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To Kalle, Verna and Frida

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ABSTRACT

The burden of influenza is substantial in all children, including those without any underlying medical conditions. Both influenza A and B viruses cause a high rate of morbidity and a large demand for hospitalization in the paediatric population. Although most children with influenza are treated as outpatients, especially among young children, influenza-attributable illnesses often cause hospitalization. Limited data exist on the clinical effectiveness of oseltamivir treatment of influenza in the youngest age group of children.

We performed a prospective cohort outpatient study to investigate the burden and clinical presentation of influenza and the impact of oseltamivir among infants during their first year of life. A substantial proportion of infants, 13.5%, were infected with influenza viruses during their first influenza season. In this special age group, oseltamivir treatment rapidly decreased the viral load in nasopharyngeal secretions as well as the duration and severity of symptoms.

We also performed a retrospective study to investigate the differences between influenza A and B infections in children <16 years of age who were hospitalized with virologically confirmed influenza at the Department of Paediatrics and Adolescent Medicine, Turku University Hospital. In the same age group, we also searched for changes in the demographic, management and clinical features of children hospitalized with influenza during the 25-year period of 1993–2018. Our results revealed that there were no significant differences between influenza A and B infections in hospitalized children when the outcomes were adjusted for age. During the 25-year period, the relative proportion of hospitalized children <2 years of age almost halved, while the proportion of children aged 6–15 years almost tripled. The median duration of hospitalization in all children shortened from 2 days to 1 day.

According to our results, the clinical severity of influenza A and B is similar in hospitalized children. Our findings strengthen the importance of influenza B in contributing to the total morbidity of influenza. The results of our outpatient studies provide new information about the incidence and clinical features of influenza among infants and demonstrate the benefit of antiviral treatment in children during their first year of life.

KEYWORDS: Influenza, children, hospitalization, outpatient, oseltamivir

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TIIVISTELMÄ

Influenssa aiheuttaa huomattavan tautitaakan myös ennestään terveille lapsille. Niin influenssa A- kuin B-virukset aiheuttavat lapsille runsaasti sairastuvuutta ja sairaalahoidon tarvetta. Vaikka suurin osa influenssaan sairastuneista lapsista hoidetaankin avohoidossa, varsinkin pienimmät lapset joutuvat usein influenssan vuoksi sairaalahoitoon. Oseltamiviirin tehosta pienten lasten influenssan hoidossa on olemassa hyvin niukasti tutkimustietoa.

Prospektiivisessa kohorttitukimuksessamme selvitimme influenssan tautitaakkaa, oirekuvaa ja oseltamiviirin tehoa lapsilla heidän ensimmäisen elinvuotensa aikana. Huomattava osa lapsista, 13,5 %, sairastui influenssaan ensimmäisen influenssakautensa aikana. Oseltamiviirihoito vähensi nopeasti virusmääriä nenäeritteissä, lyhensi influenssan kestoa ja lievitti influenssan oireita.

Retrospektiivisessä tutkimuksessa selvitimme eroja influenssa A:n ja B:n taudinkuvissa alle 16-vuotiailla lapsilla, jotka olivat joutuneet sairaalahoitoon Tyksin lasten ja nuorten klinikalle. Samassa ikäryhmässä selvitimme myös, onko vuosien 1993 ja 2018 välisenä 25-vuotiskautena tapahtunut muutoksia influenssan vuoksi sairaalaan joutuneiden lasten taudinkuvissa tai heidän hoidossaan. Tulokset osoittivat, että influenssa A:n ja B:n aiheuttamissa taudinkuvissa ei ollut eroja, kun oireet ja löydökset suhteutettiin lasten ikään. Totesimme myös, että 25 vuoden seuranta-aikana alle kaksivuotiaiden sairaalaan joutuneiden lasten suhteellinen osuus lähes puolittui, kun taas 6–15-vuotiaiden lasten osuus lähes kolminkertaistui. Samalla ajanjaksolla sairaalahoidon keskimääräinen kesto lyheni kahdesta vuorokaudesta yhteen vuorokauteen.

Tutkimuksemme osoitti, että influenssa A:n ja B:n taudinkuvat ovat sairaalassa hoidetuilla lapsilla samanlaiset. Löydöksemme vahvistavat influenssa B-virusten merkitystä influenssan lapsille aiheuttamassa kokonaistautitaakassa. Tutkimuksemme toi myös uutta tietoa pienten lasten influenssan ilmaantuvuudesta ja sen taudinkuvasta sekä oseltamiviirihoidon hyödyistä lasten ensimmäisen ikävuoden aikana.

AVAINSANAT: influenssa, lapset, sairaalahoito, avohoito, oseltamiviiri

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Abbreviations

ALRI	Acute lower respiratory infection
AOM	Acute otitis media
CEN	Cap-dependent endonuclease
CDC	Centers for Disease Control and Prevention
Ct	Cycle threshold
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
FFP2	Filtering face piece 2
H	Hemagglutinin
IAV	Inactivated influenza vaccine
VE	Vaccine effectiveness
ICU	Intensive care unit
IF	Immunofluorescence
Ig	Immunoglobulin
IQR	Interquartile range
LAIV	Live attenuated influenza vaccine
M	Matrix protein
N	Neuraminidase
NAAT	Nucleic acid amplification test
PCR	Polymerase chain reaction
PEP	Post-exposure prophylaxis
POC	Point of care
R	Reproduction number
RNA	Ribonucleic acid
RCT	Randomized controlled trial
RT-PCR	Reverse transcription-polymerase chain reaction
WHO	World Health Organization

List of Original Publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals:

I Mattila JM, Vuorinen T, Heikkinen T. Comparative severity of influenza A and B infections in hospitalized children. *Pediatr Infect Dis J*, 2020; 39(6): 489–493.

II Mattila JM, Thomas E, Lehtinen P, Vuorinen T, Waris M, Heikkinen T. Burden of influenza during the first year of life. *Influenza Other Respir Viruses*, 2021; 15(4): 506–512.

III Mattila JM, Vuorinen T, Waris M, Antikainen P, Heikkinen T. Oseltamivir treatment of influenza A and B infections in infants. *Influenza Other Respir Viruses*, 2021; 15(5):618–624.

IV Mattila JM, Vuorinen T, Heikkinen T. Trends and changes in influenza-associated hospitalizations in children during 25 years in Finland, 1993–2018. *Pediatr Infect Dis J*, 2023; 42(4):332–337.

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1 Introduction

The continuous antigenic evolution of influenza viruses and their capability to cause pandemics make influenza one of the most dangerous pathogens facing humankind. A remarkable part of the overall burden of influenza arises from seasonal epidemics that infect millions of people every year. The greatest mortality is seen among the elderly population, but children play a fundamental role in the transmission and the total burden of influenza in the community. Among children, infants <6 months of age have the highest risk for severe infections, and the highest rates of influenza hospitalization are invariably reported among this vulnerable population.

Prevention of influenza in infants is challenging. Influenza vaccines are licensed for use only for children 6 months and older, and the vaccine responses in young children are generally weaker than in older children. Influenza vaccination of pregnant mothers reduces the risk of influenza in their infants during the first months of life, but the duration of protection afforded by maternal antibodies is limited. Despite many important advances in prevention, treatment and diagnostics of influenza, antiviral treatment is currently the only way to manage influenza in young infants.

Influenza A viruses are conventionally thought to cause more severe infections than influenza B viruses, because the incidence of influenza A is higher compared to influenza B, and only A viruses are capable of causing pandemics. Influenza A viruses generally infect younger children compared to influenza B, and therefore age should always be considered when comparing any clinical features between influenza A and B.

This study aimed to investigate the clinical presentation and overall burden of influenza and the effectiveness of oseltamivir treatment in outpatient infants during their first year of life. We further sought to compare the clinical presentation, outcomes and management of children hospitalized with virologically confirmed influenza A and B infections. In addition, we estimated trends and changes in influenza-associated hospitalizations of children during a 25-year period.

2 Review of the Literature

2.1 Influenza viruses

Influenza viruses are ribonucleic acid (RNA) viruses from the orthomyxoviridae family that are classified into A, B, C and D types based on the variation in their nucleoprotein and matrix antigens. Except in humans, influenza A viruses can be found in several birds and mammals including water birds, swine, horses, bats, dogs, marine mammals and minks. Influenza B viruses are seen in humans and seals, and influenza C viruses in humans, swine and dogs. Influenza D viruses, instead, are not known to cause illness in humans. Therefore, influenza A and B viruses are the most important human viruses causing seasonal epidemics, and, of them, only influenza A is capable of causing pandemics.

2.1.1 Viral structure

Influenza viruses have a characteristic outer layer with about 500 spikes radiating outward from the lipid envelope. Rod-shaped spikes are glycoproteins called hemagglutinin and mushroom-shaped spikes are neuraminidase (N). The influenza A virus envelope also contains a third membrane protein called matrix protein (M) 2. M2 plays a role in both the assembly of the influenza A virus and the uncoating of viruses. Beneath the lipid membrane is a M1, which gives rigidity to the lipid envelope.

Hemagglutinin is a glycoprotein on the influenza viral surface that allows the virus to bind to receptors in host cell membranes. Hemagglutinin contains antigenic sites recognized by the host immune system and stimulates a host-neutralization antibody response, which makes it the main target molecule of vaccination against influenza. Another surface glycoprotein, neuraminidase, enzymatically removes sialic acid and enables the virus to be released from the infected host cell. It also prevents viral aggregation. Neuraminidase is the target molecule of antivirals like oseltamivir, which is a sialic acid analogue that inhibits neuraminidase.

Surface proteins are also major antigenic determinants that specify the subtype and the strain of the virus. Influenza A viruses can be classified into subtypes and strains based on the variation in hemagglutinin and neuraminidase antigens. There

are 18 different hemagglutinin subtypes and 11 different neuraminidase subtypes (H1 through H18 and N1 through N11, respectively). Currently, three subtypes of hemagglutinin (i.e., H1, H2 and H3) and three subtypes of neuraminidase (i.e., N1, N2 and N8) have caused sustained epidemics in human populations (e.g., H3N8, H1N1, H2N2, H3N2, H1N2). The main reservoir of influenza virus strains is in water birds, as they have almost all hemagglutinins (e.g., H1–H16) and neuraminidase (N1–N9) subtypes. Avian subtype H5 is potentially disastrous because of its high pathogenic potential. So far, it has not been able to cause significant human-to-human transmission. Influenza B viruses are not divided into subtypes but subdivided into two antigenically distinct lineages being B/Victoria and B/Yamagata. The nomenclature of influenza viruses includes virus type, geographical location where they were first isolated, the sequential number of isolation, the year of isolation, and, for type A viruses, the subtype of the hemagglutinin and neuraminidase (e.g., A/Brisbane/10/2007 [H3N2]). In association with the Covid-19 pandemic, the influenza B/Yamagata lineage has not been detected since April 2020, and A(H3N2), A(H1N1) and B/Victoria viruses have been circulating with a more narrow genetic diversity compared to the pre-pandemic era (Dhanasekaran et al., 2022).

RNA viruses, like influenza viruses, need the presence of host cell deoxyribonucleic acid (DNA) to replicate and reproduce its RNA genome. The replication cycle of influenza viruses is very quick and contains two steps being transcription of complimentary RNA followed by transcription of new viral RNA copies using the complimentary RNAs as templates.

The genomes of influenza A and B viruses are composed of eight pieces of single-stranded, negative-sense RNA encoding surface glycoproteins hemagglutinin and neuraminidase, proteins M1 and M2, RNA polymerases, structural proteins necessary to form the capsid, the nucleoprotein, non-structural protein 1 and 2 and the nuclear export protein.

2.1.2 Antigenic drift and shift

Influenza viruses have a great potential for evolution due to antigenic variation. The composition of influenza viruses changes continuously when minor mutations occur in the genes which code for surface glycoproteins. The RNA virus replication process is vulnerable for errors due to the lack of proofreading enzymes, which leads to permanent changes in replicated viral genomes and rapid viral evolution. For example, point mutations in the H and N genes can lead to changes in antigenicity that allow a virus to infect people who were either infected or vaccinated with a previously circulating virus. This process, which leads to a small but permanent change in the genetic code of influenza virus, is called antigenic drift.

Another process leading to antigenic variation is antigenic shift. Antigenic shift occurs when two influenza viruses of the same type infect the same host cell and segments from different viruses reassort and form a novel influenza virus subtype. This is possible in birds and many mammals, including humans, who may be a host for more than one influenza subtype. Direct transmission of an avian or swine influenza virus to humans is also possible. Due to a lack of pre-existing immunity, the changed influenza viruses can spread quickly and lead even to influenza pandemics. Both influenza A and B viruses can undergo antigenic drift, but an antigenic shift is not possible for influenza B viruses because they have no subtypes. Altogether, the genetic plasticity of influenza viruses has important consequences for vaccine design and the capacity for novel viruses to emerge from natural reservoirs to cause global pandemics.

2.2 Pathogenesis

Surface glycoproteins are essential in the virulence and pathogenesis of influenza. The binding of hemagglutinin to its cellular receptor, sialic acid, induces penetration into the cell by membrane fusion. Sialic acids are found on several cell types in different locations in the human body. Influenza virus-producing cells are found not only in the respiratory tract but also in the gastrointestinal tract, brain and myocardium. Inside the infected cell, the influenza virus replicates and causes the destruction of the host cell.

The human immune system responds immediately to viruses. The first defense mechanisms are mechanical: mucus and collectins protect epithelial cells against viral penetration. At the same time, several specific and non-specific response mechanisms turn on and the rapid innate cellular response induces the production of different kinds of proinflammatory cytokines and interferons. This leads to the inhibition of the viral replication and activation of the adaptive immune response with virus-specific B and T cell responses (Kuiken et al., 2012). The viral replication process in the epithelial cells leads to local inflammation. Further, the balance of viral replication and the immune response determines the symptoms of infections.

People with a previous influenza virus infection or vaccination against influenza have specific antibodies against hemagglutinin. When these antibodies match the strain causing the infection, they can, together with cell-mediated immunity, neutralize the virus and restrict or even block the infection.

2.2.1 Viral load and shedding

The susceptibility to clinical influenza infection is based on the individual immune response and specific features of the pathogenic influenza virus. If the antibody titers

of an infected person against the same or related influenza strains are high, a larger amount of virus is needed to lead to symptomatic illness (Lee et al., 2009; Morris et al., 1966). It is possible to have several different situations concerning the transmission of influenza viruses. Children can have symptomatic illness and shed the viruses forward, while on the other hand, some of the infected children can go through the illness without transmitting the disease. It is also possible that one has no clinical symptoms but still transmits the virus.

The replication of influenza viruses starts quickly after transmission. This also means that shedding of the viruses begins without delay. In a large meta-analysis of experimental influenza infection in adults, the viral shedding peaked on day 2 and lasted less than 5 days. Moreover, one-third of infected adults were asymptomatic (Carrat et al., 2008).

Due to the incubation time between the infection and the onset of symptoms, viral shedding starts before clinical symptoms appear, and asymptomatic persons can be contagious. The infectiousness of an individual can be estimated by viral shedding. The influenza viral load correlates positively with the severity of illness (Lau et al., 2010; Lee et al., 2009; Li et al., 2010). There is also evidence that, although the duration of viral shedding is shorter in asymptomatic individuals, the peak level of viral shedding is approximately the same in asymptomatic and symptomatic individuals (Loeb et al., 2012).

The total duration of influenza virus shedding is about 5 days (Carrat et al., 2008; Lau et al., 2010). There is evidence that viral shedding patterns are unequal between different influenza virus types and subtypes. The peak of viral shedding comes earlier and decreases more rapidly in influenza A compared to influenza B (Lau et al., 2010, 2013; Wang et al., 2017). Age also matters, as children tend to shed influenza viruses longer before the onset of symptoms compared to adults (Lau et al., 2013; Ng et al., 2016). Children also shed influenza viruses for a longer period (Lau et al., 2013; Ng et al., 2016). Immunosuppressive patients have been reported to have a prolonged viral shedding that can last for weeks (Antón et al., 2010).

In an double-blind, randomized controlled trial (RCT), oseltamivir significantly reduced viral shedding in children, even when treatment was started 48 hours or longer after illness onset (Fry et al., 2014). Identical results have been published also from another RCT with oseltamivir (Whitley et al., 2001). Baloxavir marboxil has also proven to diminish influenza viral shedding (Hayden et al., 2018).

2.2.2 Transmission

Influenza can be transmitted through respiratory droplets, via contact transmission and by airborne transmission through small aerosols. Different sizes of infectious respiratory particles are generated by sneezing, coughing and talking and are spread

into the air. In droplet transmission, large droplets with a particle diameter of >5 μm can be floated into nasal, oral or conjunctival mucosa of people within 1–2 meters and infect them. Droplets can also descend to a surface within 1–2 meters. Influenza viruses tolerate drying quite well and can stay alive at room temperature for some days on nonporous surfaces. That is why influenza viruses can also be transmitted by indirect contact by touching contaminated surfaces (i.e., fomites) and then touching the mouth, eyes, or nose before washing hands. Moreover, influenza can spread via small aerosols with a diameter of <5 μm over long distances (Lampejo, 2020). The Covid-19 pandemic has increased the understanding about airborne transmission of respiratory viruses. During airborne transmission, small infectious particles, aerosols, disseminate over long distances. One study has reported that half of the influenza A transmissions in a household setting occur via airborne transmission (Cowling et al., 2013). Surgical masks are needed to limit droplets, but data on the effectiveness of masks in controlling airborne transmission of influenza are limited (Leung et al., 2020; Milton et al., 2013).

The reproduction number (R) is an important transmissibility parameter, which describes, on average, how many people a typical infectious case will transmit. In a systematic review of influenza reproduction numbers, the median point estimate of R for seasonal influenza was 1.27 and for the 2009 A/H1N1 pandemic, it was 1.46 (Biggerstaff et al., 2014).

2.3 Epidemiology

2.3.1 Seasonal influenza

2.3.1.1 Seasonality

In temperate regions, influenza virus epidemics occur during the cold season. In Finland and elsewhere in the Northern hemisphere, an epidemic breaks out typically around the turn of the year. The peak of epidemics is reached characteristically 2–3 weeks after the first cases, and the duration of epidemics is 6–8 weeks (Neuzil et al., 2000). Particularly in child populations, an epidemic can last longer, and influenza viruses can circulate among children over the whole winter season (Heikkinen et al., 2003). The seasonal cycle of influenza has been recognized for several decades, but the reasons behind this seasonality are complex and multifactorial. Ambient humidity and temperature associated with the characteristic behaviour of people are suggested to prolong virus shedding and transmission (Lowen et al., 2007).

2.3.1.2 Morbidity

The expansion of the influenza epidemic fluctuates according to the virulence of the current variants, the antigenic changes that have occurred after the previous epidemic, and the level of immunity of the population against the circulating viruses. During most seasons, two or more influenza virus strains are cocirculating and causing outbreaks. In previous studies, the relative proportion of influenza B viruses has varied between seasons and geographic areas, and the proportion of B viruses tends to be higher in tropical countries compared to countries in the temperate regions. Influenza A accounts for about 80% of all seasonal influenza infections, and influenza B accounts for the remaining 20% (Caini et al., 2019; Glezen et al., 2013; Jennings et al., 2018; Moa et al., 2017; Mosnier et al., 2015; Silvennoinen et al., 2011).

Seasonal influenza causes a major burden of disease in populations every year. Children are at the highest risk, and it is estimated that about 110 million children younger than 5 years of age get influenza infection annually (Wang et al., 2020). A large review of 32 RCTs estimated that every fifth unvaccinated child and one-tenth of unvaccinated adults are infected by seasonal influenza every year (Somes et al., 2018).

The highest rates of influenza are consistently seen in the youngest children (Heikkinen et al., 2004; Nair et al., 2011; Neuzil et al., 2002; Poehling et al., 2006, 2013; Ram Purakayastha et al., 2018). One reason for the high incidence among youngest children can be the lack of pre-existing immunity. In addition, children play a central role in the transmission of influenza in the community (Glezen & Couch, 1978; Monto et al., 1970; Neuzil et al., 2002; Reichert et al., 2001).

2.3.1.3 Underlying risk conditions

Children under 6 months of age have the highest risk for severe influenza and its complications. Moreover, they are too young to be vaccinated. The risk of hospitalization and outpatient visits is increased in children up to 5 years of age (Li et al., 2021). The United States Centers for Disease Control and Prevention (CDC) considers children to have an underlying risk condition if they have a cardiac, pulmonary, endocrine, liver, kidney or a major neurologic disorder or malignancy or other immunosuppressive condition. In several studies, the proportion of children with risk conditions has been observed to increase with age (Kamidani et al., 2022; Moore et al., 2006; Silvennoinen et al., 2011). Furthermore, the risk for severe outcomes in children with underlying risk conditions is higher in low-income countries than in high-income countries (Coleman et al., 2018).

The elevated risk for hospitalization in children with known pre-existing risk conditions has been demonstrated in systematic reviews and meta-analyses (Gill et

al., 2015; Tuckerman et al., 2019). In separate studies, the proportion of children with underlying risk conditions varied based on the study design. For example, in a recent study from Norway, children with underlying risk conditions had over a 6-fold higher risk of hospitalization compared to the general population within the same age group (Hauge et al., 2020). In that study, the highest risk was observed in immunocompromised children and children with epilepsy (Hauge et al., 2020). The duration of hospitalization in previously healthy children is shorter compared to children with underlying risk conditions (Ampofo et al., 2006; Chaves et al., 2014; Moore et al., 2006). Moreover, children with underlying risk conditions have a higher risk for treatment in the intensive care units (ICUs) (Hardelid et al., 2018).

Except for underlying risk conditions listed by the CDC, there is an increased risk for influenza complications also in prematurely born children and in some ethnic minorities, for example. In one systematic review and meta-analysis, prematurity was identified as a strong risk factor for influenza-related complications in children (Gill et al., 2015).

2.3.1.4 Sex

An increased risk for severe health outcomes in boys compared to girls with influenza has been reported in a systematic review and meta-analysis (Coleman et al., 2018). Several studies show an excess number of influenza hospitalizations in boys (Chaves et al., 2014; Kamidani et al., 2022; Quach et al., 2003; Silvennoinen et al., 2011; Wang et al., 2015). The reasons behind this elevated risk in boys are complex and mostly unknown. The mechanisms may include sex-dependent differences in innate and adaptive immune responses, viral loads and time to the clearance of viruses (Ursin & Klein, 2021).

2.3.1.5 Outpatient visits

Most children with influenza are treated in outpatient settings (Heikkinen, 2006; Heikkinen et al., 2004; Poehling et al., 2006; Tsolia et al., 2006). According to a large cohort study, outpatient visits of children increased during influenza epidemics. The excess numbers of outpatient visits were highest in infants 6–12 months of age, and every year 6–15% of children visited outpatient clinics because of influenza infection (Neuzil et al., 2000).

According to one meta-analysis, influenza accounted for 23% of outpatient cases among children under 5 years old with acute respiratory infection and fever (Buchan et al., 2016). In Germany, the proportion of influenza in outpatients between 1 to 5 years old was 38% during post-pandemic influenza seasons (Streng et al., 2018).

2.3.1.6 Hospitalizations

Influenza-associated hospital admission rates vary widely depending on the medical history and age of children. Young children are more likely to experience a severe infection requiring hospitalization. The highest numbers of population-based hospitalizations are reported in children younger than 6 months of age (Heikkinen et al., 2013b; Kamidani et al., 2022; Neuzil et al., 2000; Poehling et al., 2013; Silvennoinen et al., 2011, 2012). In children under 6 months of age, sepsis-like illness is the major cause for influenza-attributable hospitalization (Silvennoinen et al., 2012). Altogether, influenza-infected children are frequently admitted due to respiratory symptoms and febrile convulsions (Poehling et al., 2006; Quach et al., 2003; Silvennoinen et al., 2012).

A systematic analysis, which consisted of data from 60 countries, estimated that influenza causes 10% of all respiratory hospitalizations in children. In that analysis, influenza was estimated to cause a total of 870,000 hospitalizations in children younger than 5 years old (Lafond et al., 2016; Wang et al., 2020). Children with severe influenza also frequently need treatment in paediatric ICU. In a large study from the years 2003–2015 in the United Kingdom, ICU admission rate for influenza was 2.6/100,000 children during the winter seasons (Hardelid et al., 2018).

2.3.1.7 Mortality

The global burden of influenza-associated mortality is greatest in the elderly. In children, the highest mortality rates among children with influenza are reported in the youngest children (Bhat et al., 2005; Shang et al., 2018).

In a review and meta-analysis by Nair and colleagues, they estimated that influenza-associated acute lower respiratory infections (ALRI) led to 28,000 to 111,500 deaths in children <5 years of age in the year 2008 (Nair et al., 2011). In the same age group, mortality was higher in low-income countries compared to high-income countries, and up to 99% of all influenza-associated deaths may occur in developing countries (Coleman et al., 2018; Nair et al., 2011). Ten years later, in 2018, the corresponding estimate in children aged <5 years was from 13,200 to 97,200 influenza virus-associated ALRI deaths globally (Wang et al., 2020).

The risk of lethal influenza infection is higher in children with high-risk conditions compared to healthy children (Tuckerman et al., 2019). However, in a large study of influenza-associated paediatric deaths, 43% of children who died were previously healthy (Wong et al., 2013).

2.3.2 Pandemic influenza

Since the 20th century, novel influenza A viruses have caused five pandemics. Compared with seasonal influenza epidemics, influenza pandemics that appear at irregular intervals are generally associated with more severe symptoms and deaths mainly in people under 65 years old. By far, the most destructive influenza pandemic has been the 1918 pandemic caused by the H1N1 influenza A virus and known as the Spanish flu. It broke out near the end of the World War I and killed more than 30–50 million people. The subsequent influenza pandemics appeared in 1957 (H2N2, the Asian flu), in 1968 (H3N2, the Hong Kong flu), in 1977 (H1N1, Russian flu) and in 2009 (H1N1, Swine flu).

2.4 Clinical presentation

2.4.1 Symptoms

Influenza symptoms range from mild to severe and can even lead to death. Influenza can be asymptomatic as well. Overall, fever is the most remarkable sign of influenza in children (Danier et al., 2019; Heinonen et al., 2012; Poehling et al., 2006; Silvennoinen et al., 2009; Teros-Jaakkola et al., 2019). In a Finnish outpatient study of 353 children, fever was the strongest predictor of influenza, with the highest odds ratio of 58.46 being observed for fever ≥40.0ºC (Heinonen et al., 2012). Furthermore, the median length of fever in influenza has been 3–4 days in outpatient children (Heikkinen et al., 2016; Silvennoinen et al., 2009).

According to an Australian study, the next common symptoms after fever in hospitalized children were cough, rhinorrhoea and vomiting (Daley et al., 2000). Correspondingly, in a Finnish prospective study among unselected outpatient children, 95% of 353 virologically confirmed children were febrile, 77% of children had cough and 78% had rhinitis. The clinical presentation of influenza was most severe in young children <3 years of age (Silvennoinen et al., 2009). Subjective symptoms, such as headache and myalgia, are difficult to confirm in young children because of their inability to describe these symptoms.

As the clinical presentation is most severe in the youngest children, sepsis-like illness is common and frequently leads to hospitalization in this age group (Boddington et al., 2021; Fell et al., 2017; Izurieta et al., 2000; Montes et al., 2005; Neuzil et al., 2000; Poehling et al., 2006, 2013; Silvennoinen et al., 2012). Sepsis suspicion also predisposes young children to invasive examinations like lumbar punctures and blood samples, together with empirical antibiotic treatment (Neuzil et al., 2000).

Besides fever and respiratory symptoms, influenza in children can manifest in many other various ways. Other possible manifestations include general symptoms like dehydration, muscle weakness, fatigue and gastrointestinal symptoms, especially in hospitalized cases (Friedman & Attia, 2004; Moore et al., 2006; Peltola et al., 2003; Quach et al., 2003; Rojo et al., 2006). Influenza does not typically provoke laryngeal symptoms, but acute expiratory wheezing or bronchiolitis occur at times (Kenmoe et al., 2020; Silvennoinen et al., 2012). On the other hand, if croup is caused by influenza, the clinical presentation has been reported to be more severe and hospitalization longer compared to croup caused by parainfluenza viruses (Peltola et al., 2002).

2.4.2 Complications

Influenza infections in children frequently lead to bacterial complications such as acute otitis media (AOM), pneumonia, sinusitis and conjunctivitis, which in most cases result in antimicrobial treatment. Influenza may also induce different neurological complications. During an ongoing seasonal epidemic, influenza infection may be behind acute neurological symptoms.

In general, influenza viruses belong to the most important viruses predisposing children to AOM (Chonmaitree et al., 2008; Heikkinen et al., 1999). Not surprisingly, AOM is the most common complication of influenza (Heikkinen et al., 2004; Neuzil et al., 2002; Silvennoinen et al., 2009). In a Finnish outpatient study, about 40% of under 3-year-old children developed AOM as a complication of their influenza infection (Heikkinen et al., 2004). According to previous studies from Finland, about half of all cases of AOM develop within 3–4 days after the onset of symptoms of a respiratory tract infection (Heikkinen & Ruuskanen, 1994; Koivunen et al., 1999).

The next most common bacterial complication is pneumonia. Ten to twenty-six percent of hospitalized children have been reported to have pneumonia as a complication of influenza (Ampofo et al., 2006; Dawood et al., 2010; Forster, 2003; Lahti et al., 2006; Peltola et al., 2003; Rojo et al., 2006; Schrag et al., 2006).

Influenza primarily affects the respiratory system, but children with influenza develop frequently different neurological manifestations. In a Finnish study of hospitalized children with confirmed influenza infections, 15% were hospitalized due to acute neurologic conditions (Silvennoinen et al., 2012). The most typical acute neurological manifestation of influenza in young children is febrile convulsion (Newland et al., 2007; Silvennoinen et al., 2012). The spectrum of other reported neurological manifestations is wide, but fortunately severe neurological conditions associated with influenza are rare. Seizures, encephalopathy, myelitis, Guillain-Barré syndrome, Reye syndrome, acute necrotizing encephalopathy and speech and

motor disorders have been diagnosed in association with influenza. Some influenza-related neurological manifestations, like encephalopathy, have high mortality (Morishima et al., 2002). The pathogenesis of influenza associated CNS complications is not fully understood, but for example, RANBP2 gene mutations are found to be associated with recurrent necrotizing encephalitis in influenza. (Neilson et al., 2009).

2.4.3 Influenza A and B infections

Influenza A viruses are traditionally thought to cause more severe infections compared to influenza B viruses. This may arise from the predominance of influenza A viruses during seasonal outbreaks and their capacity to cause pandemics. Influenza A predominates among young children, and also the risk of hospitalization is higher among young children compared to older children (Heikkinen et al., 2014; Silvennoinen et al., 2015). Only a few studies comparing influenza A and B have considered age when assessing the severity of the symptoms. However, several studies have reported rather similar severity and symptoms across these two types of influenza (Daley et al., 2000; Hong et al., 2015; Mancinelli et al., 2016; Peltola et al., 2003; Silvennoinen et al., 2015; Streng et al., 2018). On the contrary, a large multiseasonal study found that children with influenza B had significantly more headaches, abdominal pain, myositis and myalgia (Tran et al., 2016). Also in other studies, myositis and myalgia have been observed more often in association with influenza B infections (Hong et al., 2015; Peltola et al., 2003; Silvennoinen et al., 2012).

2.5 Diagnostics

The diagnosis of influenza is traditionally made on the basis of clinical signs and symptoms during the seasonal influenza epidemic. However, several studies show that influenza virus infections in children are indistinguishable from other acute respiratory infections without specific diagnostics (Buchan et al., 2016; Ohmit & Monto, 2006; Peltola et al., 2005; Zambon et al., 2001). A definitive, rapid influenza diagnosis is precious for optimizing antiviral treatment and for cohorting of hospitalized patients. A rapid, specific diagnosis also helps clinicians to avoid unnecessary hospitalizations and antibiotics. Several different diagnostic methods are currently available for influenza diagnostics, and various types of patient samples can be used. Influenza virus nucleic acids or antigens are typically detected from nasopharyngeal swabs but, for example, nasal washes, throat swabs and bronchoalveolar lavage specimens can also be used.

2.5.1 Virus culture

A virus culture is the "gold standard" in influenza diagnostics. In this traditional method, viruses from patient specimens are inoculated in cell lines and then identified based on virus-specific cytopathy evident in light microscopy. After that, viruses are recognized by virus-specific antibodies or with nucleic acid techniques. Viral culture can be done from various kinds of patient samples, and the method is very sensitive but not rapid enough for everyday clinical use. When combined with immunological techniques, influenza viruses can be identified more rapidly, even within 1–2 days.

However, a virus culture has a special value in epidemiological control. It gives possibilities to observe antigenic drift and shift and to identify influenza subtypes. Information on the strain characteristics is a requirement for developing effective influenza vaccines and for preparing for pandemics.

2.5.2 Antigen detection

Antigen detection tests use monoclonal antibodies that target the viral nucleoprotein and utilize immunological technologies. These technologies include immunofluorescence (IF), enzyme-linked immunosorbent assays (ELISA) and immunochromatographic (lateral flow) techniques. Many current antigen detection methods are available as rapid tests and give results within 10–30 minutes. Rapid antigen tests are known for their simplicity and advantages in clinical practice, especially if deployed at the point of care (POC) (Williams et al., 2014). Multiplex panels are useful in differential diagnostics of viruses cocirculating in children (Gunell et al., 2016; Tuuminen et al., 2013).

2.5.3 Nucleic acid-based tests

Molecular biology has progressed rapidly during the last years. Nucleic acid amplification tests (NAATs) are based on polymerase chain reaction and the enzymatic amplification and specified detection of the amplified DNA or RNA sequences. Today, the reverse transcription-polymerase chain reaction (RT-PCR) is the most important technique for influenza diagnosis. It is specific and superior to virus culture because of its sensitivity and rapidity.

Most NAATs for respiratory viruses are commercial multiplex panels and available in a POC setting. In viral analytics, semi-quantitative and quantitative information from NAAT is useful. The cycle threshold (Ct) is one example of the semi-quantitative information of NAAT. It is defined as a calculated cycle number at which the polymerase chain reaction (PCR) product crosses a threshold of detection. A low Ct value reflects high viral load in the sample.

2.5.4 Serology

Influenza virus infection can be diagnosed also by serological methods based on either the presence of influenza-specific immunoglobulin (Ig) M antibodies or a significant increase in the levels of IgG antibodies in the convalescent sample. The need for a second serum sample 10–14 days after a baseline sample limits the usefulness of serology in clinical practice. Serological methods are useful in retrospective diagnostics and in investigating the response to influenza vaccination (Dwyer et al., 2006; Harper et al., 2009).

2.6 Treatment

Effective antiviral drugs have a great potential to diminish the burden of influenza in the community. They can shorten the duration of symptoms, lessen symptoms and reduce the risk of complications and death. Rapid influenza diagnostic tests allow for early, specific influenza diagnoses and help to start the treatment in the early course of an infection.

The discovery of the crystal structure of neuraminidase together with an increased understanding of the major role of neuraminidase in the replication of influenza viruses has led to the development of neuraminidase inhibitors. Four different antivirals are approved for use in influenza-infected children. Three of them are neuraminidase inhibitors being oseltamivir, zanamivir and peramivir, and the fourth is a cap-dependent endonuclease (CEN) inhibitor, baloxavir marboxil. The continuous evolution of influenza viruses makes them capable of developing changes to the binding site of antivirals. The development of resistance can come up spontaneously or during antiviral treatment.

2.6.1 Adamantanes

Adamantanes, amantadine and rimantadine are historical antivirals that have been in clinical use for treatment against influenza A. The effect of adamantanes is based on inhibition of M2 protein, which prevent viral uncoating and block the endocytosis of the influenza virus (Hay et al., 1985). The effectiveness of adamantanes is restricted to influenza A viruses because influenza B viruses do not contain M2 protein. Since all currently circulating influenza A viruses are resistant to the M2 inhibitors, they are no more recommended for clinical use.

2.6.2 Neuraminidase inhibitors

2.6.2.1 Oseltamivir

Neuraminidase is one of the two anchored surface proteins of influenza A and B viruses. The activity of the neuraminidase enzyme is required for removing sialic acid on the host cell surface, which contributes to the release of viruses from the surface of infected cells (Laborda et al., 2016; Moscona, 2005). Neuraminidase inhibitors block the enzyme activity of neuraminidase, preventing the release of newly formed virions from infected cells and thereby reducing viral replication.

Oseltamivir is available by oral administration, and it is officially recommended for infants 2 weeks and older. In Finland, oseltamivir is available by prescription as capsules (30 mg, 45 mg and 75 mg) and powder for an oral suspension. For the treatment of influenza in children, the recommended dosage of oseltamivir is 3 mg/kg twice a day for 5 days with the maximum single dose of 75 mg. Oseltamivir is rapidly converted into the active neuraminidase inhibitor oseltamivir carboxylate in the liver.

Antiviral recommendations vary between different countries. The CDC of the United States recommends empiric antiviral treatment soon after the onset of symptoms of suspected influenza in children under 2 years old; all hospitalized patients; patients with severe, complicated, or progressive diseases and for persons who are at an increased risk for the complications of influenza. Additionally, United States guidelines encourage the consideration of empiric antiviral treatment also for the treatment of outpatients with influenza suspicion. The treatment of non-high-risk outpatients is recommended only if initiated within 48 hours of symptom onset (CDC, 2023a; Uyeki et al., 2019). The Finnish Institute for Health and Welfare has more conservative recommendations. They recommend antiviral treatment for influenza patients who are at risk for influenza-related complications or hospitalization (THL, 2023a).

Due to the mode of action, early administration of neuraminidase is essential to acquire the maximum effect of preventing viral replication. To gain maximal clinical effectiveness against influenza, the administration of oseltamivir should be started as soon as possible during the early phase of the illness (Aoki et al., 2003; Heinonen et al., 2010; Malosh et al., 2018). In a Finnish RCT, oseltamivir shortened the duration of influenza A illness for about 4 days when started within 24 hours of the onset of the influenza illness. In the same study, oseltamivir appeared to be more effective in unvaccinated children (Heinonen et al., 2010).

Oseltamivir is more effective against influenza A compared to influenza B in children (Heinonen et al., 2010; Kawai et al., 2006; Sugaya et al., 2007). In *in vitro* studies, influenza B viruses have demonstrated a reduced susceptibility to

neuraminidase inhibitors that might explain this difference (Burnham et al., 2013). However, there are also results indicating that early oseltamivir treatment can have an effect also on the risk of influenza B virus-associated pneumonia (Dai et al., 2020).

Oseltamivir has proven effective against the risk of developing AOM (Malosh et al., 2018). In a Finnish study, oseltamivir prevented 80% of AOM complications if initiated within 12 hours of the onset of symptoms (Heinonen et al., 2010). Moreover, oseltamivir treatment is associated with a reduced risk of influenza-related pneumonia (Dai et al., 2020; Lee et al., 2020).

Neuraminidase inhibitors are well tolerated also in children and infants. Vomiting is the most common side effect reported in children (Heinonen et al., 2010; Malosh et al., 2018; Siedler & Skopnik, 2010; Whitley et al., 2001).

Oseltamivir is widely used in hospitalized patients and thought to have several advantages, but its effectiveness in children is not undisputable (Biondi & Krysan, 2012; Bueno et al., 2013; Campbell et al., 2021; Coffin et al., 2011; Muthuri et al., 2014; Walsh et al., 2022b). In a recent United States study with 55799 children hospitalized with influenza, early oseltamivir treatment was associated with a one day shorter median length of stay in the hospital and lower odds of hospital readmissions, transfer to ICU, use of extracorporeal membrane oxygenation and death (Walsh et al., 2022b).

The real-life use of oseltamivir in children is not in concordance with antiviral treatment guidelines, as oseltamivir is underutilized in children both in outpatient and inpatient settings. In a recent Canadian study, only 41% of children hospitalized with a confirmed influenza infection got antivirals (Mehta et al., 2021). Similarly, in a recent United States outpatient study, 42% of influenza-infected children at high-risk for complications did not receive guideline-concordant antiviral treatment (Antoon et al., 2023). However, in recent years, an increasing use of oseltamivir has been reported among hospitalized patients (Walsh et al., 2022a).

2.6.2.2 Zanamivir

Zanamivir is another neuraminidase inhibitor available against influenza. Aerosol formation for oral inhalation of zanamivir is available for prevention and treatment of influenza. Zanamivir is used in influenza-infected children over 5 years of age within 36 hours of the onset of illness. The preferred dosage is 5 mg twice daily for 5 days. An intravenous formulation of zanamivir is also available, but it is indicated only for children over 6 months of age with a complicated and life-threatening influenza virus infection. The preferred dosage of intravenous zanamivir is 4 mg/kg twice a day for 5–10 days with a maximum single dose of 600 mg. In a systematic review, zanamivir treatment shortened the duration of influenza infection.

Additionally, zanamivir was better tolerated compared to oseltamivir in children (Su et al., 2022).

2.6.2.3 Peramivir

Peramivir acts as a transition state analogue inhibitor of influenza neuraminidase and thereby prevents new viral particles from leaving infected cells. It is administered as a single intravenous dose and only for the treatment of acute uncomplicated influenza in children over two years old. All these factors limit its clinical use, but it is available also in Finland. According to a review, it is potent, effective and a well-tolerated neuraminidase inhibitor and recommended as an additional option for treating uncomplicated influenza in children older than two years (Scott, 2018).

2.6.2.4 Resistance to neuraminidase inhibitors

Influenza viruses are constantly changing, and variations in the neuraminidase enzyme may lead to a reduced susceptibility to neuraminidase inhibitors. Neuraminidases in influenza A/H1N1 viruses are more sensitive to crucial changes to enzymatic function compared to influenza A/H3N2 and influenza B viruses. For example, H275Y is a known neuraminidase mutation of A/H1N1pdm09 virus that causes oseltamivir resistance. The risk of developing neuraminidase resistance is increased in children and immunocompromised patients, who potentially have persistently high viral loads (Lampejo, 2020). The CDC of the United States and Public Health England follow and keep up with current influenza resistance data (CDC, 2023b; Gov.UK, 2023). Currently, all the tested influenza viruses were susceptible to zanamivir, and the vast majority (>99%) were susceptible both to oseltamivir and peramivir (Merced-Morales et al., 2022).

2.6.3 Cap-dependent endonuclease inhibitors (CEN)

Cap-dependent endonuclease inhibitors (CEN) is an essential enzyme in the initiation of messenger RNA synthesis of influenza virus. CEN inhibitors inhibit viral replication by targeting the endonuclease function. The first CEN inhibitor, baloxavir marboxil, is metabolized to its active form baloxavir acid that is capable of blocking influenza virus replication. It is administrated as a single oral dose, but it is not yet available in Finland. A meta-analysis of baloxavir marboxil indicated less adverse events compared to oseltamivir and zanamivir (Liu et al., 2021). Recently circulating influenza viruses have shown no resistance to baloxavir marboxil (Merced-Morales et al., 2022).

2.6.4 Other antivirals

There are also new antivirals under investigation. Laninamivir and favipiravir are licensed for use in Japan (Ikematsu & Kawai, 2011; Łagocka et al., 2021). Laninamivir is a long-acting, inhaled neuraminidase, and favipiravir is an oral and intravenous antiviral that inhibits RNA-dependent RNA polymerases. New potential compounds against influenza are, for example, 1,3-dihydroxy-6-benzo [c] chromene (D715-2441) and FA-6005, and also, phytochemicals and bacterial RNA are under investigation (Świerczyńska et al., 2022).

2.7 Prevention

2.7.1 Vaccination

Vaccination is the most important way to diminish the burden of influenza. Influenza viruses change continuously and thus evade the host immune response. To provide optimal protection, the influenza vaccine composition must be updated for every influenza season. The prediction of the timing and severity of upcoming influenza epidemics is hard, and the development of effective influenza vaccinates requires continuous surveillance and analysis of the antigenic properties of different influenza strains. Every year, the World Health Organization (WHO) recommends the combination of influenza virus strains to be included in vaccines for each world hemisphere. However, the influenza vaccine production process takes several months, thus when the seasonal influenza vaccines become available, the circulating viruses may have already changed. The vaccines are safe, and they are effective for the prevention of influenza if the strains included in the vaccine are a good match with the circulating strains (Heikkinen & Heinonen, 2011; Heinonen et al 2011b).

Injectable, inactivated influenza vaccines (IAV) have been available already since the 1940s (Salk et al., 1945). The first live attenuated influenza vaccines (LAIV) administered as a nasal spray were developed in the 1960s, but eventually LAIV was not approved until in 2003. Both of those vaccine types are produced from viruses grown in embryonated eggs. In 2013, the first influenza vaccines using recombinant DNA technology were approved. Newer technologies, such as nanoparticles and gene- and vector-based technologies, are currently under investigation (Rockman et al., 2020).

The protective effect of influenza vaccines is based on vaccine-induced antibodies against the surface proteins hemagglutinin and neuraminidase. An immediate response of the innate immune system is followed by cascades of the adaptive immune system. Antibodies against one type of influenza virus do not give significant protection against different subtypes or lineages (Wiggins et al., 2021).

The immunogenicity of influenza vaccines can be measured by hemagglutination inhibition titers. The strength and duration of the antibody response depend on the person´s medical history, previous influenza infections and vaccinations. Generally, children have a weaker antibody response compared to adults (Tapia et al., 2016; Walter et al., 2010).

Recently, several meta-analyses have demonstrated the impact of influenza vaccination against hospitalization in children. Kalligeros et al. found a 57.5% and Boddington et al. found a 53.3% vaccine effectiveness in preventing hospitalizations. In both meta-analyses, the best protection among different influenza strains was reported against A/H1N1. Vaccine effectiveness was reported higher in seasons when the circulating influenza strains were antigenically matched to vaccine strains (Boddington et al., 2021; Kalligeros et al., 2020).

2.7.1.1 Inactivated influenza vaccines (IAVs)

Inactivated influenza vaccines (IAVs) contain subtypes of influenza A/H3N2 and A/H1N1 and one or two lineages of influenza B viruses. Data about the comparable severity of influenza A and B infections (Daley et al., 2000; Hong et al., 2015; Mancinelli et al., 2016; Peltola et al., 2003; Silvennoinen et al., 2015; Streng et al., 2018) support the use of quadrivalent vaccines. Moreover, the cost effectiveness of quadrivalent vaccines has been demonstrated in several studies (Dolk et al., 2016; Nagy et al., 2016; Thommes et al., 2015) and, currently in Finland, for example, all seasonal influenza vaccines are quadrivalent. Most are licensed for use in children from 6 months of age.

Inactivated influenza vaccines are effective against influenza in children. In Taiwan, during the influenza seasons between 2004–2009, the vaccine effectiveness (VE) was 51% in children 6–23 months old. Correspondingly, in Finland, during 2007–2008, the VE was 66% in children younger than 24 months of age (Heinonen, et al., 2011b; Su et al., 2015). When the antigens in the vaccine have a suboptimal match, the benefit of vaccines remains modest. Besides preventing influenza infections, influenza vaccines reduce influenza-associated hospitalizations in infected children (Boddington et al., 2021) and also reduce the development of complications, such as AOM (Clements et al., 1995; Heikkinen et al., 1991; Norhayati et al., 2017). Inactivated trivalent and quadrivalent influenza vaccines are generally well tolerated, and serious adverse events are rare. Local injection site reactions, such as erythema and swelling, and fever are the most frequent adverse events in children under 18 years (Haber et al., 2016, 2019).

2.7.1.2 Live attenuated influenza vaccine (LAIV)

The live attenuated influenza vaccine (LAIV) is made from weakened influenza viruses and administered as a nasal spray instead of by injection. These vaccines consist of cold-adapted influenza viruses, which can replicate in a temperature of 33–34ºC in the upper airways but lose their ability to replicate at higher temperatures in the lower airways (Wareing & Tannock, 2001). The LAIV is based on a modified influenza strain that does not cause disease but activates the immune system. These vaccines activate both cellular and humoral immune responses of the adaptive immune system.

The effectiveness of LAIV in children is proven in several studies, and, especially, the highest efficacy has been in children aged 6 months to 7 years (Osterholm et al., 2012). The LAIV has also been reported to reduce the incidence of all-cause AOM compared with placebo in children. In one analysis, the estimated 12-month effectiveness of LAIV against AOM was comparable to a 7-valent pneumococcal conjugate vaccine (Heikkinen, et al., 2013a). In a large RCT, the efficacy of LAIV against influenza-associated AOM was 85% compared with placebo and 54% compared with trivalent influenza vaccine (Block et al., 2011).

The adverse events associated with LAIVs in children aged 2–17 years old are mild and are rhinorrhoea, nasal congestion and sore throat (Ambrose et al., 2011). In studies in children <2 years of age, an increased risk for wheezing was reported, and therefore they are currently recommended only for children older than 2 years (Belshe et al., 2007).

2.7.1.3 Adjuvants and vaccine dose

To promote a better immune response, adjuvants can be used in influenza vaccines. Adjuvants can also reduce the amount of antigen needed per vaccine. Currently, there is only one widely used influenza vaccine with an adjuvant. That adjuvant is an oil-in-water emulsion of squalene oil, called MF59, and used in a standard dose quadrivalent influenza vaccine for people older than 65 years old (Tregoning et al., 2018). At the beginning of the A/H1N1 pandemic of 2009–2010, an AS03 adjuvant was used in a pandemic influenza vaccine. After the peak of the pandemic, an increase in narcolepsy was detected among children who had received the AS03-adjuvanted vaccine. A Finnish systematic review and meta-analysis reported a 5- to 14-fold higher relative risk of narcolepsy in children and adolescents and 2- to 7-fold higher risk in adults (Sarkanen et al., 2018).

Another way to increase the efficacy of an influenza vaccine is to increase the amount of hemagglutinin contained in the vaccine. For young children, a half dose of influenza vaccine (7.5 μg hemagglutinin per strain) has been used before, but currently, a full dose influenza vaccine (15 μg hemagglutinin per strain) is

recommended in Finland. A full dose influenza vaccine induces higher hemagglutination inhibition titers and has proven more effective compared to half-dose influenza vaccines (Jain et al., 2017; Langley et al., 2012; Pavia-Ruz et al., 2013). Several studies have shown the safety of full-dose vaccines in children (Claeys et al., 2018; Heinonen et al, 2011a). Currently, there is also a high-dose quadrivalent influenza vaccine (60 μg hemagglutinin per strain) available, but its use is limited to the elderly (Wilkinson et al., 2017).

2.7.1.4 Influenza vaccine recommendations

Influenza vaccination of healthy children began in 2003 for children 6–23 months of age in the United States, and since 2008, the recommendations have covered all healthy children 6 months and older. Worldwide, the WHO recommends vaccination of all children from 6 months to 5 years of age, children with specific chronic medical conditions, pregnant women and healthcare workers. However, many European countries do not follow the WHO influenza vaccine recommendations, and influenza vaccinations are not offered to all children. In Europe, during the influenza season of 2010–2011, only the United Kingdom, Ireland, Austria, Estonia, Finland, Latvia, Malta, Poland, Slovakia and Slovenia had a recommendation for influenza vaccination of children (Mereckiene et al., 2014). However, despite general recommendations in several countries, the coverages of influenza vaccination have remained low. This may be due to several factors, such as inadequate local arrangements for influenza vaccination of children, but perhaps the most important obstacle is that few countries have included influenza vaccines in their fully reimbursed immunization programs for children. In one study during the influenza season of 2014–2015, the highest coverage rate was 80% in Northern Ireland, but the median coverage in 13 European countries with immunization recommendations was only 10.9% (Jorgensen et al., 2018). Fully reimbursed influenza vaccinations for all children are currently included in the national vaccination programs in, for example, Finland, the United Kingdom, Austria and Ireland.

Finland was the first European country to start influenza vaccinations of healthy children in 2007. Currently, according to the Finnish national vaccine recommendations, free influenza vaccinations are offered for all children older than 6 months but younger than 7 years of age, people over 65 years old, people with an underlying risk condition, pregnant women, healthcare workers, people starting their military service and for people close to a person susceptible to serious influenza. A nasal spray vaccine is available for children aged 2–6 years, and an injectable quadrivalent vaccine is available for all children from 6 months of age. During the last years, influenza vaccine coverage among Finnish children <3 years of age has been 30–43% (THL, 2023b).

In the absence of vaccine-based prevention of influenza in young infants <6 months of age, maternal influenza vaccination during pregnancy is recommended for reducing the risk of influenza in infants. The WHO has recommended influenza vaccination for all pregnant women since 2005. In the United Kingdom, for example, the uptake of the influenza vaccine among pregnant women has been around 45% during the last years (Sebghati & Khalil, 2021). However, the duration of protection afforded by maternal antibodies is limited (Jarvis et al., 2020; Nunes et al., 2016; Omer et al., 2020; Zaman et al., 2008).

Because children are the main transmitters of influenza in the society, vaccination of children may provide indirect protection against influenza for others, especially for the elderly population. A recent large systematic review and meta-analysis suggested that influenza vaccination of children may provide indirect protection to other age groups (Yin et al., 2017).

2.7.2 Antiviral prophylaxis

Although vaccination is the cornerstone of influenza prevention during both epidemic and pandemic situations, antiviral medications can also be used in special situations as an adjunct to vaccination for controlling influenza. Antiviral chemoprophylaxis can be divided into post-exposure and seasonal prophylaxis.

2.7.2.1 Post-exposure prophylaxis (PEP)

The purpose of post-exposure prophylaxis (PEP) is to prevent the development of influenza illness after exposure, especially in hospital and family settings. Of the current antivirals, the neuraminidase inhibitors, oseltamivir and zanamivir, and the CEN inhibitor, baloxavir marboxil, can be used.

Oseltamivir is effective in PEP in households. In one study, oseltamivir was proven effective in PEP in the households where the influenza infections of the index patients were confirmed virologically, but they did not receive any antiviral therapy (Welliver et al., 2001). In another study, in which the index patients were treated with oseltamivir, oseltamivir was effective in PEP among household members aged 1 year or older. The efficacy of PEP by oseltamivir in children 1–12 years of age was 80% (Hayden et al., 2004).

Another neuraminidase inhibitor, zanamivir, also prevents influenza transmission in households (Hayden et al., 2000; Monto et al., 2002). In a double-blind, randomized study, Monto et al. showed the effectiveness of zanamivir in the PEP of influenza in persons 5 years and older. In their study, the index patients did not receive antiviral agents (Monto et al., 2002).

Occasionally, PEP of influenza is also recommended in hospital settings. PEP with oseltamivir and zanamivir for 7–10 days has been found to be effective against nosocomial infections in paediatric wards (Shinjoh et al., 2012). Furthermore, a 3-day regimen of oseltamivir as PEP has been reported to be comparable to a 7–10-day regimen in wards (Ishiguro et al., 2016).

In a recent study, also the CEN inhibitor baloxavir marboxil was established effective in the PEP of influenza in households. In that study, the index patients were treated with antiviral agents, and the risk of laboratory-confirmed influenza was reduced by 86% among those who received baloxavir marboxil within 24 hours of the onset of symptoms in index patients (Ikematsu et al., 2020).

2.7.2.2 Seasonal prophylaxis

Seasonal prophylaxis indicates the long-term use of antivirals during the influenza season. Occasionally, it is recommended for special patients like those who are immunocompromised or who have some other condition that increases the risk for a complicated influenza infection. Neuraminidase inhibitors can be used in seasonal prophylaxis (Hayden et al., 1999; Monto et al., 1999).

2.7.3 Non-pharmaceutical interventions

Non-pharmaceutical interventions include social distancing and hygienic recommendations. Avoiding contact with sick people and washing hands are traditional ways to diminish the spread of viruses. During pandemics, more restrictive measures, like school closures and remote work recommendations, may be used. Regarding children´s influenza, only limited data exist about the benefits of these prevention methods.

During the Covid-19 pandemic, the use of surgical masks increased significantly. Surgical masks are used to protect against large droplets and to diminish the touching of the face. In two RCTs, surgical masks and filtering face piece 2 (FFP2) masks were evaluated for the prevention of influenza among nurses working with patients with respiratory infections. In those studies, standard surgical masks were no worse than N95 respirators (Loeb et al., 2009; Radonovich et al., 2019). According to a recent meta-analysis, wearing a mask has a minimal effect against laboratory-confirmed influenza, but hand hygiene might offer some benefit, with an observed 11% relative reduction in respiratory infections (Jefferson et al., 2020).

3 Aims

The specific aims of the thesis were:

I To compare virologically confirmed influenza A and B infections in hospitalized children

II To estimate the burden of influenza during the first year of life

III To evaluate the effectiveness of oseltamivir in reducing the duration of illness, severity of clinical symptoms and viral load in outpatient children younger than 1 year of age with influenza A and B infection

IV To investigate trends and changes in influenza-associated hospitalizations of children during a period of 25 years

4 Materials and Methods

More detailed description of materials and methods are presented in the original publications.

4.1 Subjects, study design and data collection

This thesis is comprised of four original studies with two separate study populations.

4.1.1 Inpatient studies

Studies I and **IV** were retrospective studies conducted at the Department of Paediatrics and Adolescent Medicine, Turku University Hospital, Finland. Turku University Hospital is the sole tertiary-care hospital in Southwestern Finland and the only provider of acute paediatric hospital care for children. The study population consisted of all children <16 years of age who were hospitalized with virologically confirmed influenza A or B infection during the period of July 1, 2004, through June 30, 2018 in **Study I** and during the period of July 1, 1993 through June 30, 2018 in **Study IV**.

In **Study IV**, we analysed population-based rates of hospitalization, and to enable more reliable estimations, only children who lived within the catchment area of the hospital were included in the study. Detailed annual information on the population of children in different age groups was obtained from the official databases of Statistics Finland. The average number of children <16 years of age living in the catchment area of the hospital was 70,890 during the 25-year study period.

To find all children who were hospitalized with virologically confirmed influenza, we searched for data from four different sources: 1) the database of the Department of Virology, University of Turku, 2) the central database of Turku University Hospital, 3) the files of the paediatric infectious diseases ward and 4) the database at the paediatric ICU. The medical records of all children with an International Classification of Diseases (ICD) code related to influenza (ICD-9: 4870A, 4871A, 4878X; ICD-10: J10-J11), who were not found from the virologic databases, were thoroughly examined to confirm or rule out the viral diagnosis of

influenza. We excluded children whose viral specimens had been obtained >2 days after admission because of potential nosocomial influenza infections. Furthermore, in **Study I,** children with simultaneous influenza A and B infections were excluded. For retrieving the data on the clinical presentation, outcomes, management and duration of hospitalization, the medical records of the children were examined by a systematic hand search.

4.1.2 Outpatient studies

For **Studies II** and **III**, data were derived from a prospective cohort study performed at a primary care study clinic in Turku, Finland, from September 1, 2017, to June 30, 2018. The parents of infants born at Turku University Hospital in June to August 2017 received written information about the study at the maternal ward of the hospital soon after the child was born, and parents who wanted their child to participate signed an informed consent form. Infants were eligible for participation if they lived within the catchment area of the hospital, the parents were able to understand and communicate in Finnish language and the infant did not have any major congenital defects or serious chronic illnesses. Of all children born during the enrolment period, approximately half were enrolled in the study. The parents filled out a questionnaire regarding background information about the family, pregnancy and delivery before the start of the study.

The parents were invited to fill out daily symptom diaries, with one for September to January and another for February to June, throughout the 10-month follow-up period. Children were regarded as active participants if they visited the study clinic at least once or if their parents returned at least one of the two symptom diaries, and if the parents did not inform the study personnel that their child had been treated for a respiratory illness somewhere else than at the study clinic. Data on influenza vaccination of all family members were collected after the influenza season. Furthermore, the parents of influenza-infected children were asked to complete a separate, more detailed influenza symptom diary twice daily, in the morning and in the evening, until their child was asymptomatic, but at least for 8 days. In that detailed symptom diary, day 1 was the day of the first dose of oseltamivir treatment or the day of the first visit to the study clinic for infants who did not receive oseltamivir. At each time point, the parents filled out the infant´s measured temperature, the presence and severity of cough, rhinitis, vomiting and diarrhoea (all on a 4-point scale), the administration of oseltamivir and the doses of antipyretic or analgesic medications administered during the preceding 12 hours.

The study clinic was open every day, including weekends and holidays. All visits to the study clinic were free of charge to the families, and there was no limit for the number of visits during the study. The parents were instructed to bring their child for

clinical examination as soon as possible after the onset of fever or signs of a respiratory infection. At each visit, the children were carefully examined by a study physician who recorded the signs and symptoms, clinical findings and treatment in a structured medical record and collected nasopharyngeal specimens for virologic analyses.

All infants were routinely re-examined at the study clinic 5–7 days after the illness onset. To allow for diagnosing all complications arising at any time during the illness, the parents were encouraged to bring their child for additional re-examinations whenever they deemed it necessary. Moreover, all infants with virologically confirmed influenza were re-examined at shorter intervals, usually at intervals of 2 days. All infants who were diagnosed with influenza within 48 hours of the onset of symptoms received oseltamivir treatment. The dosage of oseltamivir was 3 mg/kg twice daily for 5 days.

4.2 Virologic methods

In inpatient **Studies I** and **IV**, viral sampling was routinely performed for the identification of respiratory viruses in children hospitalized with respiratory symptoms. In these studies, the virologic diagnosis of influenza was made either at the Department of Virology by RT-PCR, antigen detection or viral culture, or at the hospital emergency department or wards by antigen detection. In the outpatient studies (**Studies II** and **III**), two nasopharyngeal specimens were collected for viral analyses from each child at the initial visit of each respiratory infection. One of the specimens was analysed immediately at the study clinic by a rapid antigen test for 11 respiratory pathogens (mariPOC® respi test, ArcDia International Ltd, Finland). The other specimen was refrigerated and transported daily to the laboratory at the Department of Clinical Microbiology, Turku University Hospital, where it was analysed by multiplex RT-PCR assays for 16 viruses (Allplex™ Respiratory Panels 1–3, Seegene Inc, South Korea). In **Study III**, children were frequently retested by the antigen test until it turned negative. Standardized influenza A and B virus nucleoprotein antigen preparations were utilized in the calculations of antigen concentrations in specimens.

4.3 Definitions

In **Studies I** and **IV,** each study year covered the period from July 1 through June 30 of the following year. If children were hospitalized with confirmed influenza twice during the study period, they were considered as two separate children who were analysed in the age group that they belonged to on the day of each admission. To adjust for age, in **Study I**, the children were classified into 3 age cohorts (0–2, 3–9

and 10–15 years), and, in **Study IV,** into 6 age cohorts (<6 and 6–11 months and 1, 2, 3–5 and 6–15 years) based on their age at the time of admission.

The lengths of hospital and ICU stays were recorded as the numbers of nights spent in each ward. The length of stay was recorded as 1 day in the case that a child was admitted in the morning and discharged in the evening of the same day. In case a child had been admitted to another hospital before being transferred to Turku University Hospital, the length of stay was calculated by adding up the durations of the hospitalizations.

The underlying risk conditions in hospitalized children included malignancies, immunosuppressive states, and pulmonary, cardiac, endocrine, liver, kidney and major neurologic disorders. Convulsions with fever were classified as febrile convulsions except in children who had a previous diagnosis of epilepsy. The diagnosis of pneumonia required radiological confirmation of the condition.

In **Study II**, all consecutive days on which the infant had fever, rhinitis or cough were counted in the total duration of illness. The presence of middle-ear effusion and signs of inflammation of the tympanic membrane, as detected by pneumatic otoscopy, and at least one sign of an acute infection were required for the diagnosis of AOM. Children were classified as vaccinated if they had received two doses of the seasonal influenza vaccine. During the study season of 2017–2018, the trivalent influenza vaccine in Finland included the following strains: A/Michigan/45/2015, A/Hong Kong/4801/2014 and B/Brisbane/60/2008.

In **Study III,** the total influenza symptom score at each time point was calculated by summing the scores for fever, cough, rhinitis and antipyretic/analgesic medications. The scores were analysed since the evening of day 1. The duration of preceding symptoms was defined as the time between the first symptoms reported by the parents and the first visit at the study clinic. The total duration of illness was calculated as the time between the first symptoms and the first time when the following conditions were met simultaneously and lasted so for ≥24 hours: measured temperature <37.5ºC, and rhinitis and cough either absent or mild.

4.4 Statistical methods

In all studies, the *t* test was applied for comparing normally distributed continuous data, and the Mann-Whitney *U* test or the Kruskal-Wallis test was used to compare non-normally distributed continuous data. Proportions were compared by the $\chi 2$ test or Fisher's exact test. Two-sided P values <0.05 were considered to indicate statistical significance.

In **Study II**, the incidence rate of influenza was calculated by dividing the number of influenza episodes by the follow-up time. To provide a conservative annual estimate regarding a seasonal virus, the follow-up time was determined as 1

year, although the actual follow-up time was 10 months. In **Study IV**, the incidence rates of hospitalizations were calculated by dividing the numbers of hospitalizations by the numbers of children at risk and expressed per 100,000 children.

All statistical analyses were performed using StatsDirect software, versions 2.8.0, 3.2.7 or 3.3.4 (StatsDirect Ltd, UK).

4.5 Ethics

The Ethics committee of the Hospital District of Southwest Finland approved the clinical studies, and written informed consent was obtained from the parents of all participating children (**Studies II** and **III**). Based on the Finnish Medical Research Act (Finlex, 2023), an ethical review was not required for the register-based retrospective studies (**Studies I** and **IV)**.

5 Results

5.1 Influenza A and B hospitalizations (I)

During the 14-year observation period, 391 influenza-infected children were hospitalized. Influenza A was diagnosed in 279 (71.4%) and influenza B in 112 (28.6%) children. The median ages of children hospitalized with influenza A and B were 2.6 and 6.4 years, respectively (P <0.0001). Excluding the pandemic influenza A season of 2009–2010, the relative proportions of influenza A ranged from 45% to 93% and those of influenza B from 7% to 55% of hospitalizations during the different seasons (Figure 1).

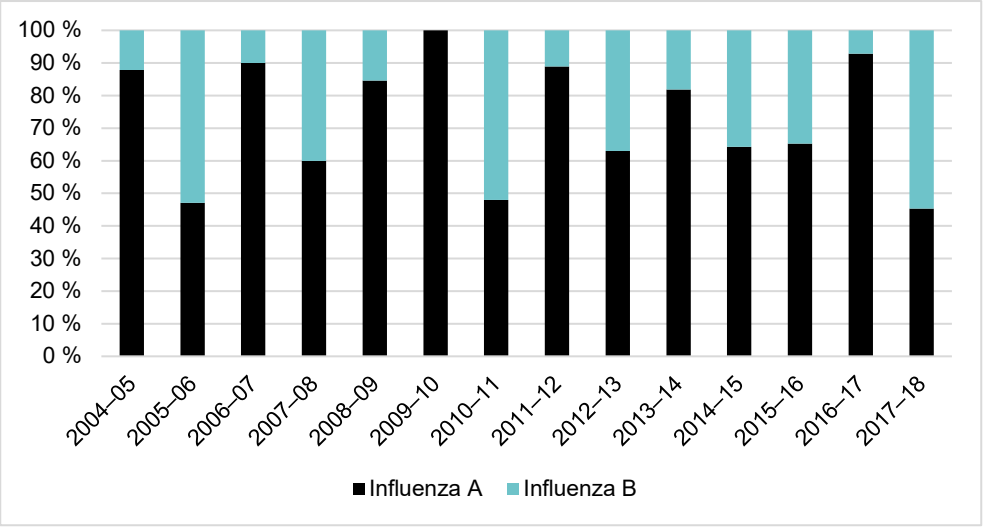

Figure 1. Relative proportions of children hospitalized with influenza A and B during each study year. Modified from Original Publication I.

5.1.1 Clinical features of influenza A and B

The mean length of stay in all children was 2.3 days (SD, 3.0) for influenza A and 2.7 days (SD, 4.1) for influenza B (P = 0.34). A total of 36 (12.9%) children with influenza A and 17 (15.2%) children with influenza B required treatment in the ICU

(P = 0.55). Lumbar puncture was performed on 16 (5.7%) influenza A -infected children and 11 (9.8%) influenza B -infected children (P = 0.15), and a blood culture was obtained from 101 (36.2%) children with influenza A and 39 (34.8%) children with influenza B (P = 0.80).

In detailed analyses within the different age groups and in all age groups combined, no statistically significant differences were observed in any signs, symptoms, laboratory findings or complications between children with influenza A and B. Moreover, there were no significant differences in any outcomes or the management of children with influenza A and B, whether analysed within age groups or in the entire group of children.

5.2 Burden of influenza during the first year of life (II)

5.2.1 Rates of influenza infections

Among the 408 actively participating children, a total of 55 episodes of laboratory-confirmed influenza were diagnosed. This corresponds to an annual incidence rate of 135/1000 children (95% CI 102–175). One child had two separate influenza illnesses, one caused by influenza A and another by influenza B viruses. Of the 54 children (13.2% of all) with at least one episode of influenza, 26 (48.1%) were boys and 28 (51.9%) were girls. Thirty-three (61.1%) children had at least one sibling, and 4 (7.4%) had received two doses of seasonal influenza vaccine. At the time of influenza diagnosis, the mean age of the children was 7.6 months (range, 4.3–11.0). The dominant virus types in Finland during the study season were A/H3N2 (41% of all) and B/Yamagata (54% of all) viruses. The monthly numbers of influenza A and B infections in the follow-up cohort are shown in Figure 2.

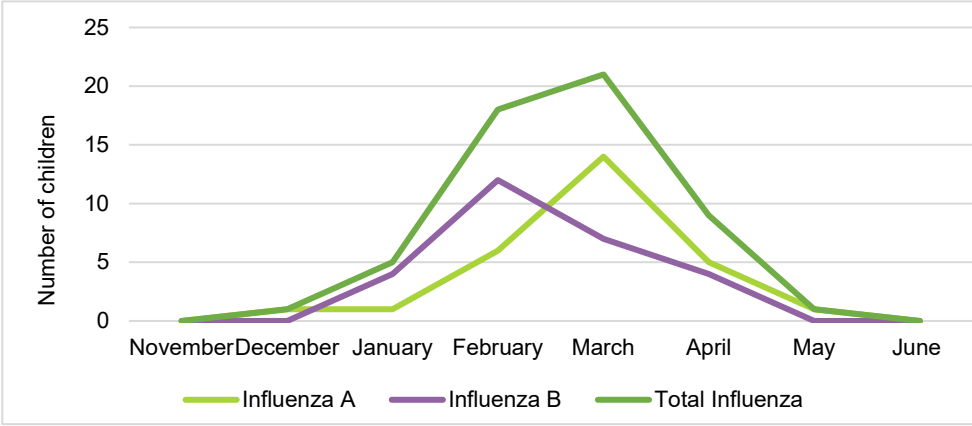

Figure 2. Influenza A and B virus infections in the study cohort during the influenza season of 2017–2018. Modified from Original Publication II.

5.2.2 Clinical symptoms and complications

Five episodes of influenza were excluded because of confirmed double viral infections in which another virus (i.e., rhinovirus or respiratory syncytial virus) dominated, and the final data consisted of 50 influenza episodes. Influenza A was detected in 26 (52.0%) and influenza B in 24 (48.0%) children.

The clinical findings in children with influenza A and B at the first examination at the study clinic are presented in Figure 3. The median duration of symptoms preceding the first visit was 13.5 hours (interquartile range, IQR, 7.5–23.8) in children with influenza A and 26.8 hours (IQR, 11.3–76.4) in those with influenza B (P = 0.035). The total median duration of influenza illness was 8.0 days (IQR, 6.0–11.3), while the median duration of fever was 3.0 days (IQR, 1.8–4.0); there were no differences between influenza A and B infections.

Overall, acute otitis media developed as a complication of influenza in 23 (46.0%) children, and 21 (42.0%) received antibiotics, all for the treatment of AOM. Eleven (47.8%) of all 23 AOM cases were diagnosed during a follow-up visit. One (2.0%) child was hospitalized because of febrile convulsion. In 37 (74.0%) children, influenza was virologically diagnosed within 48 hours of the onset of symptoms, and all these children received oseltamivir treatment.

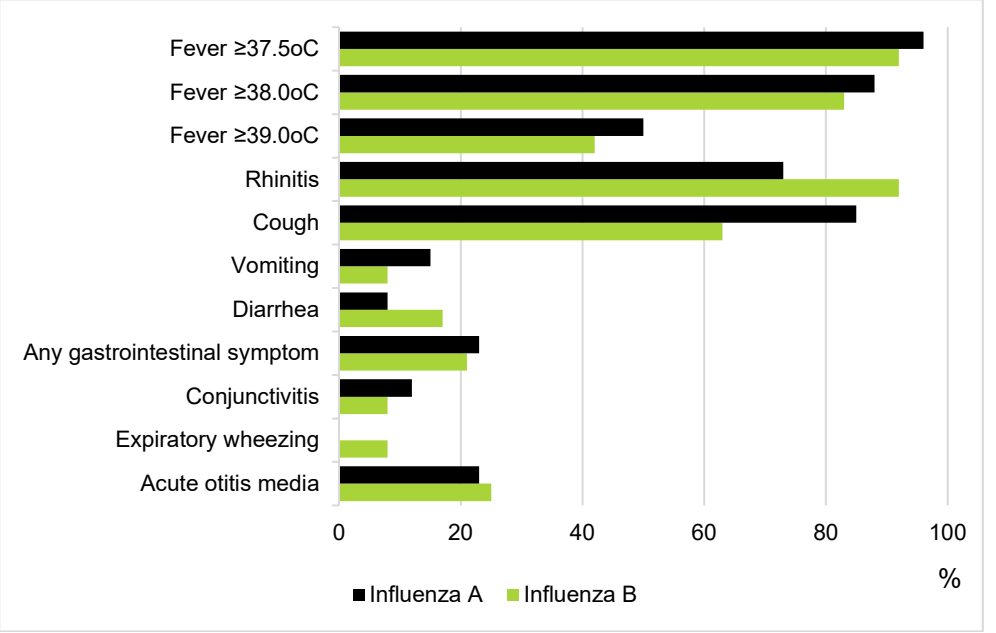

Figure 3. Signs and symptoms and clinical diagnoses at the initial visit in 50 children with influenza. Modified from Original Publication II.

5.2.3 Effectiveness of influenza vaccination

The families of 379 children provided data on their influenza vaccinations for the 2017–2018 season. A total of 54 study children had been vaccinated against influenza, and 4 (7.4%) of them were diagnosed with influenza. Among the 325 unvaccinated study children, 46 (14.2%) were diagnosed with influenza, which translates to a vaccine effectiveness of 48% (95% CI, -29%-80%; P = 0.17). A total of 123 (37.8%) of the 325 unvaccinated children lived in households in which all other family members had been vaccinated against influenza, and influenza was diagnosed in 17 (13.8%) of those children. Among the remaining 202 unvaccinated children who lived in households in which at least one of the other family members had not been vaccinated, 29 (14.4%) children were diagnosed with influenza (vaccine effectiveness 4%; 95% CI -66%-45%; P = 0.89).

5.3 Efficacy of oseltamivir treatment in infants (III)

5.3.1 Duration of symptoms

Initially, the follow-up cohort included 55 influenza episodes. After excluding double viral infections, children without an influenza symptom diary and children whose duration of illness could not be determined, the final analysis consisted of 38 infants. The mean age of these children at the diagnosis of influenza was 7.6 months (range: 4.3–10.9). Influenza A was accounted for 23 (60.5%) and influenza B for 15 (39.5%) of the cases. In 31 (81.6%) infants, influenza diagnosis was confirmed within 48 hours of symptom onset, and they all received oral oseltamivir treatment. In those children, the mean duration of symptoms preceding the initial visit to the study clinic was 15.9 (SD, 9.5) hours in influenza A cases and 19.1 (SD 12.4) hours in influenza B cases (P = 0.43).

The mean total duration of influenza A illness was 82.1 hours in infants with oseltamivir and 253.5 hours in those without (difference = 171.4 hours, P = 0.0003). The mean total duration of influenza B illness was 110.0 hours in infants with oseltamivir and 173.9 hours in those without (difference = 63.9 hours, P = 0.03). There was no significant difference in the mean total duration of illness between children with influenza A and B treated with oseltamivir (82.1 vs. 110.0 hours, P = 0.20).

5.3.2 Symptom scores

Among infants with influenza A, the total symptom scores in oseltamivir-treated infants were significantly lower at all time points between days 3 and 11 after the

onset of therapy when compared with infants without oseltamivir treatment. Among infants with influenza B, there were no significant differences in total symptom scores at any time point between infants treated with oseltamivir and those without treatment. In all children treated with oseltamivir, the symptom scores were significantly lower from the morning of day 4 through the morning of day 5 in children with influenza A compared to those with influenza B. The total influenza symptom scores at different time points are shown in Figure 4.

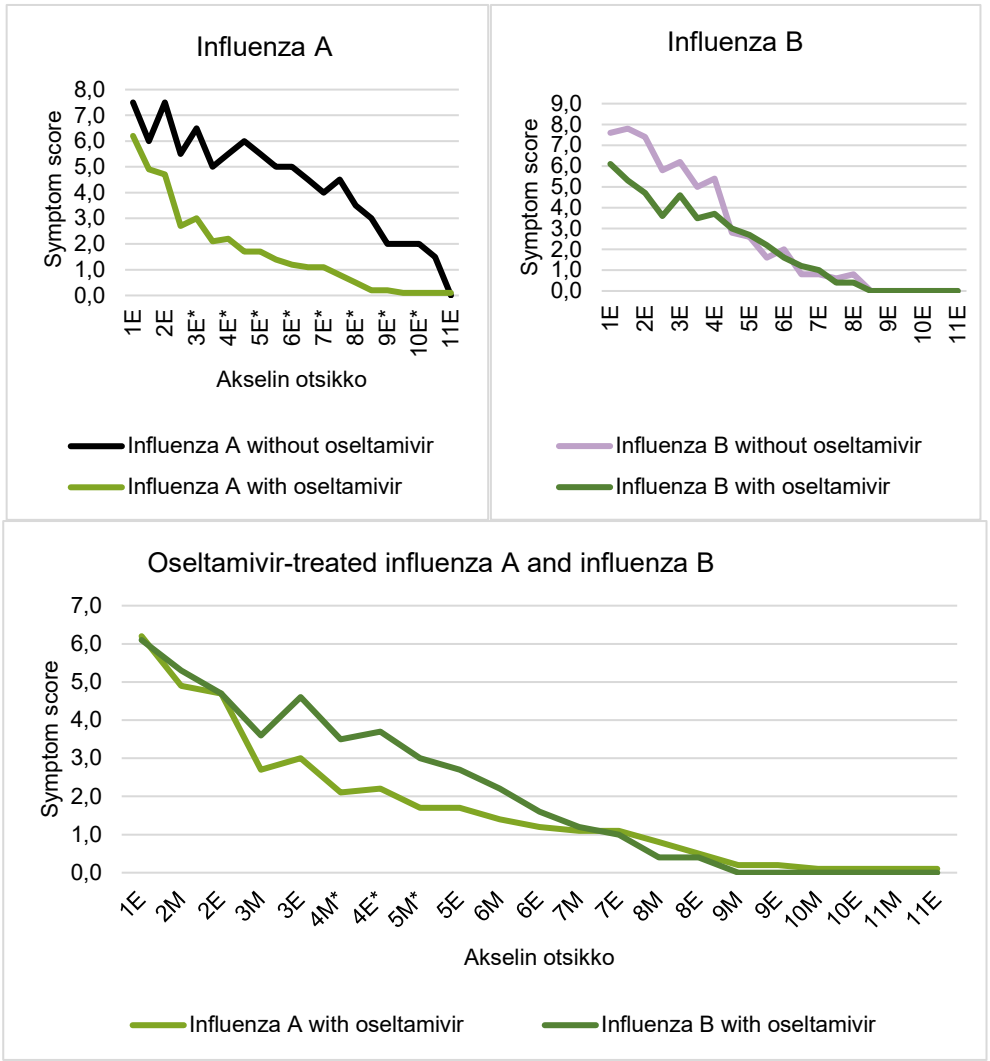

Figure 4. Total influenza symptom scores at different time points. Day 1 is the day of the first dose of oseltamivir. M is morning and E is evening. * indicate the time points at which the differences between the groups were statistically significant. Modified from Original Publication III.

5.3.3 Viral load

During the follow-up visits of children with influenza illnesses, an average of 4.1 (range, 2–8) nasopharyngeal swabs were obtained for determination of the viral antigen concentrations. In most children, regardless of the type of influenza, the antigen concentrations declined rapidly within 1–2 days after the initiation of oseltamivir treatment.

5.3.4 Adverse events

Among 7 infants not receiving oseltamivir, vomiting was reported in 2 (28.6%) and diarrhoea in 4 (57.1%) infants. Vomiting during oseltamivir treatment was reported by the parents in 15 (48.4%) and diarrhoea in 14 (45.2%) of 31 infants. However, in these infants, vomiting was already present before the first dose of oseltamivir in 5 (33.3%) of the 15 infants, and diarrhoea was present already before treatment in 4 (28.6%) of the 14 infants with diarrhoea. Two other infants had vomiting before oseltamivir treatment, but it disappeared after the first dose of the drug. In most cases, vomiting was rated as mild, and it ceased by the second day of treatment. In none of the infants, oseltamivir treatment had to be discontinued because of an adverse event.

5.4 Trends and changes in influenza-associated hospitalizations (IV)

5.4.1 Characteristics of children

During the 25-year study period, 703 children were hospitalized with laboratory-confirmed influenza infection. Influenza A was diagnosed in 536 (76.2%) children, influenza B in 159 (22.6%) children, and 8 (1.1%) children were infected with both A and B viruses simultaneously. Of all hospitalized children, 394 (56.0%) were boys and 309 (44.0%) were girls. At least one risk condition for severe influenza was present in 176 (25.0%) children, and the prevalence of such conditions increased with age. Seventy-four (10.5%) children were treated at the ICU, and 2 (0.3%) died. Between the consecutive 5-year periods of the study, a significant trend was observed regarding the age of the admitted children. Between 1993–1998 and 2013–2018, the median age of hospitalized children increased by 2 years, from 1.3 years to 3.3 years ($P < 0.0001$).

5.4.2 Incidence in different age groups

During the 25-year study period, the absolute population-based incidence rates of influenza hospitalization showed decreasing trends in all children <2 years of age. Compared with 1993–1998, the incidence rates in 2013–2018 were 27% lower among infants <6 months and 6–11 months of age (incidence rate ratio [IRR], 0.73; 95% CI, 0.42–1.27; P = 0.24 and IRR, 0.73; 95% CI, 0.34–1.51; P = 0.35, respectively). On the contrary, there were increasing trends in the incidence rates among children 6–15 years during the same periods (IRR, 2.94; 95% CI 1.70–5.32; P <0.0001) (Figure 5).

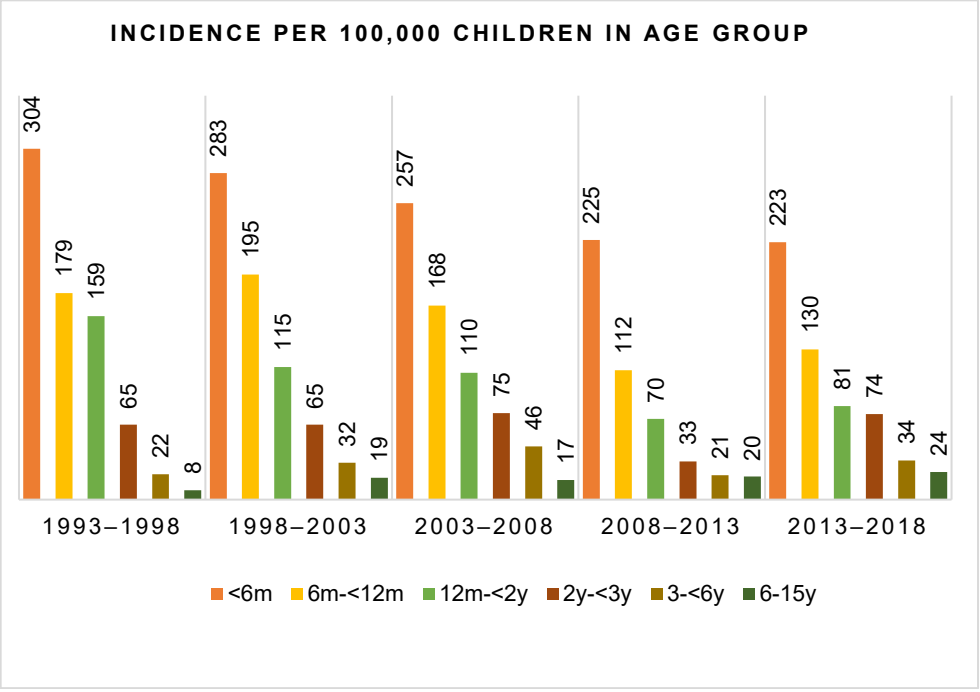

Figure 5. Population-based incidence rates of influenza-associated hospitalizations in different age groups of children during the 25-year study period. Modified from Original Publication IV.

Between 1993–1998 and 2013–2018, the relative proportion of children aged <2 years decreased from 65.2% to 36.8%, whereas the proportion of children between 6 and 15 years of age increased from 13.0% to 36.2% (P <0.0001 for both; Figure 6).

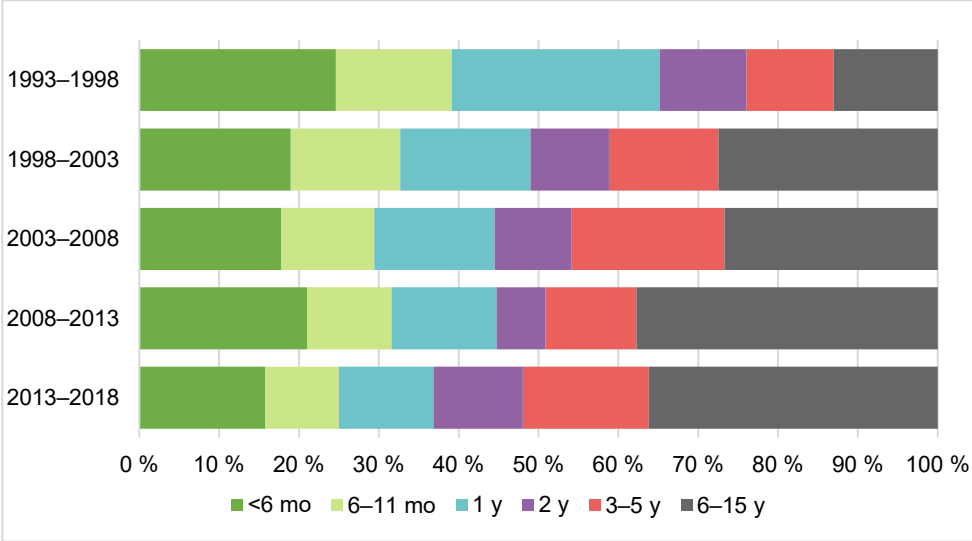

Figure 6. Relative proportions of age groups hospitalized with influenza. Modified from Original Publication IV.

The average annual incidence rate of influenza hospitalization was 43 per 100,000 (95% CI 39–48) in boys and 36 per 100,000 (95% CI 32–40) in girls (IRR, 1.21; 95% CI, 1.04–1.41; P = 0.01). In different age groups, the incidence rates were consistently higher in boys than in girls, although the differences within the age groups did not reach statistical significance (Figure 7).

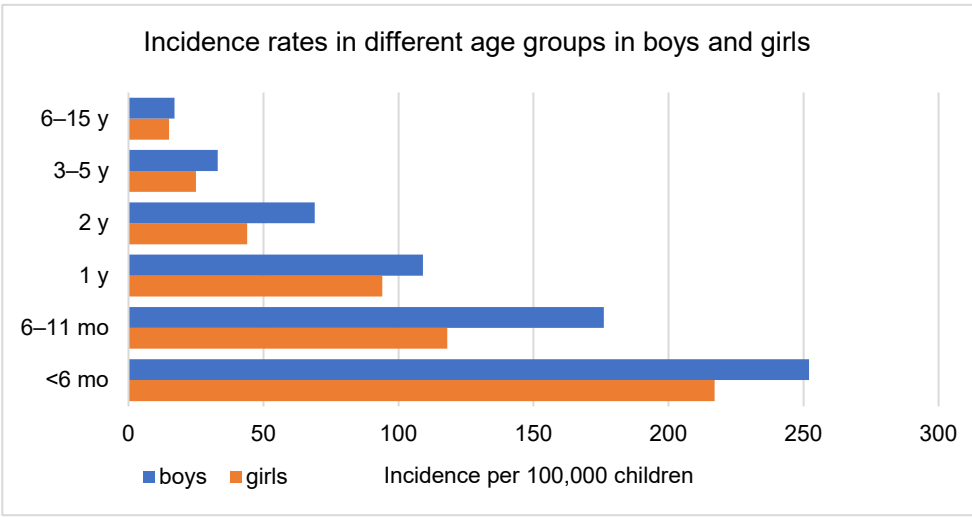

Figure 7. Average annual population-based incidence rates of influenza-associated hospitalizations in different age groups of boys and girls. Modified from Original Publication IV.

5.4.3 Length of stay and intensive care treatment

Between 1993–1998 and 2013–2018, the median duration of hospitalization shortened from 2.0 days (IQR, 1.0–4.0) to 1.0 day (IQR, 1.0–2.0; P <0.0001). There were no significant differences in the duration of hospitalization between different age groups and between boys and girls. During the 25-year study period, the proportions of children treated at the ICU varied between 6.5% and 15.1%, and there were no significant trends between the consecutive 5-year periods.

6 Discussion

6.1 Burden of influenza in children

6.1.1 Inpatients

It is estimated that influenza epidemics cause over 100 million infections and 870,000 hospitalizations for children <5 years of age every year (Wang et al., 2020). The youngest children are at the highest risk for influenza-attributable hospitalizations (Heikkinen, et al., 2013b; Izurieta et al., 2000; Neuzil et al., 2000; Poehling et al., 2006; Silvennoinen et al., 2011, 2012). Consistent with previous reports, the population-based incidence rate of hospitalization in our 25-year analysis was clearly highest in infants <6 months of age. Interestingly, during the 25-year study period, the relative proportion of children of <2 years of age almost halved, while the proportion of children aged 6–15 years almost tripled. Despite the decreasing trends in the incidence of hospitalization seen among young children, the rates are still clearly the highest among infants <6 months of age who are not eligible for influenza vaccination.

Several factors may contribute to the observed changes in the age of hospitalized children. One reason behind the higher incidence in the older children can be in the risk conditions, which are more prevalent in older children and adolescents. Another factor may be the increased use of rapid influenza diagnostic tests. POC tests help to diagnose influenza already in the outpatient setting and emergency clinics. The early diagnosis of influenza may have a greater impact on the reduction of hospitalization among previously healthy children than among those with underlying risk conditions. The rapid diagnosis of influenza can also diminish the suspicion of sepsis, which is one of the most important causes for admission among young infants with influenza (Silvennoinen et al., 2012). Moreover, seasonal influenza vaccinations are effective against hospitalizations (Boddington et al., 2021), and influenza vaccine recommendations extended to younger children could have an effect on the age distribution of influenza-associated hospitalizations. However, because in Study IV, the changes toward an older age of inpatients started already before the introduction of free seasonal influenza vaccinations for children in

Finland, it is likely that other explanations than vaccination contributed to the observed decrease in the hospitalizations of young children.

The severity of influenza can manifest in several ways in hospitalized children. The symptoms of influenza range from mild to severe and even in death. In Study I, 14% of the study children were treated in the ICU. Blood cultures were obtained from one-third of children and cerebrospinal fluid specimens for suspected meningitis were obtained from 7% of children. Interestingly, however, in our 25-year study, the rate of sepsis suspicion decreased significantly towards the end of the study period. The use of antibiotics is also frequent in influenza-infected children. According to Study I, approximately half of the children hospitalized with influenza were treated with antibiotics. Most children had some bacterial complications, but no less than one-fourth received antibiotics without any apparent bacterial infection. In previous studies from the United States and Australia, the proportions of children receiving antibiotics without a proper indication ranged from 36% to 57% (Wilkes et al., 2009; Willis et al., 2019).

The median length of stay in the hospital has shortened significantly from 2 days to 1 day between 1993–1998 and 2013–2018. The use of oseltamivir has increased during that study period, and it is possible that the early use of oseltamivir has contributed to the shortened length of stay. In previous studies, early administration of oseltamivir has shortened the duration of illness as well as reduced the severity of symptoms, which may permit an earlier discharge of children from the hospital (Heinonen et al., 2010; Malosh et al., 2018). The proportions of children treated in the ICU varied during the 25-year study period, but we did not find any clear trends regarding that outcome.

6.1.2 Outpatients

Even though influenza often results in the hospitalization of children, the majority of children with influenza are treated as outpatients (Fell et al., 2017; Teros-Jaakkola et al., 2019). Also in outpatient settings, the burden of influenza is heavy especially in the youngest age group. According to previous studies, 10–20% of infants get influenza every year (Heikkinen et al., 2004; Somes et al., 2018). In our outpatient study, 13.5% of healthy infants had a confirmed influenza illness during their first influenza season, which corroborates previous findings of the great burden of influenza among the youngest children. Bacterial complications of influenza are common also in outpatient children. In our study, AOM was diagnosed in 46% of infants with influenza, which corroborates previous findings of the frequency of AOM in influenza-infected children (Chonmaitree et al., 2008; Heikkinen et al., 2004). The median duration of influenza illness in the infants was 8 days, and the median duration of fever was 3 days. These results are concordant with earlier

findings (Heinonen et al., 2010; Whitley et al., 2001). Besides the direct health burden on children, their influenza illnesses may also result in days off from day care or school and work absenteeism for their parents.

6.2 Clinical features of influenza A and B

Influenza A viruses are conventionally thought to cause more severe infections compared to B viruses. This may be due to the generally higher incidence of influenza A during annual outbreaks and the capability of influenza A to cause pandemics. Influenza A usually predominates among young children, who are also at a higher risk for influenza-associated hospitalizations (Heikkinen et al., 2014; Silvennoinen et al., 2015). Therefore, differences in age distributions should be considered when comparing the severity of influenza A and B illnesses. Against convention, in Study I, we could not find any significant differences in the clinical presentation, outcomes or treatment between influenza A and B infections in hospitalized children. The results were similar regardless of whether the analyses were performed within different age groups or among all children. Furthermore, the proportions of influenza A and B viruses among hospitalized children were comparable to the overall distribution of A and B viruses in Finnish children. These data indicate that the risk of hospitalization was similar in influenza A and B infections (THL, 2023c). Also, the findings in our outpatient study support the view that the signs and symptoms of influenza A and B infections are similar. The only difference between influenza A and B in infants was the duration of symptoms before the initial visit to the study clinic. That duration was longer in influenza B compared to influenza A. It is possible that the early symptoms of influenza A are slightly different from those caused by B viruses. However, it must be acknowledged that the numbers of infants with influenza A and B in our outpatient study were relatively small, and therefore the results should be interpreted with caution.

Influenza B accounts for approximately one-fourth of all influenza infections, which means that the importance of influenza B in the total burden of influenza in the community is great. The data on the similarity of influenza A and B illnesses in children indicate that influenza B infections should be managed analogous to influenza A infections. During the past years, the contribution of influenza B to the total influenza burden has been increasingly recognized, and quadrivalent influenza vaccines that include both influenza B lineages have become common. Regarding the different lineages of influenza B viruses, the recent disappearance of B/Yamagata is interesting. Since March 2020, no Yamagata strains have been detected. It is kind of contradictory that now, when quadrivalent influenza vaccines have become the standard and the recommended products in many areas, the need for the Yamagata

strain in the vaccines should unexpectedly be reestimated. However, it is still too early to predict whether the Yamagata strain could reappear from somewhere.

Fever is the most remarkable sign of influenza in children (Danier et al., 2019; Heinonen et al., 2012; Poehling et al., 2006; Silvennoinen et al., 2009). Also in our outpatient study, almost all children with influenza had fever at the initial visit. The next common symptoms were rhinitis and cough, and about one-fifth of the children had gastrointestinal symptoms. The frequency of those symptoms were similar in influenza A and B infections. Our findings of the most typical symptoms of influenza are in line with previous studies (Heinonen et al., 2012; Silvennoinen et al., 2009).

6.3 Treatment

Influenza remains the only viral respiratory infection that can be effectively treated with specific antivirals. The efficacy of oseltamivir against influenza A is better compared to influenza B in children, but some effectiveness has also been reported against influenza B (Heinonen et al., 2010; Kawai et al., 2007; Sugaya et al., 2007). Because of the possibility to treat patients with an effective antiviral drug, each infant with influenza in whom the diagnosis was made within 48 hours of symptom onset was treated with oseltamivir in our outpatient study. Withholding of an effective therapy in this special age group would have been ethically questionable.

To gain the maximal benefit of oseltamivir, the treatment should be started as soon as possible but at the latest within 48 hours of the onset of symptoms (Aoki et al., 2003; Heinonen et al., 2010; Malosh et al., 2018). An increased efficacy against influenza symptoms by early administration of oseltamivir supports the widespread use of rapid influenza diagnostic tests.

In our outpatient study, the parents were encouraged to bring their child to the study clinic as soon as possible after the onset of symptoms. Because the study clinic was open every day, we had the possibility to diagnose influenza and to start oseltamivir treatment within 48 hours from the onset of symptoms. In real life, if the initial symptoms in a child are not severe, the parents often bring them to healthcare clinics when the delay after the onset of symptoms is too long for starting antiviral treatment. This may be one of the reasons why most children in an outpatient setting remain without antiviral therapy. Another important reason for the low consumption of antivirals in outpatient settings may be the lack of a virological confirmation of influenza, which is most frequently performed in hospital settings.

In our outpatient study, oseltamivir treatment shortened the duration and severity of symptoms in infants. The mean total duration of influenza A infections shortened by approximately 170 hours, which was statistically significant despite the small numbers of infants. The effect in infants with influenza B was not as good as in influenza A, but the reduction in the mean total duration of illness was still

approximately 60 hours, which was also statistically significant. In our study, there was no significant difference in the duration of illness between influenza A and B in children who were treated with oseltamivir.

Previous studies have established that early administration of oseltamivir decreases the replication of influenza viruses, which, in turn, results in decreased viral shedding (Fry et al., 2014; Whitley et al., 2001). The peak of viral shedding comes earlier and decreases more rapidly in influenza A compared to influenza B (Lau et al., 2010, 2013; B. Wang et al., 2017). In Study III, we demonstrated that in most infants, viral antigen concentrations in nasopharyngeal secretions declined rapidly within 1–2 days after the initiation of oseltamivir treatment.

The safety of oseltamivir in children has been proven in several studies (Malosh et al., 2018). Vomiting and diarrhoea are common symptoms of influenza in children, but they are also the most frequently reported adverse events of oseltamivir in young children (Heinonen et al., 2010; Malosh et al., 2018; Siedler & Skopnik, 2010; Whitley et al., 2001). It is difficult to distinguish if vomiting or diarrhoea are symptoms of influenza or a side effect of the antiviral treatment. In our outpatient study, about half of the families reported gastrointestinal symptoms in their child during oseltamivir treatment, but in many cases, those symptoms were already present before the start of the treatment. Importantly, in most infants, vomiting was mild and ceased rapidly, and no one discontinued oseltamivir treatment because of gastrointestinal events.

6.4 Prevention

Vaccination of healthy children is a sensitive topic. In the beginning of the latest A/H1N1 pandemic in 2009, an AS03 adjuvant was used in a pandemic influenza vaccine. The use of the adjuvanted vaccine was associated with an increased risk for narcolepsy, which rapidly reduced the rates of seasonal influenza vaccinations in children during the subsequent years. Vaccination is the primary method of preventing influenza, but the coverage of seasonal influenza vaccines is low compared to many other childhood vaccines. During the Covid-19 pandemic years, the awareness of prevention possibilities of infectious diseases has increased, and influenza vaccine coverage has increased again during the last years. In 2022–2023, about 40% of children <3 years and one-third of children 3–6 years of age were vaccinated against influenza in Finland (THL, 2023b). After one completely absent influenza season during the Covid-19 pandemic, influenza seems to have now returned into seasonal circulation, with an early start of the outbreak during the winter of 2022–2023 in Finland. Now when the seasonal epidemics have come back, the time for increasing the influenza vaccine coverage is perfect. This preventative

work requires activity from all healthcare professionals who have a responsibility to recommend people to take seasonal influenza vaccinations.

During the influenza season of 2017–2018, when Studies II and III were carried out, there was a mismatch between the vaccine and the circulating influenza strains, and the vaccine effectiveness was low (Baum et al., 2020; Ikonen et al., 2017). During a season when the vaccine effectiveness is generally low because of a mismatch, the vaccination of family members cannot be expected to reduce the incidence of influenza in unvaccinated groups, like young infants, and "the cocooning effect" does not work. In our study, the influenza vaccine effectiveness was 48% against influenza infection, but it was not statistically significant. It is possible that the study population was not big enough to show the effectiveness of the vaccine. Anyhow, low vaccine effectiveness in our study is concordant with the nationwide analysis conducted by the Finnish Institute for Health and Welfare (Baum et al., 2020; Ikonen et al., 2017). For the same reasons, the effectiveness against AOM was also low in our study at only 5%.

The Covid-19 pandemic has increased the pace of development of new vaccines. Messenger RNA vaccines have proved their feasibility for Covid-19, and the first messenger RNA influenza vaccine trial in rodents yielded encouraging results (Arevalo et al., 2022). High-dose quadrivalent influenza vaccines contain four times the antigen as standard dose influenza vaccines and are already used in people older than 65 years. In a phase II study in children, high-dose vaccines provided improved immunogenicity without affecting vaccine safety (Chang et al., 2021). Current influenza vaccines are strain-specific, but due to the need for broader protection, research into universal influenza vaccines is ongoing (Wang et al., 2022).

6.5 Methodological aspects

All studies in this thesis were conducted in the area of the Hospital District of Southwest Finland, where all the children´s beds are in one tertiary hospital, Turku University Hospital. This reduced the variation in clinical practices in different centers and therefore allowed for more precise analyses of the management of influenza A and B during the long study periods. On the other hand, a long study period in one center with the same viral sampling and hospitalization routines enabled reliable comparisons from one year to another. A clear limitation in the inpatient studies was the lack of the systematic vaccination status in patient medical charts. Thus, no analyses on how vaccination possibly affected hospitalizations could be done.

The studies of children hospitalized with laboratory-confirmed influenza were retrospective chart reviews with relatively large sample sizes, and the material covered all the children hospitalized during the study periods. An even longer study

period would have caused problems because the earlier recording routines have been largely different, and sampling for respiratory viruses was not yet routine in the 1980`s. The patients´ data were collected systematically from four different sources, which was a clear strength of our retrospective studies. For example, if we had searched for influenza-associated hospitalizations only based on influenza-related ICD codes, one-fourth of influenza cases would have been missed. During the 25-year study period, the proportion of ICD codes increased during the study period by 20 percentage points. However, even during the last 5-year period, about one-fifth of the influenza-admitted children had no ICD code related to influenza in their medical records.

Our outpatient material was collected during the influenza season of 2017–2018, when about half of all influenza cases were caused by influenza B viruses. Therefore, we had a good possibility to compare the features of influenza A and B in young children. The main strengths of our outpatient study were the close follow-up of a pre-enrolled cohort of infants and the active use of sensitive virologic assays to determine the aetiology of each infection. A major limitation was that the study covered only one influenza season, and that is why the size of our study population was not big enough for sufficient power to show differences in many statistical analyses. Fortunately, the outpatient study was completed before the Covid-19 pandemic. During the pandemic years, the normal epidemiology of most respiratory viruses, including influenza viruses, was distracted, and Covid-19-related restrictions might have seriously hampered the performance of the study.

6.6 Future considerations

Against convention, the severity of influenza B infections is similar to influenza A. Influenza B accounts for a substantial proportion of all influenza illnesses, especially in children and adolescents. Thus, the attitude to influenza B should be consistent with influenza A. Although influenza B viruses are not able to cause worldwide pandemics, the prevention of influenza B lineages in influenza vaccines is recommended. Currently, influenza is no more the only viral respiratory disease preventable by vaccination and, for example, vaccines against respiratory syncytial virus are under development. In the future, it may be possible to combine several viruses into the same vaccine.

Influenza viruses circulate not only in high-income countries. On the contrary, the burden of influenza disease is heavy particularly in low-income countries. In children younger than 5 years old, even 99% of influenza-associated deaths are estimated to occur in developing countries (Coleman et al., 2018; Nair et al., 2011). Vaccination against severe preventable diseases, like influenza, should be every child´s right. On the other hand, it is unfortunate that, for example in Finland, despite

effective and free-of charge influenza vaccines for children younger than 7 years of age, the vaccine coverage is low. It is possible that people have doubts about the effectiveness and safety of influenza vaccines. Increasing the vaccine coverage requires continuous work from all healthcare professionals.

The youngest children need special attention. Children <6 months of age are at the highest risk for hospitalization, and the burden in this age group is great also in the outpatient setting. Despite the special vulnerability of young infants, there are no influenza vaccines available for this age group. Currently, the only options for diminishing the burden of influenza in the youngest infants are the vaccination of pregnant mothers and other family members, rapid diagnosis of influenza and starting oseltamivir treatment as early as possible during an influenza illness. New vaccines, which are immunogenic even in young children, could be one option.

The world is becoming smaller, and diseases can spread rapidly globally. The capability of influenza to have a fast antigenic shift and drift makes it a genuine threat. Today, frequently emerging zoonotic influenza viruses call for universal influenza vaccines that would provoke a broad immune response against all strains of influenza.

7 Summary

The burden of influenza is significant in children, especially in the youngest age group. We established, in our 25-year study, that the overall incidence rate of influenza hospitalization among all children stayed quite constant across the study years. Instead, the median age of children hospitalized with laboratory-confirmed influenza increased by 2 years. Even so, the rate of hospitalization remained clearly highest among children younger than 6 months of age. In addition, we substantiated the burden of influenza in an outpatient setting among infants during their first year of life, as about one-seventh of infants were infected with laboratory-confirmed influenza during the first influenza season of their life, and almost half of them developed AOM as a complication. Children <6 months of age are not eligible for influenza vaccination, and the maternal antibodies against influenza decrease rapidly during the first months of life. Effective new strategies are needed to diminish the burden of influenza in children, especially among the youngest age group. So far, rapid influenza diagnostics and early treatment with oseltamivir help to diminish the heavy burden of influenza in this special age group.

Against convention, influenza A and B viruses cause illnesses with comparable severity in children. We demonstrated the importance of influenza B virus infections in children with influenza-associated hospitalizations as well as in children treated in outpatient clinics. In both settings, a substantial proportion of all influenza virus infections were caused by B viruses. We could not find any significant differences in the clinical presentation, outcomes or treatment between influenza A and B among children hospitalized with influenza when adjusting for age. Furthermore, in our outpatient study, we demonstrated that oseltamivir treatment is effective in infants treated in outpatient clinics, not only in influenza A, but also in influenza B virus infections. Our findings support influenza prevention strategies, in which both influenza A and B types are taken into consideration. In the treatment of both influenza A and B, rapid confirmation of influenza diagnosis enables the early use of oseltamivir to offer the best benefit from the treatment, thereby diminishing the overall burden of influenza in the community.

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