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To my family

ABSTRACT

Johanna Uitti, MD

Role of symptoms in the diagnosis and management of acute otitis media in young children

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Acute symptoms are required for the diagnosis of acute otitis media (AOM) and they guide the management together with the laterality of AOM. However, symptoms are variable in young children and not diagnostic with AOM.

We investigated ear pain with parentally used pain scales, clinical characteristics of bilateral and unilateral AOM, symptoms and nasopharyngeal microbes and the need for follow-up of children with AOM initially managed without antimicrobials. We included children (6-35 months) with respiratory tract infection (RTI) whose parents suspected AOM.

In children with RTI, either with or without AOM, ear pain was assessed as moderate/severe in 80-90% with parentally used pain scales, compared with 56-65% with parental interview. In children with bilateral vs. unilateral AOM, fever occurred in 54% vs. 36% and moderate/severe bulging of tympanic membrane in 63% vs. 40%. In children with the suspicion of AOM, respiratory symptoms were associated with the nasopharyngeal colonization of *Moraxella catarrhalis* in the presence of viruses and fever was associated with respiratory viruses. Of the children with AOM initially managed without antimicrobials who had symptomatic improvement, the otoscopic signs worsened in 3%.

Majority of children with RTI suffer from moderate/severe ear pain or distress, when assessed by parents, regardless of AOM. Without pain scales, parents may underestimate children's pain. Clinically, bilateral AOM is only slightly more severe illness than unilateral AOM. Symptoms are associated with nasopharyngeal microbes. For children with AOM who are initially managed without antimicrobials and who are symptomatically improving, close follow-up may be unnecessary.

Keywords: acute otitis media, symptoms, ear pain, pain scales, otoscopic signs, unilateral, bilateral, children, respiratory tract infection, bacteria, viruses, nasopharynx

TIIVISTELMÄ

LL Johanna Uitti

Oirekuvan rooli äkillisen välikorvatulehduksen diagnostiikassa ja hoidossa pienillä lapsilla

Turun yliopisto, Lääketieteellinen tiedekunta, Kliininen laitos, Lastentautioppi, Turun kliininen tohtoriohjelma; Lasten ja nuorten klinikka, Turun yliopistollinen keskussairaala, Turku, Suomi

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Äkillisen välikorvatulehduksen diagnoosiin vaaditaan äkilliset infektioon viittaavat oireet. Oireet ja äkillisen välikorvatulehduksen tois- tai molemminpuolisuus vaikuttavat välikorvatulehduksen hoitoon. Oireet ovat kuitenkin vaihtelevia pienillä lapsilla ja yksikään oireista ei ole diagnostinen äkilliselle välikorvatulehdukselle.

Tutkimme korvakipua vanhempien käyttämillä kipumittareilla, tois- ja molemminpuolisen välikorvatulehduksen kliinisiä piirteitä, oireita ja nenänielun mikrobeita, sekä seurantakäynnin tarvetta 2-3 päivän kuluttua äkillisen välikorvatulehduksen diagnoosista niillä lapsilla, joita seurataan ilman mikrobilääkehoitoa. Tutkimukseen otettiin mukaan 6-35 kuukauden ikäisiä lapsia, joilla oli hengitystieinfektio ja joilla vanhemmat epäilivät äkillistä välikorvatulehdusta.

Hengitystieinfektiota sairastavilla lapsilla, joilla joko oli tai ei ollut äkillistä välikorvatulehdusta, vanhemmat arvioivat kipumittareita käyttäessään korvakivun kohtalaiseksi/vaikeaksi 80-90%:lla. Kun vanhempia haastateltiin, vastaava luku oli 56-65%. Molemminpuolista välikorvatulehdusta ja toispuolista välikorvatulehdusta sairastavilla lapsilla oli kuumetta 54%:lla ja 36%:lla ja tärykalvon kohtalaista tai merkittävää pullotusta 63%:lla ja 40%:lla. Lapsilla, joilla epäiltiin äkillistä välikorvatulehdusta, flunssaoireet olivat yhteydessä nenänielun *Moraxella catarrhalis*-bakteerikantajuuteen virusten ohella ja kuumeella oli yhteys hengitystieviruksiin. Äkillistä välikorvatulehdusta sairastavilla lapsilla, joita seurattiin ilman mikrobilääkehoitoa ja joiden yleisvointi parani, korvatulehduslöydökset pahenivat 3%:lla.

Valtaosa hengitystieinfektiota sairastavista lapsista kärsii vanhempien arvion mukaan kohtalaisesta tai vaikeasta korvakivusta tai tuskaisuudesta, riippumatta siitä, onko lapsella äkillistä välikorvatulehdusta vai ei. Ilman kipumittareita vanhemmat saattavat aliarvioivat lastensa kipua. Molemminpuolinen välikorvatulehdus on kliinisesti vain lievästi vaikeampi kuin toispuolinen välikorvatulehdus. Oireet ovat yhteydessä nenänielun mikrobeihin. Lapset, joiden äkillistä välikorvatulehdusta seurataan ilman mikrobilääkehoitoa ja joiden yleisvointi on paranemassa, eivät välttämättä tarvitse seurantakäyntiä 2-3 päivän kuluttua diagnoosista.

Avainsanat: äkillinen välikorvatulehdus, oireet, korvakipu, kipumittarit, tärykalvolöydökset, toispuoleinen välikorvatulehdus, molemminpuolinen välikorvatulehdus, lapset, hengitystieinfektio, bakteerit, virukset, nenänielu

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ABBREVIATIONS

AAP	American Academy of Pediatrics
AOM	acute otitis media
AOM-SOS	AOM severity of symptom scale
AOM-FS	AOM faces scale
AOM-Si	AOM total severity index
CI	confidence interval
dB	decibel
ET	Eustachian tube
ETG-5	ear treatment Group – five symptoms
FLACC	the Face, Legs, Activity, Cry, Consolability Scale
FPS-R	the Faces Pain Scale-Revised
HBoV	human bocavirus
HMPV	human metapneumovirus
IL	interleukin
MEE	middle ear effusion
OM-3	otitis media symptom questionnaire, 3 items
OME	otitis media with effusion
OR	odds ratio
OS-8	otoscopy scale, 8 grades of severity
PCR	polymerase chain reaction
RR	relative risk
RSV	respiratory syncytial virus
RTI	respiratory tract infection
TLR	Toll-like receptor
TM	tympanic membrane
TNF	tumor necrosis factor

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to in the text by the Roman numerals I-IV. Previously unpublished data are also included.

I. Uitti JM, Salanterä S, Laine MK, Tähtinen PA, Ruohola A. Are parentally used pain scales and symptoms useful for detecting pain in young children with the suspicion of acute otitis media? *Submitted.*

II. Uitti JM, Laine MK, Tähtinen PA, Ruuskanen O, Ruohola A. Symptoms and otoscopic signs in bilateral and unilateral acute otitis media. *Pediatrics*. 2013;131:e398-405.

III. Uitti JM, Tähtinen PA, Laine MK, Huovinen P, Ruuskanen O, Ruohola A. Role of nasopharyngeal bacteria and respiratory viruses in acute symptoms of young children. *Pediatr Infect Dis J.* 2015;34:1056-62.

IV. Uitti JM, Tähtinen PA, Laine MK, Ruohola A. Close follow-up in children with acute otitis media initially managed without antimicrobials. *JAMA Pediatr*. 2016;170:1107-1108.

1 INTRODUCTION

Symptoms of young children commonly raise the parental suspicion of acute otitis media (AOM). Unfortunately, the spectrum of symptoms is variable in young children with AOM and none of the symptoms is shown to be diagnostic to AOM in children at otitis-prone age (Laine et al. 2010). Ear pain has been considered as the main symptom for AOM, although only half of the children with AOM have been shown to suffer from ear pain (Arola et al. 1990, Niemelä et al. 1994, Heikkinen and Ruuskanen 1995, Kontiokari et al. 1998, McCormick et al. 2016). In young children under two years of age, ear pain is even more uncommon, occurring in less than half of the children with AOM (Arola et al. 1990, Niemelä et al. 1994, Kontiokari et al. 1998, McCormick et al. 2016). Moreover, the Finnish study comparing symptoms in young children with the suspicion of AOM concluded that ear pain was equally common in children with AOM and in those without AOM (Laine et al. 2010). It must be noted that no research has been conducted to assess children's ear pain by using pain scales, which would be a more accurate, structured and scientific method to grade pain intensity.

Despite the fact that none of the symptoms is specific for AOM, acute symptoms are still required for the diagnosis of AOM. Moreover, symptoms of ear pain and fever are of paramount importance, when assessing the severity of AOM, according to American Academy of Pediatrics (AAP) guideline (Lieberthal et al. 2013). The severity, in turn, guides the management of AOM. In case of severe AOM (children have moderate/severe ear pain or ear pain lasts at least 48 hours or fever is at least 39 °C), immediate treatment with antimicrobials is recommended (Lieberthal et al. 2013).

Besides symptoms, another aspect guiding the management of AOM is the bilaterality of AOM. It is commonly held that young children with bilateral AOM have more severe illness than those with unilateral AOM, requiring more active antimicrobial treatment and/or follow-up (Appelman et al. 2006, Tan et al. 2008, Marchisio et al. 2010, Solen and Hermansson 2011, Lieberthal et al. 2013). This conclusion is drawn from the meta-analysis of Rovers et al., which suggested young children with bilateral AOM to benefit more from antimicrobial treatment than those with unilateral AOM (Rovers et al. 2006). In addition, microbiological studies have shown that bacterial pathogens are slightly more often isolated in the middle ear effusion (MEE) of children with bilateral AOM, compared to those with unilateral AOM (McCormick et al. 2007, Leibovitz et al. 2007), thus reflecting the more treatable status of bilateral AOM, compared to unilateral AOM. However, when comparing the illness severity between bilateral and uni-

lateral AOM from the symptomatic point of view, many questions still remain to be answered.

Symptoms of AOM and respiratory tract infection (RTI) are overlapping (Laine et al. 2010), probably due to the fact that practically all children with AOM have a concurrent respiratory virus infection (Ruohola et al. 2013) causing RTI. Nevertheless, in children with RTI, symptoms vary tremendously, not just between individuals but also within individuals. The cause behind the symptomatic variation is not completely understood. There is some evidence that nasopharyngeal bacteria and respiratory viruses may play a role in the manifestation of symptoms in children with RTI or AOM (Arola et al. 1990, McCormick et al. 2000, Cohen et al. 2006, Beder et al. 2009, Rodrigues et al. 2013). However, comprehensive studies investigating the individual role of nasopharyngeal bacteria and respiratory viruses behind the symptoms are missing.

Symptoms and a child's overall symptomatic condition play the key role in close follow-up of AOM, especially in children managed with initial observation without antimicrobial agents. Several guidelines require that if the child's overall symptomatic condition worsens within two to three days after the AOM diagnosis, parents should contact the physician and antimicrobials should be initiated (Marchisio et al. 2010, Lee et al. 2012, Lieberthal et al. 2013, Heikkinen et al. 2017). Hence, guideline makers make an assumption that the child's deteriorating overall condition is due to the worsening of AOM and otoscopic signs. Interestingly, no research has been done on the relationship between the child's overall symptomatic condition and development of otoscopic signs.

To summarize, acute symptoms are an essential part of the diagnostic criteria of AOM and symptoms also guide the management and close follow-up of AOM. Nevertheless, there are still gaps in the research literature related to the symptoms associated to AOM in young children. This thesis aims to fill those gaps by investigating whether AOM has any effect on the symptoms in young children so that the symptoms could reliably guide the management of AOM. We also wanted to find the factors contributing to the symptom development and see whether the child's overall symptomatic condition can be used in the follow-up of children with AOM initially managed without antimicrobials. To be precise, we investigated whether pain scales could be used by parents to detect ear pain in young children with the suspicion of AOM. We also studied whether bilateral AOM is a clinically more severe illness than unilateral AOM. In addition, we looked into the relation of nasopharyngeal bacteria and respiratory viruses with acute symptoms in children with RTI, either with or without AOM. Finally, we examined the need for follow-up in children with AOM initially observed without antimicrobials.

2 REVIEW OF LITERATURE

2.1 Definitions

Otitis media (OM) is defined as an inflammation of the middle ear without reference to etiology or pathogenesis, whereas the definition of *acute otitis media* (AOM) includes rapid onset of signs and symptoms of acute infection within the middle ear and the presence of middle ear effusion (MEE). MEE, on the other hand, is defined as fluid in the middle ear, regardless of etiology, pathogenesis, pathology or duration. MEE can be either serous, mucoid, purulent or a combination of these. *Otitis media with effusion* (OME) is defined as an inflammation of the middle ear with MEE, with no signs or symptoms of acute infection present (Bluestone and Klein 2007). However, as OME frequently complicates symptomatic viral respiratory tract infection (RTI), it can also be defined as MEE or air-fluid bubble detected by pneumatic otoscopy without signs of tympanic membrane (TM) inflammation (Chonmaitree et al. 2008). *Otorrhea* is defined as discharge from the middle ear (Bluestone and Klein 2007). *Recurrent AOM* is defined as three or more well-documented and separate AOM episodes in the preceding six months or four or more episodes in the preceding 12 months with at least one episode in the past six months (Lieberthal et al. 2013).

2.2 Epidemiology

AOM causes significant burden in young children. The risk of AOM increases at the age of 6-12 months and roughly half of the children have experienced their first AOM episode by one year of age (Teele et al. 1989, Alho et al. 1991, Chonmaitree et al. 2016, Kaur et al. 2017). Further, 60-80% of children will have suffered from AOM at least once before three years of age (Teele et al. 1989, Kaur et al. 2017). In children under three years, AOM is one of the most common reasons for physician visits and the major reason for the prescription of antimicrobials (Vergison et al. 2010). As a result, young children with the history of AOM episodes are shown to have significantly poorer quality of life than those without any such history (Kujala et al. 2017).

Children are predisposed to AOM due to viral RTI and 27-37% of children with viral RTI develop concurrent AOM (Chonmaitree et al. 2008, Chonmaitree et al. 2015). The average day of AOM diagnosis is 3-6 days after RTI (Arola et al. 1990, Heikkinen and Ruuskanen 1994, Heikkinen and Ruuskanen 1995, Koivunen et al. 1999, Kalu et al. 2011, McCormick et al. 2016, Chonmaitree et al.

2016). Recurrent viral RTIs increase the incidence of AOM in young children (Toivonen et al. 2016). The occurrence of AOM reflects the seasonal variation of viral RTIs (Ruuskanen et al. 1989), peaking in autumn and spring (Vesa et al. 2001, Stockmann et al. 2013).

2.3 Etiology

2.3.1 Bacteria

Bacteria are considered to be the main causative agents of AOM. The main AOM pathogens are *Streptococcus pneumoniae, Haemophilus influenzae* and *Moraxella catarrhalis,* which account for 73% or 86% of the bacterial etiology of AOM, depending on the detection method (culture or a combination of culture and polymerase chain reaction [PCR] technique) (Ruohola et al. 2006). *S. pneumoniae* has traditionally been the most common AOM pathogen, followed by *H. influenzae* and *M. catarrhalis*. In children with AOM, the worldwide average detection rates of AOM pathogens from MEE using culture are as follows: *S. pneumoniae* 26-30%, *H. influenzae* 22-28% and *M. catarrhalis* 3-14% (Ngo et al. 2016).

The detection rates of bacterial AOM pathogens from MEE vary between different geographical regions (Ngo et al. 2016). However, it is of note that tympanocentesis is nowadays rarely performed in children with AOM, especially in Western countries where the data on the AOM etiology are largely based on the studies from the 1980s and 1990s with a few exceptions (Ngo et al. 2016). In Finland, *S. pneumoniae* and *H. influenzae* are detected at the same rate by culture as worldwide (Heikkinen et al. 1999, Kilpi et al. 2001). In contrast, the detection rate of *M. catarrhalis* from MEE appears to be higher in Finland compared to the worldwide average detection rate. When using bacterial culture, Kilpi et al. detected *M. catarrhalis* from MEE in 23% of the AOM events (Kilpi et al. 2001). When using the PCR technique along with bacterial culture, Ruohola et al. detected M. catarrhalis in 28% of the cases with AOM with tympanostomy tube otorrhea (Ruohola et al. 2006), whereas Sillanpää et al. detected *M. catarrhalis* in 47% of the MEE samples in young children with AOM (Sillanpää et al. 2016). Of note, bacteria have also been detected by PCR from culture-negative MEE in children with OME (Post et al. 1995).

S. pneumoniae has been considered as the most virulent AOM pathogen. McCracken et al. showed that, with no antimicrobial treatment, *S. pneumoniae* was the most persistant pathogen in the MEE whereas *M. catarrhalis* was most likely to spontaneously eradicate from MEE after a few days of the AOM diag-

nosis (McCracken 1994). In 2000, the United States introduced the pneumococcal conjugate vaccine. Since then, countries all around the world have followed its course, including Finland in 2010. After the introduction of pneumococcal conjugate vaccine, the pneumococcal serotypes included in the vaccine, have been replaced by new serotypes. In addition, the predominant AOM pathogen has changed from *S. pneumoniae* to *non-typeable H. influenza*e in many countries (Ngo et al. 2016). Interestingly, a surge in *H. influenzae* detection rates was reported in MEE in children with AOM in 2016. *H. influenzae* was shown to account for 60% of the AOM cases (Kaur et al. 2017). In addition, both *H. influenzae* and *S. pneumoniae* are nowadays considered as the predominant bacteria causing recurrent and persistent OM (Pichichero 2000, Kaur et al. 2013).

Other less commonly isolated bacteria from MEE in children with AOM are *Staphylococcus aureus* and *Streptococcus pyogenes* (Bluestone and Klein 2007).

2.3.2 Viruses

Respiratory viruses have a pivotal role in the pathogenesis of AOM. They induce symptomatic RTI, which predisposes children to the development of AOM (Ruuskanen et al. 1989, Winther et al. 2006, Chonmaitree et al. 2008, Chonmaitree et al. 2015). In children with RTI, multiple respiratory viruses have concurrently been detected in 20-40% of the cases (Nascimento-Carvalho and Ruuskanen 2016). Respiratory viruses and their clinical manifestations are described in more detail in chapter 2.4.4.

Respiratory viruses not only pave the way for the nasopharyngeal bacteria to enter the middle ear, but respiratory viruses may be causative agents of AOM. Viruses have been detected from MEE in 70% of the AOM cases (Ruohola et al. 2006), but they seem to be seldom detected as the sole pathogens. Viruses have been detected in combination with bacteria in two thirds of the cases (Heikkinen et al. 1999, Ruohola et al. 2006). Viruses in MEE may explain the prolongation of symptoms and treatment failure in children with AOM treated with antimicrobials (Arola et al. 1990, Sung et al. 1993).

Rhinovirus is the most common causative agent for RTI, occurring throughout the year (Mäkelä et al. 1998, Vesa et al. 2001, Heikkinen and Järvinen 2003). Rhinovirus seems to play a dominant role already from birth, whereas other viruses are generally detected after six months of age (Sarna et al. 2017). Thus, rhinovirus is causing the greatest symptomatic burden of RTI for young children and it is the most common virus associated with AOM. In children with RTI caused by rhinovirus, AOM has been diagnosed in 30-64% of the children, re-

spectively (Vesa et al. 2001, Chonmaitree et al. 2008, Ruohola et al. 2013). On the other hand, in children with AOM, rhinovirus has been concurrently detected in the nasopharynx and in the MEE in 20-36% (Pitkäranta et al. 1998, Nokso-Koivisto et al. 2004). Of note, rhinovirus is frequently detected in asymptomatic children by PCR (Jartti et al. 2008, van der Zalm et al. 2009).

Respiratory syncytial virus (RSV) is considered as the most "ototropic" respiratory virus. It is more likely to invade the middle ear and cause AOM compared to other viruses (Heikkinen et al. 1999, Nokso-Koivisto et al. 2015). RSV epidemics have clear seasonal occurrence (Jha et al 2016). During the epidemics, 47-58% of the children infected with RSV are reported to develop AOM (Ruuskanen et al. 1989, Vesa et al. 2001, Chonmaitree et al. 2008). High viral load of RSV and the nasopharyngeal carriage of *S. pneumoniae* have been shown to increase the risk of AOM by 4-fold in children with RTI (Pettigrew et al. 2011). On the other hand, RSV has been shown to increase the risk of AOM by 6-fold even without the nasopharyngeal carriage of bacterial pathogens (Ruohola et al. 2013). In children with AOM, RSV has been concurrently detected in the nasopharynx and in the MEE in up to 59-74%, thus reflecting the ototropic nature of RSV (Heikkinen et al. 1999, Nokso-Koivisto et al. 2004).

Influenza viruses are strongly associated with the development of AOM and influenza vaccines have effectively prevented AOM during influenza epidemics (Block et al. 2011, Heikkinen et al. 2013). Influenza viruses also have a strong seasonal nature, they circulate in the northern hemisphere communities annually in winter months, between December and May, and the duration of epidemics is around 6-8 weeks (Peltola et al. 2003). During epidemics, 34-41% of the children infected with influenza virus are reported to develop AOM (Ruuskanen et al. 1989, Vesa et al. 2001, Chonmaitree et al. 2008, Antonova et al. 2012, Haas et al. 2016). Influenza viruses are considered as ototropic, because they have been detected from MEE in around 40% of the children with AOM infected with influenza virus (Heikkinen et al. 1999, Nokso-Koivisto et al. 2004). Influenza virus infection is also often complicated by secondary bacterial infection of *S. pneumoniae* (Morris et al. 2017).

Parainfluenza viruses seem to resemble influenza viruses regarding their ability to invade the middle ear. According to the study of Heikkinen et al., of the 29 children with AOM infected with parainfluenza virus, 15 (52%) had parainfluenza virus also in the MEE (Heikkinen et al. 1999). As opposed to influenza virus, parainfluenza viruses, occurring in the spring, early summer and fall, have less seasonal variation (Hodinka 2016). Among children with RTI caused by parainfluenza virus, one third has been diagnosed with AOM (Ruuskanen et al. 1989, Vesa et al. 2001, Chonmaitree et al. 2008).

Coronaviruses have also been associated with AOM. Of the children infected with coronavirus, 10-50% developed AOM (Chonmaitree et al. 2008, Ruohola et al. 2013). In children with AOM, coronaviruses have concurrently been detected from the nasopharynx and MEE in 5% of the cases (Pitkäranta et al. 1998).

Human metapneumovirus (HMPV) infection may increase the AOM risk in young children; the study of Heikkinen et al. showed AOM to develop in 61% of the HMPV-infected children (Heikkinen et al. 2008). In contrast, Williams et al. found only 6% of the children with HMPV to develop AOM (Williams et al. 2006). In children with AOM, HMPV has been detected from MEE only in 3% of the cases (Ruohola et al. 2006).

Enteroviruses seem to be related to the development of AOM, because 6-34% of the enterovirus-infected children are reported to have AOM (Chonmaitree et al. 2008, Ruohola et al. 2013). Enteroviruses have concurrently been detected from the nasopharynx and MEE in 11-42% of the AOM cases, respectively (Heikkinen et al. 1999, Nokso-Koivisto et al. 2004). In MEE, enteroviruses are detected in 10% of the children with AOM (Ruohola et al. 2006).

Human bocavirus (HBoV), which was discovered only a decade ago (Allander et al. 2005), has been linked to the development of AOM by several studies. Of the HBoV species 1-4, HBoV1 is known to affect the respiratory tract (Jartti et al. 2012). Indeed, HBoV1 infection, as indicated by seroconversion, has recently been associated with AOM in children (Meriluoto et al. 2012). Further support is provided by the study of Nokso-Koivisto et al., which showed 45% of the HBoV1-positive children with RTI to develop AOM. When HBoV1 was the only virus detected from the nasopharynx, the rate of AOM complicating RTI rose to 52% (Nokso-Koivisto et al. 2014). HBoV is also slightly more commonly detected in the nasopharynx in children with AOM than in those without AOM (28% vs. 20%) (Ruohola et al. 2013). HBoV may have a synergistic relationship with *H. influenzae*, since their presence in the nasopharynx together has been shown to increase the risk for AOM more than the individual presence of HBoV or *H. influenzae* (Pettigrew et al. 2011). Compared to other respiratory viruses, HBoV has a tendency towards a prolonged shedding after acute infection (Blessing et al. 2009, Martin et al. 2010). HBoV has also been frequently detected in asymptomatic children by PCR, and co-detections with other viruses are highly common (Weissbrich et al. 2006, Martin et al. 2010). This must be taken into account when interpreting the clinical significance of the HBoV detected from the nasopharynx by PCR.

Adenovirus infection may promote the development of AOM, because 5-47% of the children with RTI caused by adenovirus have been diagnosed with AOM (Ruuskanen et al. 1989, Vesa et al. 2001, Chonmaitree et al. 2008, Ruohola et al.

2013). The presence of adenovirus in the nasopharynx has been shown to independently increase the risk of AOM by up to 3-fold in children with RTI (Pettigrew et al. 2011). In MEE, however, adenovirus is seldom detected in children with AOM (Heikkinen et al. 1999, Ruohola et al. 2006, Bulut et al. 2007).

2.4 Pathogenesis

2.4.1 Anatomy

AOM is the disease of the middle ear. TM separates the middle ear from the external ear canal. Middle ear is connected with nasopharynx via The Eustachian tube (ET). The anatomic differences of the ET between adults and young children may partly explain the high incidence of AOM at early age. First, the ET is approximately half shorter in infants than in adults, leading to impaired protective function of ET and allowing nasopharyngeal secretions to reflux or insufflate into the middle ear (Bluestone and Klein 2007, Takasaki et al. 2007). Second, the angle of the ET is more horizontal in infants than in adults, which may impair clearance of the ET and promote nasopharyngeal secretions to enter the middle ear (Bluestone and Klein 2007, Takasaki et al. 2007). Third, the tubal lumen of the ET may not open or dilate effectively in infants and young children due to the increased compliance of the ET, which is related to greater cartilage cell density in infants and young children, compared to adults. This, in turn, may cause functional obstruction of the ET when the muscles around the ET contract. The greater cartilage cell density of the ET may likewise be related to the increased distensibility of the ET, which can promote insufflation of nasopharyngeal secretions into the middle ear (Bluestone and Klein 2007). The ET has an intimate anatomical relation with the adenoid. The adenoid, on the other hand, may act as a microbial reservoir for pathogens that eventually reach the middle ear through the ET. The function of the ET may be impaired due to the adenoid hypertrophy causing negative pressure to the middle ear cavity and eventually mucosal transudation (Buzatto et al. 2017). Moreover, the smaller size of a nasopharynx may contribute to the development of AOM (Niemelä et al. 1994, Renko et al. 2007).

The lumen of the ET is lined with the respiratory mucosa and the same mucous membrane is continued in the nasopharynx and middle ear (Bluestone and Klein 2007). AOM begins to develop when the mucosa of the middle ear gets inflamed. The inflamed mucosa of the middle ear and the medial aspect of TM cause pain, which is mediated by the glossopharyngeal nerve. In case of bulging TM, pain sensation is mediated by the auriculotemporal branch of the trigeminal nerve, and

the complex of the facial, glossopharyngeal, and vagus nerves (Shah and Blevins 2003, Majumdar et al. 2009).

2.4.2 Function of the Eustachian tube

The main function of the ET is to ventilate the middle ear by equilibrating pressures between atmosphere and middle ear. In addition, the ET protects the middle ear from nasopharyngeal sound pressure and secretions. The ET clears the secretions produced within the middle ear and thus serves as the drainage between the middle ear and nasopharynx. The function of the ET is disturbed due to RTI, which results in congestion of the respiratory mucosa in the ET and nasopharynx. Congestion of the mucosa obstructs the ET and leads to the development of negative middle-ear pressure. If the obstruction of the ET is prolonged, bacteria and viruses are aspirated from the nasopharynx into the middle ear. Due to the obstruction, middle-ear effusion accumulates in the middle ear and provides the optimal environment for potential pathogens to proliferate in the secretions, thus causing AOM (Bluestone and Klein 2007).

2.4.3 Nasopharyngeal colonization

Nasopharynx acts as a reservoir for bacteria to enter the middle ear, causing AOM. Colonization of the nasopharynx by bacterial AOM pathogens is considered as a mandatory step before pathogens can invade the middle ear, because if no pathogens are present in the nasopharynx, it is unlikely that they are found in the middle ear either (Faden et al. 1990).

Predominant bacterial AOM pathogens *S. pneumoniae, non-typeable H. influenzae* and *M. catarrhalis* may be carried asymptomatically in the nasopharynx from the early infancy (Faden et al. 1997). According to Faden et al., by six months of age, 68% of children were colonized with one or more of the three major pathogens, *M. catarrhalis* being the most common colonizer (55%), followed by *S. pneumoniae* (38%) and nontypeable *H. influenzae* (19%). By one year of age, the rates were 72%, 54% and 33%, respectively (Faden et al. 1997). However, colonization rates decrease among older children. By the age of seven, the colonization rates for *M. catarrhalis, S. pneumoniae* and *H. influenzae* were 40%, 21% and 30%, respectively (Christenson et al. 1997).

Nasopharyngeal bacterial colonization rates have geographical variation. This is probably due to different genetic background variables and socioeconomic conditions, including housing, access to health care, poor hygiene, family size, over-

crowded living conditions, day care contact and number of siblings (Garcia-Rodriguez and Fresnadillo Martinez 2002).

Colonization rates are different in healthy periods and during RTI (either with or without AOM), because the nasopharyngeal colonization of bacterial AOM pathogens is increased during viral RTI (Faden et al. 1990, Syrjänen et al. 2001, Teo et al. 2015). On the other hand, in children with RTI, the bacterial AOM pathogens are more commonly found in the nasopharynx of children with complicating AOM, compared to those without AOM (Revai et al. 2008, Ruohola et al. 2013). The risk of AOM complicating viral RTI has been shown to increase with the nasopharyngeal colonization of *H. influenzae* (Pettigrew et al. 2011). Furthermore, nasopharyngeal co-colonization by *M. catarrhalis* with either *S. pneumoniae* or *H. influenzae* has been shown to increase the risk of AOM, compared to when children were colonized by *S. pneumoniae* or *H. influenzae* alone (Ruohola et al. 2013). This suggests that *M. catarrhalis* may have a more active role in the development of AOM than previously thought.

The early nasopharyngeal colonization of bacterial AOM pathogens has been associated with early onset of AOM in children (Faden et al. 1997), whereas early onset of AOM increases the risk for recurrent AOM (Kvaerner et al. 1997).

2.4.4 Viral respiratory tract infection

AOM is normally preceded by viral RTI, but only symptomatic RTI has been shown to increase the risk of AOM, as opposed to asymptomatic RTI (Chonmaitree et al. 2015).

The etiology of RTI is diverse. Several respiratory viruses are found to be causative agents of RTI and are thus also associated with the development of AOM, as previously described in chapter 2.3.2. Regardless of etiology, the hallmark symptoms of RTI or common cold include nasal stuffiness/congestion and discharge/rhinitis, sneezing, sore throat and cough (Heikkinen and Järvinen 2003). In addition to common cold, respiratory viruses are associated with other clinical manifestations (Waris et al. 2017) as presented in Table 1.

Table 1. Respiratory viruses and their clinical manifestations in children. The strength of an association is presented with + signs. Modified from the review of Waris et al. 2017.

Virus	Common cold	AOM	Pharyngitis/ tonsillitis	Laryngitis	Bronchiolitis	Acute wheezing	Pneumonia
RNA viruses							
Rhinovirus	++++	+++	++	+	++	++++	++++
RSV	+++	++++	+	+	++++	+++	++++
Influenza viruses	++	+++	++	++	+	+	+
Parainfluenza viruses	++	++	++	++++	+	+	+
Coronaviruses	+	++	+	+	+	+	+
HMPV	++	++	+	+	+	++	++
Enteroviruses	++	++	++	+	+	+	+
DNA viruses							
HBoV	+++	+++	+++	+	++	+++	++
Adenovirus	++	++	+++	+	+	+	++

Symptomatic RTI is an essential part of the pathogenesis of AOM. Symptomatic RTI begins to develop, when a child is infected by a respiratory virus or multiple viruses. Viruses multiply locally in respiratory epithelium of the large and small airways without causing systemic infection, but resulting in inflammation to the mucosa (Hodinka 2016). Viral infection increases bacterial colonization and adherence in the nasopharyngeal mucosa and generates host immune and inflammatory responses, including the generation of cytokines, chemokines and inflammatory mediators (Patel et al. 2009, Nokso-Koivisto et al. 2015). The host immune response is suggested to alter the properties of mucus and lead to the diminished mucociliary clearance of the nasopharynx and ET (Nokso-Koivisto et al. 2015, Schilder et al. 2016), causing congestion of the respiratory mucosa and ET dysfunction, which are a crucial part of the AOM pathogenesis (See chapter 2.4.2). The risk for development of AOM complicating RTI is associated with the severity of nasopharyngeal inflammatory injury. Ede et al. have shown that high concentrations of lactate dehydrogenase in the nasopharyngeal samples, which indicate the more severe inflammatory cellular injury, are significantly associated with the risk for development of AOM (Ede et al. 2013).

2.4.5 Immunology

The immune system protects its host from invaders, such as viruses and bacteria. When a pathogen enters the pharynx through a nose or a mouth, it encounters the palatine tonsils and the adenoid, which are a part of the immunocompetent lymphoid tissue of the upper respiratory tract. The palatine tonsils and the adenoid

serve as an important first-line defense against pathogens, thus acting as gatekeepers. In addition to tonsils and the adenoid, immunocompetent lymphoid tissue is present in the mucosa of the upper respiratory tract and in the middle ear (Bluestone and Klein 2007).

The exposure of a pathogen activates a complex interplay of innate and adaptive immune mechanisms (Mittal et al. 2014). The innate immune system serves as the first line, non-specific defense mechanism against pathogens (Mittal et al. 2014) and plays a crucial role in the pathogenesis of OM. In the middle ear, the innate immune system detects pathogens by using pattern recognition receptors, such as Toll-like receptors (TLR) that recognize the molecular signature of pathogens (Mittal et al. 2014). Not only bacteria, but viruses are recognized through activation of TLRs (Beutler 2009, Carty and Bowie 2010). The activation of these receptors leads to the mobilization of other innate immune molecules, such as cytokines, chemokines, interferons, as well as proteases, defensins, collectins, lysozyme, lactoferrin, and other antimicrobial intermediates (Leichtle et al. 2011). This results in the initiation of inflammation and other mechanisms critical not only for the clearance of invading microorganisms and the restoration of tissue homeostasis but also for the activation and sensitization of the adaptive immune system (Kurabi et al. 2016). Thus, in children with AOM, the MEE contains the major classes of immunoglobulins, immunoglobulin A being the most predominant, as well as the products of the innate immune system, such as cytokines (Bluestone and Klein 2007).

Interestingly, in children with AOM, cytokine and chemokine levels have been shown to be higher in culture positive MEE samples than in children with culture negative MEE samples, indicating higher proinflammatory responses when bacterial otopathogens are present in the MEE (Kaur et al. 2015). Indeed, children who had culture-positive MEE at the time of spontaneous perforation of TM had higher levels of interleukin (IL)-1β, tumor necrosis factor (TNF), IL-8 and IL-10 than children with culture-negative MEE (Skovbjerg et al. 2010). Of the bacterial pathogens in the MEE in children with AOM, *S. pneumoniae* seems to elicit higher IL-10 response than *H. influenzae* or *M. catarrhalis* in serum (Liu et al. 2013). In MEE, however, the levels of inflammatory mediators, including IL-10, seem not to be different between *S. pneumoniae* and *H. influenzae* (Skovbjerg et al. 2010). When respiratory viruses are detected from MEE together with bacteria, inflammatory mediator concentrations have been shown to be higher than when only bacteria were present in MEE (Chonmaitree et al. 1996), thus supporting the active role of viruses in the pathogenesis of AOM. Moreover, a higher cytokine concentration of IL-1β in the nasopharyngeal sample has been associated with an increased rate of AOM development in young children with RTI (Patel et al. 2009).

As the innate immunity requires a cascade of events to function properly, it is also vulnerable for distractions, which may result in higher morbidity of OM. Polymorphisms in the genes encoding TLR2, TLR4 and TLR9 have been shown to result in more profound, persistent inflammation with impaired bacterial clearance in the middle ear, when OM was induced by *H. influenzae* in the animal model (Leichtle et al. 2009, Leichtle et al. 2012). TLR2 and TLR4 polymorphism have also been shown to be associated with recurrent AOM in children (Emonts et al. 2007, Toivonen et al. 2017). Moreover, single-nucleotide polymorphism of the genes that encode acute phase cytokines, such as TNF-α $^{-308}$ and IL-6^{-174}, has been associated with the risk for OM susceptibility and increased risk for AOM complicating RTI (Patel et al. 2006, Revai et al. 2009). Those children who develop AOM with bulging TMs as a complication and have IL-1β^{+3953} polymorphism are shown to have more severe symptoms (McCormick et al. 2011).

The innate immunity is suggested to have a central role regarding the development of symptoms during respiratory viral infections. This conclusion is drawn from the observations that respiratory viruses mostly cause similar clinical syndromes and sequelae, despite their distinct virion and genome structures, unique entry receptors, and modes of replication. Thus, the host response to infection seems to primarily account for the clinical and pathologic changes observed during respiratory viral infections rather than direct viral injury of respiratory cells (Newton et al. 2016). Patel et al. showed that in young children during RTI, all of the investigated viruses in the nasopharyngeal sample, namely rhinovirus, adenovirus, enterovirus, parainfluenza virus, RSV and influenza virus, induced significant quantities of nasopharyngeal cytokines, such as IL-1β, IL-6 and TNF-α, which correlated with each other, thus suggesting a common pathway in acute inflammation during viral RTI (Patel et al. 2009). The nasopharyngeal IL-6 concentration was inversely correlated with the duration of RTI symptoms, i.e. nasal congestion, rhinitis, cough and/or sore throat with or without fever, prior to nasopharyngeal sample collection (Patel et al. 2009), thus possibly explaining the more severe symptoms in the early course of RTI. Kaiser et al. showed that higher IL-6 plasma concentrations were associated with more severe symptom scores (nasal congestion, sore throat, cough, aches and pain, fatigue, headache, chills and sweats) and with higher fever in adults with naturally occurring influenza (Kaiser et al. 2001). Indeed, IL-6 is considered as the pyrogenic cytokine, because it is an important mediator of fever induction and supposed to have a requisite role in sustaining fever (Evans et al. 2015). In line with this, Patel et al. reported that the concentrations of IL-6 were positively correlated with the duration of fever and higher cytokine concentrations of IL-6 were detected in febrile children with RTI, as compared with children without fever (Patel et al. 2009).

Respiratory viruses may induce different amounts of cytokines. The study of Patel et al. showed that in RTI caused by influenza or adenovirus, the mean concentrations of IL-6 in nasopharyngeal samples were significantly higher compared with RTI caused by rhinovirus or enterovirus (Patel et al. 2009). In children with AOM due to RSV, cytokine concentrations of IL-6, monocyte chemoattractant protein-1, IL-10, interferon-γ and IL-8 from the blood were significantly higher than in those with AOM due to other viruses (non-RSV) (Patel et al. 2009). It would be logical to assume that respiratory viruses have different symptom profiles due to the different production of cytokines. To complicate the matter, though, Laham et al. reported that infants with hMPV and infants with RSV, who were experiencing their first RTI, had similar clinical manifestations, although RSV elicited significantly more profound production of cytokines (IL-6 and IL-8) in the nasopharynx as compared with hMPV (Laham et al. 2004). Thus, the explanation for the variation of symptoms in children with RTI is still far from being clear.

2.5 Risk factors

Respiratory virus infection is a prerequisite step before the development of AOM. After the exposure of the respiratory virus infection, risk factors begin to play their part in the pathogenesis of AOM, as presented in Figure 1. The most important risk factor for AOM is day care outside the home (Uhari et al. 1996). Other important risk factors are parental smoking (Uhari et al. 1996), short or lack of breast-feeding (Uhari et al. 1996, Kaur et al. 2017) and having siblings (Ladomenou et al. 2010). Not mentioned in the Figure 1, the use of a pacifier has been shown to increase the risk for AOM, especially for recurrent AOM (Niemelä et al. 1994, Niemelä et al. 2000, Rovers et al. 2008). Moreover, diet may modify the AOM risk, because frequent consumption of fruits and berries has been shown to be associated with the decreased AOM risk (Tapiainen et al. 2014). Just recently, maternal use of antimicrobials during pregnancy was suggested to be associated with an increased risk of OM and tympanostomy tube insertions in the offspring during the first three years of life (Pedersen et al. 2017).

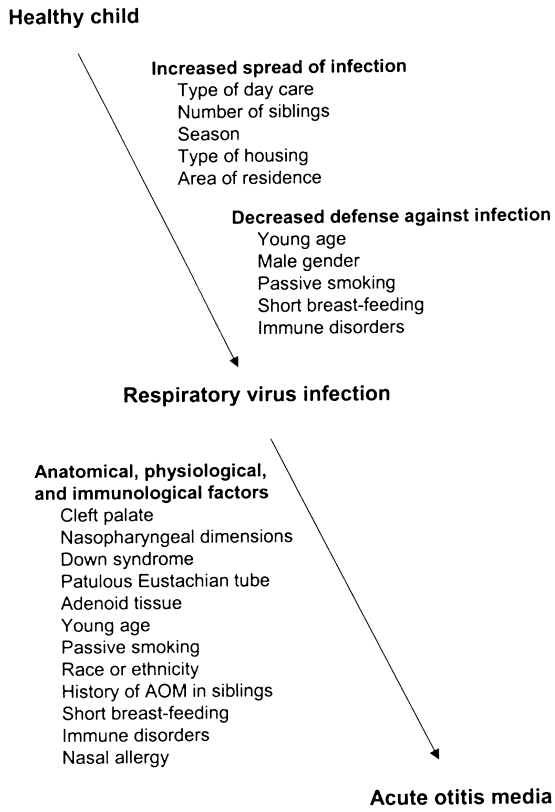


Figure 1. Proposed sites of influence of some risk factors for AOM (Heikkinen and Chonmaitree 2003).

2.6 Symptoms

Acute symptoms in young children often raise the parental suspicion of AOM and makes parents to seek medical care for their child. If some specific symptoms could be appointed for AOM, it would help parents to decide whether their child needs to see the physician. Acute symptoms are also an essential part of the diagnostic criteria for AOM. The severity of AOM, guiding the management of AOM, is defined by the presence and severity of certain symptoms, namely ear pain and fever (Lieberthal et al. 2013). For the obvious need, symptoms have been the target for research for the past decades.

Over 50 years ago, John Dixon Coffey Jr. (Coffey 1966) described the symptoms of OM in children as follows:

> *"The patients in this study presented many complaints, occasionally gastrointestinal, but usually respiratory. These included cough, "cold", fever, hoarsness, restlessness, and crying out after cough-*

> *ing or while attempting to nurse the bottle. Ear pain was not mentioned in 75% of the patients. The average age of those who did complain of ear pain was 4 3/12 years."*

The study of Coffey is the first to describe the symptoms related to OM. Worthy of note, he includes not only the children with AOM, but also those with OME. The symptom profiles of AOM and OME are, however, different because children with AOM have acute symptoms or signs of infection, unlike children with OME (Bluestone and Klein 2007, Lieberthal et al. 2013). To complicate the matter, OME may occur during symptomatic RTI (Chonmaitree et al. 2008); OME may precede and predispose children to AOM and OME occurs as the aftermath of an episode of AOM (Alho et al. 1995, Koopman et al. 2008, Armengol et al. 2011).

To specifically study the symptoms of AOM, children with OME should be carefully excluded in order to avoid the dilution of the results. Furthermore, as AOM is preceded by RTI, it is essential to have a comparison group of children with RTI when investigating symptoms of AOM. It is likewise evident that the child's age affects the symptom profile, and it is the children under three years of age that encounter AOM the most frequently. Therefore, this thesis pursues to take a closer view on symptoms in young children.

Table 2 shows the results of the six studies of prospective design investigating the occurrence of symptoms in children with AOM in an outpatient setting. Apart from the study of Arola et al., the other five studies compared symptoms between AOM and RTI, or between AOM and another acute infectious disease, as in the study of Niemelä et al. The study of Kontiokari et al. was unique in a way that each child served as his/her own control. On the other hand, the study of Laine et al. had different study inclusion criteria, allowing only children with parental suspicion of AOM to participate, resulting in markedly higher rates of parentally reported ear pain in both children with and without AOM (Laine et al. 2010), as compared with other studies. Hence, the incidence rates of ear pain in the study of Laine et al. are not comparable with other studies. It is also worthy of note that during the past 20 years, the diagnostic criteria of AOM have been modified. Therefore, the older studies are not entirely comparable to the studies performed in the 2010s, when bulging of TM has become a major determinant in the diagnosis of AOM. Furthermore, the older studies included children with a wider age range, whereas the studies in the 2010s have focused on investigating only young children. These factors have to be taken into account when interpreting the results. Otherwise, all six studies are very comprehensive regarding the symptoms of AOM.

Table 2. Occurrence of symptoms in children with AOM and in children with RTI without AOM.

	Arola et. al. 1990	**Niemelä et al. 1994**		**Heikkinen and Ruuskanen 1995**		**Kontiokari et al. 1998**		**Laine et al. 2010**		**McCormick et al. 2016**	
Inclusion criteria	Diagnosis of AOM	Any kinds of acute symptoms		Symptoms of RTI		Symptoms of RTI		Parental suspicion of AOM		Symptoms of RTI	
Study design	Case series, descriptive: symptoms in children with AOM.	Comparison of symptoms in children with AOM vs. in children with other acute infectious disease		Comparison of symptoms in children with AOM vs. in children with RTI		Comparison of symptoms during an episode with AOM vs. without AOM. Each child served as his/her own control.		Comparison of symptoms in children with AOM vs. in children with RTI		Comparison of symptoms in children with AOM vs. in children with RTI	
Diagnostic criteria for AOM	1. Acute symptoms and signs 2. MEE detected by pneumatic otoscopy	1. Acute symptoms 2. MEE detected by pneumatic otoscopy		1. Acute symptoms 2. Bulging or opacification of TM 3. Mobility of TM either absent or markedly decreased		1. Acute symptoms 2. Otorrhea or air-fluid level behind TM or a cloudy or red TM and/or impaired mobility of TM		1. Acute symptoms 2. MEE detected by pneumatic otoscopy (At least 2 of the following signs: bulging position; decreased/absent mobility; abnormal color or opacity; or air-fluid interfaces) 3. At least 1 acute inflammatory sign of TM		1. Acute symptoms 2. MEE detected by pneumatic otoscopy 3. Abnormal, inflamed TM (mild, moderate, or severe bulging, loss of landmarks, and opacification)	
Study population	**AOM**	**AOM**	**Others**	**AOM**	**RTI**	**AOM**	**RTI**	**AOM**	**RTI**	**AOM**	**RTI**
No. of children	363	191	163	121	181	138	138	237	232	63	297
Age range, years	0-11.6	0-15.0		0.6-4.2		0.6-6.9		0.5-3.0		0-1	
Mean age of children, years	2.5	3.0	4.8	2.1		3.7		1.3	1.3	-	-
Symptom, %											
Parentally reported ear pain	47	54	18	60	8	59[1]	15[1]	92	92	40	18
Ear rubbing	-	42	13	-	-	-	-	70	78	-	-
Fever (≥38 °C)	55	40	52	69[2]	77[2]	42	28	43	35	10	6
Irritability	55	-	-	-	-	39	30	87	93	59	55
Excessive crying	-	55	31	-	-	-	-	87	88	-	-
Restless sleep	-	-	-	64	49	33	14	87	86	51	46
Decreased activity	-	28	34	-	-	-	-	47	45	-	-
Poor appetite	50	36	34	-	-	-	-	63	64	35	30
Rhinitis (cloudy, obstructive or purulent)	90	99	79	96	92	91	76	94	95	79	78
Nasal congestion	-	-	-	-	-	-	-	75	74	83	93
Cough	78	47	55	83	83	-	-	79	74	83	84
Hoarse voice	-	-	-	-	-	-	-	34	35	-	-
Wheezing	-	6	4	-	-	-	-	-	-	-	-
Sore throat	-	13	26	-	-	14	6	-	-	8	10
Conjunctivitis/ red, watery eyes	-	6	10	-	-	15	7	19	14	46	42
Mucus vomiting	-	-	-	-	-	-	-	11	10	-	-
Vomiting	-	11	11	-	-	-	-	1	2	-	-
Diarrhea	10	8	7	-	-	-	-	13	10	-	-

[1]Older children with verbal skills reported ear pain themselves and parents reported ear pain in young, preverbal children

[2]Fever defined as >37.5 °C

In children with the parental suspicion of AOM, the suspicion was correct in 51-76% of the cases based on overall symptoms (Arola et al. 1990, Niemelä et al. 1994, Kontiokari et al. 1998, Laine et al. 2010).

2.6.1 Ear-related symptoms

Ear rubbing

Ear rubbing has been traditionally held as the sign of AOM by parents as they easily pay attention to this phenomenon (Rothman et al. 2003). Niemelä et al. found ear rubbing to increase the likelihood of AOM (relative risk [RR]: 5.0; confidence interval [CI] 2.9 to 8.6), regardless of the child's age (Niemelä et al. 1994). However, other studies are not supporting this. Baker found ear rubbing not to associate with ear infections (Baker 1992) and Laine et al. showed ear rubbing to be more common in young children with RTI than in those with AOM (Table 2).

Hearing loss

Hearing loss and its association with AOM has not been investigated in young children, probably due to the fact that hearing loss is not at all easy for parents to evaluate, especially in preverbal children. Therefore hearing loss is not mentioned in Table 2. However, hearing loss is considered as one of the main concerns in children with prolonged MEE and it is likely that hearing loss also occurs in children with acute MEE, i.e. in children with AOM. In fact, Koivunen et al. have shown that transmission of acoustic energy to and from the middle ear is altered in children experiencing any form of otitis media with effusion and that the amount of MEE has significant effect on the acoustic transmission of the middle ear (Koivunen et al. 2000). Sabo et al. compared the mean hearing thresholds between children younger than 3 years who had unilateral or bilateral MEE continuously at least for eight weeks and children with no MEE with visual reinforcement audiometry. The mean hearing threshold levels were 23-34 desibels (dB) in bilateral MEE, 18-23 dB in unilateral MEE and 15-19 dB when no MEE was detected (Sabo et al. 2003). Thus, it may be presumed that the hearing thresholds are similarly altered also in children with bilateral and unilateral AOM.

Ear pain

Ear pain is considered as the main symptom of AOM and parents find it as one of the greatest burden in young children with AOM (Barber et al. 2014). Niemelä et al. showed ear pain, as reported by parents, to increase the risk for AOM by 5-

fold (RR: 5.4; [CI] 3.3 to 8.9) (Niemelä et al. 1994), whereas Kontiokari et al. found ear pain to increase the likelihood of AOM by 20-fold (RR: 21.3; CI 1.1 to 11) (Kontiokari et al. 1998). The latter may be explained by the fact that children were asked to describe their pain and only if they were verbally unable, parents assessed their pain. Moreover, children served as their own controls and also the mean age of children was higher in the study of Kontiokari than in the other studies. In the study of Heikkinen et al., the positive predictive value and specificity of ear pain for AOM were 83% and 92%, respectively, when pain was reported by parents (Heikkinen and Ruuskanen 1995). In addition, McCormick et al. suggested the severity of ear pain to predict AOM, when pain was assessed by parents, but no RRs were reported. Only the study of Laine did not find parentally reported ear pain to predict AOM, but it may be partly due to their study design (Laine et al. 2010).

The predictive value of ear pain is, however, hampered with the fact that not everybody with AOM complains ear pain. Heikkinen et al. found parentally reported ear pain to have only 60% sensitivity for AOM (Heikkinen and Ruuskanen 1995). Hayden et al. studied ear pain in children with AOM with completely bulging TMs and reported ear pain in 83% of the AOM cases, but their study included also older children (Hayden and Schwartz 1985). Ear pain was assessed by the child and/or parent (Hayden and Schwartz 1985). Pukander et al. investigated children up to 16 years of age diagnosed with AOM. Of the AOM attacks, 75% had moderate or severe bulging of TM. Pukander reported ear pain to be related with 73.5% of the attacks of AOM, but their study did not specify who assessed the occurrence of ear pain (Pukander 1983). Worthy of note, studies not requiring bulging of TM as the diagnostic criteria of AOM, reported lower ear pain rates of 47-60% in children with AOM (Arola et al. 1990, Niemelä et al. 1994, Heikkinen and Ruuskanen 1995, Kontiokari et al. 1998). Therefore, another aspect possibly affecting the reported rates of ear pain is the severity of otoscopic signs. Redness of the completely bulging TM in children with AOM has been shown to increase the likelihood of ear pain (Hayden and Schwartz 1985).

Child's age affects the reported rates of ear pain. Of children younger than two years, Hayden et al. reported ear pain in 75% of AOM cases (Hayden and Schwartz 1985), when pain was assessed by the child and/or parent, as compared with 27-50% in other studies (Arola et al. 1990, Niemelä et al. 1994, Kontiokari et al. 1998, McCormick et al. 2016). In children older than two years of age, nearly all the AOM cases (93%) were reported to have ear pain by Hayden et al. (Hayden and Schwartz 1985), compared to 60-72% in other studies (Arola et al. 1990, Niemelä et al. 1994, Heikkinen and Ruuskanen 1995, Kontiokari et al. 1998). Older children are naturally more capable of expressing their pain, which presumably explains their higher incidence rates.

Worthy of note, not only children with AOM complain about ear pain; also children with RTI or other illnesses are reported to suffer from ear pain (Table 2). A possible explanation is that pain sensation in the middle ear is mediated by branches of trigeminal, glossopharyngeal and vagus nerves, which also supply sensations from the face, mouth, pharynx and larynx (Shah and Blevins 2003, Majumdar et al. 2009). Ingvarsson et al. investigated children up to 15 years of age who either complained ear pain by themselves or their parents reported ear pain due to changes in the child's symptoms. All children were suspected to have AOM, but only 46% had AOM with bulging TM. In the rest of the children, the complaint of ear pain was referred pain due to discomfort when swallowing (tonsillitis), nasal obstruction, or throat pain (pharyngitis). Other reasons were general irritability due to fever, teething or moderate hearing loss due to secretory OM (Ingvarsson 1982). Thus, not all ear pain is due to infectious process in the middle ear.

As can clearly be seen, ear pain is a very complex concept and its assessment is challenging. Due to its subjective nature, young and preverbal children are at risk of suffering from unrecognized ear pain, despite all the efforts of parents. Since the presence of ear pain in young and preverbal children is always based on parental assumptions, it is subject to possible major bias. Shaikh et al. showed that sosioeconomic status of parents, namely higher level of parental education and private insurance, were associated with higher reported pain levels in children (Shaikh et al. 2010). Moreover, parents who thought their children do not experience pain as intensively as adults, identified less pain behaviors in children 1-6 years of age (Kankkunen et al. 2003). Hence, parents' perception of their children's pain may contribute to the reported ear pain rates in young children.

Severity of ear pain

Only three studies have investigated the severity of ear pain in children with AOM. All the studies had stringent diagnostic criteria of AOM. First, McCormick et al. investigated children with AOM up to one year of age and showed that among the children with ear pain, parents reported it severe in 4% (1/25), moderate in 44% (11/25) and mild in 52% (13/25) of the children. The severity of ear pain was assessed by parents via questionnaire. Second, Laine et al. investigated children with AOM at otitis-prone age and showed that among those with ear pain, parents reported it severe in 24% (53/219), moderate in 42% (93/219) and mild in 33% (73/219) (Laine et al. 2010). The severity of ear pain was assessed by parents via interview. Third, Hayden et al. included older children in their study and used the strict ear pain classification for children with AOM. Ear pain was assessed by the child and/or parent and classified as severe if the pain had caused at least some crying; moderate if the child was extremely fussy but

not crying; and mild if the child was slightly fussy or querulous. The results for severe, moderate and mild ear pain among children with ear pain were as follows: 51% (142/277), 26% (72/277) and 23% (63/277), respectively (Hayden and Schwartz 1985). According to Hayden et al., in children with AOM who suffered from ear pain, the pain was considered severe in half of the children. In contrast, Laine et al. reported severe ear pain only in one quarter of the children and in the study of McCormick et al., severe ear pain in children with AOM was a rarity. The wide age range of children between the studies will definitely contribute to the results. However, it remains to be speculated, whether the difference between the results is also due to different pain assessment methods used in the studies.

Howie et al. used another ear pain classification in children with AOM. Ear pain was classified as severe if the child had awakened crying two or more times during the preceding night in apparent pain, or cried one-half hours or more during the day. Pain was classified as moderate if the child had awakened crying only once or cried less than one-half hour during daytime; and mild if the child mentioned an earache or was fussy without crying (Howie et al. 1970). To the best of our knowledge, however, the classifications of ear pain either by Howie et al. or by Hayden et al. have not been implemented for further studies. Thus, there is a need to classify the severity of ear pain reliably and in a standardized way.

2.6.1.1 Pain scales

"Assessing pain does not cause pain!"

Renee C.B. Manworren and Jennifer Stinson, 2016

To systematically assess acute pain of young children, use of several validated pain scales is recommended, because otherwise pain tends to be underestimated (Manworren and Stinson 2016). If children cannot provide self-reports of pain due to their young age or disabilities, observational and behavioral tools can be used. However, these pain assessment tools are only indirect measures of pain; they do not indicate the intensity of pain but rather the intensity of pain-related distress and pain reactivity. Behaviors associated with acute pain in young children are shown in Table 3. It is suggested that children may use behaviors, such as increased activity or sleeping, as methods of distraction to cope with pain. Thus, these behaviors should be interpreted as indicators of pain (Kankkunen et al. 2003).

Table 3. Behaviors associated with acute pain in preverbal and nonverbal pediatric patients. (Manworren and Stinson 2016)

Vocalizations (e.g. crying)
Facial expressions (e.g. quivering chin and nasolabial furrowing)
Large body movements (e.g. withdrawal of the affected limb, touching the affected area, and movement or tensing of limbs and torso
Changes in social behavior or appetite
Changes in sleep/wake state or cognitive functions
Behavioral responses to interventions

Children's behavior varies between individuals, and children are often more expressive in the presence of parents than strangers (von Baeyer and Spagrud 2003). Therefore, parents are held as the most reliable proxy for assessing their child's pain, because they are more able to discriminate their child's behavior from other aberrant behavior (Schechter et al. 2002, von Baeyer and Spagrud 2007). The age of the child may systematically influence the parental assessment of pain intensity. The study of Pillai Riddell et al. showed that when parents viewed videotapes of the vigorous behavioral responses of healthy infants (2, 4, 6, 12 and 18 months of age) to a routine immunization injection, parents judged older infants to have more pain compared to younger ones, although the behavioral reaction to pain stimuli was similar and standardized across all the age groups (Pillai Riddell and Craig 2007). Hence, young children or infants seem to be at the greatest risk of suffering from unrecognized pain.

Although pain scales in children have been the target of interest for the past decades, no research has been conducted towards reliable and standardized ear pain assessment in children with AOM. Only Shaikh et al. have used two validated pain scales when they investigated pain management in children under three years of age with AOM undergoing diagnostic tympanocentesis (Shaikh et al. 2011). Global mood scale was used by a research assistant. It is a 7-point validated scale measuring the intensity of pain-related behaviors. In addition, Visual Analog Scale was used by physician, parent and the study nurse to indicate their perceptions of the child's pain and distress following tympanocentesis. However, research regarding pain scales and non-procedural ear pain is lacking. Especially for children under three years of age, who carry the greatest burden of AOM and consequently suffer from ear pain, no validated pain scales for parental observation are available.

2.6.2 Fever

Fever is held as the sign of severe AOM (Lieberthal et al. 2013) and fever is a common cause for parents to suspect AOM in their child. Kontiokari et al.

showed fever to slightly increase the probability of AOM (RR: 1.8; CI 1.1 to 3.2), whereas Niemelä et al. showed quite the contrary (RR: 0.6; CI 0.4 to 1.0) (Niemelä et al. 1994). It is worthy of note that fever occurs only in 40-70% of the children with AOM (Table 2). Schwartz et al. reported fever only in 23% of the children with AOM, but they included children up to 17 years of age and thus the result cannot be generalized to young children with AOM (Schwartz et al. 1981). In young children, the occurrence of fever is similar in children with AOM and in children with RTI (Table 2). However, fever may occur at different times during the course of RTI symptoms between the groups. For instance, children with RTI were shown to be more often febrile at the beginning of RTI symptoms (days 1-2), whereas children with AOM were more often febrile between days 3 and 9 from the beginning of RTI symptoms, peaking at days 3 to 6 (Kontiokari et al. 1998). Interestingly, the peak day of AOM diagnosis is likewise day 3 after RTI onset, with the median/mean day of 3-6 (Arola et al. 1990, Heikkinen and Ruuskanen 1994, Koivunen et al. 1999, Kalu et al. 2011, Chonmaitree et al. 2016), which reflects possible causality between fever and AOM. Nevertheless, on day 3 after onset of RTI, OME is also being most commonly diagnosed (Chonmaitree et al. 2008). As a conclusion, fever is not a reliable symptom when diagnosing AOM in children with viral RTI, because it rather reflects the underlying viral infection (Putto et al. 1986, Arola et al. 1990).

2.6.3 Non-specific symptoms

Non-specific symptoms, such as *irritability, excessive crying, restless sleep, decreased activity* and *poor appetite*, may generally be interpreted as signs of pain or discomfort due to AOM. Parental suspicion of AOM is most commonly raised due to non-specific symptoms of the child, especially restless sleep or irritability (Laine et al. 2010). In fact, Kontiokari et al. showed restless sleep and irritability to increase the likelihood of AOM (RR for restless sleep: 2.6; CI 1.1 to 6.9; RR for irritability: 1.7; CI 1.0 to 3.2), whereas Niemelä et al. showed excessive crying to increase the likelihood of AOM (RR 2.8; CI 1.8 to 4.3). However, when Kontiokari et al. and Niemelä et al. analyzed symptoms and their likelihood for AOM separately in the age groups, the associations were no longer detected in children younger than two years (Niemelä et al. 1994, Kontiokari et al. 1998). On the contrary, McCormick et al. showed that in infants up to 12 months, the combination of non-specific symptoms, namely poor appetite, restless sleep and irritability, boosted with fever and ear pain, were associated with the prediction of AOM (McCormick et al. 2016). It must be noted though that also children with RTI have non-specific symptoms, occurring at similar rate as in children with AOM (Table 2), thus hampering their predictive value. Rothman et al. showed that non-specific symptoms, when not combined, are not reliable predictors for

AOM (Rothman et al. 2003). In the study of Laine et al., irritability was shown to decrease the probability for AOM (RR: 0.7 [95% CI: 0.6-0.9]) and non-specific symptoms were found to be equally severe in children with and without AOM (Laine et al. 2010). Thus, non-specific symptoms are not useful at differentiating children with AOM from those without AOM.

2.6.4 Respiratory symptoms

Practically all the children with AOM have at least some of the respiratory symptoms, namely *rhinitis, nasal congestion, cough, hoarse voice, conjunctivitis* or *mucus vomiting*. Rhinitis and cough are the most common symptoms in children with AOM, but also in children with RTI (Table 2). The association between conjunctivitis and AOM is well known (Bodor 1982, Bodor et al. 1985), but it occurs only in 6-19% of the children with AOM. Mucus vomiting may be easily misinterpreted as actual vomiting and it may explain the higher rates of vomiting in previous studies in children with AOM (Niemelä et al. 1994, Spiro et al. 2006), compared to the study of Laine et al. (Laine et al. 2010).

2.6.5 Gastrointestinal symptoms

Vomiting is relatively rare in children with AOM, as previously mentioned, and *diarrhea* is encountered only in 1 out of 10 children with AOM (Niemelä et al. 1994, Spiro et al. 2006, Laine et al. 2010).

2.6.6 Duration of symptoms

The mean duration of symptoms preceding the diagnosis of AOM has been shown to be approximately 3-6 days (Arola et al. 1990, Heikkinen and Ruuskanen 1994, Heikkinen and Ruuskanen 1995, Koivunen et al. 1999, Kalu et al. 2011, McCormick et al. 2016, Chonmaitree et al. 2016). On the other hand, in case of distinct ear pain, four out of five children are brought to the physician in less than 24 hours after the pain onset (Ingvarsson 1982, Pukander 1983). When comparing the duration of individual symptoms before the child was brought to the physician due to the parental suspicion of AOM, rhinitis was shown to last approximately one day longer in children with AOM than in children without AOM (Laine et al. 2010). In general, the duration of symptoms did not have any predictive value for AOM (Laine et al. 2010).

2.6.7 Severity of symptoms and viral load

The knowledge on the severity of individual symptoms in children with AOM remains rather limited. The severity of ear pain was already reviewed separately in chapter 2.6.1. As for the remaining symptoms, two studies have reported the severity of symptoms in children with and without AOM. First, Laine et al. investigated children aged 6-35 months with parental suspicion of AOM and showed that the severity of fever, ear-related, non-specific, respiratory and gastrointestinal symptoms, was similar in children with and without AOM. The only exception was conjunctivitis that was more severe in children with AOM than those without AOM (Laine et al. 2010). Second, McCormick et al. investigated infants up to one year of age. The infants were brought to the study clinic when their parents noted the onset of RTI symptoms. McCormick et al. suggested that when combining the severity of cough and ear pain with the child's age and current day care attendance, the risk for AOM would be increased (McCormick et al. 2016).

Higher viral load has been suggested to be associated with more severe symptoms. Chonmaitree et al. showed viral loads in the nasopharyngeal specimens to be significantly higher in children with symptomatic RTI compared to asymptomatic children, but viral loads did not differentiate children with symptomatic RTI from those with accompanying AOM (Chonmaitree et al. 2015). Jansen et al. investigated children admitted to the hospital with RTI, and showed that decreased viral loads were associated with clinical improvement (Jansen et al. 2010). Regarding individual respiratory viruses, evidence is conflicting whether viral load has any effect on the illness severity. In addition, the mechanism behind the phenomenon is not fully understood. It could be speculated whether higher viral load provokes a more robust cytokine storm and this would lead to the development of more severe symptoms. The study of Piedra et al. showed that higher RSV gene copy numbers were correlated with a stronger innate immune response early in the course of bronchiolitis, but as a surprise, the disease was less severe in those children. The researchers thus suggested that the increased amount of cytokines early in infection could be protective (Piedra et al. 2017). However, more studies are needed to further investigate whether a viral load is associated with the severity of symptoms.

Multiple respiratory virus detection and its relation to symptom or illness severity has been the target of research in recent years. However, contradictory results have been published. Hence, the real clinical role of multiple respiratory virus detection still remains to be clarified (Nascimento-Carvalho and Ruuskanen 2016).

2.6.8 Symptom severity scores

Symptom severity scores were initially developed to provide a valid and reliable method for measuring symptom severity of AOM in clinical practice, as well as in treatment trials. For example, scores could help physicians to determine, whether children with AOM could be managed either with immediate antimicrobials or with initial observation without antimicrobials, based on their symptom severity. In case of initial observation, scores could serve as follow-up tools for parents and physicians (Friedman et al. 2006, Shaikh et al. 2009).

In 1991, Kaleida et al. described AOM in children as non-severe or severe, depending on the measured temperature and otalgia. They used the otalgia scoring system that took into account the estimated parental anxiety and reliability and assigned 1, 3, or 12 points, respectively, for each hour of ear pain or apparent discomfort (ear rubbing or irritability in infants) rated as mild, moderate, or severe. AOM was classified as severe if the child had attained an otalgia point score of ≥12 or if his/her temperature had reached 39 °C orally or 39.5 °C rectally within the 24-hour period before presentation (Kaleida et al. 1991).

The AAP AOM guideline defines children to have *severe AOM* if they have moderate or severe ear pain or ear pain lasts at least 48 hours or fever is at least 39 °C. Moreover, the AAP guideline endorses the assessment of pain to be included in the management of AOM (Lieberthal et al. 2013). Unfortunately, the AAP guideline does not provide any ear pain scoring system, or instruct how to actually assess the severity of ear pain in children.

Symptom scores. Table 4, Table 5 and Table 6 summarize the most commonly or recently used symptom severity and otoscopic scores, as well as their combinations. Table 7 shows which symptoms and otoscopic scores are included into the scores, allowing easier comparison between the symptom severity scales.

Table 4. Symptom scores.

Symptom scores	Author and year	Symptoms	Grading
OM-3	McCormick et al. 2003	1. Physical suffering (ear pain, ear discomfort, high fever, or poor balance) 2. Emotional distress (irritability, frustration, sadness, restlessness, or poor appetite) 3. Limitations in activity (playing, sleeping, doing things with friends/family, attending school or day care)	7-point scale: 1 = not present, not a problem 7 = an extreme problem Max. 21 points
ETG-5	Kalu et al. 2011	1. Fever 2. Ear pain 3. Irritability 4. Poor feeding 5. Restless sleep	0 = none 1 = mild (<38 °C) 2 = moderate (38-39 °C) 3 = severe (>39 °C) Max. 15 points
AOM-SOS	Shaikh et al. 2009	1. Ear rubbing 2. Excessive crying 3. Irritability 4. Restless sleep 5. Decreased activity 6. Poor appetite 7. Fever	0 = none 1 = a little 2 = a lot Max. 14 points
AAP Severity of AOM	Lieberthal et al. 2013	1. Fever 2. Ear pain	*Non-severe AOM* AOM with mild ear pain and a temperature <39 °C *Severe AOM* AOM with moderate or severe ear pain or fever ≥39 °C
AOM-FS	Friedman et al. 2006	See Figure 2 (pp. 39)	Max. 7 points

Table 5. Otoscopic scores.

Otoscopic scores	Author and year	Otoscopic signs of tympanic membrane (TM), grading
OS-8	Friedman et al. 2006	0 = normal ear, no AOM 1 = erythema only, no effusion (myringitis) 2 = erythema, air-fluid level, clear fluid 3 = erythema, complete effusion, no opacification 4 = erythema, opacification with air-fluid level or air bubble(s), mild or no bulging TM 5 = erythema, opacification, complete effusion, mild or no bulging TM 6 = erythema, bulging, rounded donut appearance of TM 7 = erythema, bulging, complete effusion, and opacification with bulla formation

Table 6. Scores combining symptoms and otoscopic signs.

Scores combining symptoms and otoscopic signs	Author and year	Symptoms and otoscopic signs	Grading
AOM-Si	Friedman et al. 2006	AOM-FS + OS-8 score	Max. 14 points
COS	Polachek et al. 2004	1. Temperature 2. Irritability 3. Redness of TM 4. Bulging of TM	0 = absent (<38 °C) 1 = mild (38-38-5 °C) 2 = moderate (38.6-39 °C) 3 = severe (>39 °C) Max. 12 points
10-point AOM scoring instrument	Casey et al. 2011	1. Fevers at home 2. Level of ear pain 3. Irritability 4. Temperature now 5. Erythema of TM 6. Mobility of TM 7. Effusion color of TM 8. Position of TM 9. Otorrhea	0 = none/mild, 1 = moderate/severe 0 = none/mild, 1 = moderate/severe 0 = none/mild, 1 = moderate/severe 0 = none/mild, 1 = moderate, 2 = severe 0 = none/mild, 1 = moderate/severe 0 = normal, 1 = decreased/absent 0 = normal/orange, 1 = yellow/green/white 0 = neutral/retracted, 1 = full/bulging 0 = no, 1 = yes

Table 7. Symptoms and otoscopic signs included into the symptom severity scores.

Symptoms	OM-3	ETG-5	AOM-SOS	AAP Severity of AOM	AOM-FS	OS-8	AOM-Si	COS	10-point AOM scoring instrument
Ear pain	x	x		x	x		x		x
Ear rubbing	x		x		x		x		
Fever	x	x	x	x	x		x	x	x
Irritability	x	x	x		x		x	x	x
Excessive crying	x		x		x		x		
Restless sleep	x	x	x		x		x		
Decreased activity	x		x		x		x		
Poor appetite	x	x	x		x		x		
Otoscopic signs									
Position of TM						x	x	x	x
Color of TM						x	x		x
Erythema/redness of TM						x	x	x	x
Mobility of TM									x
Translucency of TM						x	x		
Otorrhea									x

Of the scores based solely on symptoms, only *the AOM severity of symptom scale* (*AOM-SOS*), developed by Shaikh et al. (Shaikh et al. 2009), does not include ear pain. The scale originally included a question about child's ear pain (Shaikh et al. 2009). However, Shaikh et al. have themselves stated that the question on ear pain requires parents to interpret and synthesize information from a variety of sources, and may be more prone to variability. Furthermore, in their study, 5 out of the 10 parents considered ear pain as difficult to understand or assess. Thus, the question on ear pain was removed from the final version (Shaikh et al. 2009). Other questions in the symptom scale are related to observable behavior and thus, easier for parents to assess. AOM-SOS has been successfully used in treatment trials as a follow-up tool (Hoberman et al. 2011, Hoberman et al. 2016).

Since all the symptom severity scores were with a written multiple choice format, *the AOM faces scale* (*The AOM-FS*) was developed by McCormick et al. for parental use to assess the severity of AOM (Figure 2) (Friedman et al. 2006). The AOM-FS examines parents' perception of the AOM severity in children or the child's overall condition (range of 1-7). The parental perception, on the other hand, is influenced by the child's symptoms. Indeed, the AOM-FS has been shown to have a good correlation between OM-3 and ETG-5, which are symptom-specific scores (Friedman et al. 2006). Since its publication, the AOM-FS has been used in clinical trials (Laine et al. 2010, Kalu et al. 2011).

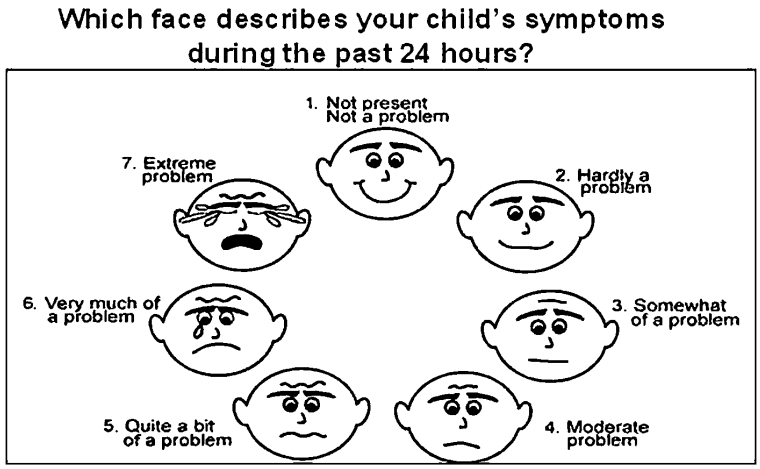


Figure 2. AOM faces scale. Copyright permission granted by David P. McCormick, M.D., UTMB.

The only score that grades purely otoscopic signs is the *OS-8 scale* (*otoscopy scale, 8 grades of severity*). Originally developed by McCormick et al. (McCormick et al. 2003), the OS-8 scale grades the TM and middle ear appearance as

seen during otoscopy with a range of 0-7. The OS-8 scale has been used in numerous studies over the years and it has undergone just a few modifications with the wording of the grades (McCormick et al. 2005, Friedman et al. 2006, McCormick et al. 2007, Kalu et al. 2011). The detailed grading of the OS-8 scale is described in Table 5.

A few scores have combined both symptoms and otoscopic signs. *The AOM total severity index* (*AOM-Si*) sums up the scores from the AOM-FS and the OS-8 (maximum of 14 points) (Friedman et al. 2006).

The clinical/otologic score (*COS*), originally developed by Dagan et al., included fever, irritability, ear rubbing, redness of TM and bulging of TM, and scored from 0 to 3 (as none, mild, moderate, severe) with maximum of 15 points (Dagan et al. 1998). Polachek et al. later modified the score by excluding ear rubbing, thus leaving the score with maximum of 12 points (Polachek et al. 2004). The clinical/otologic score has been shown to be statistically higher in children with AOM with culture-positive MEE (*H. influenzae*, *S. pneumoniae* or both), compared with children with culture-negative MEE (the mean score of 9.3 vs. 8.4; P=0.01) (Leibovitz et al. 2003).

The 10-point AOM scoring instrument is the most recently developed tool by Casey et al. (Casey et al. 2011). It includes the assessment of ear pain, fever and irritability, and the evaluation of otoscopic signs, such as erythema, mobility, effusion color, position of TM and otorrhea. In children with AOM treated with antimicrobials, the 10-point AOM scoring instrument has been shown to differentiate clinical cure from clinical failure after three weeks of the AOM diagnosis (Casey et al. 2011).

Although several symptom scores have been shown to be reliable follow-up tools in children with AOM, they are not useful to discriminate children with AOM from those without AOM (Laine et al. 2010). Moreover, fever is included in all the symptom scores, although it is not a specific symptom for AOM in young children (Niemelä et al. 1994, Heikkinen and Ruuskanen 1995, Laine et al. 2010, McCormick et al. 2016), probably due to the underlying viral infection (Putto et al. 1986, Arola et al. 1990). Symptom scores may also be considered as too complex to be used in everyday clinical practice. Therefore, the clinical significance of symptom scores remains controversial.

2.6.9 Association with bacteria and respiratory viruses

Specific associations between symptoms and bacteria or respiratory viruses would aid physicians to draw conclusions about the etiology of AOM. This, in turn, would ease the management of AOM.

Table 8 shows the occurrence of symptoms according to the pathogenic bacteria and respiratory viruses in outpatient children with AOM. The percentages are combined from several studies (Coffey 1966, Howie et al. 1970, Schwartz et al. 1981, Bodor et al. 1985, Arola et al. 1990, Rodriguez and Schwartz 1999, Palmu et al. 2004, Beder et al. 2009). Most of the studies have focused on investigating bacteria from MEE, with the exception of the studies of Arola et al. and Beder et al., investigating respiratory viruses from nasopharyngeal sample. In addition, Cohen et al. used nasopharyngeal samples to investigate the association between bacteria and symptoms (Cohen et al. 2006). However, as their study reported the associations with odds ratios (OR), it was excluded from Table 8. Studies of McCormick et al. and Leibovitz et al. were likewise excluded from Table 8 because they reported symptom scores instead of individual symptoms (McCormick et al. 2000, Leibovitz et al. 2003). Although these studies are excluded from Table 8, their results are described in the text below.

Table 8. Occurrence of symptoms according to the bacterial pathogens and respiratory viruses detected from the nasopharynx or from middle ear effusion in outpatient children with AOM.

Symptom	Sp	Hi	Mc	HRV	HBoV	RSV
Ear pain	24-49%	13-33%	20-26%	-	-	-
Fever	19-60%	10-40%	0-39%	45%	93%	74%
Conjunctivitis	15%	54-100%	18%	-	-	-
Cough	-	-	-	72%	-	96%
Vomiting	-	-	-	8%	-	24%
Diarrhea	-	-	-	6%	-	9%

Sp=*S. pneumoniae*, Hi=*H. influenzae*, Mc=*M. catarrhalis*, HRV= human rhinovirus, HBoV=human bocavirus, RSV=respiratory syncytial virus

Ear pain has been positively associated with *S. pneumoniae* in children with AOM. Ear pain is also associated with *H. influenzae* and *M. catarrhalis*, but to a lesser extent (Howie et al. 1970, Palmu et al. 2004, Cohen et al. 2006). *Irritability* is suggested as less probable in children with AOM with the nasopharyngeal carriage of *M. catarrhalis* (Cohen et al. 2006). On the other hand, irritability and *ear rubbing* reached similar severity scores in children with AOM, caused by either *S. pneumoniae* or *H. influenzae* (Leibovitz et al. 2003).

Fever has been shown to have a positive association with *S. pneumoniae*, detected either from MEE or nasopharynx, in children with AOM, whereas fever is less likely to be present with *H. influenzae* (Howie et al. 1970, Rodriguez and Schwartz 1999, Palmu et al. 2004, Cohen et al. 2006). On the contrary, Schwartz and his co-workers found no difference in febrile response between *S. pneumoniae* and *H. influenzae* detected from MEE in children with AOM (Schwartz et al. 1981). Similarly, Leibovitz et al. reported no difference in febrile response between *S. pneumoniae*, *H. influenzae* and *M. catarrhalis* in children with AOM (Leibovitz et al. 2003). These studies, however, investigated only bacteria. Conversely, Arola et al. investigated only respiratory viruses together with symptoms and found fever to be more common in RSV-positive children with AOM, compared to the rhinovirus-positive or virus-negative children with AOM (Arola et al. 1990). It is important to note, though, that Arola et al. used viral culture to detect rhinoviruses and therefore they missed approximately 50% of rhinoviruses (personal communication with professor Olli Ruuskanen, MD). Beder et al. focused on investigating HBoV and showed fever to be more common in children with AOM if HBoV was found in the nasopharynx, compared with the children with AOM and no HBoV in the nasopharynx (Beder et al. 2009). However, the proportion of children harboring HBoV in their nasopharynx was very low, only 6%. In addition, the detection of HBoV was positively correlated with the detection of *S.pneumoniae* in MEE of children with AOM (Beder et al. 2009), which may confound the results.

Rhinitis with duration of two to four weeks was associated with *H. influenzae* in children with AOM nearly 50 years ago (Howie et al. 1970). *Cough* and *vomiting* have been associated with RSV in children with AOM (Arola et al. 1990), although vomiting is more likely to be defined as mucus vomiting when related to RSV.

The association between conjunctivitis and *H. influenzae* seems evident in children with AOM. The association was originally discovered by Coffey (Coffey 1966) and later referred to as the "otitis-conjunctivitis syndrome" by Bodor et al. (Bodor 1982, Bodor et al. 1985). Later studies have further confirmed the association of eye-associated symptoms and *H. influenzae* (Palmu et al. 2004, Cohen et al. 2006).

It is noteworthy that the studies have mainly focused on investigating either bacteria or respiratory viruses. The only exception is McCormick et al. that investigated both bacteria and respiratory viruses as well as their association with symptoms in children with AOM. Instead of analyzing each bacterium or respiratory virus separately, they grouped different AOM etiologies as bacterial, bacterial/viral, viral or no pathogen. However, they found no difference in the symptom

scores between nine symptoms (fever, ear pain or rubbing, irritability, poor appetite, rhinitis, nasal congestion, cough, sneezing, watery eyes) and different AOM etiologies (McCormick et al. 2000).

Thus, symptoms and their association with microbes in children with AOM are still far from being resolved.

2.6.10 Laterality of acute otitis media

Bilateral AOM is reported in 40-76% of the children with AOM (Pukander 1983, Kilpi et al. 2001, Palmu et al. 2004, McCormick et al. 2007, Leibovitz et al. 2007, Tähtinen et al. 2011, Hoberman et al. 2011). Children with bilateral AOM tend to be younger than children with unilateral AOM (Howie et al. 1970, Pukander 1983, Hayden and Schwartz 1985, McCormick et al. 2007, Leibovitz et al. 2007). Children with bilateral MEE are also shown to experience more severe hearing loss than children with unilateral MEE (Sabo et al. 2003).

In several national guidelines, bilateral AOM is considered a more severe illness than unilateral AOM, and more active antimicrobial treatment is recommended for children with bilateral AOM (Tan et al. 2008, Marchisio et al. 2010, Solen and Hermansson 2011, Lieberthal et al. 2013, Heikkinen et al. 2017). The recommendation is largely based on the meta-analysis of Rovers et al. which suggested children younger than two years of age with bilateral AOM to benefit more from antimicrobial treatment than those with unilateral AOM (Rovers et al. 2006).

Microbiological studies indicate that children with bilateral AOM have positive bacterial culture in MEE slightly more often than children with unilateral AOM (70-83% vs. 57-67%) (McCormick et al. 2007, Leibovitz et al. 2007). When comparing etiologies between bilateral and unilateral AOM, *H. influenzae* has been more commonly found in MEE of children with bilateral AOM (Howie et al. 1970, McCormick et al. 2007, Leibovitz et al. 2007). In children with bilateral AOM, *S. pneumoniae*, *H. influenzae* and *M. catarrhalis* have been detected at similar rates from MEE (Kilpi et al. 2001, McCormick et al. 2007).

However, research comparing clinical characteristics between children with bilateral and unilateral AOM remains limited. Hayden et al. did not find ear pain to be related to the laterality of AOM (Hayden and Schwartz 1985). McCormick et al. compared symptoms (fever, ear pain, irritability, feeding and sleeping) by using the ETG-5 score and found no difference in the scores between bilateral and unilateral AOM. Likewise, occurrence of fever was similar between the groups, but severe otoscopic signs, namely erythema, opacification and bulging of TM

were more commonly detected in children with bilateral AOM (McCormick et al. 2007). Leibovitz et al. compared bilateral and unilateral AOM by using the clinical/otologic score, a combination of otoscopic signs (bulging and redness of TM) and symptoms (fever and irritability). They found bilateral AOM to reach a somewhat higher mean score than unilateral AOM (8.3 vs. 7.8) (Leibovitz et al. 2007). Nevertheless, the previous studies do not shed the light on whether bilateral AOM is clinically more severe illness than unilateral AOM, because the detailed information about symptoms in children with bilateral and unilateral AOM is missing.

2.7 Diagnostics

> *"The number of cases (of AOM) found varies directly with the diligence with which they are sought."*
>
> *John Dixon Coffey, Jr., 1966*

2.7.1 Signs of tympanic membrane

The diagnostic criteria of AOM require that along with acute symptoms, signs of TM inflammation, as well as MEE are detected. Thus, the examination of TM is a crucial part in the diagnostics of AOM. The examination is often performed with pneumatic otoscopy and it should contain the assessment of the following aspects of TM: the position (normal, retracted, full, bulging), color (gray, yellow, pink, amber, white, red, blue), translucency (translucent, semiopaque, opaque) and mobility (normal, increased, decreased, absent) (Lieberthal et al. 2013).

Bulging of TM has been shown to reliably predict the presence of MEE (Karma et al. 1989). On the other hand, MEE is also detected in children with OME, not just in children with AOM. Experts consider bulging of TM as the most accurate sign for distinguishing AOM from OME (Shaikh et al. 2011). Furthermore, a bulging TM increases the probability that bacteria are found in the MEE (Halsted et al. 1968, McCormick et al. 2000, Leibovitz et al. 2003, Palmu et al. 2004), although it does not help distinguishing the etiology of AOM (Leibovitz et al. 2003, Palmu et al. 2004). In line with this, two recent AOM treatment trials, which included only children with AOM with bulging TMs, showed antimicrobials to be more effective than placebo (Tähtinen et al. 2011, Hoberman et al. 2011).

Distinctly red (moderately or strongly red or haemorrhagic) *TM* has been shown to increase the probability of AOM, compared to slightly red TM (Rothman et al.

2003). Redness of TM has been shown to be slightly more severe in children with culture-positive AOM than in children with culture-negative AOM (the mean clinical/otologic scores 2.4 for *H. influenzae* and 2.3 for *S. pneumoniae* vs. 2.1 for no pathogen) (Leibovitz et al. 2003), although the difference was clinically modest. When comparing redness of TM between bacterial etiologies of AOM, Leibovitz et al. found no difference in redness of TM between the etiologies (Leibovitz et al. 2003), whereas Rodriguez et al. reported redness of TM to be more commonly associated with *S. pneumoniae* than with *H. influenzae* or *M. catarrhalis* (Rodriguez and Schwartz 1999). Hemorrhagic myringitis is associated with a higher isolation rate of *S.pneumoniae* in MEE, compared to AOM without hemorrhagic redness (44% vs. 15%) (Palmu et al. 2001). On the other hand, 82% of the children with hemorrhagic myringitis are shown to ultimately develop MEE (Palmu et al. 2001). Therefore, intense erythema of TM may be considered as an early sign of AOM if it is accompanied with recent onset of ear pain, according to the AAP guideline (Lieberthal et al. 2013). In general, redness of TM has been reported in 18-46% of the cases with AOM (Karma et al. 1989, Arola et al. 1990), whereas distinctly red TM has been reported only in 14-24% of the cases with AOM (Karma et al. 1989). Nevertheless, redness of TM may be due to other reasons than AOM, such as crying (Ingvarsson 1982, Isaacson 2016) or struggling of a child during examination (Karma et al. 1989). Hence, TM redness cannot be regarded as a reliable indicator of AOM (Karma et al. 1989, Rothman et al. 2003).

An opaque or *cloudy TM* is another sign predicting the presence of MEE (Karma et al. 1989) and experts consider the opacification of TM as the best sign to differentiate OME from no effusion (Shaikh et al. 2011). However, an opaque or cloudy TM is not associated with increased detection of bacteria in the MEE, nor does it help distinguish between different etiologies of AOM (Palmu et al. 2004). Therefore, an opaque or cloudy TM cannot reliably differentiate AOM from OME.

Distinctly immobile TM has been shown to predict the presence of MEE (Karma et al. 1989). Palmu et al. showed that children with culture-positive MEE had more frequently immobile TMs, compared to children with culture-negative MEE. However, bacterial etiologies of AOM cannot be distinguished based on immobility (Palmu et al. 2004).

Purulent effusion behind TM may indicate pathogenic bacterial growth in MEE. McCormick et al. described the tendency of TMs to be yellow/red when any pathogen was cultured from MEE (McCormick et al. 2000). Holder et al. showed *H. influenzae, S. pneumoniae* and *M. catarrhalis* to be over two times more frequently detected in purulent effusions than in non-purulent effusions in children

undergoing routine tympanostomy tube placement (Holder et al. 2015). According to Palmu et al., MEE was purulent in 64% if it contained mixed culture of the main pathogens. On the contrary, MEE was purulent in 47% if the culture was negative (Palmu et al. 2004). However, purulent effusion was detected at the similar rate between all the bacterial pathogens (Palmu et al. 2004).

Bulla formation on TM is considered as one of the otoscopic signs of AOM. MEE has been detected by myringotomy in 97-100% of the cases of bullous myringitis (Coffey 1966, Palmu et al. 2001), whereas with the tympanogram, the B-curve has been detected in 62-78% suggesting the presence of MEE (McCormick et al. 2003, Kotikoski et al. 2003). In addition, bulging TM was reported in 97% of the cases of bullous myringitis (McCormick et al. 2003). In children with bullous myringitis, *S. pneumoniae* has been more commonly isolated in MEE, compared to children with AOM without bulla formation (32% vs. 15% and 74% vs. 30%, respectively) (Palmu et al. 2001, Rosenblut et al. 2001). Hence, it is not at all surprising that AOM with bulla formation is related to more severe form of symptoms. Children with bulla formation have more often physical suffering, such as ear pain, fever, restless sleep, excessive crying or poor appetite than children with AOM without bulla formation (McCormick et al. 2003, Kotikoski et al. 2003). However, bulla formation is not very frequently observed; it has been reported only in 5-16% of the AOM cases (Arola et al. 1990, Rosenblut et al. 2001, Kotikoski et al. 2003). Despite its rare occurrence, bulla formation of TM is considered as the most severe otoscopic sign of AOM and is thus included in the OS-8 score (Friedman et al. 2006).

Spontaneous perforation of TM leading to *otorrhea* is considered as a complication of AOM. Of the otorrhea samples taken from children with AOM and spontaneous perforation, 47-69% were culture-positive (Skovbjerg et al. 2010, Grevers et al. 2012, Marchisio et al. 2013). Of the culture-positive samples, *H. influenzae* seems to be most commonly isolated (32-54%), followed by *S. pneumoniae* (23-45%) and *S. pyogenes* (6-19%) (Leibovitz et al. 2009, Skovbjerg et al. 2010, Marchisio et al. 2013). Despite the relatively rare isolation rate, *S. pyogenes* has been proposed to be more locally aggressive than the other AOM pathogens, because those children with AOM, who have *S. pyogenes* in MEE, have a greater tendency for spontaneous perforation of TM, compared to children with other AOM pathogens isolated in MEE (Segal et al. 2005).

The combination of otoscopic signs of severe bulging, severe redness and opacity of TM have been associated with the increased detection of bacteria, or bacteria and viruses in MEE in children with AOM (Yano et al. 2009).

Diagnostic criteria for AOM

The AAP guideline of the diagnosis and management of AOM recommends diagnosing AOM in children with moderate or severe bulging of TM or with new onset of otorrhea not due to acute otitis externa. Moreover, AOM may be diagnosed in children with mild bulging of TM and recent (< 48 hours) onset of ear pain (holding, rubbing of the ear in a nonverbal child) or with intense erythema of TM (Lieberthal et al. 2013).

As a comparison, the Finnish national AOM guideline (Käypä Hoito) (Heikkinen et al. 2017) has the following diagnostic criteria for AOM: First, the child needs to have at least one acute symptom referring to either general or localized infection, such as rhinitis, cough, fever, ear pain, impaired hearing and excessive crying. Second, MEE has to be detected and signs of TM inflammation have to be present. Detailed descriptions of the otoscopic signs suggestive for AOM are provided in Table 9.

Table 9. Otoscopic signs suggestive of AOM, according to Finnish national guideline of the diagnosis and management of AOM (Heikkinen et al. 2017).

Otoscopic sign	***Signs suggestive of AOM***
Position or shape	Full or bulging
Color	Red, yellow, white
Transparency	Opaque
Light reflex	Widened or absent
Mobility	Impaired or absent

In addition, new onset of otorrhea, either through a tympanostomy tube or due to the perforation of TM, is considered as a sign of AOM**,** according to the Finnish national guideline (Heikkinen et al. 2017).

Peculiarly, bulla formation of TM is not mentioned in either of the guidelines, although several researches consider it as a sign of severe form of AOM (Palmu et al. 2001, Rosenblut et al. 2001, Kotikoski et al. 2003, McCormick et al. 2003).

2.7.2 Diagnostic tools

Pneumatic otoscopy is the most important diagnostic tool in the hands of physicians. The otorhinolaryngologists prefer using the otomicroscopy, but it is not mandatory or practical for the diagnosis of AOM. The most common problem encountered during the visualization of the TM, is obstructive cerumen in the ear canal. Thus, when teaching medical students to perform pneumatic otoscopy in

young children, it is as important to teach the careful cerumen removal technique. It is of note that pneumatic otoscopy is always subjective and prone to interpretation that has been shown to vary according to the examiner's experience (Shaikh et al. 2016). Hence, adjunct tools are needed to aid in the diagnostics of AOM. Tympanometry is an objective tool that provides quantitative information on the function of structures and the presence of fluid in the middle ear (Onusko 2004). Tympanometry has 90-94% sensitivity but lower specificity (50-75%) to detect MEE (Takata et al. 2003). On the other hand, a normal tympanogram, i.e. C or A curve, is significantly associated with a lower weight of MEE found during myringotomy (Koivunen et al. 2000). Thus, normal tympanogram excludes the presence of MEE rather reliably. However, tympanometry cannot distinguish between OME and AOM (Helenius et al. 2012). Thus, tympanometry may only serve as a complementary diagnostic tool, while pneumatic otoscopy remains the diagnostic gold standard for AOM.

2.8 Management

2.8.1 Symptomatic treatment

The treatment of pain is the key to successful management of AOM. Unfortunately, it appears that Finnish physicians too rarely prescribe or offer pain medication for children with AOM (Pulkki et al. 2006). The management of pain, especially during the first 24 hours of an episode of AOM, is essential, regardless of the use of antimicrobial treatment (Lieberthal et al. 2013). Acetaminophen and ibuprofen are considered equally effective in relieving short-term pain in children with AOM (Bertin et al. 1996) and their use is recommended for children with AOM (Lieberthal et al. 2013, Heikkinen et al. 2017). Interestingly, a recent study showed the combination of acetaminophen and ibuprofen to be equally effective with the opioid analgesics in reducing pain in adults with acute extremity pain (Chang et al. 2017), which suggests that the combination therapy might be useful in children as well.

Naproxen and its effect on pain in children with AOM are studied only by Varsano et al. In their randomized, double blind study, children between 1 and 12 years of age with AOM received either naproxen 7mg/kg 3 times per day or placebo. In addition, all the children were given a regular dose of amoxicillin for the treatment of AOM. Interestingly, no significant differences were seen in the duration of ear pain or in sleep disturbances between children receiving naproxen and children receiving placebo (Varsano et al. 1989). It is possible that the study population was too small (47 children) to reach a statistically significant differ-

ence in the duration of symptoms between the groups. In Finland, however, naproxen is recommended to be used in children with AOM (Heikkinen et al. 2017).

The optimal dosage of pain medications in children has not been clear, especially regarding acetaminophen. Doses of 10-20mg/kg have been used, but it is nowadays believed that 10mg/kg has no analgesic effect (Mason 2017). Just recently, Korppi et al. published a review of recommended pain medications and their optimum doses, as well as their duration to peak analgesic effect and the duration of total analgesic effect (Table 10) (Korppi and Vilo 2017). Anaesthetic ear drops may bring extra pain relief in older children with AOM, when combined with oral pain medication (Hoberman et al. 1997, Foxlee et al. 2006, Bolt et al. 2008). It is likely that also younger children would benefit from anaesthetic ear drops, but unfortunately no research has been performed in children younger than three years of age with AOM.

Table 10. Recommended pain medications and their optimal dosing, the duration to peak analgesic effect and the duration of analgesic effect, according to Korppi et al.

Peroral pain medication	Recommended dose per day	Maximum dose per day	Duration to peak analgesic effect (Time to peak drug concentration), hours	Duration of total analgesic effect, hours
Acetaminophen	15mg/kg x 4(first dose 20mg/kg)	80mg/kg	0.5-1	6
Ibuprofen	10mg/kg x 3	40mg/kg	1-1.5	8
Naproxen	5mg/kg x 2	15mg/kg	1-1.5	12

2.8.2 Antimicrobial treatment

AOM treatment trials

The effect of antimicrobial treatment in young children with AOM has long remained controversial. This has been due to multiple flaws in the study designs (Pichichero and Casey 2008). First, stringent diagnostic criteria of AOM have not been used, enabling children with OME or only with red ear to be included in the studies. In addition, diagnostic criteria of AOM have varied between the studies, thus complicating the comparison of the results (Kaleida et al. 1991, Appelman et al. 1991, Burke et al. 1991, Damoiseaux et al. 2000, Little et al. 2001, McCormick et al. 2005, Le Saux et al. 2005, Spiro et al. 2006). Second, only the study of Damoiseaux was focused on investigating children under two years of age (Damoiseaux et al. 2000), who have the highest incidence of AOM and for

whom AOM is the main reason for the prescriptions of antimicrobials (Vergison et al. 2010). Third, some studies have focused on investigating the effect of antimicrobials in children with non-severe symptoms (Burke et al. 1991, Kaleida et al. 1991, McCormick et al. 2005), which will ultimately have an effect on the treatment outcomes as the severe cases of AOM are excluded. Fourth, the daily dosage of amoxicillin has varied from 40mg/kg to 90mg/kg between the studies (Kaleida et al. 1991, Damoiseaux et al. 2000, McCormick et al. 2005, Le Saux et al. 2005) or the suboptimal antimicrobial dosage has been used (Appelman et al. 1991, Burke et al. 1991, Little et al. 2001). Likewise, the duration of antimicrobial treatment has varied from 3 to 14 days. Fifth, treatment outcomes have been variable between the studies. Due to all the above-mentioned differences between the studies, it has been difficult to draw uniform conclusions from the effectiveness of antimicrobial treatment in children with AOM.

Due to the uncertainty of the AOM diagnostics and effect of antimicrobials in the management of AOM, the observation option, also known as watchful waiting or wait-and-see approaches or as a safety-net prescription, was introduced by AAP AOM guideline in 2004 to diminish the use of antimicrobials (American Academy of Pediatrics Subcommittee on Management of Acute Otitis Media 2004). With this approach, children of six months to two years of age with non-severe illness and uncertain diagnosis of AOM are not initially prescribed antimicrobials, but they are closely followed. In case of worsening of symptoms or overall condition of the child within two to three days, antimicrobials are prescribed. This is also known as delayed antimicrobial prescribing. The recommendation of the observation option is based on the studies of Little et al., McCormick et al. and Spiro et al., which showed the resolution of symptoms to be faster with the immediate antimicrobial treatment than with the observation option. However, all the studies concluded that despite the beneficial effect of immediate antimicrobials in the resolution of children's symptoms, the benefit was only modest and antimicrobials also have side effects. Hence, the observation option was considered as a feasible tool and suggested as an alternative option in the management of AOM (Little et al. 2001, McCormick et al. 2005, Spiro et al. 2006).

Meta-analyses have been performed by Rovers et al. and Venekamp et al. in order to identify which subgroups would reach the greatest benefit from antimicrobial treatment. Both analyses suggested that antimicrobials seem to be most beneficial in children younger than two years with bilateral AOM and in children with otorrhea (Rovers et al. 2006, Venekamp et al. 2015). However, the results must be interpreted with caution, because these reviews included studies with methodological flaws, as described above.

In 2011, two studies of the efficacy of antimicrobial treatment in young children with AOM were published. Studies of Hoberman et al. and Tähtinen et al. were randomized, double-blind and placebo-controlled trials. They used stringent diagnostic criteria for AOM and thus, most of the children had bulging TMs. Furthermore, they required the presence of acute symptoms and no exclusion was made due to symptom severity (Hoberman et al. 2011, Tähtinen et al. 2011).

Hoberman et al. investigated children under two years of age with AOM (Hoberman et al. 2011). The children were randomly assigned to receive either amoxicillin-clavulanate (a daily dose of 90mg/kg and 6.4mg/kg) or placebo for 10 days. The primary outcomes were the time to resolution of symptoms and the symptom burden over time. Children who received amoxicillin-clavulanate reached a sustained symptomatic response significantly faster and their symptom burden was lower than in children who received placebo (the 7-day weighted mean AOM-SOS score 2.79 vs. 3.42, P=0.01). Moreover, clinical failure, defined as the persistence of symptoms and otoscopic signs, was less common in children receiving amoxicillin-clavulanate than in children receiving placebo (at or before the day 10-12 visit, 16% vs. 51%, P<0.001). Interestingly, bilateral AOM, severe bulging of TM and severe symptoms at entry (AOM-SOS score >8) increased the probability of treatment failure.

Tähtinen et al. investigated children younger than three years of age with AOM (Tähtinen et al. 2011). The children were randomly assigned to receive either amoxicillin-clavulanate (a daily dose of 40mg/kg and 5.7mg/kg) or placebo for seven days. The primary outcome was the time to treatment failure, which was dependent on the child's overall condition (no improvement by day 3, or worsening of the overall condition at any time) and otoscopic signs (no improvement by day 8, or perforation of TM at any time). Treatment failure occurred significantly less often in children who received amoxicillin-clavulanate than in children who received placebo (19% vs. 45%, P<0.001). In addition, treatment with amoxicillin-clavulanate significantly accelerated the resolution of fever, poor appetite, decreased activity and irritability. The effect of treatment on the resolution of symptoms was seen on the second study day at the latest. Unlike in the study of Hoberman et al., bilateral AOM was not associated with higher treatment failure rates compared with unilateral AOM.

Antimicrobial treatment and duration of ear pain

Some studies have found antimicrobial treatment to shorten the duration of ear pain. Le Saux et al. reported that in the first two days after treatment, ear pain was present in 22-32% of the children in the amoxicillin group as compared with 33-42% of the children in the placebo group (Le Saux et al. 2005). Little et al. found the mean duration of ear pain to be 2.5 days in children who received im-

mediate antimicrobials, as compared with 3.6 days in children allocated to receive delayed antimicrobials after 72 hours in case of no improvement in the symptomatic condition (Little et al. 2001). According to the study of Spiro et al, prolonged ear pain tends to be the major reason for parents to fill the wait-and-see prescription (Spiro et al. 2006). Tapiainen et al. reported that five days after administration of the study drug, none of the children receiving amoxicillin-clavulanate suffered from ear pain, as compared with 17% of the children receiving placebo (Tapiainen et al. 2014). Rovers et al. performed a meta-analysis and reported that relative to placebo, overall RR for children who had pain on 3-7 days with antimicrobials was 0.86, resulting in a number-needed-to-treat of 10 children. Children younger than two years with bilateral AOM seemed to reach the greatest benefit from antimicrobials, because ear pain was less common on days 3-7 in the antimicrobial group than in the placebo group (55% vs. 30%), resulting in a number-needed-to-treat of five children (Rovers et al. 2006). On the other hand, Tähtinen et al. found no difference in the resolution of ear pain between children receiving antimicrobials or placebo, although they investigated only children younger than three years of age and used more stringent diagnostic criteria than other studies (Tähtinen et al. 2011). Hence, the administration of analgesics remains the cornerstone in the treatment of pain, although antimicrobial treatment would be initiated.

Antimicrobial treatment and duration of MEE

Antimicrobial treatment has been reported to shorten the duration of MEE in children younger than 15 years. According to Tapiainen et al., the mean duration of MEE was 19 days in children receiving amoxicillin-clavulanate and 33 days in children receiving placebo (Tapiainen et al. 2014). Thus, antimicrobial treatment may reduce the possible concomitant hearing impairment due to MEE in those children. Unfortunately, in young children, the effect of antimicrobial treatment on the duration of MEE is rather modest. Tapiainen et al. showed that in children younger than two years, the mean duration of MEE was 32 and 40 days in the amoxicillin-clavulanate and placebo groups, respectively (Tapiainen et al. 2014). Correspondingly, Ruohola et al. found the mean duration of MEE to be 36 and 42 days in the amoxicillin-clavulanate and placebo groups in children under two years (Ruohola et al. 2017). Interestingly, the accelerating effect of antimicrobial treatment on the resolution of MEE seems to last only for two to three weeks, because thereafter no significant treatment effect was observed (Kaleida et al. 1991, Ruohola et al. 2017). As a matter of fact, Ruohola et al. showed that in children younger than three years, the major reason for inadequate resolution and, thus for the persistence of MEE, is the recurrence of AOM before MEE resolution (Ruohola et al. 2017). Hence, it seems that in young children with AOM,

antimicrobial treatment does not significantly reduce the persistence of MEE and possible concomitant hearing impairment.

Duration of antimicrobial treatment

Hoberman et al. showed the 10-day treatment of amoxicillin-clavulanate to be superior compared with the 5-day treatment when clinical failure, worsening or lack of nearly complete resolution of symptoms and otoscopic signs by the end of treatment, was used as outcome (16% vs. 34%, respectively) (Hoberman et al. 2016). However, the mean AOM-SOS scores by the end of treatment (days 12-14) were clinically not that different (1.63 and 1.99 in the 10-day and 5-day groups, respectively). This leaves room for debate on whether the 10-day treatment is clinically more beneficial than the 5-day treatment after all.

Prognostic factors for treatment failure

Recently, Tähtinen et al. published a secondary analysis of their original study, in which they described prognostic factors for treatment failure in AOM (Tähtinen et al. 2017). It showed that children with severe bulging of TM benefited most from antimicrobial treatment (treatment failure in 64% of children in the placebo group vs. 11% in the antimicrobial treatment group). Interestingly, the occurrence of symptoms (fever, ear pain, ear rubbing, decreased activity, severe illness according to AAP), or bilaterality of AOM did not increase the risk for treatment failure.

2.8.2.1 Treatment guidelines for AOM

Immediate antimicrobial treatment

AAP guideline (updated in 2013) recommends antimicrobial treatment for children with *severe AOM* (moderate or severe ear pain or ear pain for at least 48 hours, or temperature of at least 39 °C), regardless of age. In addition, *children under two years of age with bilateral AOM* should be managed with antimicrobials, regardless of illness severity. Otherwise, *children with non-severe AOM* (mild ear pain for less than 48 hours and fever less than 39 °C) can be managed either with immediate antimicrobials or with initial observation (Lieberthal et al. 2013).

Finnish national guideline (updated in 2017) recommends antimicrobial treatment mainly for all AOM cases. The guideline does not strictly state, which of the AOM cases should be managed with antimicrobials, but the guideline brings forth the following factors, which might support the management with antimi-

crobials in children with AOM: under two years of age, bulging of TM, bilateral AOM and otorrhea (Heikkinen et al. 2017).

In case of immediate antimicrobial treatment, amoxicillin is the first drug of choice, according to the AAP guideline. The recommended dose of amoxicillin is 80-90mg/kg per day in two divided doses for 10 days (Lieberthal et al. 2013). In case of AOM with concurrent purulent conjunctivitis, the AAP guideline recommends prescribing amoxicillin-clavulanate with the dose of 90mg/kg per day in two divided doses for 10 days. The Finnish guideline recommends primarily either the regular dose of amoxicillin 40mg/kg per day or amoxicillin-clavulanate 40mg/5.7mg/kg per day in two or three divided doses for five to seven days.

Initial observation without antimicrobial treatment and close follow-up

According to the AAP guideline, initial observation without antimicrobial treatment serves as one of the management options in children under two years of age with non-severe (the presence of mild ear pain and a temperature below 39 °C), unilateral AOM, as well as in children over two years of age with non-severe AOM (Lieberthal et al. 2013). The Finnish guideline on the management of AOM likewise allows children with AOM to be managed with initial observation, but instead of specifying any subgroups, the guideline gives physician a mandate to make the final decision about the management of AOM in children (Heikkinen et al. 2017).

If the option of initial observation is chosen in children with AOM, the decision has to be made in good agreement with parents and close follow-up must be provided by the physician. If the child's overall condition worsens or fails to improve within two to three days, the AAP guideline recommends that antimicrobial treatment has to be initiated, but the guideline is ambiguous of its execution, e.g. whether re-examination is needed (Lieberthal et al. 2013). The Finnish guideline, on the other hand, recommends actual re-examination by the physician if the child's overall condition worsens or fails to improve within two to three days (Heikkinen et al. 2017).

2.9 Complications

Suppurative complications are a rare sight in Western countries. In developing countries, however, suppurative complications are still widely present due to the shortage of antimicrobials (Vergison et al. 2010).

Acute mastoiditis remains as the main complication of AOM. The most common pathogens related to acute mastoiditis are *S. pneumoniae*, followed by *S. py-*

ogenes (Groth et al. 2012, Gorphe et al. 2012, Halgrimson et al. 2014, Laulajainen-Hongisto et al. 2014, Schilder et al. 2017). The average incidence of acute mastoiditis is suggested to be 1.0-4.8/100,000 children per year in children younger than 18 years (Groth et al. 2012, Anthonsen et al. 2013, Laulajainen-Hongisto et al. 2014, Halgrimson et al. 2014, Tawfik et al. 2017). However, differing trends in the incidence of acute mastoiditis have been recently described between the studies (Schilder et al. 2017). Moreover, concerns have been raised whether the introduction of initial observation or watchful waiting in the management of AOM would increase the risk of developing acute mastoiditis. Thompson et al. showed that antimicrobial treatment for AOM halved the risk of acute mastoiditis (Thompson et al. 2009). However, to prevent acute mastoiditis after AOM, the number-needed-to-treat was over 4,000 (Petersen et al. 2007). Furthermore, antimicrobials were given in 33-81% of the children before the diagnosis of acute mastoiditis (Groth et al. 2012, Gorphe et al. 2012, Anthonsen et al. 2013, Giannakopoulos et al. 2014, Laulajainen-Hongisto et al. 2014, Halgrimson et al. 2014, Tamir et al. 2014, Garcia et al. 2017). Thus, the administration of antimicrobials for AOM seems not to eliminate the risk of developing acute mastoiditis (Schilder et al. 2017).

Spontaneous perforation of TM, described in more detail in chapter 2.7.1, is also considered as a complication of AOM. Antimicrobial treatment of AOM is shown to reduce the risk of spontaneous TM perforation (RR 0.37; 95% CI 0.18 to 0.76) (Venekamp et al. 2015).

3 AIMS OF THE STUDY

The aim of the study was to investigate the role of symptoms in the diagnosis and management of AOM in young, mostly preverbal children.

The specific aims of the individual four studies were:

I To study whether parentally used pain scales are able to detect ear pain in young children, whose parents suspect them to have AOM, and which symptoms are associated with parentally assessed moderate/severe pain.

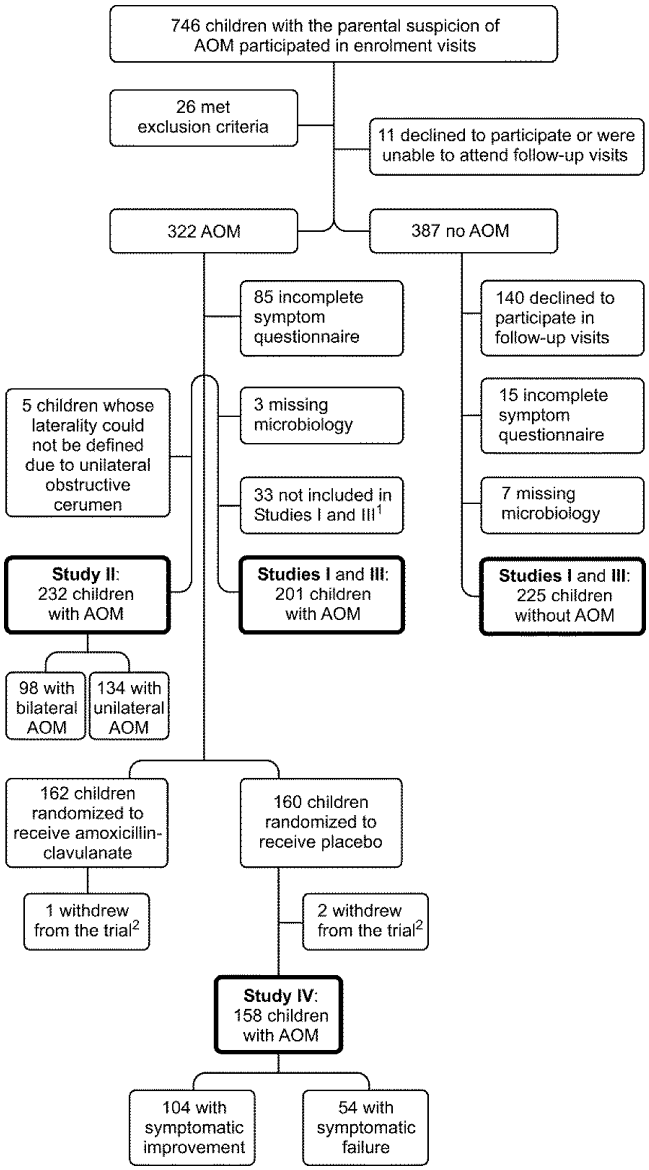
II To study whether bilateral AOM is clinically more severe illness compared to unilateral AOM in young children.

III To study whether the variation of acute symptoms is associated with the nasopharyngeal bacteria and/or respiratory viruses in young children, whose parents suspect them to have AOM.

IV To study whether close follow-up with re-examination is needed for children with AOM who are initially managed without antimicrobial treatment and whose overall symptomatic condition is improving.

4 MATERIALS AND METHODS

4.1 Study population and study design



[1] 33 children did not have AOM at the very first visit at study clinic when the symptom questionnaire was introduced to the parents for the first time. They were thus included in the non-AOM group in these analyses. However, children ended up developing AOM a couple of days or few months later and then they participated in the AOM treatment trial.

[2] Children withdrew from the trial within 48 hours and did not attend follow-up visits.

Figure 3. Flow chart of the study population in Studies I-IV.

This study is part of a project examining the diagnostics and management of AOM at the primary care level in Turku, Finland (Tähtinen et al. 2011). Patients were enrolled in the study between March 2006 and December 2008. Written informed consent was obtained from parents of all children before they could participate in the study. The study protocol was approved by The Ethics Committee of the Hospital District of Southwest Finland.

Children aged 6-35 months were eligible when they had parental suspicion of AOM. Therefore, all the children had acute symptoms of RTI. The flow chart of the study population is presented in Figure 3. Children were excluded from the study if they had ongoing antimicrobial treatment; AOM with spontaneous perforation of TM; systemic or nasal steroid therapy within the four preceding days; antihistamine or oseltamivir therapy within the three preceding days; allergy to penicillin or amoxicillin; tympanostomy tube present in TM; severe infection requiring systemic antimicrobial treatment; documented Epstein-Barr virus infection within the seven preceding days; known immunodeficiency, Down syndrome or other condition affecting middle ear diseases (e.g. cleft palate); severe vomiting or another symptoms disrupting per oral dosage; poor parental cooperation due to language or other reasons or use of any investigational drugs during the four preceding weeks.

4.1.1 Diagnostic criteria for AOM

We had stringent diagnostic criteria for AOM. First, middle ear effusion (MEE) had to be detected by pneumatic otoscopy. In order to detect MEE, at least two of the following signs had to be present on TM: bulging position, decreased or absent mobility, abnormal color or opacity not due to scarring or air-fluid interfaces. Second, at least one acute inflammatory sign of TM had to be observed, such as distinct erythematous patches/streaks or increased vascularity over full/bulging/yellow convexity. Third, the child needed to have symptoms of an acute infection.

4.1.1.1 Bilateral and unilateral AOM

The child had bilateral AOM if AOM was diagnosed in both ears. The unilateral AOM was diagnosed if the other ear had AOM and the contralateral side was either completely healthy or had OME.

In Study II, we included only children with AOM and divided them into two groups, based on the laterality of AOM on day 1: children with bilateral AOM and children with unilateral AOM.

4.1.2 Children with AOM and children without AOM

For the Studies I and III, children were further divided into two cohorts, based on the diagnosis by the study physician on day 1: children with AOM and children without AOM. Children who did not fulfill the diagnostic criteria of AOM had either OME or healthy ears. Children without AOM had one follow-up visit in approximately two weeks. In addition, parents were encouraged to contact the study clinic if they had any concerns about their child's well-being and additional visits were arranged. The follow-up for children with AOM is described in Section 4.3 of this Thesis.

4.2 The enrolment visit (day 1)

Studies I, II and III

The studies I, II and III were cross-sectional. The focus of the Studies was on the child's symptoms and clinical findings on day 1 when parents had brought their child to the study clinic due to the suspicion of AOM. All the children were symptomatic due to RTI.

4.2.1 Symptom questionnaire

Before examining the child, the study physician interviewed the parents about the symptoms of their child by using a standardized, structured symptom questionnaire. The parents were asked to assess the occurrence, duration and severity of respiratory, ear-related, nonspecific and gastrointestinal symptoms of their child (Figure 4). In addition, fever was the target of interest. The child was defined as febrile if temperature was ≥38 °C within the preceding 24 hours. However, in terms of the occurrence and duration of fever, we accepted parents' report of their child suffering from fever even though temperature was not measured with a thermometer. When analyzing the highest measured temperature within 24 hours, we included only those children whose temperature was actually measured to be ≥38 °C either at home or at the study clinic. The duration of each symptom was estimated with the accuracy of 0.5 days by parents.

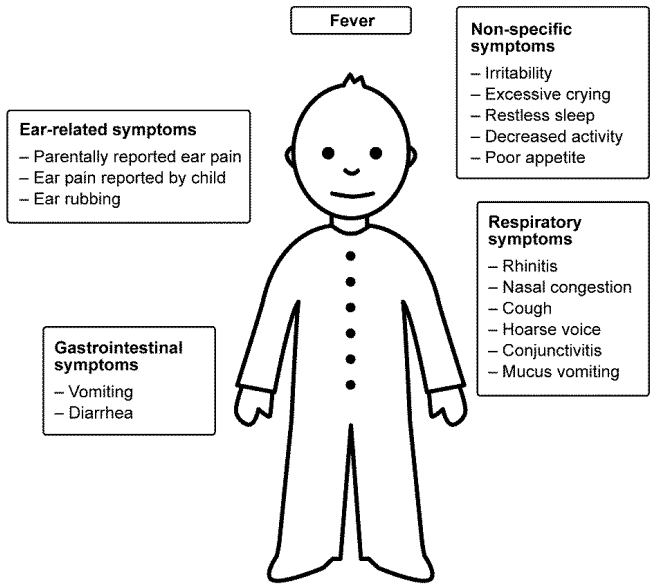


Figure 4. A child's symptoms, interviewed by the study physician and assessed by the parents. Figure by Timo Kattelus.

The study physician asked parents to assess the severity of each symptom of their child. The severity of symptoms was mainly categorized as mild or severe. However, as shown in Table 11, for a few symptoms, we also had three severity categories: mild, moderate and severe. The variability in the symptom severity categories between symptoms was due to the calculation of the symptom scores (See chapters 2.6.8. and 4.2.6).

Table 11. Symptom severity categories for each symptom, marked as x.

Symptom	Mild	Moderate	Severe
Parentally reported ear pain (based on the child's behavior, parents have suspected/reported their child of having ear pain)	x	x	x
Ear pain reported by child (the child has verbally expressed of having ear pain, for example, by saying that the ear is aching or pointing to the ear and saying it hurts)	x	x	x
Ear rubbing	x	x	x
Irritability	x	x	x
Excessive crying	x	N/A	x
Restless sleep	x	N/A	x
Decreased activity	x	N/A	x
Poor appetite	x	N/A	x
Rhinitis	x	N/A	x
Nasal congestion	x	N/A	x
Cough	x	N/A	x
Hoarse voice	x	N/A	x
Conjunctivitis	x	N/A	x
Mucus vomiting (retching and throwing up swallowed mucus)	x	N/A	x
Vomiting (throwing up partially digested foods and drinks)	x	N/A	x
Diarrhea	x	N/A	x

N/A = not available

4.2.2 Pain scales (Study I)

After the study physician had interviewed the parents about the occurrence, duration and severity of their child's ear pain (parentally reported ear pain and ear pain reported by child), the pain scales were introduced to the parents.

It is of note that none of the existing pain scales are designed or adapted to detect pain associated with ear infections. Moreover, none of the existing pain observation tools are validated to obtain parent measures of acute and non-surgical pain of young children. Therefore, we selected two well-established pain scales and adapted them for parent observation. First, we chose the Faces Pain Scale-Revised (FPS-R), because it is easy to comprehend and does not require a lot of time or special skills (Herr et al. 2004, Tsze et al. 2013). The FPS-R is originally designed and validated to be a self-report measure to assess the intensity of children's pain from age 4-5 onward (Hicks et al. 2001). Despite its lack of validation for the observational use, the FPS-R has been adapted for global observational ratings by parents and nurses in several previous studies (Manne et al. 1992, Chambers et al. 1998, von Baeyer and Spagrud 2007, Moon et al. 2008, Berberich and Landman 2009, Garten et al. 2010).

Second, we wanted to choose a pain scale that is based on child's observable behavior. Thus, we used the Face, Legs, Activity, Cry, Consolability (FLACC) Scale, a well-established and validated tool, suitable for children of 0-18 years (von Baeyer and Spagrud 2007, Manworren and Stinson 2016). It has low burden, excellent inter-rater reliability, and moderate concurrent validity and it is recommended for pain evaluation in brief painful events (von Baeyer and Spagrud 2007). The FLACC Scale was initially developed to evaluate postoperative pain in young children (Merkel et al. 1997), but it has further been validated for all kinds of pain in preverbal children by nurses (Manworren and Hynan 2003), and it has also been adapted for parental use (Voepel-Lewis et al. 2005). Although there seems to be discrepancies between pain researchers about its clinical utility (Crellin et al. 2015), the FLACC Scale was recently shown to be an appropriate observational tool for assessing acute pain of children from six months to five years of age in a pediatric emergency department (Kochman et al. 2017).

4.2.2.1 Faces Pain Scale-Revised (FPS-R)

First, the study physician showed the FPS-R to the parents (Figure 5). The FPS-R consists of six horizontally positioned faces, representing increasing levels of pain from left ("no pain") to right ("very much pain"), scored from 0-2-4-6-8-10 (Hicks et al. 2001). Scores 0 and 2 were classified as "none or mild", 4 and 6 as "moderate" and 8 and 10 as "severe" pain, respectively (McConahay et al. 2006,

Tsze et al. 2013). The study physician asked parents to show the face which best reflected their child's pain at its worst within the preceding 24 hours.

Figure 5. "Faces Pain Scale – Revised (FPS-R)". www.iasp-pain.org/fpsr. Copyright ©2001, International Association for the Study of Pain®. Reproduced with permission.

4.2.2.2 Face, Legs, Activity, Cry, Consolability (FLACC) Scale

After the pain assessment with the FPS-R, the study physician showed the FLACC Behavioral Scale (Merkel et al. 1997) to the parents (Table 12). The FLACC Scale includes five behavioral categories: facial expression, leg movement, bodily activity, cry or verbalization, and consolability. The parents rated their child's pain at its worst within the preceding 24 hours in each category on a scale of 0 to 2, thus an overall pain score ranging from 0 to 10. The scores from 0 to 3 were classified as "none or mild", from 4 to 6 as "moderate" and from 7 to 10 as "severe" pain or discomfort, respectively (Malviya et al. 2006, McConahay et al. 2006). We used the Finnish translation of the FLACC Scale.

Table 12. FLACC Scale. Each of the five categories Face; Legs; Activity; Cry; Consolability is scored from 0-2 resulting in a total score between 0-10 (Merkel et al. 1997).

Categories	***Scoring***		
	0	***1***	***2***
Face	No particular expression or smile	Occasional grimace or frown, withdrawn, disinterested	Frequent to constant quivering chin, clenched jaw
Legs	Normal position or relaxed	Uneasy, restless, tense	Kicking, or legs drawn up
Activity	Lying quietly, normal position, moves easily	Squirming, shifting back and forth, tense	Arched, rigid or jerking
Cry	No cry (awake or asleep)	Moans or whimpers; occasional complaint	Crying steadily, screams or sobs, frequent complaints
Consolability	Content, relaxed	Reassured by occasional touching, hugging or being talked to, distractable	Difficult to console or comfort

4.2.3 AOM-faces scale

Just before the examination of the child, the study physician asked parents to assess their child's overall condition at its worst within the preceding 24 hours and at study clinic, by using the AOM-faces scale (See Figure 2, page 39).

4.2.4 Examination

After the symptom survey, the study physician performed clinical examination on the child, including careful otoscopic and tympanometric examinations.

4.2.4.1 Otoscopic signs

The study physicians recorded otoscopic signs systematically. Special attention was paid to the following aspects of TM: position, light reflex, translucency, color, increased vascularity, effusion and mobility. The detailed information about the classification of otoscopic signs is presented in Table 13. Figure 6, Figure 7 and Figure 8 illustrate full/slight, moderate and severe bulging of TM, respectively.

Table 13. Classification of otoscopic signs from tympanic membrane (TM) and their descriptions.

Sign	Classification	Description
Position	Retracted	Lateral process of malleus pulled forward
	Normal	Slight concave position
	Full/slight bulging	Convexity increased up to the edges of TM
	Moderate/severe bulging	Convexity increased beyond the edges of TM
Light reflex	Clear and visible	
	Dull, widened	
	Absent	
Translucency	Translucent	Landmarks are visible
	Semiopaque	Parts of TM are opaque
	Opaque	Entire TM is opaque
Color	Pearly gray	
	Grayish	
	Pink	
	Yellow	
	Red	At least part of TM is distinctly red
The degree of TM vascularity	Not increased	
	Increased	
	Strongly increased	
	Hemorrhagic redness	
Effusion	Clear	Transparent effusion without any color
	Serous	Transparent effusion with amber color
	Cloudy	Nontransparent effusion
	Purulent	Nontransparent effusion with obvious yellow color
Mobility	Normal	
	Decreased	
	Immobile	

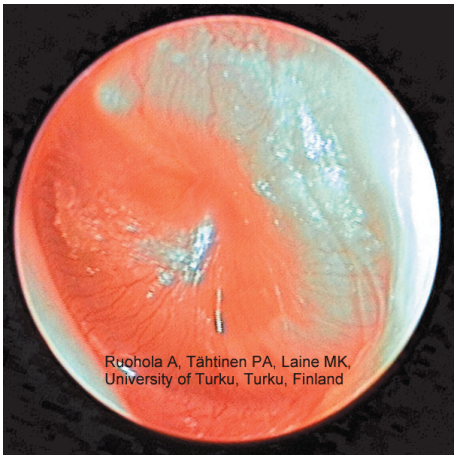


Figure 6. Full/slight bulging of TM. Published with the copyright holder permission.

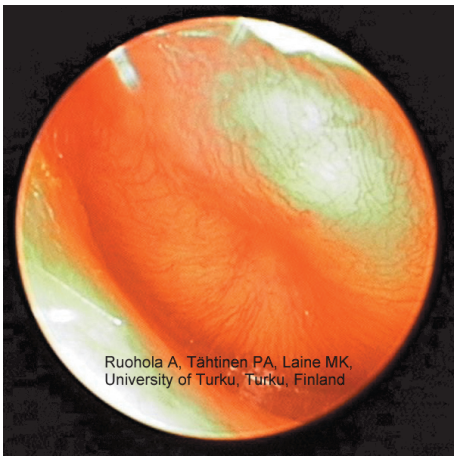


Figure 7. Moderate bulging of TM. Published with the copyright holder permission.

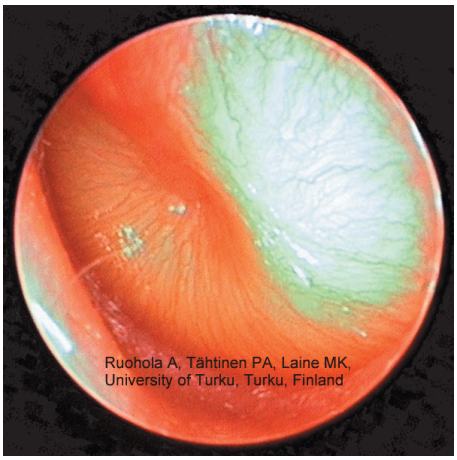


Figure 8. Severe bulging of TM. Published with the copyright holder permission.

4.2.5 Nasopharyngeal sampling, bacterial and viral analyses (Study III)

Nasopharyngeal samples were collected by the study physician with dacron swabs through the anterior nostrils from an average depth of 6.5 cm. Microbes were released from the swab into 0.9% NaCl suspension. Bacterial cultures were done immediately after sampling at the study clinic and S. *pneumoniae, H. influenzae* and *M. catarrhalis* were identified by standard microbiological methods (American Society for Microbiology. 2003). Viruses were analyzed with PCR and viral antigens were also used for virus detection (Ruohola et al. 2013). We detected the following viruses: adenovirus, coronavirus 229E/NL63 and OC43/HKU1, enteroviruses, HBoV, HMPV, influenza A and B viruses, parainfluenza virus (types 1-3), rhinovirus (types A-C) and RSV (types A and B).

4.2.6 Symptom scores (Studies II and III)

We used six symptom scores that are described in more detail in chapter 2.6.8: AOM-SOS, AAP severity of AOM, AOM-FS, OS-8, AOM-Si and the clinical/otologic score. We calculated the scores using the data from symptom questionnaires and from our systematically recorded otoscopic signs.

First, we defined the illness severity of AOM according to the AAP guideline (Lieberthal et al. 2013). The child was considered to have severe AOM if ear pain, reported by parents and/or by child, was moderate/severe and/or the highest measured temperature was ≥39 °C within 24 hours. Otherwise the child was considered to have nonsevere AOM.

Second, to determine the severity of AOM, we used the clinical/otologic score (Dagan et al. 1998, Polachek et al. 2004). The score consisted of four items: temperature, irritability, redness of TM and bulging position of TM. Each item was scored from 0 to 3, for a total range of 0-12.

Third, we assessed the severity of AOM with the AOM-SOS (version 3.0) (Shaikh et al. 2009), which included ear rubbing, excessive crying, irritability, restless sleep, decreased activity, poor appetite, and fever scored as 0 (none), 1 (a little, including our categories mild and moderate), or 2 (a lot, including our category severe). We classified temperature <38 °C as 0 (none), 38.0-38.9 °C as 1 (a little), and >39 °C as 2 (a lot). A score range of 0-14 was the result.

Fourth, child's overall condition was assessed with AOM-FS (range, 0-7) (Friedman et al. 2006) by parents (Figure 2, pp. 39). They assessed their child's overall condition at its worst within the preceding 24 hours, as well as at study clinic.

Fifth, the otoscopic signs were graded with the OS-8 score (range 0-7) (McCormick et al. 2003) (Table 5, pp. 37).

Sixth, to determine the severity of AOM, we combined the two latter scores, namely the AOM-FS and OS-8, to calculate the AOM-Si score (McCormick et al. 2005). The AOM-Si score (range, 1-14) was calculated by summing up the highest AOM-FS at its worst within 24 hours and the highest OS-8 score.

In Study II, we used all the above mentioned symptom severity scores. In Study III, we used the AOM-SOS and AOM-FS.

4.3 Follow-up in children with AOM (days 2-8)

Study IV

Children diagnosed with AOM on day 1 participated in the randomized, double-blind, placebo-controlled trial of the efficacy of antimicrobial treatment, in which they received either amoxicillin-clavulanate (40/5.7mg per kilogram divided into 2 daily doses) or placebo for seven days (Tähtinen et al. 2011). The scheduled study visits were on day 2 or 3 and after one week of the diagnosis of AOM. Moreover, parents were told to contact the study clinic whenever they had any concerns about their child's well-being and additional study visits were arranged. At each visit, the study physician asked parents to assess their child's overall condition as healthy, better, no improvement, or worse. Then the physician examined the child and recorded the otoscopic signs as completely resolved, better, no improvement, worse, or perforation of the TM. The entire follow-up lasted approximately two months, including scheduled study visits two weeks later as well as one month and two months of the diagnosis of AOM.

In Study IV, we included children who received placebo. Children were further distributed into two groups, based on the parental assessment of their overall condition: children with symptomatic improvement and children with symptomatic failure. First, symptomatic improvement was defined as improving overall condition within two to three days and not deteriorating within one week of the diagnosis of AOM. Second, symptomatic failure was defined as overall condition not improving within two to three days or deteriorating within one week of the diagnosis of AOM. Between the two groups, we compared the otoscopic signs at the end-of-treatment visit, which was one week (day 8) later or earlier if the study drug was ceased and an open-label antimicrobial treatment was initiated due to symptomatic treatment failure. The treatment trial defined symptomatic treatment failure as a composite outcome consisting of six independent compo-

nents: no improvement in overall condition by the first scheduled visit (day 3), a worsening in the child's overall condition at any time, no improvement in otoscopic signs by the end-of-treatment visit on day 8, perforation of the TM at any time, severe infection necessitating systemic open-label antimicrobial treatment at any time, and any other reason for stopping the study drug at any time (Tähtinen et al. 2011).

4.4 Statistical analysis

Statistical analyses were performed in Study II using SPSS 16.0 statistical package (SPSS Inc., Chicago, Illinois, USA), and SPSS version 22.0 (IBM SPSS Statistics, IBM Corporation, Armonk, NY) in Studies I, III and IV.

Study I

We used the χ^2 test or Fisher's test as applicable to compare the severity of pain between three parental pain assessment methods (the FPS-R, the FLACC Scale and the parental interview) and between children with and without AOM, respectively. In addition, we used multivariable logistic regression models. First, we calculated the ORs with 95% CI for AOM. We adjusted multivariable models by age (1 month as a unit), use of analgesics (yes vs. no) and the duration of parental suspicion of AOM (1 hour as a unit). Second, we calculated the ORs (with 95% CI) for moderate/severe pain. Symptoms (yes vs. no) were included into the models as covariates. We adjusted multivariable models by age (1 month as a unit), diagnosis of AOM (yes vs. no), and use of analgesics (yes vs. no).

Study II

We used the χ^2 test or Fisher's test as applicable to compare symptoms and otoscopic signs between children with bilateral and unilateral AOM, respectively. We compared the medians using the Mann-Whitney U test. Logistic regression model was used to calculate the ORs with 95% CI. We adjusted logistic regression model by age (1 month as a unit).

Study III

In the analyses, the following viruses were grouped: parainfluenza viruses (types 1-3), coronaviruses (229E/NL63 and OC43/HKU1) and influenza viruses (A and B). In order to study associations between symptoms and nasopharyngeal pathogenic bacteria and respiratory viruses, we used the χ^2 test. In addition, we used multivariable logistic regression models. From the multivariable models, 14 out of 426 children were excluded due to partly missing microbiologic data. With the

multivariable models, we calculated the ORs with 95% CI for each symptom. Nasopharyngeal pathogenic bacteria (*S. pneumoniae, H. influenzae and M. catarrhalis*) and respiratory viruses (rhinovirus, HBoV, RSV, parainfluenza viruses, coronaviruses, adenovirus, enteroviruses, HMPV, influenza viruses) were included into the models as covariates. We adjusted the models by age (1 month as a unit), diagnosis of AOM (yes vs. no) and use of antipyretics (yes vs. no). We also studied the association between *M. catarrhalis* and respiratory viruses. Thus, the OR with 95% CI for *M. catarrhalis* was calculated.

Study IV

We used the χ^2 test to compare the otoscopic signs between children with symptomatic improvement and children with symptomatic failure. We calculated OR with 95% CI for the worsening of otoscopic signs in children with symptomatic improvement by using a binary logistic regression model.

5 RESULTS

5.1 Detection of ear pain with parentally used pain scales and symptoms associated with moderate/severe pain (Study I)

The flow chart of the study population is presented in Figure 3 (pp. 57). Of 426 children, 201 (47%) had AOM and 225 (53%) had no AOM. The distributions of the FPS-R and FLACC Scale scores in children with AOM (AOM group) and without AOM (non-AOM group) are presented in Figure 9 and in Figure 10.

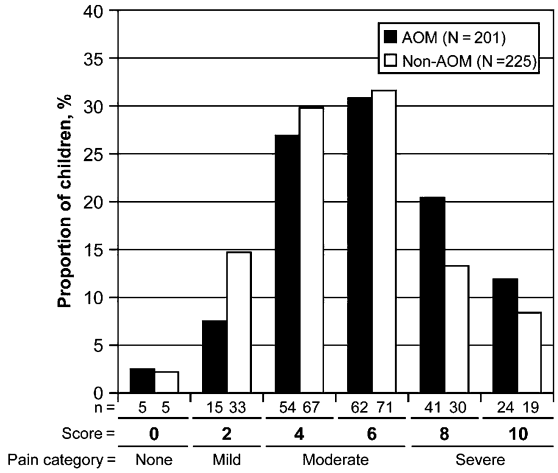


Figure 9. Distribution of the Faces Pain Scale-Revised (FPS-R) scores in children in the AOM group and in children in the non-AOM group. The numbers below the bars show the number of children with the score, indicating the numerator (n).

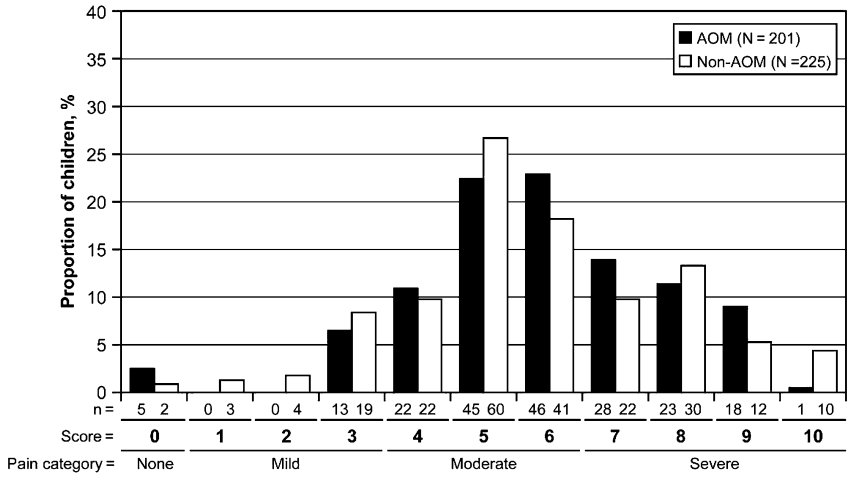
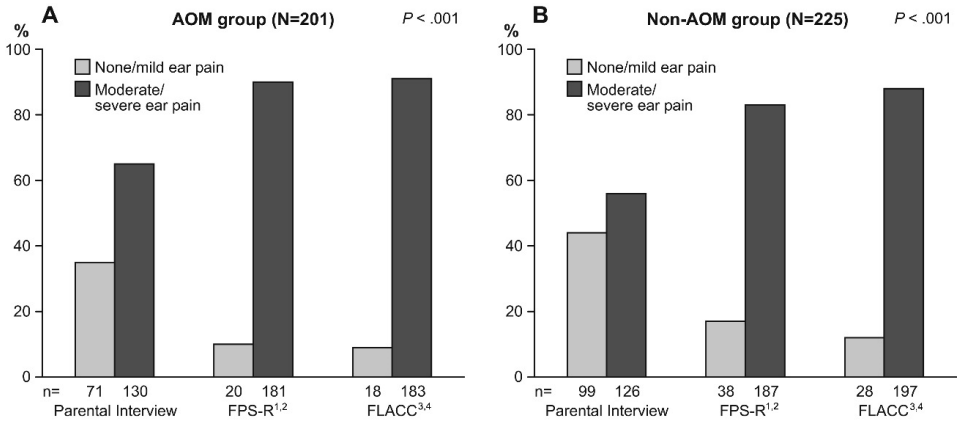


Figure 10. Distribution of the Face, Legs, Activity, Cry, Consolability (FLACC) Scale scores in children in the AOM group and in children in the non-AOM group. The numbers below the bars show the number of children with the score, indicating the numerator (n).

Severity of ear pain in the AOM group and in the non-AOM group

In both groups, parents assessed their child's ear pain significantly more often as moderate/severe with the pain scales (the FPS-R and the FLACC Scale) than with the parental interview (Figure 11).



[1] Scores 0 and 2 were classified as none/mild pain, and scores 4, 6, 8 and 10 as moderate/severe pain.

[2] P < 0.001 for the comparison between none/mild pain and moderate/severe ear pain, assessed by parents with the FPS-R and via interview.

[3] Scores from 0 to 3 were classified as none/mild pain, and scores from 4 to 10 as moderate/severe pain.

[4] P < 0.001 for the comparison between none/mild pain and moderate/severe ear pain, assessed by parents with the FLACC Scale and via interview.

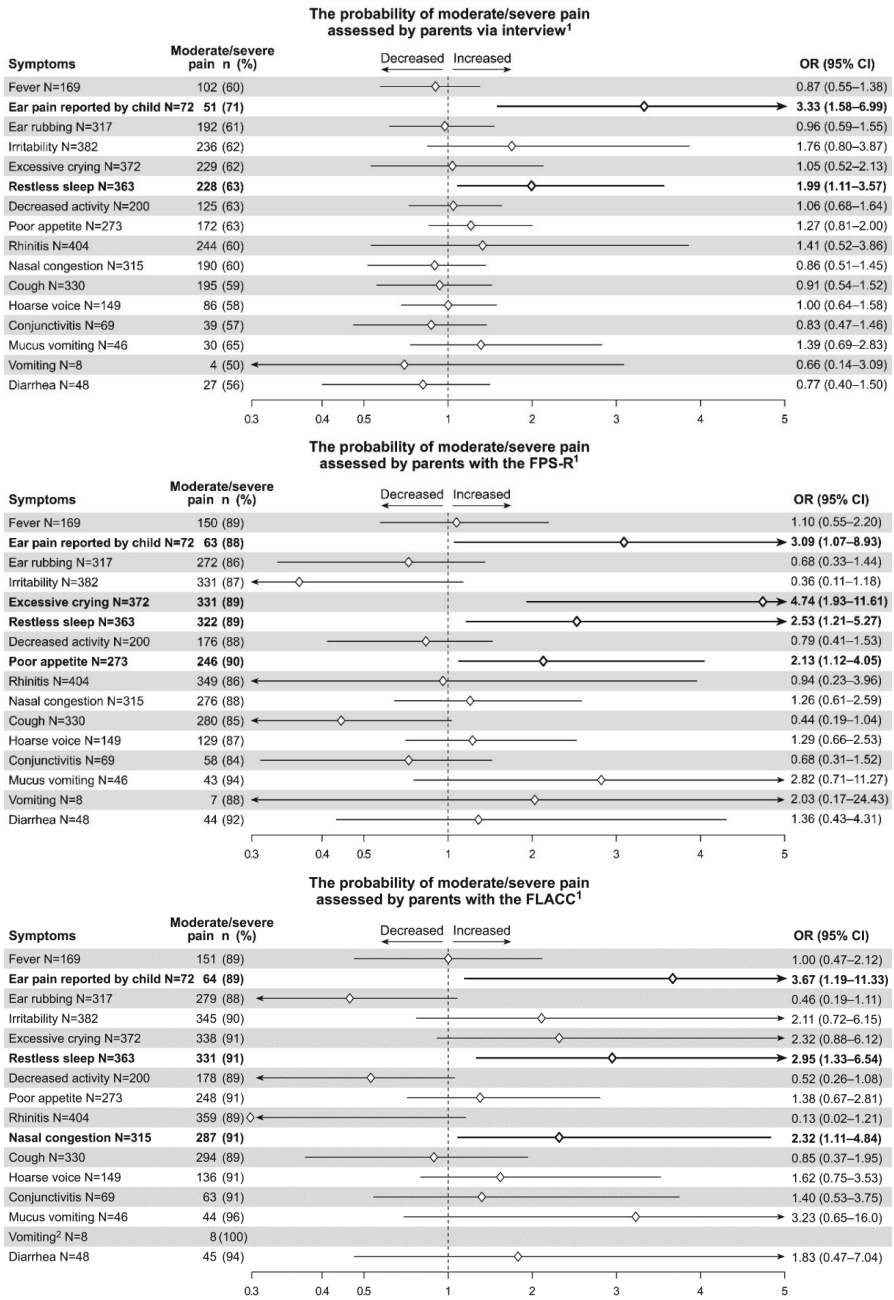
Figure 11. The occurrence of none/mild and moderate/severe pain in the AOM group (A) and in the non-AOM group (B), assessed by parents via interview, with the Faces Pain Scale-Revised (FPS-R) and Face, Legs, Activity, Cry, Consolability (FLACC) Scales. The figure has been published in Original Publication I.

Comparison of moderate/severe ear pain between AOM group and non-AOM group

First, when parents were interviewed about their child's pain, moderate/severe ear pain was reported in 65% (130/201) of the children in the AOM group, compared with 56% (126/225) in the non-AOM group (P=0.07). The adjusted OR for AOM was 1.32 (95% CI, 0.88-1.98) in children with moderate/severe ear pain, assessed by parents via interview. Second, when parents assessed their child's ear pain with the FPS-R, moderate/severe ear pain was reported in 90% (181/201) of the children in the AOM group, compared with 83% (187/225) in the non-AOM group (P=0.04). The adjusted OR for AOM was 1.75 (95% CI, 0.97-3.15) in children with moderate/severe ear pain, assessed by parents with the FPS-R. Third, when parents assessed their child's pain with the FLACC Scale, moderate/severe ear pain was reported in 91% (183/201) of the children in the AOM group, compared with 88% (197/225) in the non-AOM group (P=0.25). The adjusted OR for AOM was 1.46 (95% CI, 0.77-2.75) in children with moderate/severe ear pain, assessed by parents with the FLACC Scale.

Association of individual symptoms with moderate/severe ear pain in all the 426 children with the suspicion of AOM

The associations between symptoms and moderate/severe pain in all children are displayed in Figure 12. All the pain assessment methods (parental interview, the parental pain assessment with the FPS-R and with the FLACC Scale) showed that the presence of ear pain, as reported by the child, and the presence of restless sleep, as reported by parents, had significant associations with parentally assessed moderate/severe pain.



[1] Diamonds indicate OR, lines 95% CI, arrows are added when CI is beyond the scale. The figure is modified from the submitted manuscript.

[2] The association of moderate/severe pain with the symptom was 100%.

Figure 12. The occurrence and probability of moderate/severe pain, assessed by parents via interview, with the FPS-R and FLACC Scales, in relation to the presence of 15 parentally reported symptoms and ear pain reported by children in 426 children with the suspicion of AOM, analyzed with a multivariable logistic regression model and adjusted for age, diagnosis of AOM and use of analgesics. The figure has been published in Original Publication I.

5.2 Symptoms and otoscopic signs of bilateral and unilateral AOM (Study II)

Study population consisted of 232 children (Figure 3, pp. 57). Of those, 98 had bilateral AOM and 134 children had unilateral AOM. Children with bilateral AOM were younger than children with unilateral AOM, age < 24 months was recorded in 87% vs. 75% of the children with bilateral and unilateral AOM, respectively (P=0.032). The occurrence of symptoms was similar in children with bilateral and unilateral AOM, apart from two symptoms (Figure 13). First, ear pain reported by child was less common in children with bilateral AOM than in children with unilateral AOM (11% vs. 25%; P=0.010). However, when adjusting with age, the difference was no longer detected between the groups (adjusted OR, 0.64; 95% CI, 0.24-1.69; P=0.368). Second, fever was more frequently observed in children with bilateral AOM than in children with unilateral AOM (54% vs. 36%; P=0.006), age-adjusted OR for fever in the bilateral AOM group was 2.26 (95% CI, 1.31-3.91; P=0.003). The highest measured mean temperature was 38.8 °C in children with bilateral and 38.6 °C in children with unilateral AOM (P=0.035). Similarly, of the febrile children, temperature ≥39 °C was measured in 44% (21/48) and 20% (8/41) of the children with bilateral and unilateral AOM, respectively (P=0.015).

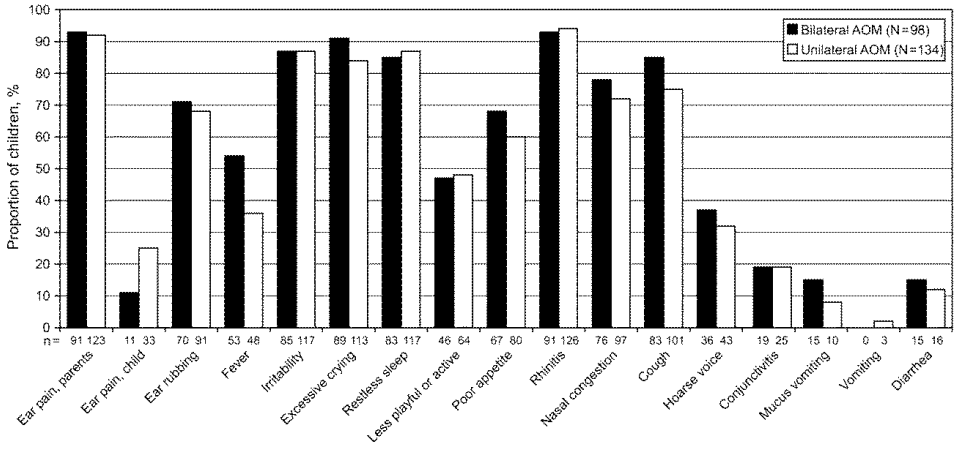


Figure 13. Occurrence of symptoms in children with bilateral and unilateral AOM. The figure has been published in Original Publication II. Reproduced with permission from Journal *Pediatrics*, Vol. 131, Pages e398-e405, Copyright © 2017 by the AAP.

Otoscopic signs of TM in children with bilateral and unilateral AOM are shown in Table 14. Briefly, moderate/severe bulging of TM and purulent effusion were more often detected in children with bilateral AOM than in children with unilateral AOM. On the other hand, bulla formation and hemorrhagic redness were equally seldom observed in children with bilateral and unilateral AOM.

Table 14. Otoscopic signs of tympanic membrane in children with bilateral and unilateral AOM.

	Bilateral AOM (N=98)	Unilateral AOM (N=134)	P
Moderate/severe bulging, n (%)	62 (63)	54 (40)	0.001
Purulent effusion, n (%)	87 (89)	95 (71)	0.001
Bulla formation, n (%)	11 (11)	13 (10)	0.707
Hemorrhagic redness, n (%)	7 (7)	14 (10)	0.386

The OS-8 score, grading the severity of otoscopic signs, ranged from 4 to 7. Scores 6 or 7 were recorded more often in children with bilateral AOM than in children with unilateral AOM (59% vs. 37%; P=0.001). The symptom score results are presented in Figure 14. The median clinical/otologic score was statistically significantly higher in children with bilateral AOM than in children with unilateral AOM (4.5 and 4.0; P=0.003). Of the other symptom scores, the median scores did not significantly differ between children with bilateral and unilateral AOM. According to the AAP guideline definition for illness severity classification, 69 (70%) children with bilateral and 90 (67%) with unilateral AOM had severe illness (P=0.599).

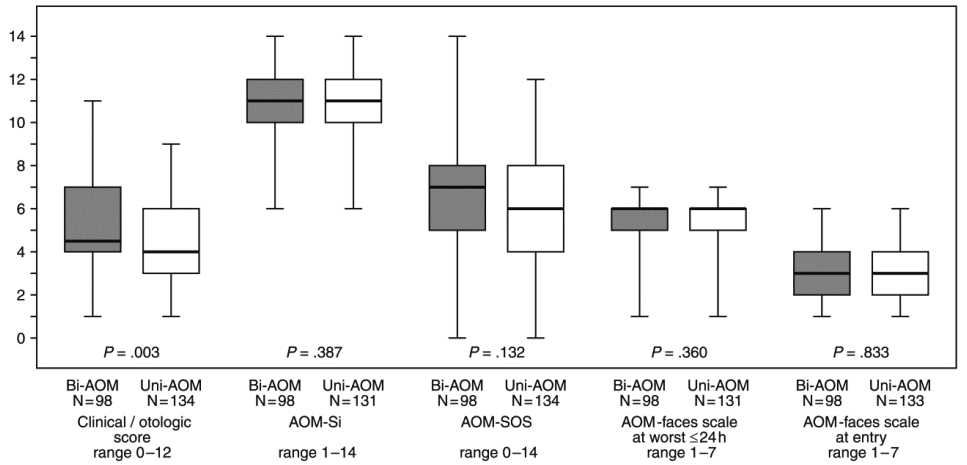


Figure 14. Distributions of the score values in bilateral (Bi-AOM) and unilateral (Uni-AOM) AOM. The box plots show the 25th, 50th (median) and 75th quartiles together with the minimum and maximum values of each score. The figure has been published in Original Publication II. Reproduced with permission from Journal *Pediatrics*, Vol. 131, Pages e398-e405, Copyright © 2017 by the AAP.

5.3 Role of nasopharyngeal bacteria and respiratory viruses in acute symptoms of young children with the suspicion of AOM (Study III)

Detection of the nasopharyngeal pathogenic bacteria and respiratory viruses

The study population was comprised of 426 children. Of those, 201 (47%) had AOM (Figure 3, pp. 57). First, the detection rate for any of the pathogenic bacteria (*S. pneumoniae, H. influenzae and/or M.catarrhalis*) was 87% in 426 children (372/426). *M. catarrhalis* was the most common bacterium, detected in 77% (287/372) of the children, followed by *S. pneumoniae* in 58% (215/372) and *H. influenzae* in 20% (75/372) of the children. Second, the detection rate for any of the respiratory viruses was 89% (379/426 children). Rhinovirus was the most prevalent virus found in 75% (286/379) of the children. The detection rates for the rest of the viruses are shown in Table 15. Among children with any of the respiratory viruses detected, a single virus was detected in 57% (216/379) and multiple viruses in 43% (163/379) of the children.

Among children with any nasopharyngeal pathogenic bacteria detected, the co-detection rate of respiratory viruses was 88% (328/372). Among children with any respiratory viruses detected, the co-detection rate of nasopharyngeal pathogenic bacteria was 87% (328/379). Correspondingly, among children with *M. catarrhalis* detected, respiratory viruses were co-detected in 89% (256/287). No significant associations between *M. catarrhalis* and respiratory viruses were found (Table 15).

Table 15. Association between *M. catarrhalis* and respiratory viruses. Unpublished results.

Respiratory viruses	Odds ratio for M. catarrhalis	95% CI
Rhinovirus (n=286)	0.91	0.57-1.47
Human bocavirus (n=108)	1.06	0.64-1.74
Respiratory syncytial virus (n=36)	0.91	0.43-1.92
Parainfluenza viruses (n=43)	1.12	0.56-2.26
Coronaviruses (n=33)	1.09	0.49-2.43
Adenovirus (n=32)	0.59	0.28-1.25
Enteroviruses (n=20)	2.61	0.73-9.36
Human metapneumovirus (n=20)	0.71	0.26-1.91
Influenza viruses (n=14)	0.95	0.30-3.00

Occurrence of symptoms

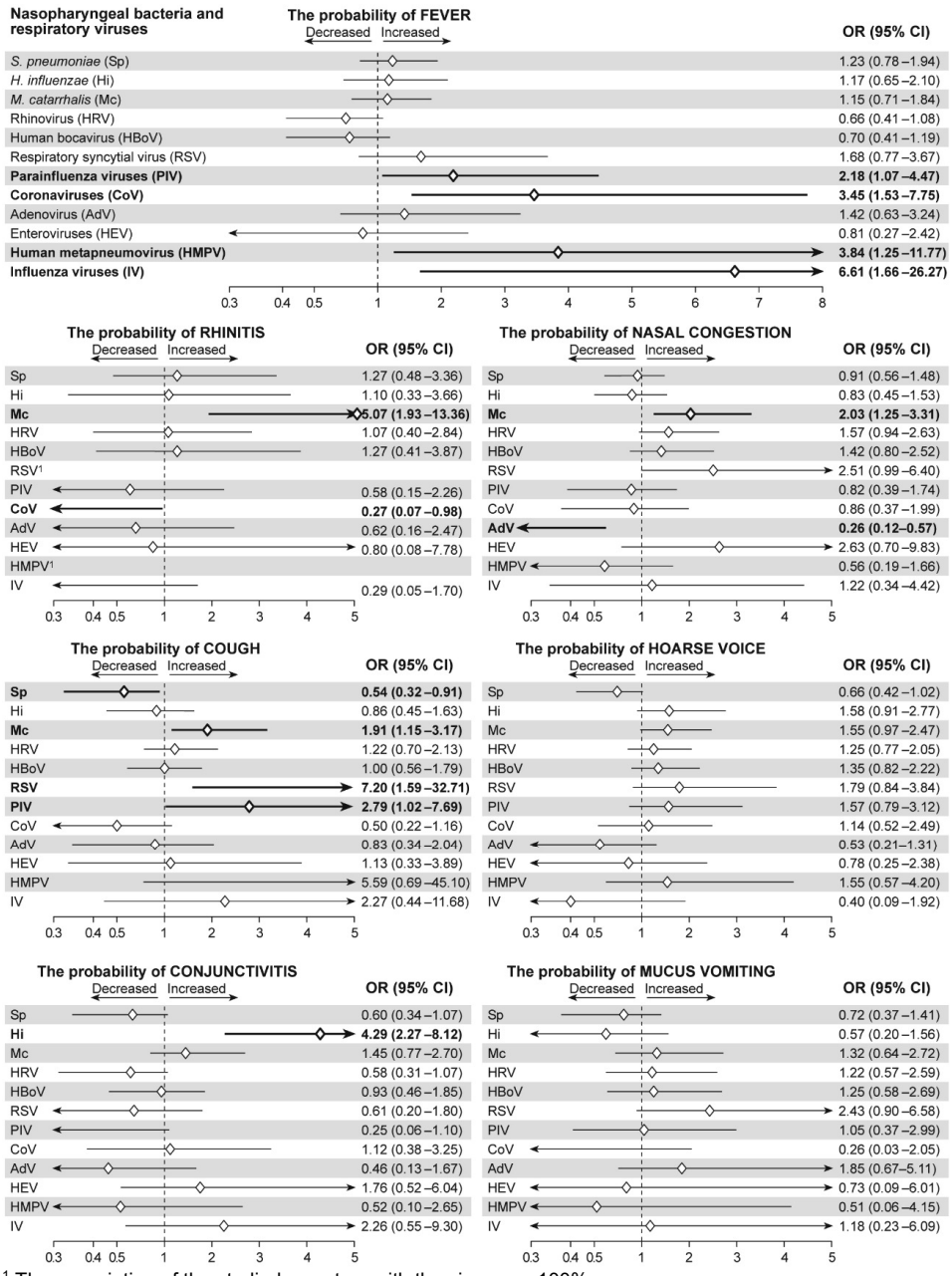
Fever occurred in 40% (169/426) of the children. In relation to the detection of respiratory viruses, fever occurred in 49% (79/163) of the children with multiple

respiratory viruses, in 33% (71/216) of the children with single virus and in 40% (19/47) of the children with no viruses detected (P=0.009). In relation to the detection of pathogenic bacteria, fever occurred in 43% (79/183) of the children with multiple bacteria, in 39% (74/189) of the children with a single bacterium and in 30% (16/54) of the children with no bacteria (P=0.199). Symptoms of RTI, namely rhinitis, nasal congestion and cough occurred in 95% (404/426), 74% (315/426) and 78% (330/426) of the children.

Probability of symptoms

The probability of fever and six respiratory symptoms in relation to nasopharyngeal pathogenic bacteria and respiratory viruses are presented in Figure 15. Fever was positively associated with influenza viruses, HMPV, coronaviruses and parainfluenza viruses. Symptoms of common cold, such as rhinitis, nasal congestion and cough, were positively associated with *M. catarrhalis*. Moreover, cough had positive associations with RSV and parainfluenza viruses and a negative association with *S. pneumoniae.* Finally, rhinitis had a negative association with coronaviruses and nasal congestion had a negative association with adenovirus.

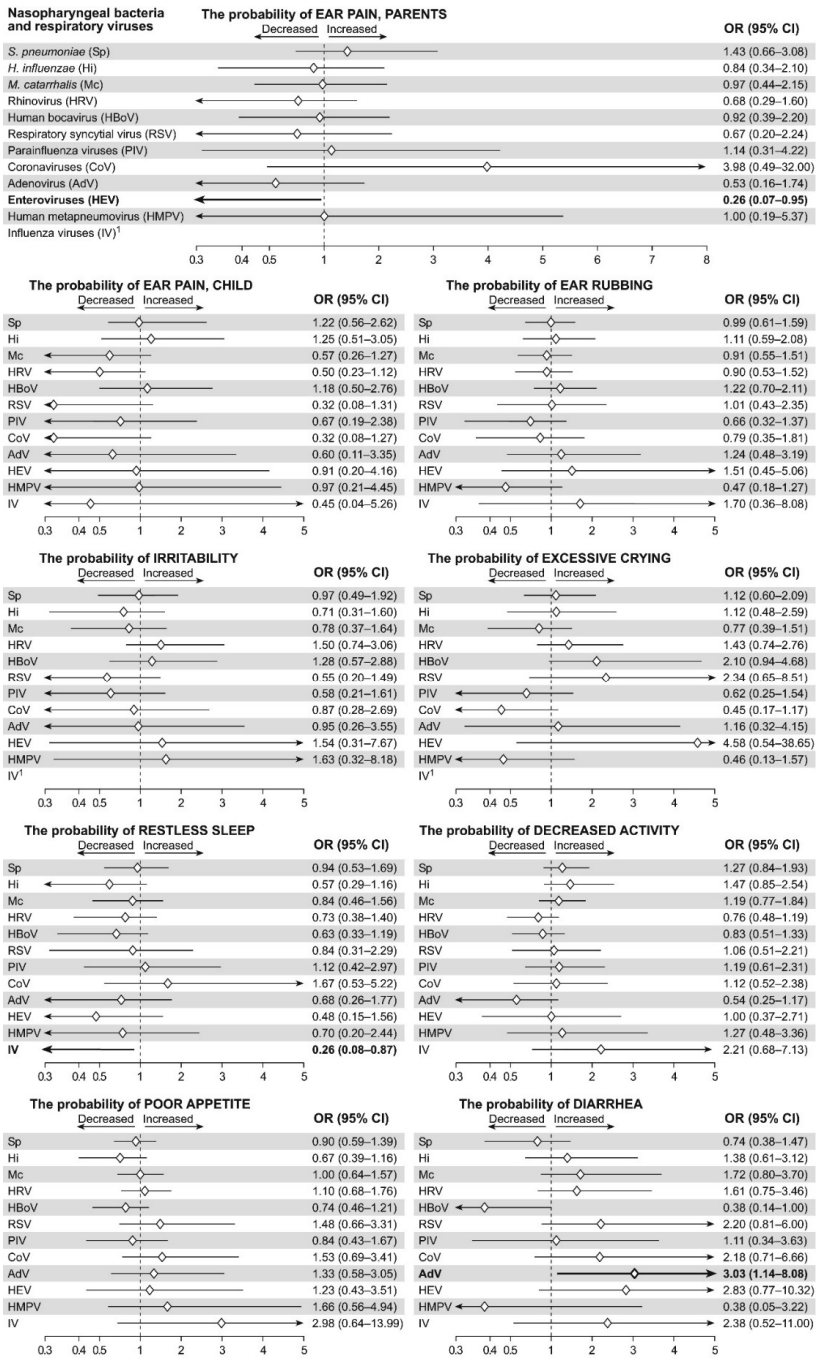
The probability of ear-related, non-specific and gastrointestinal symptoms in relation to nasopharyngeal pathogenic bacteria and respiratory viruses are shown in Figure 16. Briefly, parentally reported ear pain was negatively associated with enteroviruses, restless sleep was negatively associated with influenza viruses and diarrhea was positively associated with adenovirus.



[1] The association of the studied symptom with the virus was 100%.

The figure has been published in Original Publication III. Reproduced with permission from *The Pediatric Infectious Disease Journal*, Vol. 34, Pages 1056-1062.

Figure 15. The probability of fever and 6 respiratory symptoms in relation to nasopharyngeal pathogenic bacteria and respiratory viruses in young children, analyzed with multivariable logistic regression model. Diamonds indicate odds ratio (OR), lines indicate 95% confidence interval (CI) and arrows are added when CI is beyond the scale.



[1] The association of the studied symptom with the virus was 100%.

The figure has been published in Original Publication III. Reproduced with permission from *The Pediatric Infectious Disease Journal*, Vol. 34, Pages 1056-1062.

Figure 16. The probability of ear-related, non-specific and gastrointestinal symptoms in relation to nasopharyngeal pathogenic bacteria and respiratory viruses in young children, analyzed with multivariable logistic regression model. Diamonds indicate odds ratio (OR), lines 95% confidence intervals (95% CI), arrows are added when CI is beyond the scale.

5.4 Close follow-up in children with AOM initially managed without antimicrobials (Study IV)

In Study IV, we included 158 children receiving placebo. Children were further divided into two groups, based on their overall symptomatic condition (Figure 3, pp. 57). In children with symptomatic improvement, worsening of otoscopic signs, including perforation of TM, occurred in 3% (3/104) of the children. On the contrary, in children with symptomatic failure, otoscopic signs worsened or TM perforated in 30% of the children (P<0.001) (Table 16). In children with symptomatic improvement, the OR for the worsening of otoscopic signs during the 1-week follow-up was 0.07 (95% CI, 0.02-0.26). The development of contralateral AOM was similar between the groups.

Table 16. Development of otoscopic signs during follow-up at one week. The table is modified from the Original Publication IV and contains partly unpublished results.

	Children with symptomatic improvement (N=104)	**Children with symptomatic failure (N=54)**	**P**
Development of otoscopic signs during follow-up at one week, n (%)[1]			< .001
Worse or perforation[2]	3 (3)	16 (30)	
No improvement	15 (14)	26 (48)	
Completely resolved or better	86 (83)	12 (22)	
Development of contralateral AOM, n (%)[3]	19 (18)	10 (19)	.88

[1]Otoscopic signs were recorded before the 1-week control visit in 4 children with symptomatic improvement and in 36 children with symptomatic failure due to the cessation of the study drug.

[2]Perforation occurred in 2 children with symptomatic improvement and in 3 children with symptomatic failure. In children with symptomatic improvement, the perforation was diagnosed at a scheduled follow-up visit 48 hours after the day of diagnosis with AOM. The parents had not noticed any ear discharge and assessed their child's overall condition as better than at the time of diagnosis.

[3]Data were missing in 2/54 children with symptomatic failure due to obstructive cerumen.

6 DISCUSSION

6.1 Detection of ear pain with parentally used pain scales and symptoms associated with moderate/severe pain (Study I)

The basis for this study was the fact that no research is conducted to measure ear pain with pain scales, although their use is highly recommended (American Academy of Pediatrics. Committee on Psychosocial Aspects of Child and Family Health and Task Force on Pain in Infants, Children, and Adolescents 2001) and reliable assessment of ear pain (as mild, moderate or severe) is required for the optimal management of AOM (Lieberthal et al. 2013). The assessment of ear pain tends to be particularly tricky in children younger than three years, who are mainly preverbal and unable to express their pain verbally. Young children with AOM may thus be at risk of suffering from undetected pain if proper pain assessment tools are not used. Therefore, we adapted two pain scales for parent observation and investigated whether they are useful in detecting ear pain in young children with the parental suspicion of AOM.

Parentally assessed ear pain via physician's interview, with the FPS-R and FLACC Scale in children with RTI

The main finding of our study was that parents may underestimate pain in young children with RTI, either with or without AOM, if they do not use pain scales. When parents assessed their children's pain with pain scales, the great majority of children with RTI seemed to suffer from moderate/severe pain. In contrast, when parents assessed their child's pain via physician's interview, moderate/severe pain was reported only in two thirds of the children with RTI. The difference in the results between the pain assessment methods is fairly obvious and thus, explanations are needed. Children's self-reports, held as the primary source for estimates of pain intensity, were not available (Twycross et al. 2015). Thus, the ultimate truth of the pain intensity in young children remains a mystery. Parents may overestimate their child's pain with the faces scales and FLACC Scale (Chambers et al. 1999, Voepel-Lewis et al. 2005), although underestimation with the faces scales and parental interview has likewise been reported (Bellman and Paley 1993, Chambers et al. 1998). However, parents are considered as most reliable proxy for assessing their children's pain if the child's self-report is not possible, because children express their motions more often in the presence of parents than strangers (von Baeyer and Spagrud 2003), such as health care professionals. Parents are likewise more able to discriminate their child's pain behavior from other aberrant behavior, because they are familiar with their child's normal behavior (Schechter et al. 2002, von Baeyer and Spagrud 2007).

In the light of the recent study of Tsze et al., it could be speculated that our way of classifying children to have moderate pain with the FPS-R scores of 4 and 6 is an overstatement and that only children with the FPS-R scores of 6 should be classified as having moderate pain instead. However, considerable overlap of scores associated with mild and moderate pain could be seen in their study (Tsze et al. 2016) weakening its strength. In our study, moderate/severe pain was detected at the similar rate with both the FPS-R and FLACC Scale, thus suggesting the reliability of our pain category classification for moderate pain with the FPS-R.

On the whole, there seems to be relatively pervasive and systematic tendency for proxy judgments to underestimate the pain experience of others (American Academy of Pediatrics. Committee on Psychosocial Aspects of Child and Family Health and Task Force on Pain in Infants, Children, and Adolescents 2001). Young age of the child may also influence the parental assessment of pain. The study of Pillai Riddell showed that parents interpret younger infants to have less pain compared to older ones, although the behavioral reaction to pain stimuli would be similar and standardized across all age groups (Pillai Riddell and Craig 2007). In line with this, in children younger than two years of age with AOM, parentally reported ear pain rates are shown to be slightly lower than in older children with AOM (Arola et al. 1990, Niemelä et al. 1994, Heikkinen and Ruuskanen 1995, Kontiokari et al. 1998, McCormick et al. 2016). However, it is nowadays generally accepted that young preverbal children may experience an equal amount of pain as older children capable of expressing their pain verbally (Schechter et al. 2002).Taken these together, there is a great concern that parents who do not use pain scales may underestimate the pain of young preverbal children, which may lead to undertreatment of pain. Pain experiences in early childhood have been shown to induce long-term alterations in pain sensitivity, leading children to be hypersensitive to pain later in life (Taddio et al. 1997, Hermann et al. 2006, Wollgarten-Hadamek et al. 2011). Therefore, pain should be systematically assessed with pain scales and actively managed in all young children with RTI. Last but not least, the treatment of pain is considered as a fundamental right of human beings (Mann et al. 1994).

Ear pain in children with and without AOM

Our study further confirmed our previous findings that ear pain is not a differentiating symptom in the diagnostics of AOM in children with parental suspicion of AOM (Laine et al. 2010). Even though pain scales, namely the FPS-R and FLACC Scale, were used by parents, the severity of ear pain was similar in children with RTI either with or without AOM. Indeed, we found children without AOM to suffer from a great amount of distress or pain, when parents assessed

their children's pain using pain scales. At first sight, this may seem unexpected. On second thought, however, symptoms of RTI may likewise cause severe distress in young children. The high frequency of pain or distress has also been described in young adults during common cold by Puhakka et al. (Puhakka et al. 1998). In their study, three out of four young adults suffered from pain, such as sore throat and headache during the first week of viral RTI. We found nasal congestion to be significantly associated with moderate/severe pain, when parents used the FLACC Scale, designed to measure not only pain but also distress behavior. Furthermore, children with RTI may likewise experience ear pain due to the blocked ear and dysfunction of the ET, or ear pain may be referral pain due to pharyngitis, tonsillitis or headache (Kim et al. 2015). Therefore, our study suggests that when parents bring their children to see the physician and suspect them to have AOM, analgesics should be actively offered for children with AOM, but also for children in whom AOM is not diagnosed. Furthermore, our results propose that as ear pain is not a specific symptom of AOM, it should not be used to grade the severity of AOM in young children (Lieberthal et al. 2013).

Association of individual symptoms with parentally assessed moderate/severe pain

As our study shows, ear pain is a complex concept. Our study highlights the difficulties that parents of young children face when interpreting which of the child's symptoms are reflection of pain, due to either ear pain or distress from RTI. In our study, the key symptoms associating with the parental assessment of the child to suffer from moderate/severe pain were ear pain reported by child and restless sleep. These symptoms stood out, regardless of the pain evaluation method. In line with this, restless sleep or fussiness have also previously been related as suggestive of ear pain in preverbal children (Shaikh et al. 2010, Lieberthal et al. 2013). Barber et al. found disturbed sleep and pain to cause significant burden related to young children with AOM (Barber et al. 2014). In contrast, restless sleep has not been shown to resolve significantly faster with the antimicrobial treatment in children with AOM, compared to the treatment with the placebo (Tähtinen et al. 2011). Thus, it seems that restless sleep may reflect general pain and distress due to symptoms of RTI, rather than AOM-related ear pain specifically. Nevertheless, it can be speculated that parents are sensitized to all the disturbing behavior taking place during the night. This may lead parents to falsely interpret restless sleep as a sign of pain, although physicians have conventionally associated it with the child's developmental phases, such as fast motoric development. Our study also indicated that parents associated excessive crying and poor appetite with moderate/severe pain, when they assessed their child's pain with the FPS-R. This was unexpected as poor appetite is commonly held as a sign of worsening in the child's overall condition. Indeed, poor appetite may be

the sign of pain or discomfort due to AOM, because Tähtinen et al. showed poor appetite to resolve faster with the antimicrobial treatment in children with AOM, compared to treatment with placebo (Tähtinen et al. 2011). I suggest that symptoms, such as ear pain reported by child, restless sleep, poor appetite and nasal congestion could be considered as indicators or "red flags" for moderate/severe pain in young children with the suspicion of AOM. In the presence of these symptoms, treatment with analgesics should be given.

To conclude, ear pain seems to be underestimated by parents without the use of pain scales. What is more, ear pain is equally severe in children with and without AOM, although parents assessed their child's pain with pain scales. These results indicate that ear pain cannot be regarded as a specific symptom for AOM in young children. Correspondingly, as opposed to the AAP guideline (Lieberthal et al. 2013), our study addresses the fact that the presence or absence of ear pain or the severity of ear pain should not define the management of AOM in young children. Finally, children without AOM seem to suffer from an unexpected amount of distress and pain when their parents suspect them to have AOM. This should not be disregarded.

6.2 Symptoms and otoscopic signs of bilateral and unilateral AOM (Study II)

The basis for this study was the general conception that bilateral AOM is clinically more severe illness than unilateral AOM and therefore more active antimicrobial treatment and/or follow-up is recommended in young children with bilateral AOM by several national guidelines (Tan et al. 2008, Marchisio et al. 2010, Solen and Hermansson 2011, Lieberthal et al. 2013, Heikkinen et al. 2017). However, clinical characteristics in children with bilateral and unilateral AOM had not been comprehensively investigated. We investigated individual symptoms and otoscopic signs in children with bilateral and unilateral AOM and we also used symptom scores to compare the severity of illness in children with bilateral and unilateral AOM (See Methods 4.2.6). Ultimately, we wanted to test the hypothesis that bilateral AOM is clinically more severe illness than unilateral AOM.

Symptoms

The main finding of our study was that the symptoms did not differ between children with bilateral and unilateral AOM. It could be debated that a child's verbally expressed ear pain was more common in children with unilateral AOM than in children with bilateral AOM. However, children with unilateral AOM

were older in our study, which makes them more capable of expressing their pain. Indeed, after adjusting for age, the association between child's verbal expression of ear pain and unilateral AOM was no longer detected. Our study also showed that parents assessed ear pain more often as severe in children with unilateral AOM compared with children with bilateral AOM. However, this may also be explained by the better verbal skills of children with unilateral AOM. Certainly, if children are able to verbally express their pain, it seems natural that parents automatically interpret pain to be severe. On the other hand, moderate/severe pain, which is one of the criteria for severe illness according to the AAP guideline (Lieberthal et al. 2013), was equally commonly reported in children with bilateral and unilateral AOM (66% and 69%, respectively). Hence, ear pain was not a differentiating symptom between children with bilateral and unilateral AOM.

Fever was the only symptom that differed between children with bilateral and children with unilateral AOM. It occurred more often in children with bilateral AOM and fever was also slightly higher in children with bilateral AOM than in those with unilateral AOM. Fever and its relation to bilateral AOM have not been previously reported. On the other hand, Leibovitz et al. did not investigate fever at all, because all children were febrile according to their diagnostic criteria of AOM (Leibovitz et al. 2007). Then again, McCormick et al. reported only the temperature that was measured at study clinic and many children had received antipyretic medication before the measurement (McCormick et al. 2007), which may obviously have an effect on the results. We, however, investigated the occurrence of fever by interviewing parents and also accepted if they assessed their child to have fever without measuring it. For the highest measured temperature, however, we included only children whose temperature was actually measured.

Fever has been traditionally held as the sign of a bacterial etiology in children with AOM (Howie et al. 1970, Rodriguez and Schwartz 1999, Palmu et al. 2004). On the other hand, fever is shown to be a sign for viral infection during RTI (Putto et al. 1986, Arola et al. 1990). Nevertheless, the resolution of fever has been shown to be accelerated with the antimicrobial treatment in children with AOM, compared to children receiving placebo (Tähtinen et al. 2011). Hence, fever has been commonly held as the sign of severity in children with AOM (Lieberthal et al. 2013). Since children with bilateral AOM had more often fever, this supports the hypothesis that bilateral AOM would be clinically more severe illness than unilateral AOM. But at the end of the day, only half of the children with bilateral AOM had fever. Thus, fever cannot be regarded as the dominating symptom in children with bilateral AOM. On the whole, it seems that the symptomatic burden is only slightly greater in children with bilateral AOM, compared to children with unilateral AOM.

Otoscopic signs

Moderate/severe bulging of TM and purulent effusion were significantly more often detected in children with bilateral than with unilateral AOM. Moreover, children with bilateral AOM had higher OS-8 scores in our study, as well as in the study of McCormick et al., which mainly reflects the degree of TM bulging (McCormick et al. 2007). Indeed, a bulging TM increases the odds that MEE contains bacteria (Halsted et al. 1968, McCormick et al. 2000, Leibovitz et al. 2003, Palmu et al. 2004). Furthermore, antimicrobial treatment has been shown to be most beneficial in children with severe bulging of TM (Hoberman et al. 2011, Tähtinen et al. 2017). Similarly, purulent effusion is suggested to indicate bacterial growth in MEE (McCormick et al. 2000, Palmu et al. 2004, Holder et al. 2015). Since moderate/severe bulging of TM and purulent effusion behind TM are indicators of bacterial etiology of AOM, these findings support the hypothesis that bilateral AOM is more often of bacterial etiology, compared to unilateral AOM (Howie et al. 1970, Leibovitz et al. 2007, McCormick et al. 2007). Nevertheless, other severe otoscopic signs suggesting bacterial etiology, namely bulla formation and hemorrhagic redness (Palmu et al. 2001, Rosenblut et al. 2001), were equally seldom recorded in children with bilateral and unilateral AOM. Of note, bulla formation in children with AOM is related to the greater symptom burden than AOM without bulla formation (Kotikoski et al. 2003, McCormick et al. 2003). Thus, it seems that severe otoscopic signs are not entirely related to bilateral AOM.

Symptom scores

The symptom scores did not differentiate children with bilateral from children with unilateral AOM. Not even the severity of illness according to the definition of the AAP guideline was different between the groups. Only the clinical/otologic score reached a statistically significant difference, being slightly higher in children with bilateral than in those with unilateral AOM (4.5 vs. 4.0). This is in line with the study of Leibovitz et al. (Leibovitz et al. 2007). The higher clinical/otologic scores in children with bilateral AOM probably reflect the degree of TM bulging. In addition, the difference in the clinical/otologic scores between the groups was clinically modest in both studies and as Leibovitz et al. have themselves stated, this score seems to have only limited clinical use (Leibovitz et al. 2003, Leibovitz et al. 2007). Hence, based on the symptom score results, illness severity was similar between children with bilateral AOM and unilateral AOM. It may be debatable though if the symptom score results reflect equal severity of bilateral and unilateral AOM or whether the scores are simply unable to detect differences.

Our results suggest that bilateral AOM is clinically only slightly more severe illness than unilateral AOM. Worth noting, AOM is a dynamic disease and otoscopic signs change on a daily basis (Isaacson 1996, Kalu et al. 2011). Children with unilateral AOM may develop bilateral AOM (Ruohola et al. 2003, Tähtinen et al. 2011). Of the bilateral AOM episodes, inflammation of both TMs has been shown to be at different stages in over half of the cases (Kalu et al. 2011). Also symptoms and their severity may change daily. Thus, the illness severity assessment only once at the time of diagnosis of AOM, as we did, does not reflect the whole spectrum of the illness. On the other hand, Rovers et al. investigated symptoms of pain and fever on 3-7 days after the diagnosis and treatment of AOM and concluded young children with bilateral AOM to benefit more from antimicrobial treatment, regarding the resolution of symptoms, than young children with unilateral AOM. Also the study of Hoberman et al. found bilaterality of AOM to predict treatment failure in young children (Hoberman et al. 2011), whereas the study of Tähtinen et al. had discordant results (Tähtinen et al. 2011, Tähtinen et al. 2017). As a matter of fact, when Hoberman and Tähtinen combined their study results afterwards, they concluded that all the children younger than two years of age would benefit from antimicrobial treatment, irrespective of laterality (Hoberman et al. 2013). Besides the age, also severe bulging, exposure to other children and the symptomatic burden have been shown to be stronger predictors of treatment failure than bilaterality (Hoberman et al. 2011, Tähtinen et al. 2017).

That said, it seems rather justified to state that laterality of AOM should not be used alone as a determining factor to assess the severity of AOM and to decide the management of AOM. We would rather suggest that each child with AOM should be managed after careful deliberation by the physician, taking into account the overall symptomatic condition of the child and otoscopic signs.

6.3 Role of nasopharyngeal bacteria and respiratory viruses in acute symptoms of young children with the suspicion of AOM (Study III)

The basis of this study was that the spectrum of symptoms is variable in young children with RTI. Even though viral RTI would be complicated by AOM, the presence of AOM cannot explain the variation of symptoms because, as Studies I and II have further confirmed, AOM or its laterality cause no specific symptoms in young children. In contrast, symptoms of AOM and RTI are overlapping, probably due to the underlying viral infection (Ruohola et al. 2013). Hence, we hypothesized that the variation of symptoms would be associated with respiratory

viruses and nasopharyngeal bacteria. None of the previous studies had investigated individual symptoms and their association with both nasopharyngeal bacteria and respiratory viruses. The ultimate aim of the study was to investigate whether the variation of symptoms would be explained by the nasopharyngeal bacteria and respiratory viruses in young children with the suspicion of AOM.

Symptoms of RTI and *M. catarrhalis*

Our study showed that symptoms of RTI, namely rhinitis, nasal congestion and cough, were significantly associated with *M. catarrhalis* in the presence of respiratory viruses. This was an unexpected finding since only respiratory viruses had commonly been blamed for these symptoms (Heikkinen and Järvinen 2003). In our study, only cough was associated with *M. catarrhalis* but also with respiratory viruses, namely with RSV and parainfluenza viruses. Indeed, these viruses are notorious for their tendency to induce troublesome cough for young children (Frost et al. 2014, Hodinka 2016). Consequently, the relation between cough and respiratory viruses can be seen to reflect the overall reliability of our results.

After closer consideration, the association between *M. catarrhalis* and respiratory symptoms is not entirely new. It was already 20 years ago when Kaiser et al. showed that in adults harboring either *S. pneumoniae*, *H. influenzae* or *M. catarrhalis* in their nasopharynx during symptoms of common cold or acute sinusitis, the resolution of symptoms was significantly faster with antimicrobial treatment compared with placebo (Kaiser et al. 1996, Kaiser et al. 2001). The researchers already then suggested that these bacteria may have a pathogenic role in the early stage of common cold (Kaiser et al. 2001).

Since then, there has been more and more evidence supporting the hypothesis that instead of being innocent bystanders, nasopharyngeal bacteria seem to actively contribute to the pathogenesis and development of symptoms in RTI. Obasi et al. investigated adults with RTI and found the increased detection of nasopharyngeal pathogenic bacteria to be related with virus-negative RTI, suggesting that bacterial pathogens contribute to the development of RTI (Obasi et al. 2014). In line with this, the probability of RTI has been shown to increase by 7-fold in children up to one year of age if any of the bacterial pathogen species (*Streptococcus*, *Haemophilus* or *Moraxella*) dominates the nasopharyngeal microbiome, even when adjusted with the detection of viruses (Teo et al. 2015). Early hypopharyngeal bacterial colonization in neonates at one month of age has been associated with an increased risk of pneumonia, bronchiolitis, recurrent wheeze and later development of asthma in early childhood (Bisgaard et al. 2007, Vissing et al. 2013).

The bacterial colonization may likewise negatively affect the severity and duration of wheezing in young children (Jartti et al. 2011). Symptoms, such as wheezing, are largely due to host immune response to infection (Bruder et al. 2006). The host immune response, on the other hand, has been proposed to be altered depending on the bacterial colonization. The study of Foslgaard et al. showed that in asymptomatic neonates of one month of age, bacterial colonization of *M. catarrhalis*, *H. influenzae*, or *S. aureus* triggered a clear topical inflammatory immune response (Folsgaard et al. 2013). Thus, the exposure to bacteria in early life seems to play a crucial part in the maintenance of respiratory health (Man et al. 2017). In summary, it seems nowadays evident that bacteria in the respiratory microbiota have an active role in the immunity and development of symptoms in the respiratory tract.

Of the bacteria, a few studies have investigated the role of *M. catarrhalis* separately. There is evidence that the colonization of *M. catarrhalis* is associated with prolonged cough and increased risk of bacterial complications, such as bacterial sinusitis in children (Darelid et al. 1993, Gunnarsson et al. 2000, Marom et al. 2014), which is in line with our findings. The nasopharyngeal carriage of *M. catarrhalis* during healthy stage has been shown to increase the risk of RTI and to expose infants to suffer from their first RTI at younger age (Teo et al. 2015, Chonmaitree et al. 2016, Chonmaitree et al. 2017). Accordingly, a recent study from Netherlands suggested that colonization of *Moraxella*-dominated strain very early in life is associated with higher number of RTIs (Bosch et al. 2017). On the other hand, the same study group showed that in children who acquired *Moraxella*-dominated colonization later in the infancy, the frequency of parental-reported respiratory infections tended to be lower (Biesbroek et al. 2014). This could be explained by the fact that children who acquired *Moraxella* colonization later in the infancy could also be colonized with *Corynebacterium* and *Dolosigranulum* species, whereas early-life colonization of *Moraxella* species was associated with diminished and less prolonged establishment of these bacteria. *Corynebacterium* and *Dolosigranulum* species are associated with breast-feeding and lower rate of parental-reported respiratory infections (Biesbroek et al. 2014) and thus these bacteria are suggested to be related to better respiratory health in the future (Bosch et al. 2017). Finally, Rodrigues et al. found an association between nasal symptoms and *H. influenzae* in children attending day care. However, it should be noted that they did not investigate *M. catarrhalis* at all. Interestingly, it has been previously suggested that *M. catarrhalis* may have a competitive association with *H. influenzae* in the nasopharynx of young children during RTI, either with or without AOM (Pettigrew et al. 2008, Xu et al. 2012). All in all, it seems plausible that *M. catarrhalis* may contribute to the symptoms of RTI.

M. catarrhalis and respiratory viruses

It is of note that nearly all the children colonized with *M. catarrhalis* were also co-infected with respiratory viruses in our study. Interestingly, respiratory viruses may alter the composition of the nasopharyngeal microbiome (Tregoning and Schwarze 2010) and respiratory viral infections may be associated with different nasopharyngeal microbial profiles (Rosas-Salazar et al. 2016). Furthermore, respiratory viruses seem to increase the density of bacterial pathogens in the nasopharynx during periods of both symptomatic and asymptomatic viral infections (DeMuri et al. 2017). All of these findings together suggest that respiratory viruses need bacteria, particularly *M. catarrhalis*, in the development of viral infections and that they significantly interact with each other. This is further supported by the fact that the colonization of *M. catarrhalis* in healthy children has similar seasonal variation with respiratory viral infections, they both peak during cooler months (Heikkinen and Järvinen 2003, Verhaegh et al. 2011, Teo et al. 2015). Cold shock response of *M. catarrhalis* may partly explain this phenomenon, since cold temperature in the upper respiratory tract is shown to increase the adherence of *M. catarrhalis in vitro* (Heiniger et al. 2005). Thus, it is open to debate whether the increased colonization of *M. catarrhalis* in autumn and winter makes children more prone to be infected with respiratory viruses.

Indeed, Heinrich et al. showed expression of rhinovirus to increase in *M. catarrhalis* infected cells, compared with uninfected cells via a significant down-regulation of TLR3 by *M. catarrhalis* in human bronchial epithelial cells (Heinrich et al. 2016). Chonmaitree et al. showed the risk for RTI to increase in young children in the presence of rhinovirus and *M. catarrhalis* and in the presence of HMPV and *M. catarrhalis* in the nasopharynx. However, the risk for RTI was rather unchanged even in the absence of *M. catarrhalis* (Chonmaitree et al. 2016). The association between rhinovirus and *M. catarrhalis* has likewise been described in asymptomatic children who are otitis-prone (Pitkäranta et al. 2006, Moore et al. 2010). Although we did not find any specific associations between *M. catarrhalis* and respiratory viruses in our study, a growing body of evidence from other studies suggests that *M. catarrhalis* seems to play an active role with respiratory viruses in the nasopharynx. Accordingly, interactions between *M. catarrhalis* and respiratory viruses in the nasopharynx have been shown to alter the risk for both RTI and AOM in infants (Chonmaitree et al. 2016). Taken together, we are not suggesting that respiratory symptoms would be entirely due to *M. catarrhalis*. In contrast, there seems to be a complex interplay between *M. catarrhalis* and respiratory viruses in the nasopharynx, which ultimately leads to the development of respiratory symptoms.

Fever and respiratory viruses

Another important finding in our study was that fever was associated with several respiratory viruses instead of nasopharyngeal bacteria. This result contradicts with previous studies which showed fever in children with AOM to be associated with the detection of *S. pneumoniae* either from MEE or from nasopharynx (Howie et al. 1970, Rodriguez and Schwartz 1999, Palmu et al. 2004, Cohen et al. 2006). Worth noticing, these studies did not investigate respiratory viruses. We, instead, investigated both nasopharyngeal bacteria and respiratory viruses and found fever to be associated with influenza viruses, HMPV, coronaviruses and parainfluenza viruses. These respiratory viruses are certainly well-known for burdening children with fever (Putto et al. 1986, Heikkinen et al. 2008, Debiaggi et al. 2012, Heinonen et al. 2012, Frost et al. 2014). The association between respiratory viruses and fever is supported by the study of Yano et al. which showed that among children with AOM, respiratory viruses were more often detected from nasopharyngeal sample and MEE in children with high fever, compared with children with less fever. The detection rates of bacteria did not have an association with the body temperature in their study (Yano et al. 2009). This supports the fact that viruses play a key role in the development of fever.

To crown it all, we showed that children with multiple viruses suffered more often from fever than children with a single virus. In line with our study, Franz et al. found hospitalized children with RSV viral co-infections to more often have fever compared with the children with RSV as the only virus during lower respiratory tract infection (Franz et al. 2010). However, the detection of multiple viruses seem not to be associated with more severe illness or worsen the prognosis of the disease as compared with the detection of a single virus (Marguet et al. 2009, Brand et al. 2012, Papenburg et al. 2012, Martin et al. 2012, Rotzen-Östlund et al. 2014, Nascimento-Carvalho and Ruuskanen 2016). Although not affecting the illness severity, multiple viruses have more often been detected in children with symptomatic respiratory infection episodes as opposed to asymptomatic episodes (van der Zalm et al. 2009, Chonmaitree et al. 2015). The association of fever and multiple viruses in our study suggests that every respiratory virus may have its own role in the nasopharynx and in the development of fever. It is worth speculating whether each respiratory virus could produce an individual inflammatory host response or if the synergistic effect of the viruses could cause greater febrile response.

It needs to be acknowledged that even though nasopharyngeal bacteria did not associate to fever in our study, their role in the pathogenesis of fever cannot be completely disputed. We previously showed that antimicrobials shorten the duration of fever significantly in children with AOM (Tähtinen et al. 2011). This

phenomenon suggests that fever is somewhat related to the presence of bacteria. Indeed, increased bacterial colonization in the nasopharynx at the time of an acute infection (Faden et al. 1990, Syrjänen et al. 2001, Teo et al. 2015) lets us presume that bacteria are not just innocent bystanders (Folsgaard et al. 2013, Man et al. 2017). Interestingly, animal and in vitro studies have shown positive associations between *S. pneumoniae* and parainfluenza viruses, between *S. pneumoniae* and HMPV, between *S. pneumoniae* and influenza viruses, and between *S. pneumoniae* and RSV (Bosch et al. 2013). The association of *influenza* viruses and *S. pneumoniae* seems nowadays particularly evident (Morris et al. 2017). Hence, our study further proposes that respiratory viruses have the leading role in the development of fever, but nasopharyngeal bacteria, perhaps *S. pneumoniae*, may play a supportive role.

In conclusion, our study showed that the variation of acute symptoms in young children with the suspicion of AOM may be partly explained by the presence of nasopharyngeal bacteria or respiratory viruses. Respiratory symptoms, such as rhinitis, nasal congestion and cough, were associated with *M. catarrhalis* with the presence of respiratory viruses. Fever, on the other hand, was associated with several respiratory viruses. This finding lets us propose that fever is primarily a sign of viral infection in children with RTI, regardless of the diagnosis of AOM. Overall, symptoms seem to be a result of a complex interplay with the bacteria and respiratory viruses.

6.4 Close follow-up in children with AOM initially managed without antimicrobials (Study IV)

The basis of the study was the recommendation that if initial observation without antimicrobial treatment is chosen for the management of AOM in children, close follow-up is required by several national guidelines (Heikkinen et al. 2010, Marchisio et al. 2010, Lee et al. 2012, Lieberthal et al. 2013). According to the guidelines, if the child's overall symptomatic condition worsens within two to three days after the diagnosis of AOM, parents should either contact the physician or bring their child to the clinic in order to initiate antimicrobial treatment. Hence, guideline makers hypothesize that the deterioration of the child's overall condition would be due to the worsening of AOM and otoscopic signs, although no studies have been conducted to test the hypothesis. Consequently, we investigated the relation of the child's overall symptomatic condition and the development of otoscopic signs in children with AOM not managed with antimicrobials. Our ultimate aim was to study whether close follow-up with re-examination is

needed for children with AOM who are initially managed without antimicrobial treatment and whose overall symptomatic condition is improving.

Our study supported the hypothesis that the resolution of otoscopic signs is related to the recovery of the child's overall condition. When parents assessed that their child's overall condition was improving, the probability for the worsening of otoscopic signs was very low. Indeed, otoscopic signs worsened only in 3 (3%) children with symptomatic improvement, including 2 children with perforation of TM. In contrast, in children with symptomatic failure, otoscopic signs worsened in 16 (30%) children, including 3 children with perforation of TM. Our results are concordant with the study of Harabuchi et al. that showed a direct relationship between resolution of TM scores and symptom scores after two days of the AOM diagnosis, when all children were managed with antimicrobials (Harabuchi et al. 2001).

Our findings suggest that children whose overall symptomatic condition is improving may not need a routine follow-up visit. Thus, it appears that a telephone call between the physician and parents might be sufficient to ensure that the child is improving. Maybe not even a phone call is necessary if the physician judges parents to be reliable evaluators of their child's overall condition. In case of TM perforation in children with symptomatic improvement, it is presumable that parents would notice purulent otorrhea and seek medical care in any case. Instead, our study evidently indicates that parents should contact the physician if their child has symptomatic failure. Children with symptomatic failure seem to be at risk for worsening of otoscopic signs requiring antimicrobial treatment. The prescription can be filled by parents either independently or after the telephone consultation with the physician. Moreover, the physician needs to make sure that symptomatic failure is not due to severe bacterial illness, for example septicaemia or pneumonia.

Interestingly, the development of contralateral AOM seemed not to affect the child's overall condition. This supports our previous findings that the symptomatic burden in children with bilateral or unilateral AOM is similar (Study II) and that AOM does not cause specific symptoms by itself (Study I) (Laine et al. 2010), but symptoms may be explained by the underlying virus infection and nasopharyngeal bacteria (Study III).

All in all, our study showed that if children with AOM are managed with initial observation without antimicrobial treatment, close follow-up with routine re-examination seems not necessary in children with symptomatic improvement. Only those with symptomatic failure would need re-examination, but symptomatic failure occurs only in the minority of children. Thus, implementation of this

finding into general practice would decrease physician office visits and thus save health care resources, as well as ease the burden of families.

6.5 Role of symptoms and laterality of AOM in the management of AOM

Definition of the illness severity of AOM

If the severity of illness defines the management of AOM, it would be of paramount importance that the severity of illness would be defined as symptoms specific for AOM worsening its prognosis. The careful definition of severe illness would ensure that children receive the optimal management. Study I showed that ear pain, used as one of the criteria for the severity of AOM by the AAP guideline (Lieberthal et al. 2013), could not differentiate children with AOM from those without it, although we used pain scales for the detection of pain. Study III showed that fever, used as another criterion for the severity of AOM (Lieberthal et al. 2013), was primarily a sign for viral infection in children with and without AOM.

In line with the results of this thesis, the study of Tähtinen et al. showed that the presence of ear pain or fever at the time of the diagnosis of AOM did not increase the risk for treatment failure (Tähtinen et al. 2017). Moreover, severe AOM, which is a combination of moderate/severe ear pain and/or high fever, was not the prognostic factor for treatment failure (Tähtinen et al. 2017). Accordingly, Hoberman et al. have shown that regardless of the severity of AOM, clinical failure occurred approximately in half of the children without antimicrobial treatment (61% and 43% in children with severe AOM and non-severe AOM, respectively) (Hoberman et al. 2011).

Taken these together, ear pain and fever are not specific symptoms for AOM and they do not seem to affect the prognosis of AOM. Following that logic, the severity of AOM should not be based on the presence of ear pain or fever. However, it remains to be determined whether the occurrence of fever after several days of the beginning of the RTI symptoms would be specific for the development of AOM (Kontiokari et al. 1998). Due to our cross-sectional study design, we could not investigate this. Future studies with prospective designs are needed to investigate whether fever is associated with the development of AOM during the later course of viral RTI.

Laterality of AOM

Another factor defining the management of AOM is laterality of AOM, according to several guidelines. However, Study II gives evidence that bilateral AOM is clinically only slightly more severe illness than unilateral AOM. Moreover, our study and the study of McCormick et al. showed that bilateral AOM was more often associated with moderate/severe bulging of TM than unilateral AOM (McCormick et al. 2007). Severe bulging of TM, on the other hand, seems to be the strongest predictor of treatment failure (Tähtinen et al. 2017), regardless of the laterality (Hoberman et al. 2013). It is likely that the tight relationship between severe bulging of TM and bilateral AOM explains the previous assumptions of bilaterality being the predictor of treatment failure (Tähtinen et al. 2017). Thus, when deciding the management of AOM, it seems more reasonable to pay attention to the degree of TM bulging, instead of the laterality of AOM.

Individual variation of symptoms

When assessing symptoms and the illness severity of a child, it is fundamental to acknowledge that every child is unique and the host response to nasopharyngeal bacteria and respiratory viruses may thus be variable between individuals. This leads to the individual variation of symptoms. It can be hypothesized that gene-environmental interactions are crucial in this regard, as in other illnesses (Kantor et al. 2017). It is likely that every clinician has encountered children younger than three years whose parents suspect them to have AOM due to the presence of certain symptoms, for instance holding the ear or being restless at night. In most of the cases, parents may be right and AOM is diagnosed. Supporting this, Kontiokari et al. showed parental suspicion of AOM to be correct in 71% of the cases when the child was concomitantly suffering from ear pain and restless sleep (Kontiokari et al. 1998). It is notable that in their study, each child served as his/her own control and thus the study design takes into account the individual variation of symptoms. It would be insulting to claim that these parents are just guessing right every time. Indeed, some children may express the presence of AOM every time with a specific symptom familiar to the parents, e.g. holding the ear, while others do that with some other symptom, e.g. being restless at night. Some children, on the other hand, may have no specific symptoms that would lead parents to suspect AOM. This individual variation of symptoms may be lost when the hundreds of cases are combined in the dataset in clinical research. Thus, although AOM does not cause any specific symptoms which could be generalized to a wide range of children, it is important to notice that conclusions drawn from clinical research are always general estimations of the truth, not the absolute truth on the individual level. It is crucial to listen to the parents, to

acknowledge and respect the individual variation of symptoms when applying the results of this thesis to the general practice.

Child's overall symptomatic condition, when assessed by parents, seems an appropriate and reliable way to measure the success of management as well as the illness severity, because it takes into account the individual variation. As a matter of fact, parental concern is considered as one of the "red flags" for serious infection in children (Van den Bruel et al. 2010). Symptom scores, on the other hand, ignore the individual variation of symptoms because they are built from specific symptoms and cannot be modified depending on the current symptoms of the child. Thus, symptom scores may give false results of the illness severity, because the presence of certain symptoms does not necessarily indicate that the child's overall condition has worsened. Moreover, symptom scores may be rather complex to use in clinical practice. Therefore, the child's overall symptomatic condition seems to be more a useful measure of the illness severity, when assessed by parents.

6.6 Methodological limitations and strengths

Our recruitment methodology was based on parental suspicion of AOM. Therefore children without AOM may have had more severe symptoms than if the children had been recruited based on the presence of RTI only. Our recruitment methodology may likewise explain why the great majority of children were reported to have parentally reported ear pain. In line with this, it is probable that the occurrence of other ear-related or non-specific symptoms that parents might consider as reflectors of pain are higher in our study population than in children without the suspicion of AOM. However, our study represents well those children who seek medical care, because the parental suspicion of AOM is one of the main reasons for physician visits. Moreover, we recruited children from the primary care level where children with AOM are mainly encountered.

The obvious limitation regarding the pain assessment with pain scales is that no observational pain measurement tools are validated for ear pain or for parental use. Thus we can only present preliminary results. Nevertheless, we may draw cautious conclusions that pain seems to be underestimated in young children with RTI, regardless of the diagnosis of AOM, if the pain scales are not used, because both of the pain scales gave similar results.

The pain scales were explained to the parents quite briefly at the study visit, which may have left parents a possibility of misunderstanding of matching the child's facial expression to the faces in the FPS-R figure. The faces in the FPS-R

may resemble children's faces when they cry or feel disgusted by food. This might explain the significant associations of excessive crying and poor appetite with moderate/severe pain with the FPS-R. Moreover, to our experience, parents considered the FLACC Scale as challenging, because they had to recall their child's behavior in each of the five behavioral categories, possibly causing recall bias. However, these problems reflect the real life in clinical practice and can be seen as a strength, especially if planning to implement the use of the FPS-R or FLACC Scale in daily clinical practice.

The data on the parental education level or occupation was missing, which may be seen as a limitation because higher level of parental education has been shown to be associated with higher reported pain levels (Shaikh et al. 2010). However, our study population came from all the postal code regions of Turku area, which reflects the sosioeconomic heterogeneity of the population.

Instead of filling in questionnaires, parents answered the study physician's questions regarding the child's symptoms. This may be seen as a limitation, because parents did not have that much time to think of their answers. On the other hand, it can also be considered as a strength, because our way of asking symptoms mimics the situation in real life at the physician's office. Parents could also ask clarifications which could possibly minimize misinterpretations. Moreover, the structured symptom questionnaire allowed us to study the symptoms thoroughly, which is one of the main strengths of this thesis.

The study physician investigated otoscopic signs with pneumatic otoscopy which is always a subjective interpretation of the signs of TM. The assessment of the color of effusion may be particularly tricky because it may be hampered by the opacity of TM. This, on the other hand, may have led to overestimation of cloudy instead of purulent effusion. The subjectivity of the otoscopic signs was minimized by using the video otoscopy allowing the study physicians to assess the findings together. In addition, tympanometry was used to aid in the diagnostics of AOM. We also recorded the otoscopic signs systematically and our diagnostic criteria for AOM were stringent, both main strengths of this thesis.

We may have failed to detect significant associations between symptoms and respiratory viruses in Study III because the detection rate of certain viruses was too low, although we included several hundreds of children in our study. In addition, we did not have a control group of healthy, asymptomatic children. However, our microbiology was comprehensive and thus the detection rate of viruses was high.

Finally, Studies I-III had cross-sectional designs. This means that symptoms were investigated and nasopharyngeal samples were taken only on day 1 when

the study physician investigated whether a child had or had not AOM. Therefore, we could only investigate associations between symptoms and bacteria and respiratory viruses, not causality. Moreover, we cannot describe the whole symptomatic course of the AOM episode but only the symptoms present at the time of the diagnosis. On the other hand, symptomatic condition of a child at the time of the diagnosis of AOM defines the management of AOM. Thus, our study offers valuable information for the physicians who treat children with AOM at the primary care level.

6.7 Future considerations

New pain assessment tools are desperately needed for young children with RTI. It is unfortunate that currently ear pain and its systematic assessment in young children seem to be undervalued. This is rather alarming, taken into account the number of children seeking medical attention due to the suspicion of AOM. Especially young, preverbal children are at great risk of suffering from undetected and undertreated pain, which may lead to hypersensitivity to pain later in life (Taddio et al. 1997, Hermann et al. 2006, Wollgarten-Hadamek et al. 2011). This is ethically unbearable. Therefore, future studies should focus on investigating pain scales in young children with the suspicion of AOM and to validate pain scales for these purposes. Moreover, physicians should actively assess and grade pain in all young children with RTI and manage it accordingly. By doing so, unnecessary suffering of young children could be avoided.

Future guidelines of AOM may be modified by the results of this thesis regarding the assessment of illness severity currently guiding the management of AOM. I suggest that when AOM guidelines are next updated, the severity of AOM should not be based on the presence of specific symptoms. On the contrary, every child deserves to be evaluated individually, taking into account his/her overall condition. Furthermore, bilateral AOM is only a slightly more severe illness than unilateral AOM. Thus, future guidelines should put less emphasis on the laterality of AOM and focus on recommending antimicrobial treatment in children with severe bulging of TM, because it is a more reliable predictor of treatment failure in children with AOM. Finally, future guidelines should not recommend routine close follow-up visits for all children with AOM initially observed without antimicrobial treatment. Only those with symptomatic failure would need reassessment. Implementing this finding in general practice would cut down physician visits, reduce health care costs and most importantly, decrease the burden of families.

Nasopharyngeal bacterial colonization and microbiota seem to have a central role in regulating respiratory health (Bomar et al. 2017). There is evidence that the early nasopharyngeal colonization by *Corynebacterium* and *Dolosigranulum* species would be protective for respiratory health (Bosch et al. 2017). Hence, *Corynebacterium* and *Dolosigranulum* species could be given to newborns in a droplet form regularly for a few weeks. If the colonization of these bacterial species could be maintained and early colonization of *M. catarrhalis* prevented, it might ease the overall burden of RTIs and thus improve the quality of life in children. The vaccine for *M. catarrhalis* would serve the same purpose. However, more research is needed before implementing these innovations in practice.

7 SUMMARY AND CONCLUSIONS

This thesis established our previous finding that none of the symptoms is specific for AOM in young children (Laine et al. 2010). First, Study I evidently showed that ear pain, considered as the most specific symptom of AOM, does not differentiate young children with AOM from those without AOM, even though we used pain scales considered as the most reliable method of assessing pain. Children with RTI, regardless of the presence of AOM, seemed to suffer from remarkable pain and distress. The "red flags" for indicating moderate/severe pain were the presence of ear pain reported by child, restless sleep, excessive crying, poor appetite and nasal congestion. Study II showed that bilateral AOM is clinically only a slightly more severe illness than unilateral AOM. This indicates that the laterality of AOM does not significantly contribute to the symptom burden of the children with AOM. These findings together suggest that AOM may not be the main determinant of symptoms in young children during RTI. On the contrary, the symptoms seem to occur irrespective of AOM. Indeed, Study III suggests that the variation of symptoms is dependent on the nasopharyngeal bacteria and respiratory viruses. Study IV showed that the improvement in the child's overall symptomatic condition was related to the resolution of otoscopic signs. Thus, close follow-up with re-examination is not needed in children with AOM, who are initially observed without antimicrobial treatment and whose overall symptomatic condition is improving.

To summarize, it seems justified to say that no specific symptoms should be required when assessing the illness severity of AOM. Furthermore, laterality of AOM may not be useful when assessing the severity of AOM. I rather recommend that the severity assessment should be based on a careful overall view of the child's symptomatic condition, with the help of parents. Moreover, it is of utmost importance that the physician would actively assess the severity of pain and distress in children with the suspicion of AOM with pain scales or by asking if the child has restless sleep, excessive crying, poor appetite or nasal congestion. After the pain assessment, the physician should manage pain actively, even though AOM would not be diagnosed. Finally, if AOM is diagnosed and both physician and parents end up managing the child with initial observation without antimicrobial treatment, close follow-up with re-examination is not needed if the child's overall symptomatic condition is improving.

The following conclusions can be given to the original study questions:

I Are parents able to detect ear pain with pain scales in young children with the suspicion of AOM? Which symptoms are associated with parentally assessed moderate/severe pain?

Yes, parents are able to detect ear pain and distress with pain scales. Without the use of pain scales, parents seem to underestimate the severity of pain. Pain is similarly moderate/severe in children with and without AOM. Symptoms associated with parentally assessed moderate/severe pain are ear pain reported by child, restless sleep, excessive crying, poor appetite and nasal congestion.

II Is bilateral AOM clinically more severe illness than unilateral AOM?

Yes, but only slightly. Only fever, moderate/severe bulging of TM and purulent effusion behind TM are more common in children with bilateral AOM than in children with unilateral AOM.

III Is the variation of acute symptoms associated with the nasopharyngeal bacteria and/or respiratory viruses in young children whose parents suspect them to have AOM?

Yes. Rhinitis, nasal congestion and cough are associated with *M. catarrhalis*, in the presence of respiratory viruses. Fever is associated with influenza viruses, HMPV, coronaviruses and parainfluenza viruses.

IV Is close follow-up with re-examination needed for children with AOM who are initially managed without antimicrobial treatment and whose overall symptomatic condition is improving?

No. The probability for the worsening of otoscopic signs is very low in children with symptomatic improvement.

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