Objective: To study the combined role of immune status and eustachian tube function in the development of recurrent bilateral otitis media with effusion (OME).

Design: Prospective cohort study.

Setting: Three academic and general hospitals.

Patients: Children aged 2 to 7 years with a first clinical episode of bilateral OME that persisted for at least 3 months; 136 (81%) of 168 eligible children participated. All children received bilateral tympanostomy tubes at study entry.

Main Outcome Measure: Recurrence of bilateral OME within 6 months after tube extrusion.

Results: Univariate analyses of various immunologic factors (IgA, IgG1, IgG2, IgG3, IgG4, mannose-binding lectin, and the FcγRIIa-H/R131 genotype) and eustachian tube function (forced response test) did not show any significant associations with bilateral OME recurrence. Multivariate analyses showed that children with closing pressures higher than the 75th percentile and IgA or IgG2 levels below the 50th percentile of the cohort were more likely to develop recurrent OME than children with closing pressures higher than the 75th percentile and IgA or IgG2 levels above the 50th percentile. The corresponding risk ratios were 6.3 (95% confidence interval [CI], 1.0-40.1) for IgA level and 3.0 (95% CI, 1.1-8.2) for IgG2 level. The multivariate analyses also revealed that increasing serum levels of functional mannose-binding lectin were associated with decreasing probabilities of developing recurrent OME (odds ratio, 0.7; 95% CI, 0.6-1.0).

Conclusion: Recurrence of bilateral OME after tympanostomy tube placement is more likely in children with a combination of low IgA or low IgG2 levels with poor eustachian tube function and decreased levels of mannose-binding lectin.


Otitis Media with Effusion (OME) is a highly prevalent condition and the most important cause of hearing loss in childhood. In most children, the natural course is self-limiting and favorable; however, approximately 10% of children experience recurrent or persistent OME.1 Adverse effects of OME associated with hearing loss, such as impairment of speech and language development, mainly occur in children with recurrent or persistent OME.2-4 Because therapeutic interventions (eg, insertion of tympanostomy tubes) should be focused on this particular group of children, it is relevant to distinguish children who are prone to recurrent or persistent OME from the total group of children with OME early in the disease.

At a young age, eustachian tube (ET) function and the inflammatory response to viruses and bacteria that enter the upper airway are 2 core elements in the development of OME.5 Clinical studies have thus far shown only subtle differences in parameters of the immune system6-8 and ET function9,10 in children with and without recurrent or persistent OME. Apparently, the discriminative capacity of a single parameter of either ET function or the immune system is insufficient to predict the recurrence of a multifactorial-generated condition such as OME. Combining parameters of ET function with immunologic parameters may have more predictive power. Interaction between ET dysfunction and immunosuppression in the development of OME has been demonstrated only in a single experimental animal study.11

CME course available at www.archoto.com

To test the hypothesis that the prognosis of OME depends on a combination of
ET function and immune status, we conducted a prospective cohort study in children treated with tympanostomy tubes for their first clinical episode of OME. At the time of tympanostomy tube insertion, several factors were measured that are considered to be involved in the immune response to upper respiratory tract pathogens. Passive ET function was determined in the presence of tympanostomy tubes. Both ET function and immunologic parameters were analyzed in relation to bilateral OME recurrence after spontaneous tympanostomy tube extrusion.

**METHODS**

**PATIENTS**

In the Netherlands, health insurance companies require referral by a general physician (GP) before refunding specialist care costs. Therefore, nearly all patients with OME are first seen by their GP. In Dutch general practice, the diagnosis of OME by GPs is generally based on the combination of medical history and otoscopic evidence of fluid in the middle ear. Otoscopic competence is an important issue in the training of GPs, including validation by otomicroscopy and otolaryngologic examination. According to the guidelines of the Dutch College of General Practitioners, children with chronic OME should be referred to an otologist only after repeated observations of middle ear effusion for at least 3 months. Children were eligible for the study if they were aged 2 to 7 years, had a GP-documented first clinical episode of OME that persisted for at least 3 months, and had been referred for the first time to the department of otorhinolaryngology of 1 of the 3 participating hospitals in Nijmegen or Winterswijk (the Netherlands) between December 1, 1999, and March 31, 2002. The otolaryngologist confirmed the presence of bilateral OME. Because of compliance with the ET function test, a minimum age of 24 months was required for participation in the study.

Children with Down syndrome, cleft palate, cystic fibrosis, or daily treatment with inhalation or topical corticosteroids for at least 1 month per year were excluded from the study, as were children with documented immunodeficiency, previous adenoidectomy, myringotomy, or treatment with tympanostomy tubes. All of the children who fulfilled the inclusion criteria were sequentially asked to participate in the study. The medical ethics committees of the 3 participating hospitals approved the study protocol. Signed informed consent was obtained from the parents or legal guardians.

**STUDY DESIGN**

At study entry, all children received the same type of tympanostomy tube bilaterally while under general anesthesia. Adenoidectomy was not performed. Blood samples were obtained, and serum and DNA samples were isolated and stored at –20°C. Checkup visits were scheduled for 1 week after tube insertion and every 3 months thereafter. An otologist (N.X.H.) performed otomicroscopy to assess tympanostomy tube patency or extrusion and recurrence of OME. The ET function tests were performed on ears with patent tympanostomy tubes. The primary outcome measure was the number of children who developed bilateral recurrence of OME within the follow-up period. The risk period for the recurrence of OME started at the first checkup at which tube extrusion was observed (per ear). Follow-up ended at the checkup at which OME was diagnosed, 6 months after the observation of spontaneous tympanostomy tube extrusion, or at the predetermined end point of the study.

At study entry and the checkup visits, OME was defined according to the Maastricht Otitis Media With Effusion Study algorithm, which is based on the results of tympanometry and nonpneumatic otoscopy. Tympograms were classified in accordance with the criteria of Jerger. According to the algorithm, OME was considered present when tympanometry resulted in a type B or C2 tympanogram combined with otoscopic findings that suggested the presence of effusion in the middle ear (eg, glue and fluid lines or bubbles) and no signs of acute ear infection. If tympanometry could not be performed, otomicroscopic findings that suggested effusion in the middle ear were used to diagnose OME.

**TESTS FOR ET FUNCTION**

Tympanometry and ET function tests were performed with the TYMP 87 analyzer (Rexton Danplex A/S, Copenhagen, Denmark). The ET function tests were performed at each checkup with patent tympanostomy tubes as described previously. The forced response test was used to assess the ET ventilation function, with opening pressure and closing pressure as outcome variables. These pressures reflect the resistance to passive tubal opening when middle ear pressure is increased experimentally and the forces that act to close the open tube.

**TESTS FOR IMMUNOLOGIC STATUS**

Serum immunoglobulin concentrations of IgA, IgG1, IgG2, IgG3, and IgG4 were determined by radial immunodiffusion. To determine functional mannose-binding lectin (MBL) levels, an assay was used based on the principle of yeast-induced by-stander lysis of chicken erythrocytes. To determine the presence of FcyRIIa-H/R131, genomic DNA was extracted from whole blood using a QIAamp DNA Blood Kit (Qiagen, Westburg, the Netherlands). The FcyRIIa-H/R131 genotypes were determined by means of polymerase chain reaction amplification methods as described previously.

**OTHER RISK FACTORS**

Questionnaire information on potential risk factors for OME recurrence was obtained at the first checkup visit: gestational age, birth weight, incubator care with or without artificial ventilation or nasogastric feeding tubes, breastfeeding, siblings, habitual sniffling, day care, family history of otitis media, exposure to smoking at home, and diagnosis of otitis media by a GP or an otologist both in the first year of life and in the year before study entry.

**STATISTICAL ANALYSES**

All analyses were performed using a statistical software program (SAS version 8.0; SAS Institute Inc, Cary, NC). Children with bilateral OME recurrence were compared with the rest of the cohort regarding ET function and immunologic status. If the follow-up data to classify OME status were inadequate, the child was excluded. A single measure was defined for both the opening and closing pressures in each child, based on the mean pressure from all test results obtained at the 3 monthly checkups with patent tympanostomy tubes.

Mean differences and confidence intervals (CIs) were calculated for continuous variables. Odds ratios (ORs) and corresponding CIs were calculated for categorical variables. To investigate whether a combination of ET function and immunologic status made a greater contribution to bilateral OME recurrence than the separate effects of the 2 parameters, interaction terms were included in the multivariate logistic regres-
sion models. Potential confounders were also included in these models. The full model was reduced through manual stepwise backward regression analyses to obtain the final model that included the variables that showed significant associations (P < .05) with bilateral OME recurrence. The ORs obtained for continuous independent variables reflect the association with bilateral OME recurrence per unit increase in these independent variables. The final prognostic model included all of the variables that contributed to the prediction of bilateral recurrence of OME. Model discrimination was measured by the area under the receiver operating characteristic curve (c-index).

RESULTS

A total of 136 of the 168 eligible children participated in this study. The reasons for refusal to participate in the study were not related to immune status or ET function. In most of the included children, the diagnosis of bilateral OME at study entry was confirmed by a bilateral type B tympanogram. Fifteen children were lost to follow-up for practical reasons. At the predetermined end of the study (August 15, 2003), 31 children had incomplete follow-up in either 1 or 2 ears. Ten of these children did not develop OME in the one ear with complete follow-up. Of the 90 children who had complete follow-up, OME recurred bilaterally in 56 children, whereas 34 children with complete follow-up had unilateral or no recurrence (Figure 1). The median time of documentation of tympanostomy tube extrusion was 9 months (range, 3–27 months) after insertion in children with and without bilateral OME recurrence. Children with bilateral OME recurrence were comparable with those without, except for age at the start of follow-up, habitual snifing, and the proportion of children with either low birth weight (<2500 g), low gestational age (<37 weeks), or incubator care with or without artificial ventilation or nasogastric tubes for feeding (Table 1).

UNIVARIATE ASSOCIATIONS

Based on univariate analyses, no statistically significant associations were found between bilateral OME recurrence and levels of IgA and IgG subclasses, functional MBL, FcγRIIa-H/R131 genotype, or ET function test results (Table 2).

MULTIVARIATE ASSOCIATIONS: INTERACTION BETWEEN IMMUNOGLOBULINS AND CLOSING PRESSURE

Based on the concept that OME is a multifactorial disease, we studied potential interactions between ET function and immunologic parameters related to bilateral OME recurrence. Backward regression analyses on the full multivariate model that contained all immunologic parameters, ET function, and their combined effects provided the final multivariate model that included all of the variables with an independent significant association with OME recurrence.

Multivariate logistic regression analyses showed that higher MBL levels were associated with a lower probability of developing recurrent bilateral OME (OR, 0.7; 95% CI, 0.6–1.0). No significant associations were found between bilateral OME recurrence and the separate effects of decreasing levels of IgA (OR, 0.5; 95% CI, 0.2–1.3), IgG1 (OR, 0.9; 95% CI, 0.7–1.2), and IgG2 (OR, 0.7; 95% CI, 0.3–1.7) and closing pressure (OR, 1.0; 95% CI, 0.99–1.01). However, the association among IgG1, IgG2, and IgA levels and bilateral OME recurrence varied according to the closing pressure. To investigate these interactions, immunoglobulin levels and closing pressure were dichotomized around the median value (immunoglobulins) and the 75th percentile (closing pressure) of the data from the full cohort.

Figure 2 shows the proportion of children with bilateral OME recurrence within combinations of closing pressure and IgA, IgG1, or IgG2. A remarkably high proportion of OME recurrence was found within the category of children with high closing pressures and low IgA or IgG2 levels. The corresponding risk ratios are 6.3 (95% CI, 1.0–40.1) for IgA and 3.0 (95% CI, 1.1–8.2) for IgG2 when compared with the children with high closing pressures and high IgA or IgG2 levels. The IgG1 level had no such interaction with closing pressure (relative risk, 1.0; 95% CI, 0.5–2.2).

PREDICTIVE POWER

All variables significantly associated with bilateral OME recurrence were entered into a predictive model. To ob-
tain the most informative and efficient model, this predictive model was reduced until its corresponding c-index was less than 5% of the value of the predictive model that contained all significant variables. The final predictive model included IgA, IgG2, closing pressures, their interactions, and MBL functionality. This model had a c-index of 0.79 and can be described as follows (Pc indicates closing pressure):

Natural Logarithm (Odds Bilateral OMERecurrence = −8.6 − 0.3 MBL + 2.0 IgA + 5.0 IgG2 + 0.1 Pc − 0.02 IgA × Pc − 0.05 IgG2 × Pc).

The predicted probability of bilateral OME recurrence corresponded fairly well with the actual recurrence in our study population (Figure 3). Using a cutoff point of 0.61 (based on the maximum sum of sensitivity and specificity in the receiver operating characteristic curve), 80% of the children without bilateral OME recurrence and 65% of those with bilateral OME recurrence were correctly classified.

COMMENT

This study yielded several unique results related to OME recurrence in a population that was homogeneous with respect to OME status at baseline. First, ET dysfunction in the presence of decreased activity of several parameters of the immune system played a role in the development of OME recurrence. Children with a combination of high closing pressure and low IgA or IgG2 levels were more likely to develop recurrent bilateral OME than

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Full Cohort (n = 136)</th>
<th>Bilateral OME Recurrence (n = 56)</th>
<th>No Bilateral OME Recurrence (n = 44)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boys, No. (%)</td>
<td>68 (50)</td>
<td>30 (54)</td>
<td>21 (48)</td>
</tr>
<tr>
<td>Age at start of follow-up, median (range), y</td>
<td>6.3 (3.4-9.1)*</td>
<td>6.0 (3.5-8.3)</td>
<td>6.4 (3.4-9.1)</td>
</tr>
<tr>
<td>Birth weight, mean (range), g</td>
<td>3444 (1380-5200)</td>
<td>3615 (2575-5200)</td>
<td>3277 (1380-5010)</td>
</tr>
<tr>
<td>Breastfed for &gt;4 mo, No. (%)</td>
<td>48 (36)</td>
<td>21 (38)</td>
<td>16 (36)</td>
</tr>
<tr>
<td>LBW incubator neonate score, No. (%)</td>
<td>27 (21)†</td>
<td>4 (7)</td>
<td>11 (28)</td>
</tr>
<tr>
<td>Diagnosis of otitis media in first year of life, No. (%)</td>
<td>20 (17)†</td>
<td>6 (13)§</td>
<td>6 (16)§</td>
</tr>
<tr>
<td>Diagnosis of otitis media in year before study entry, No. (%)</td>
<td>55 (43)†</td>
<td>23 (46)§</td>
<td>19 (44)</td>
</tr>
<tr>
<td>Habitual sniffing, No. (%)</td>
<td>31 (23)</td>
<td>14 (25)</td>
<td>3 (7)</td>
</tr>
<tr>
<td>Attending day care at study entry, No. (%)</td>
<td>28 (21)</td>
<td>10 (18)</td>
<td>10 (23)</td>
</tr>
<tr>
<td>Siblings present, No. (%)</td>
<td>125 (93)</td>
<td>52 (93)</td>
<td>42 (95)</td>
</tr>
<tr>
<td>Family with history of otitis media, No. (%)</td>
<td>70 (61)†</td>
<td>32 (65)‡</td>
<td>25 (64)</td>
</tr>
<tr>
<td>Exposure to smoking at home, No. (%)</td>
<td>46 (34)</td>
<td>20 (36)</td>
<td>11 (25)</td>
</tr>
</tbody>
</table>

Abbreviations: LBW, low birth weight; OME, otitis media with effusion.
*Based on 100 children with a known clinical outcome.
†A child was assigned a positive score on this variable when at least 1 of the following variables was present: gestational age younger than 37 weeks, birth weight less than 2500 g, and/or incubator care and/or treated with artificial ventilation or nasogastric tubes in the postnatal period.
‡A total of 5% to 16% of the patients had missing data on this variable.
§A total of 10% to 18% of the patients had missing data on this variable.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Bilateral OME Recurrence</th>
<th>No Bilateral OME Recurrence</th>
<th>Mean Difference or OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgA, g/L</td>
<td>0.95 (0.10-2.40) (n = 56)</td>
<td>1.10 (0.25-2.25) (n = 42)</td>
<td>−0.19 (−0.38 to 0.01)</td>
</tr>
<tr>
<td>IgG1, g/L</td>
<td>7.54 (5.08-11.44) (n = 56)</td>
<td>8.67 (4.12-14.03) (n = 42)</td>
<td>−0.65 (−1.38 to 0.09)</td>
</tr>
<tr>
<td>IgG2, g/L</td>
<td>1.25 (0.55-2.95) (n = 56)</td>
<td>1.40 (0.05-2.60) (n = 42)</td>
<td>−0.09 (−0.30 to 0.12)</td>
</tr>
<tr>
<td>IgG3, g/L</td>
<td>0.40 (0.15-0.85) (n = 56)</td>
<td>0.40 (0.15-0.90) (n = 42)</td>
<td>0.01 (−0.07 to 0.09)</td>
</tr>
<tr>
<td>IgG4, g/L</td>
<td>0.25 (0.05-1.45) (n = 56)</td>
<td>0.40 (0.05-3.00) (n = 42)</td>
<td>−0.18 (−0.38 to 0.02)</td>
</tr>
<tr>
<td>Functional MBL, mg/L</td>
<td>1.38 (0.07-11.36) (n = 56)</td>
<td>1.77 (0.09-10.88) (n = 43)</td>
<td>−0.51 (−1.41 to 0.39)</td>
</tr>
<tr>
<td>Eustachian tube</td>
<td>336 (184-541) (n = 48)</td>
<td>329 (154-508) (n = 43)</td>
<td>23 (−14 to 60)</td>
</tr>
<tr>
<td>Po, daPa</td>
<td>103 (20-231) (n = 48)</td>
<td>98 (29-209) (n = 43)</td>
<td>4 (−14 to 22)</td>
</tr>
<tr>
<td>FcγRlla genotype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FcγRlla-R/R131</td>
<td>34</td>
<td></td>
<td>2.57† (0.72 to 9.17)</td>
</tr>
<tr>
<td>FcγRlla-R/H131</td>
<td>49</td>
<td></td>
<td>1.04† (0.36 to 3.05)</td>
</tr>
<tr>
<td>FcγRlla-H/H131</td>
<td>17</td>
<td></td>
<td>1.00† (Reference)</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; daPa, decaPascals; OME, otitis media with effusion; OR, odds ratio; Pc, closing pressure; Po, opening pressure.
*All data are given as median (range) except for FcγRlla genotypes, which are percentages.
†These data are ORs.
children with high closing pressure and high IgA or IgG2 levels. The closing pressure measured by the forced response test reflected the extraluminal forces that are part of the total closing forces of the ET (ie, the forces that keep the ET closed at rest). To open the ET actively, these forces must be overcome. Extraluminal forces are brought about by the elasticity of the cartilage and pressure from other surrounding tissues. High closing pressure thus indicates poor ET ventilation.22 Lower serum levels of IgG2 and IgA suggest decreased antibody response to pathogens that enter the middle ear cavity.12,23 In other words, middle ear effusion may develop because of diminished immune response and may persist because of impaired ET ventilation.

Second, this study showed significant associations between low functional MBL levels and bilateral OME recurrence. Low serum MBL levels reduce effective opsonization of pathogens and interaction with receptors on macrophages24 and may therefore lead to higher susceptibility to OME. Previous studies have shown that median MBL levels were lower in children with OME than in children without OME.25 Associations have also been reported between severe recurrent otitis media and MBL variant alleles known to be associated with low MBL serum levels.26

Third, univariate analyses showed a nonsignificant trend that supported our a priori hypotheses: children homozygous for the polymorphic form of the FcγRIIa receptor, with the lower binding affinity for IgG2 opsonized bacteria (FcγRIIa-R/R131), such as Streptococcus pneumoniae, were more likely to develop bilateral OME recurrence than children with the FcγRIIa-H/H131 genotype.13 The same nonsignificant trend was present in the full multivariate model. More elaborate analyses were also performed on the pressure equilibration test (active ventilatory ET function), sniff test (protective ET function),27 and serum immunoglobulins IgM and IgG, but no significant associations or interactions with OME recurrence were found (data not shown).

Because OME is a multifactorial-generated condition, it is possible that factors other than the 2 primarily addressed in this study play a role, such as low birth weight, (family) history of otitis media, day care attendance, siblings, habitual sniffing, and exposure to smoking at home.3 In our cohort, either these factors were equal at baseline or their effect on recurrent OME could not be confirmed in the multivariate analyses.

Our study results were obtained from a population of 100 children with a known clinical outcome. We believe that this population is representative of the complete cohort of children with a history of at least 3 months of bilateral OME because the predetermined end point
of the follow-up period and the reasons for loss to follow-up were unrelated to the clinical outcomes. In addition, the baseline characteristics of these 100 children were comparable with those of the full cohort population. Most of our cohort developed OME recurrence; only 28% of the ears remained effusion free in the 6-month period after tympanostomy tube extrusion. Previous studies of children treated with tympanostomy tubes for persistent OME with a comparable follow-up period demonstrated similar high recurrence rates.28,29

As far as we know, this is the first prospective study to examine baseline immunologic parameters and ET function test results in relation with bilateral OME recurrence. The discriminative power of the prognostic model with functional MBL levels, IgA, IgG2, closing pressure, and the interaction among IgA, IgG2, and closing pressure was fairly good, as reflected by a c-index of 0.8. This model requires external validation with independent data. Even if the model shows high external validity, its application to clinical practice is limited because closing pressures can be measured only as part of the forced response test in ears with patent tympanostomy tubes. Alternative function tests that enable analysis of ET function in children with an intact tympanic membrane (eg, in a pressure chamber or with sonotonometer) would add to the clinical value of the model.

Submitted for Publication: March 4, 2005; final revision received April 29, 2005; accepted April 29, 2005.

Correspondence: Gerhard A. Zielhuis, PhD, Department of Epidemiology and Biostatistics (HP 252), Radboud University Nijmegen Medical Centre, PO Box 9101, 6500 HB Nijmegen, the Netherlands (g.zielhuis@epib.umcn.nl).

Financial Disclosure: None.

Funding/Support: This study was supported by grant 904-61-092 from ZonMW, the Netherlands Organization for Health Research and Development, The Hague.

Acknowledgment: We thank the parents and children who took part in this study; the ear, nose, and throat surgeons and staff at the Departments of Otorhinolaryngology of the Radboud University Nijmegen Medical Centre, the Canisius-Wilhelmina Hospital Nijmegen, and the District Hospital Queen Beatrix Winterswijk; Piet Aerts and Hans van Dijk, MD, PhD, from the Eijkman Centre for Microbiology, Infectious Diseases and Inflammation at the University Medical Centre Utrecht for performing serum MBL testing; and Sonja van Oosterhout from the Department of Epidemiology and Biostatistics, Radboud University Nijmegen Medical Centre, for study management and data entry.

REFERENCES