Non Myr</td>
<td>Myr</td>
<td>Non Myr</td>
<td>Myr</td>
</tr>
<tr>
<td>Day 4 (n=16)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1/16</td>
</tr>
<tr>
<td>Day 7 (n=12)</td>
<td>+/4</td>
<td>++/2</td>
<td>+/5</td>
<td>++/1</td>
<td>+/3</td>
</tr>
<tr>
<td>Day 14 (n=8)</td>
<td>+/-2</td>
<td>no</td>
<td>+/-2</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>Day 28 (n=4)</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
</tr>
</tbody>
</table>

Myr – myringotomated animals; Non Myr – non-myringotomated animals; n – number of rats; –, +, ++, +++/+x – represent different levels of sign intensity, expressed in the number of inoculated ears. For additional explanation see text.

which is in agreement with prior investigations (21, 22).

The clinically observed TM changes were more prominent in the PnC type 3-infected animals, e.g. bulging, vascular reaction, quantity and quality of effusion, compared with those of the NTHi-infected ears. This is also in accordance with earlier observations (25).

In myringotomized ears, the fluid in the tympanic cavity was observed longer and TMs seemed to be more affected than the non-myringotomized ears, e.g. thicker and with larger vessels in both PnC type 3 and NTHi inoculated animals. In a study where myringotomy was performed during PnC induced AOM, it was shown that the infection resolved more slowly in myringotomized ears compared to non-myringotomized ears (26). Another study concluded that myringotomy in AOM provoked a delayed recovery from the inflammatory process within the TM (27).

In our study, otomicroscopically visible myringosclerosis was not found up to 28 days after NTHi induced AOM, whether myringotomy was performed or not. Scar formation on day 14 was observed in both groups of NTHi-infected animals and may be a sign of incipient myringosclerosis. In PnC type 3 inoculated and myringotomized ears scar formation in most cases was seen. However, a formation of a sclerotic plaque was observed in only one myringotomized and some non-myringotomized animals on day 14, persisting on day 28.

The site of myringosclerosis formation was not restricted to the site of myringotomy, and in many cases it was present only in other areas of the TM. Furthermore, there was a tendency to more extensive myringosclerosis in ears with a relatively mild inflammatory reaction (e.g. lower intensity and shorter duration of pathological signs) following inoculation. Previous studies about 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. It seems possible that the inflammation of the connective tissue layer induced by the infection interferes with the process of myringosclerosis formation (28). In addition, the development of myringosclerosis in non-infected myringotomized TMs has been associated with exposure to a hyperoxic environment (29).

The present findings have shown that NTHi and PnC type 3 both induce AOM, but with different clinical courses, provoking different degrees of myringosclerosis plaque formation and scarring of the TM. A detailed histological evaluation of these TMs is necessary, in order to elucidate structural changes which might be clinically invisible in the otomicroscope.

**Conclusions**

The present clinical study demonstrates that *Streptococcus pneumoniae* type 3 provokes a severe clinical course of acute otitis media, which heals with scarring and myringosclerosis formation. Myringosclerosis develops more often and more extensively in non-
myringotomized ears. In comparison, non-typeable *Haemophilus influenzae* induces a milder course of acute otitis media, which is followed by tympanic membrane scar formation only.

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**Miringosklerozės vystymasis Streptococcus pneumoniae ir netipinio Haemophilus influenzae sukeltą ūminio vidurinės ausies uždėgimo metu.**

**Klinikinis otomikroskopinis tyrimas naudojant žiurkės modelį**

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**Raktažodžiai:** ūminis vidurinės ausies uždėgimas, bakteriologija, miringotomija, miringosklerozė, eksperimentinis gyvybinis modelis.

**Santrauka. Darbo tikslas.** Ištirti miringosklerozės atsiradimą ūminio, sukeltu skirtingų bakterijų, vidurinės ausies uždėgimo metu taikant miringotomiją ir be jos.


**Išvados.** 3 serotipai *Streptococcus pneumoniae* sukelia stipresnį ūminį vidurinės ausies uždėgimą, kai gyjant būgneliuose formuojasi randai ir miringosklerozinės plėšėsi. Kliniškai matoma miringosklerozė vystosi po 3 serotipai *Streptococcus pneumoniae* sukelto ūminės ausies infekcijos.

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**References**


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