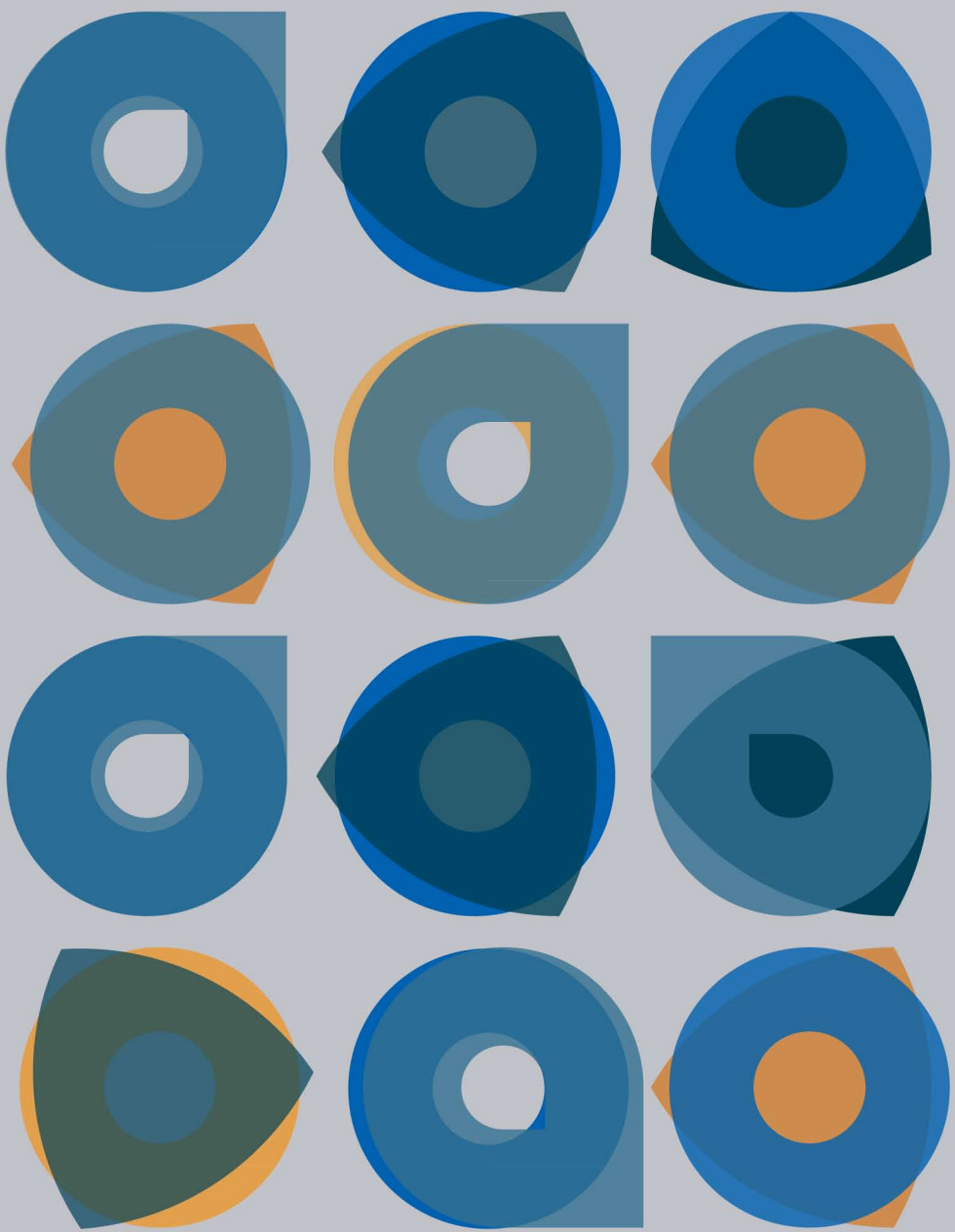
The Benefits and Risks of Pandemic Influenza Vaccines

Leonoor Wijnans



The Benefits and Risks of Pandemic Influenza Vaccines

Leonoor Wijnans

Cover design by Viv Cherry

Layout by Leonoor Wijnans

Printing by Gildeprint te Enschede

The work described in this thesis was conducted at the department of Medical Informatics of the Erasmus University Medical Centre, Rotterdam, The Netherlands in collaboration with Pharmacotherapeuticgroup IV of the Dutch Medicines Evaluation Board (CBG-MEB), Utrecht, The Netherlands. The CBG-MEB is dedicated to ensure that licensed medicinal products have a positive benefit-risk during their whole life cycle. This role requires intensive collaboration with academic and clinical partners in order to develop new assessment and decision-making methods, to engage with the clinic and to strengthen regulatory science. This thesis aims to go beyond its scientific merits as such by delivering science, learning and insight to promote public health.

Part of this research was conducted with EC-funded projects: I-MOVE, VAESCO

Financial support for printing this thesis was generously provided by the Interdisciplinary Processing of Clinical Information group of the department of Medical Informatics, Erasmus University Medical Centre and the Dutch Medicines Evaluation Board.

The Benefits and Risks of Pandemic Influenza Vaccines

De baten en risicos van pandemische influenza vaccins

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de
rector magnificus

Prof.dr. H.A.P. Pols

en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op

woensdag 16 december 2015 om 11.30 uur

door

Eleonora Gabriëlle Wijnans

geboren te Utrecht

Erasmus University Rotterdam

Erasmus

Promotiecommissie:

Promotor:	Prof.dr. M.C.J.M. Sturkenboom
Overige leden:	Prof.dr. M.G. Koopmans
	Prof.dr. H.G.M. Leufkens
	Prof. dr. G. F. Rimmelzwaan
Copromotoren:	Dr. A.C.G. Voordouw
	Dr. D.M. Weibel

CONTENTS

Appendices

Chapter 1. General Introduction

March 2009 saw the emergence of a pandemic influenza virus in Mexico and the United States (1). In the months following, vaccines were developed in order to protect the population against the potential effects of this novel influenza virus, now known as the H1N1pdm09 virus. At the level of the drug regulatory agencies much effort was put into ensuring timely licensing of these vaccines. Due to the nature of pandemic influenza, uncertainties surrounding the benefit risk balance for pandemic influenza vaccines remained at the time of licensing - in particular regarding those vaccines with novel constructs or with constructs for which experience was limited. These uncertainties were addressed in the post-licensure phase through additional studies or alternative monitoring efforts. Whilst the monitoring of the effectiveness and safety of vaccines is challenging under most circumstances this is particularly the case when vaccination is rolled out on an unprecedented scale within a developing pandemic as was seen in the 2009/2010 H1N1 pandemic.

This thesis deals with the evaluation of the benefits and risks of H1N1pdm09 vaccines after licensing. The general introduction provides a background on pandemic influenza, influenza vaccines and touches on the regulation of pandemic influenza vaccines in Europe highlighting the importance of thorough monitoring of the benefit risk balance of pandemic influenza vaccines post-licensure and illustrating the challenges that are faced during the post-licensure evaluation of pandemic influenza vaccines.

Pandemic Influenza

Influenza is an acute viral disease of the respiratory tract caused by enveloped ribonucleic acid (RNA) viruses of the family of Orthomyxoviridae. Influenza viruses belong to three genera: influenza A, B and C. Influenza B and C predominantly infect humans, whilst influenza A viruses also infects a wide variety of animals including birds, pigs, horses, whales and seals. This makes that influenza A viruses are capable of causing pandemics.

Influenza A viruses are formed of an envelope in which two main glycoproteins are embedded: haemagglutinin (HA) and neuraminidase (NA). The envelope surrounds the viral RNA genome which consists of eight strands of RNA, each encoding for one or two proteins. There is one RNA strand which codes for the haemagglutinin surface protein and one which codes for the neuraminidase surface protein. Influenza A viruses are characterised by the HA and NA - for example an 'H1N1 virus' or 'H3N2 virus'. Up to now, 18 HA and 11 NA subtypes have been identified (2). Of these sustained transmission in humans is seen for three HA subtypes (H1, H2, H3) and two NA subtypes (N1, N2) (3, 4). Other subtypes primarily infect birds, however they can occasionally infect mammals as illustrated by the H5N1 and H7N9 infections in humans (5-7).

Protection against infection with influenza is mediated through antibodies against the haemagglutinin and neuraminidase surface proteins. As antibodies latch on to surface

proteins they prevent the influenza viruses from infecting cells and thus protect the tissue, organs and organisms from becoming diseased (8). Influenza viruses need to infect host cells in order to propagate. The viruses use the infrastructure of the host cell to copy the genome and produce new viruses. The copying of the genome is however an error-prone process and point mutations in genes coding for H and N arise frequently. As these mutations can result in a change in the shape of the H or N surface proteins, whereby existing antibodies are no longer able to prevent that virus from infecting cells, new antigenic variants can emerge which are capable of evading the protective immune response. This process is called antigenic drift and is the reason why we can get infected with influenza every few years and why influenza vaccines need updating almost annually.

Pandemics arise not from antigenic drift but from the reassortment of viruses, a process called antigenic shift which is described in more detail below, or from the introduction of a zoonotic – or otherwise new - influenza virus in the human population. When a host cell is infected simultaneously by two distinct influenza viruses re-assortment of the two viruses can take place. In other words, the viruses could exchange their RNA strands and by doing so acquire new antigenic properties – antigenic shift. In theory the newly formed virus could exhibit surface proteins which are new to the human population whilst maintaining other characteristics enabling human to human transmission (4). As the surface proteins would be new to humans, there would be no pre-existing antibodies and thus humans would be immunologically naïve. Consequently the virus could spread freely throughout the population in what is commonly understood as a pandemic: 'an epidemic occurring worldwide, or over a very wide area, crossing international boundaries and usually affecting a large number of people' (9).

During the 20th century, four influenza pandemics have been documented (10). The 1918 influenza A(H1N1) pandemic, mostly referred to as the Spanish flu, has been the most deadly influenza pandemic ever documented resulting in an estimated 40 million deaths, with high death rates among healthy adults. The Asian (H2N2) and Hong-Kong (H3N2) pandemics of 1957 and 1968 were associated with considerable morbidity, although unlike the 1918 pandemic, deaths were mostly in young children and elderly. The 1977 pandemic concerned influenza A(H1N1) virus that was antigenically similar to viruses which circulated in the 1950s. As a consequence, people who were over 20 years of age at the time of the 1977 pandemic possessed antibodies to the virus and thus infection and illness was mostly confined to children and adolescents. It is now thought that this virus was accidentally released from a laboratory freezer (10).

Whilst the 1957 and 1968 pandemics were classic examples of reassortment of human influenza viruses with avian influenza viruses, the 1918 pandemic influenza strain was not the result of reassortment. Genetic sequencing has pointed out that all eight genes of the

H1N1 virus are closely related to avian influenza viruses (11, 12). Therefore rather than originating from reassortment of an avian influenza strain with a human influenza strain, it appears likely that avian influenza viruses infected humans and adapted to enable person to person transmission eventually resulting in a pandemic (13-15).

Pandemic preparedness

At the end of the 20th century, reports of highly pathogenic H5N1, and to a lesser degree H9N2, avian influenza transmission from birds to people (16, 17) demonstrated that a threat of an influenza pandemic as experienced in 1918 still existed (18). Although there has always been academic interest in pandemic influenza, it was never high on the agenda of policy (19). With an identifiable risk emerging, pandemic influenza moved up the priority lists and *pandemic preparedness* became a topic of contemporary concern.

The first pandemic preparedness plan was drafted by the WHO in 1999 (20). Increased efforts were put into establishing and upgrading surveillance systems, stockpiling of antiviral drugs, scaling up vaccine production, preparing for the licensing of potential pandemic influenza vaccines and planning for distribution of drugs and vaccines (21-24). It took just over 10 years before these pandemic preparedness plans were put to the test. The first pandemic of the 21st century was however not caused by a highly pathogenic H5N1 influenza strain or re-assortment thereof.

The first pandemic of the 21st century

In March 2009 transmission of a novel influenza A(H1N1) strain - H1N1pdm09 - was reported in Mexico and the United States (US) (1). With increased circulation of H1N1pdm09 information on its origin and structure became available, leading to evidence that at least parts of the virus had been experienced previously by a part of the human population (25). The H1N1pdm09 virus induced a recall response boosting pre-existing heterosubtypic antibody responses to the HA surface protein resulting in protection in older people (25, 26).

Despite the fact that parts of the population were not immunologically naïve, the H1N1pdm09 virus could not be considered similar to a regular seasonal influenza epidemic. The virus proved lethal in previously healthy individuals as well as in those with pre-existing risk factors, and affecting mainly the younger part of the population (27-29).

As the virus spread across the world, vaccines were being produced and licensed at an unprecedented scale and speed. In Europe adjuvanted and non-adjuvanted H1N1pdm09 vaccines were licensed through fast track procedures (30), with the first centrally registered vaccines licensed at the end of September 2009 (31).

Influenza vaccines

Discoveries that heralded the development of influenza vaccines include the isolation of the influenza A virus in the 1930-s (32), the propagation of influenza viruses in embryonated hen's eggs (33) and the uncovering of the role of viral haemagglutination in influenza transmission (34, 35). These important initial steps eventually resulted in the characterisation of influenza viruses and the immune response to immunisation, as well as the development and testing of different vaccine constructs and ultimately the production and use of influenza vaccines.

Influenza vaccines are based either on inactivated virus, i.e. dead virus material, or on live viruses that have been attenuated such that they can no longer cause disease. Conventionally, influenza vaccines are produced using an egg-based platform. There are now several influenza vaccines which are produced with a cell culture platform, i.e. in which the virus is propagated on cell lines (36).

Inactivated vaccines

Vaccines based upon inactivated virus include split vaccines, subunit vaccines, and inactivated whole-virus vaccines. In primed populations these vaccines are generally given as a single dose containing 15µg of each virus strain. For seasonal influenza vaccines which generally contain three different strains (H1N1, H3N2 and B) this would amount to 45 µg. Recently quadrivalent seasonal influenza vaccines have been approved containing an additional B-strain (37-40).

Live vaccines

Whilst live attenuated influenza vaccines (LAIVs) were being developed alongside the inactivated vaccines, the use of LAIVs was restricted to Russia until 2003 when a LAIV was licensed in the US (41). In Europe, the first LAIV was not licensed until 2010 (42). As LAIVs are delivered intranasally to the mucosal surfaces of the upper respiratory tract it is thought that the immune response to these vaccines mimics the immune response following natural infection resulting in broader and longer protection than inactivated vaccines which are delivered intramuscularly.

Adjuvanted vaccines

It is commonly accepted that influenza vaccines are the most effective intervention to prevent influenza (43). It is similarly recognised that the protective efficacy of conventional vaccines can be improved and might not be satisfactory for specific target groups. Additionally, the production capacity for influenza vaccines worldwide is limited and would not serve the entire population at risk when faced with a pandemic. Developments to improve the immune

response to conventional influenza vaccines include high-dose vaccines, intradermal vaccination and adjuvanted vaccines.

High-dose vaccines, with an increased HA content, have been developed to improve immunogenicity and thus assumedly efficacy, however these do not address the production capacity limitations. Developments that do address this include the delivery of the vaccine through alternative routes, notably intradermal vaccination, and the introduction of adjuvants into vaccines. Adjuvants, as the term is used here, are components that are added to vaccines to enhance the immune response. An example of a widely used adjuvant is aluminium salts.

Experience with adjuvanted influenza vaccines goes back to the 1950-s when mineral-in-oil adjuvanted influenza vaccines were used on a large scale. These were abandoned as their use was associated with severe local reactions including cysts and abscess formation (44). It was not until 1997 that the first adjuvanted influenza vaccine, an oil-in-water (MF59™) adjuvanted seasonal influenza vaccine, was licensed for use in older adults in Europe (45). Increased awareness of a potential pandemic threat combined with the limited production capacity for influenza vaccines facilitated the development and licensing of several adjuvanted pandemic influenza vaccines (46). These played a prominent role during the 2009/2010 influenza A(H1N1) pandemic in Europe (47).

Licensing of Pandemic Influenza Vaccines in Europe

Vaccination is thought to be the most efficient method to protect populations during pandemics (48). In order to be successful it is crucial that safe and efficacious vaccines become available early in the pandemic. The regulators play an important role in this process as they are responsible for the evaluation of the benefits and risks of new vaccines and ultimately for the licensing. Under normal circumstances the regulatory evaluation of a new vaccine can easily take up to a year, if not longer. Such a period would create an unacceptable delay for pandemic influenza vaccine availability. In order to ensure that this process would be timely new regulatory pathways were put in place in response to the emergence of H5N1 as a pandemic threat.

In Europe a 'mock-up vaccine' concept was introduced (49). This concept enabled the licensing of pandemic vaccines before the actual emergence of a pandemic virus. The idea was to license a vaccine based on a strain for which the population could be assumed to be immunologically naïve, say the H5N1 virus. For this mock-up vaccine, containing a potential pandemic influenza strain, a dossier would be formed which would consist of data on quality aspects, immunogenicity and on safety – the core dossier. This data formed the basis for dosing regimens, for possible limitations of use and for the safety information on the product. If a pandemic virus were then to emerge and the WHO were to raise the pandemic

alertness to level 3, vaccine manufacturers would replace the strain in the mock-up vaccine with the new pandemic influenza strain. This process is in many ways similar to the annual strain update for seasonal influenza vaccines. In theory, the data in the core dossier would still be representative for the 'new' pandemic vaccine. In support of the strain change the manufacturer would only need to submit data demonstrating that the 'quality' of the vaccine is unchanged. Once the strain variation would be accepted, the use of the vaccine would have to be monitored through an extensive risk management plan, collecting additional information on the safety and effectiveness of the pandemic vaccines in real life.

At the start of the 2009/2010 H1N1 pandemic three mock-up constructs were licensed in Europe: Foclivia, Adjupanrix and Pandemic influenza Vaccine H5N1 Baxter. These constructs formed the basis for the licensing of three H1N1pdm09 vaccines: Focetria, Pandemrix and Celvapan (50).

Although an elegant solution to the regulatory challenge of ensuring the timely benefit risk assessment and licensing of pandemic influenza vaccines when faced with an emerging threat, there are limitations regarding the demonstration of benefits and risks of new pandemic influenza vaccines for regulatory assessment.

Determining Benefit of Pandemic Influenza Vaccines

To determine whether a vaccine protects against an influenza infection and the complications thereof, the influenza virus has to be known and circulating in the population. Efficacy of a pandemic vaccine can thus only be determined in clinical trials conducted when the pandemic influenza virus is spreading, by which the vaccine ought to be licensed to obtain maximal benefit of vaccination. Mock-up vaccines contain strains for which the population is assumed to be naïve, a strain that is not circulating in the population. The regulatory challenge is how to ensure that these vaccines will provide benefit to the recipient without having data on the efficacy of that vaccine. This could be done by measuring the immune response (antibody production) following immunization, i.e. the immunogenicity.

The core dossier for the mock-up vaccines, Foclivia, Adjupanrix and Pandemic influenza Vaccine H5N1 Baxter, contained data on the antibody response following vaccination in predominantly healthy adults (51-53). Whether the antibody response of the mock-up vaccine was sufficient for licensure was determined by applying a set of criteria labelled the Committee for Human Medicinal Products (CHMP) criteria (see Table 1.1). These criteria are based upon the assumption that a serological threshold value, i.e. a serum HI titre of ≥1:40, correlates to protection achieved in 50% of vaccinated subjects.

Table 1.1 European CHMP criteria for evaluation of influenza vaccine immunogenicity

	adults	elderly (>60 years)
GMT increase	2.5	2
Seroconversion /significant increase*	40%	30%
Seroprotection*	70%	60%

* In haemagglutination inhibition tests seroconversion corresponds to: negative prevaccination serum (HI<1:10), postvaccination serum HI≥1:40; Prevaccination serum >1:10, significant increase: at least a fourfold increase in titre. Seroprotection corresponds to the % with serum HI≥1:40. Alternative criteria have been defined for the SRH assay.

The European regulatory guideline for pandemic influenza vaccines that set the criteria that a candidate pandemic influenza vaccine had to meet in order to gain licensure at the time of the H1N1pdm09 pandemic stipulated that all three criteria (seroprotection rate, geometric mean titre (GMT) increase and response rate) should be fulfilled - thereby setting the benchmark for efficacy for these vaccines (54).

A major drawback of relying on anti haemagglutinin antibodies to assess the immune response to influenza vaccination is that the two available assays used to quantify these antibodies, the haemagglutination inhibition (HI) assay and the single radial haemolysis (SRH) assay, are not standardised and that there is considerable variability within and between laboratories (55).

Based upon years of experience with the conventional seasonal influenza vaccines the consensus is that these vaccines provide benefit for the majority of recipients (43). As it is known that the HA antibodies play an important role in conveying protection it is therefore not unreasonable to assume that eliciting an HA-response following vaccination can be related to efficacy at least for HA-based vaccines. It has been recognised however that a cut-off such as the HI≥1:40 threshold is unlikely to form an absolute correlate of protection – certainly not across all populations and for all different vaccines (56, 57). One study in children for example found that an HI titre >1:110 would predict a clinical protection level of 50%, while a titre of 1:330 would predict 80% protection (58).

The immunogenicity evaluated using the CHMP criteria provided evidence that the vaccines would elicit an immune response which broadly could be related to protection. However, uncertainty remained what level of protection could be achieved with the different vaccines and as to whether these responses would translate into clinical protection against disease in the wide range of target groups for pandemic vaccination, in particular the young children and elderly. Additionally, whereas the bulk of the data in the core dossier was obtained with H5N1-vaccines, at the time of licensure of the pandemic vaccines it was unclear how this related to the H1N1pdm09 containing vaccines. There was little immunogenicity data with the H1N1pdm09 vaccines and, due to the difficulties with the assays cross study,

comparisons could not be made hence it was unclear whether the dose recommendations as determined for the H5N1-vaccine were also applicable for the H1N1pdm09-vaccines.

Determining the safety of pandemic influenza vaccines

Considering the decades of use of seasonal inactivated trivalent influenza vaccines in millions of people worldwide, there is substantial experience with conventional influenza vaccines which supports the safety profile for non-adjuvanted pandemic influenza vaccines based upon similar constructs (59). Nonetheless, it has to be recognised that the introduction of a new viral strain that into the human population through a process of antigenic shift or adaptation could result in a vaccine with a different reactogenicity profile. Potentially, this vaccine could also induce serious adverse events that are not associated with the seasonal variants of the vaccine.

The mock-up vaccines licensed in Europe at the time of the emergence of the H1N1pdm09 virus comprised adjuvanted and cell-based vaccines for which the experience was far more limited and the uncertainties thus greater (50). The safety profile of these vaccines was based upon non-clinical testing and on data derived from clinical studies in around 3,000 – 5,000 healthy adults (51-53). For the MF59 adjuvanted vaccine – Foclivia/Focetria- a similar adjuvanted seasonal vaccine had been used for over a decade in elderly in Italy, providing additional data regarding the safety of this vaccine construct (52).

No safety data with the mock-up vaccines had been collected in persons at particular risk for influenza complications and only limited data was available for children, with no data available for children under three years of age. Furthermore, although data in 3000 persons would be sufficient to rule out the occurrence of common adverse events that occur in approximately 1 in 1000 persons, it cannot exclude adverse events which are rare – i.e. occur less frequent. If these adverse events are serious they will impact the benefit risk balance and will necessitate a re-evaluation of the use of the vaccine.

Concluding remarks

As outlined in the sections above, at the time of licensure of the pandemic influenza vaccines in Europe several uncertainties remained regarding the benefits and risks of these vaccines. Due to the nature of pandemics, this will always be the case. Pandemic influenza vaccines can only be produced and tested once the virus is spreading – hence there is never sufficient time for a full evaluation of the efficacy and safety of new pandemic influenza vaccines pre-licensure.

To address uncertainties surrounding the benefit risk of pandemic influenza vaccines extensive post licensure monitoring programmes need to be implemented to confirm the

benefits of the vaccines and, most importantly, to rapidly identify risks and establish the association with vaccination. However, once licensed, depending on the availability, pandemic influenza vaccines are rolled out quickly and large populations are vaccinated in a relatively short space of time. This provides an extremely challenging setting for conducting observational studies which are ultimately the backbone of post-licensure monitoring and evaluation of vaccines.

Aims and outline of this thesis

The work described in this thesis was conducted in the wake of the 2009/2010 H1N1 pandemic and consists of several studies that evaluate the benefits and risks of pandemic influenza vaccines used across Europe. The experience with the H1N1pdm09 vaccines is collated and lessons drawn to improve the monitoring of the benefits and risks of pandemic influenza vaccines.

In the first section different observational study designs are used to evaluate the benefits of pandemic influenza vaccines. In **chapter 2** a study is presented which evaluated the effectiveness of Focetria, an MF59 adjuvanted pandemic influenza vaccine, against medically attended influenza-like illness and reverse transcriptase polymerase chain reaction (RT-PCR) confirmed influenza in the at-risk population and persons over 60 in the Netherlands through a cohort with a nested case control design. This study was conducted within the framework of Influenza - Monitoring Vaccine Effectiveness (I-MOVE) project and sponsored by the European Centre for Disease Control (ECDC). **Chapter 3** consists of a study that evaluated the effectiveness of Focetria against hospitalization through a matched case-control study.

The second section of this thesis deals with the risks of pandemic influenza vaccines, starting with a review of available pre- and post-licensure data regarding the safety of the H1N1pdm09 vaccines in children in **chapter 4**. In **chapter 5** the association between Bell's palsy and influenza A(H1N1)pdm09 containing vaccines is evaluated in a self-controlled case series. Chapters 6 and 7 focus on the evaluation of a particular safety signal that emerged in the aftermath of the H1N1pdm09 vaccination campaigns in Europe, where a potential association between Pandemrix, an AS03-adjuvanted H1N1pdm09 vaccine, and narcolepsy was seen. **Chapter 6** presents a multi-national study into the diagnosis rates of narcolepsy in seven European countries prior to, during and after the H1N1pdm09 pandemic. This study was a collaborative study conducted within the Vaccine Adverse Event Surveillance & Communication (VAESCO) network – a European research network aiming to develop guidelines and a sustainable infrastructure for post licensure vaccine safety assessment in the European Region. In **chapter 7** the potential role of bias in studies looking

into the association between Pandemrix and narcolepsy is evaluated through a simulation study.

The third section of this thesis considers how we can draw on the experience gained during the 2009/2010 influenza A(H1N1) pandemic to improve the monitoring of influenza vaccines and whether we can use the large amounts of data generated on the safety and efficacy of different pandemic influenza vaccine constructs to improve influenza vaccination. In **chapter 8** the evidence surrounding adjuvanted influenza vaccines in children younger than three years of age is brought together in order to see whether these vaccines could fulfil an urgent need in providing safe and efficacious influenza vaccines for an age group where the efficacy of conventional influenza vaccines is limited. **Chapter 9** reflects on the proposed regulatory guidance for the assessment of new influenza vaccines in Europe. Finally, a discussion of the main findings presented in this thesis and of their potential implications is included in **chapter 10.**

References

1. Fraser C, Donnelly CA, Cauchemez S, Hanage WP, Van Kerkhove MD, Hollingsworth TD, et al. Pandemic potential of a strain of influenza A (H1N1): early findings. Science. 2009 Jun 19;324(5934):1557-61. PubMed PMID: 19433588. Pubmed Central PMCID: 3735127.

2. Tong S, Zhu X, Li Y, Shi M, Zhang J, Bourgeois M, et al. New world bats harbor diverse influenza A viruses. PLoS pathogens. 2013;9(10):e1003657. PubMed PMID: 24130481. Pubmed Central PMCID: 3794996.

3. Palese P, Shaw M. Orthomyxoviridae: The Viruses and their Replication. . In: Knipe D, Howley P, editors. Fields Virology Philadelphia: Lippincott Williams & Wilkins; 2007.

4. Bouvier NM, Palese P. The biology of influenza viruses. Vaccine. 2008 Sep 12;26 Suppl 4:D49-53. PubMed PMID: 19230160. Pubmed Central PMCID: 3074182.

5. Wang H, Feng Z, Shu Y, Yu H, Zhou L, Zu R, et al. Probable limited person-to-person transmission of highly pathogenic avian influenza A (H5N1) virus in China. Lancet. 2008 Apr 26;371(9622):1427-34. PubMed PMID: 18400288.

6. Ungchusak K, Auewarakul P, Dowell SF, Kitphati R, Auwanit W, Puthavathana P, et al. Probable person-to-person transmission of avian influenza A (H5N1). The New England journal of medicine. 2005 Jan 27;352(4):333-40. PubMed PMID: 15668219.

7. Gao R, Cao B, Hu Y, Feng Z, Wang D, Hu W, et al. Human infection with a novel avian-origin influenza A (H7N9) virus. The New England journal of medicine. 2013 May 16;368(20):1888-97. PubMed PMID: 23577628.

8. Couch RB, Kasel JA. Immunity to influenza in man. Annual review of microbiology. 1983;37:529-49. PubMed PMID: 6357060.

9. Last J. A dictionary of epidemiology New York: Oxford University Press; 2001.

10. Oxford JS. Influenza A pandemics of the 20th century with special reference to 1918: virology, pathology and epidemiology. Reviews in medical virology. 2000 Mar-Apr;10(2):119-33. PubMed PMID: 10713598.

11. Reid AH, Fanning TG, Hultin JV, Taubenberger JK. Origin and evolution of the 1918 "Spanish" influenza virus hemagglutinin gene. Proceedings of the National Academy of Sciences of the United States of America. 1999 Feb 16;96(4):1651-6. PubMed PMID: 9990079. Pubmed Central PMCID: 15547.

12. Taubenberger JK, Reid AH, Lourens RM, Wang R, Jin G, Fanning TG. Characterization of the 1918 influenza virus polymerase genes. Nature. 2005 Oct 6;437(7060):889-93. PubMed PMID: 16208372.

13. Belshe RB. The origins of pandemic influenza--lessons from the 1918 virus. The New England journal of medicine. 2005 Nov 24;353(21):2209-11. PubMed PMID: 16306515.

14. Reid AH, Taubenberger JK, Fanning TG. Evidence of an absence: the genetic origins of the 1918 pandemic influenza virus. Nature reviews Microbiology. 2004 Nov;2(11):909-14. PubMed PMID: 15494747.

15. Taubenberger JK, Reid AH, Janczewski TA, Fanning TG. Integrating historical, clinical and molecular genetic data in order to explain the origin and virulence of the 1918 Spanish influenza virus. Philosophical transactions of the Royal Society of London Series B, Biological sciences. 2001 Dec 29;356(1416):1829-39. PubMed PMID: 11779381. Pubmed Central PMCID: 1088558.

16. Yuen KY, Chan PK, Peiris M, Tsang DN, Que TL, Shortridge KF, et al. Clinical features and rapid viral diagnosis of human disease associated with avian influenza A H5N1 virus. Lancet. 1998 Feb 14;351(9101):467-71. PubMed PMID: 9482437.

17. Peiris, Yuen. Human infection with influenza H9N2 Lancet. 1999;354(9182):916-7.

18. Belshe. influenza as a zoonosis, how likely is a pandemic. Lancet. 1998;351(9101).

19. Rowe PM. Pandemic influenza (un)preparedness described. The Lancet. 1995 12/30/;346(8991–8992):1699.

20. World Health Organisation. Influenza pandemic preparedness plan. Responding to an influenza pandemic or its threat: The role of WHO and guidelines for national or regional planning. Geneva: 1999.

21. Cox NJ, Tamblyn SE, Tam T. Influenza pandemic planning. Vaccine. 2003 May 1;21(16):1801-3. PubMed PMID: 12686098.

22. Jennings LC, Monto AS, Chan PK, Szucs TD, Nicholson KG. Stockpiling prepandemic influenza vaccines: a new cornerstone of pandemic preparedness plans. The Lancet infectious diseases. 2008 Oct;8(10):650-8. PubMed PMID: 18922487.

23. Webby RJ, Webster RG. Are we ready for pandemic influenza? Science. 2003 Nov 28;302(5650):1519-22. PubMed PMID: 14645836.

24. Committee for Proprietary Medicinal Products. Guideline on submission of marketing-authorisation applications for pandemic influenza vaccines through the centralised procedure. (CPMP/VEG/4986/03). In: Agency EM, editor. 2004.

25. Hancock K, Veguilla V, Lu X, Zhong W, Butler EN, Sun H, et al. Cross-reactive antibody responses to the 2009 pandemic H1N1 influenza virus. The New England journal of medicine. 2009 Nov 12;361(20):1945-52. PubMed PMID: 19745214.

26. Pica N, Hai R, Krammer F, Wang TT, Maamary J, Eggink D, et al. Hemagglutinin stalk antibodies elicited by the 2009 pandemic influenza virus as a mechanism for the extinction of seasonal H1N1 viruses. Proceedings of the National Academy of Sciences of the United States of America. 2012 Feb 14;109(7):2573-8. PubMed PMID: 22308500. Pubmed Central PMCID: 3289326.

27. Donaldson LJ, Rutter PD, Ellis BM, Greaves FE, Mytton OT, Pebody RG, et al. Mortality from pandemic A/H1N1 2009 influenza in England: public health surveillance study. BMJ. 2009;339:b5213. PubMed PMID: 20007665. Pubmed Central PMCID: 2791802.

28. Dominguez-Cherit G, Lapinsky SE, Macias AE, Pinto R, Espinosa-Perez L, de la Torre A, et al. Critically Ill patients with 2009 influenza A(H1N1) in Mexico. JAMA : the journal of the American Medical Association. 2009 Nov 4;302(17):1880-7. PubMed PMID: 19822626.

29. Louie JK, Acosta M, Winter K, Jean C, Gavali S, Schechter R, et al. Factors associated with death or hospitalization due to pandemic 2009 influenza A(H1N1) infection in California. JAMA : the journal of the American Medical Association. 2009 Nov 4;302(17):1896-902. PubMed PMID: 19887665.

30. Johansen K, Nicoll A, Ciancio BC, Kramarz P. Pandemic influenza A(H1N1) 2009 vaccines in the European Union. Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin. 2009;14(41):19361. PubMed PMID: 19883538.

31. European Medicines Agency. Pandemic influenza A(H1N1)v vaccines authorised via the core dossier procedure. Explanatory note on scientific considerations regarding the licensing of pandemic A(H1N1)v vaccines. EMEA/608259/2009 rev. 2009.

32. Smith W, Andrewes CH, Laidlaw PP. Virus obtained from influenza patients. The Lancet. 1933;222(5732):66-8.

33. Burnet FM. Influenza virus infections of the chick embryo by the amniotic route. Aust J Exp Biol 1940;18:353-60.

34. Hirst GK. Agglutination of red cells by allantoic fluid of chick embryos infected with influenza with influenza virus. Science. 1941;94(22-23).

35. McClelland L, Hare R. The adsorption of influenza virus by red cells and a new in vitro method of measuring antibodies for influenza virus. . Canad J Publ Hlth. 1941;32:530-8.

36. Milian E, Kamen AA. Current and Emerging Cell Culture Manufacturing Technologies for Influenza Vaccines. BioMed research international. 2015;2015:504831. PubMed PMID: 25815321. Pubmed Central PMCID: 4359798.

37. FDA. Fluarix Quadrivalent 2012 [cited 2015 09-02-2015]. Available from: www.fda.gov/biologicsbloodvacciens/vaccines/approvedproducts/ucm342391.htm.

38. EMA. Fluenz Tetra - influenza vaccine (live attenuated, nasal) 2013 [cited 2015 09-02-2015]. Available from: www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/002617/human_med_001713.jsp&mid=WC0b01ac058001d124.

39. FDA. Flumist Quadrivalent 2012 [cited 2015 09-02-2015]. Available from: www.fda.gov/biologicsbloodvacciens/vaccines/approvedproducts/ucm293952.htm.

40. FDA. Fluzone Quadrivalent 2013 [cited 2015 09-02-2015]. Available from: www.fda.gov/biologicsbloodvacciens/vaccines/approvedproducts/ucm356091.htm.

41. Belshe RB, Ambrose CS, Yi T. Safety and efficacy of live attenuated influenza vaccine in children 2–7 years of age. Vaccine. 2008;26, Supplement 4(0):D10-D6.

42. European Medicines Agency. European Public assessment report – Fluenz 2011 [cited 2013 05-01]. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/001101/WC500103711.pdf.

43. World Health Organisation. Vaccines against influenza: WHO position paper - November 2012. Wkly Epidemiol Rec. 2012 Nov 23;87(47):461-76. PubMed PMID: 23210147. Epub 2012/12/06.

44. Stuart-Harris CH. Adjuvant influenza vaccines. Bull World Health Organ. 1969;41(3):617-21. PubMed PMID: 5309486. Pubmed Central PMCID: 2427695. Epub 1969/01/01. eng.

45. O'Hagan DT, Ott GS, Nest GV, Rappuoli R, Giudice GD. The history of MF59((R)) adjuvant: a phoenix that arose from the ashes. Expert Rev Vaccines. 2013 Jan;12(1):13-30. PubMed PMID: 23256736. Epub 2012/12/22. eng.

46. Kieny MP, Costa A, Homback J, Carrasco P, Pervikov Y, Salisbury D, et al. A global pandemic influenza vaccine action plan. Vaccine. 2006;24(40-41):6367-70.

47. Mereckiene J, Cotter S, Weber JT, Nicholl A, D'Ancona F, Lopalco PL, et al. Influenza A(H1N1)pdm09 vaccination policies and coverage in Europe. Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin. 2012;17(4):pii=20064.

48. Ferguson NM, Cummings DA, Fraser C, Cajka JC, Cooley PC, Burke DS. Strategies for mitigating an influenza pandemic Nature 2006;442:448–52.

49. European Medicines Agency. Vaccines for pandemic influenza [cited 2015 07-02-2015]. Available from: www.ema.europa.eu/ema/index.jsp?curl=pages/special_topics/general/general_content_000462.jsp&mid=WC0b01ac058004b9ac.

50. European Medicines Agency. Pandemic report and lessons learned. Outcome of the European Medicines Agency's activities during the 2009(H1N1) flu pandemic. . 2011.

51. European Medicines Agency. European Public Assessment Report for Pandemrix: influenza vaccine (H1N1)v (split virion, inactivated, adjuvanted) A/California/7/2009 (H1N1)v like strain (x-179a). Last updated 10/03/2015 2009 [cited 2015 04/04]. Available from: http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/000832/human_med_000965.jsp&mid=WC0b01ac058001d124.

52. European Medicines Agency. European Public Assessment Report for Focetria: influenza vaccine (H1N1) (surface antigen, inactivated, adjuvanted). Last updated 05/10/2010 2009 [cited 2015 04/04]. Available from: http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/000710/human_med_000796.jsp&mid=WC0b01ac058001d124.

53. European Medicines Agency. European public assessment report for Celvapan: influenza vaccine (H1N1)v (whole virion, inactivated, prepared in cell culture) 2009 [cited 2015 04/04]. Available from: http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/000982/human_med_000690.jsp&mid=WC0b01ac058001d124.

54. European Medicines Agency. Dossier structure and content for pandemic-influenza marketing authorisation application. 2009.

55. Wagner R, Gopfert C, Hammann J, Neumann B, Wood J, Newman R, et al. Enhancing the reproducibility of serological methods used to evaluate immunogenicity of pandemic H1N1 influenza vaccines-an effective EU regulatory approach. Vaccine. 2012 Jun 8;30(27):4113-22. PubMed PMID: 22446639. Epub 2012/03/27. eng.

56. Reber A, Katz J. Immunological assessment of influenza vaccines and immune correlates of protection. Expert Rev Vaccines. 2013;12(5):519-36.

57. Ohmit SE, Petrie JG, Cross RT, Johnson E, Monto AS. Influenza hemagglutination-inhibition antibody titer as a correlate of vaccine-induced protection. The Journal of Infectious Diseases. 2011;204:1879-85.

58. Black S, Nicolay U, Vesikari T, Knuf M, Del Giudice G, Della Cioppa G, et al. Hemagglutination inhibition antibody titers as a correlate of protection for inactivated influenza vaccines in children. Pediatr Infect Dis J. 2011 Dec;30(12):1081-5. PubMed PMID: 21983214. Epub 2011/10/11. eng.

59. Vellozzi C, Burwen DR, Dobardzic A, Ball R, Walton K, Haber P. Safety of trivalent inactivated influenza vaccines in adults: background for pandemic influenza vaccine safety monitoring. Vaccine. 2009 Mar 26;27(15):2114-20. PubMed PMID: 19356614.

Chapter 2. Effectiveness of MF59™ adjuvanted A(H1N1)pdm09 vaccine in risk groups and the elderly in the Netherlands

Leonoor Wijnans

Jeanne Dieleman

Bettie Voordouw

Miriam Sturkenboom

Published in: Plos ONE. Apr 30;8(4):e63156. doi: 10.1371/journal.pone.0063156.

Abstract

Background

The aim of the present study was to estimate the effectiveness of the MF59™-adjuvanted influenza A(H1N1)pdm09 vaccine against medically attended influenza-like illness and RT-PCR confirmed influenza in the at-risk population and persons over 60 in the Netherlands.

Methods

We conducted a retrospective cohort study in a Dutch based GP medical record database between 30 November 2009 and 1 March 2010 to estimate the vaccine effectiveness against influenza-like illness. Within the cohort we nested a test negative case-control study to estimate the effectiveness against laboratory confirmed influenza.

Results

The crude effectiveness in preventing diagnosed or possible influenza-like illness was 17.3% (95%CI: -8.5% - 36.9%). Of the measured covariates, age, the severity of disease and health seeking behaviour through devised proxies confounded the association between vaccination and influenza-like illness. The adjusted vaccine effectiveness was 20.8% (95%CI: -5.4% - 40.5%) and varied by age, being highest in adults up to 50 years (59%, 95%CI: 23% - 78%), and non-detectable in adults over 50 years.

The number of cases in the nested case-control study was too limited to validly estimate the VE against confirmed influenza.

Conclusions

With our study we demonstrated that the approach of combining a cohort study in a primary health care database with field sampling is a feasible and useful option to monitor VE of influenza vaccines in the future

.

Introduction

Vaccination is regarded as one of the most efficient interventions that protect the population at risk of serious health complications during influenza pandemics 1. During the H1N1-influenza pandemic of 2009/2010 mass vaccination campaigns with new influenza vaccines were set out throughout the world. In order to reduce the amount of antigen needed for vaccinating entire populations oil-in-water adjuvanted vaccines were used for the first time on a large scale in Europe 2. In the Netherlands general practitioners (GPs) were provided with MF59™-adjuvanted influenza A(H1N1)pdm09 vaccines to vaccinate persons at risk due to underlying comorbidities and persons over 60 years of age. These persons were offered two doses of the vaccine.

The MF59-adjuvanted influenza A(H1N1)pdm09 vaccine was licensed based on immunogenicity and safety of vaccines with avian influenza strains, allowing for fast track roll out of vaccines upon the emerging pandemic 2. Estimates of the effectiveness of the vaccine in targeted risk groups are scarce to date 3-5. Steens et al. reported no significant vaccine effectiveness (VE) (19%, 95%CI: -28% - 49%) against influenza A(H1N1)pdm09 - infection related hospitalisation in a matched case control study in targeted risk groups in the Netherlands 4. Castilla et al. 5 conducted a cohort study in all non-institutionalized persons in a region in Spain where children (1-17 years) and persons aged over 60 received the MF59 TM-adjuvanted influenza A(H1N1)pdm09 vaccine. They found no evidence of effectiveness of vaccination against medically attended influenza-like illness (ILI) in children (VE: 12%; 95%CI: -142% - 68%) and in the elderly (VE: 25%; 95%CI: -19% - 53%). Data on effectiveness of vaccination programmes with adjuvanted vaccines in different target groups is essential to inform future decisions and recommendations for vaccination programmes and possible complementary or alternative public health measures in order to mitigate the potential impact of influenza epidemics and pandemics. The aim of our study was to estimate the effectiveness of the MF59™-adjuvanted influenza A(H1N1)pdm09 vaccine against medically attended ILI and against laboratory confirmed A(H1N1)pdm09 infection in the population that was indicated for vaccination by the GP in the Netherlands.

Methods

We conducted a retrospective cohort study in a Dutch GP medical record database, in which we nested a case control study to determine effectiveness of the MF59™-adjuvanted influenza A(H1N1)pdm09 vaccine against RT-PCR confirmed influenza infection.

IPCI database:

Our cohort was identified within the Integrated Primary Care Information (IPCI) database. More detailed information on IPCI has been published elsewhere [6]. In short, IPCI contains longitudinal data from anonymized computer-based medical records of Dutch GPs from 1996 onwards. In the Netherlands, almost all residents are registered with a GP or practice, which serves as the gatekeeper to and from all medical care in the Netherlands. The age and gender distribution of the population in IPCI is representative of the Netherlands and of community dwelling persons. Currently, IPCI contains information on over 1,100,000 patients from over 200 participating GP practices located throughout the Netherlands. IPCI includes anonymous demographic information as well as information on signs, symptoms and diagnoses, both coded through the International classification of primary care (ICPC) and as free text, prescriptions (ATC coded), annual vaccinations against influenza and non-childhood vaccines, hospital admissions, referrals to secondary care, letters from specialists, and laboratory test results. Records have good validity for prescriptions, hospitalizations, influenza vaccination and influenza related outcomes [6-8]. The IPCI database complies with European Union guidelines on the use of medical data for medical research [6]. Approval for this study was obtained by the Scientific and Ethical Advisory Board of the IPCI project and by the Medical Ethical committee of Erasmus MC. Informed consent was obtained from all patients participating in the nested case control study.

Study population

Cohort : We defined a cohort within the IPCI database of persons who were eligible for A(H1N1)pdm09 vaccination through the GP due to an underlying medical condition or age >60 years and who had at least one year of valid database history. As pregnancy is not consistently recorded from the start of pregnancy, only pregnant women indicated for A(H1N1)pdm09 vaccination due to underlying medical conditions were included in the cohort. Eligibility for vaccination was assessed from the electronic patient records using free text and ICPC-code searches followed by manual verification in the full electronic medical record.

We excluded GPs with incomplete or unreliable registration of vaccination defined as a coverage of influenza A(H1N1)pdm09 vaccine in persons >60 years lower than 50%, or with unreliable vaccination dates. In addition, we excluded persons with a contraindication to influenza vaccination and persons who had visited the GP for ILI between start of circulation of H1N1 in the Netherlands (week 28) and start of follow-up (week 49).

Nested case control: Practices included in the cohort study were invited to participate in the case control study. Cases and controls were obtained from cohort members who visited the

GP for ILI during the study period. Controls were to be matched to cases by GP practice and time of presentation.

Study period

Cohort: Follow-up started on 30 November 2009 (week 49), two weeks after the majority of GP practices had administered the 1st dose. Follow-up ended at death, first ILI, transferring out of the practice, or end of the study period (1 March 2010).

Nested case control: The swab schedule for the nested case control study was planned to start two weeks after start vaccination as indicated by participating GPs. Swabbing started on 9 November 2009 and ended on 3 March 2010.

Study endpoint

Cohort: The outcome of interest was medically attended ILI using the European ILI case definition 9: a sudden onset of symptoms combined with 1) at least one of the following symptoms: fever or feverishness, malaise, headache, or myalgia; and 2) at least one of the following three respiratory symptoms: cough, sore throat, shortness of breath.

ILI cases were extracted from the IPCI database by using an extensive string search including free text terms combined with ICPC-codes (R80, R81, R74, R78) reflecting the symptoms and diagnosis of ILI. Obvious negations were excluded. All identified ILI cases from week 30 onwards were manually validated against the full electronic patient record to check whether they met the case definition, validation was done while being blinded to exposure.

Nested case control: The primary outcome in the case control study was RT-PCR confirmed influenza in persons presenting to the GP with ILI. A nasopharyngeal swab was taken from cohort members with ILI symptoms during the influenza season. Nasopharyngeal swabs were sent to the virology department of the Erasmus-MC for RT-PCR analysis. All persons with samples tested positive for influenza infection were classified as cases. Cases were sub-typed as influenza A(H1N1)pdm09, H1N1, H3N2 or B. Persons with ILI but no detectable influenza were classified as controls.

Exposures

The primary exposure of interest in this study was vaccination with MF59™-adjuvanted influenza A(H1N1)pdm09 vaccine. Persons having received at least a first dose of vaccine at the start of follow-up (cohort) or at time of swabbing (nested case control) were considered exposed, regardless of the time since vaccination. Vaccination status was determined through GP-specific free text searches and ICPC-codes in the full electronic

patient record followed by random manual verification to assess and increase the specificity of the final search. Distinction between seasonal influenza vaccination and doses of H1N1-vaccinations were based on free text wording and calendar dates. Information on the following covariates at baseline was collected from the electronic patient record for each individual in the cohort: age, gender, presence of co-morbidity (diabetes, respiratory, cardiovascular, renal insufficiencies, immune-compromised or malignancies; identified through free text searches and ICPC-codes followed by manual verification against the electronic records), seasonal influenza vaccination history, use of oseltamivir, zanamivir, amantadine, rimantadine, health care utilization (defined as number of GP-visits in previous year) and severity of underlying comorbidity (estimated by the number of different drugs prescribed in previous year identified by number of different ATC-codes).

Participants in the nested case control study had a unique study ID that was linked to their unique patient identifier in the IPCI database. Information on exposure and covariates was extracted from the IPCI-database.

Statistical methods

Cohort: Descriptive analyses and univariate analysis were performed to compare study population baseline characteristics between vaccinated and unvaccinated patients. We estimated crude and adjusted estimates for VE (1-relative risk*100%) for ILI through univariate and multivariate Cox-proportional hazard analysis. We used subject time, which was calendar time, as the time axis. Variables were included in the multivariate analysis if they changed the crude point-estimate by more than 10%.

Nested case control: Crude odds ratios with 95% confidence intervals were obtained by using conditional logistic regression analysis. The crude VE was computed as VE = 1 – OR.

Sensitivity analyses: In the cohort, misclassification of exposure was investigated by varying the start of the follow-up period (starting at week 47 and week 51 instead of 49), and varying the definition of exposure. In this analysis persons were considered exposed if they were vaccinated >14 days prior to baseline or >7 days prior to baseline. All other persons were considered unexposed. Additionally, we conducted a post hoc analysis in which vaccination was considered as a time dependent variable, meaning the exposure status was determined when an outcome occurred. Persons were considered exposed 14 days after vaccination. In this analysis baseline could be brought back to 01-10-2009, which increased the number of cases. As vaccination was time dependent misclassification was also minimized.

Statistical significance was accepted at a p-value <0.05. All analyses were done using SPSS (SPSS Inc., Chicago, IL, USA) version 15.0 for Windows.

Results

Study population

Cohort : At the start of follow-up there were 191,518 persons who had an indication for influenza A(H1N1)pdm09 vaccination in 205 GP practices contributing data to IPCI. Of these, 68,642 persons from 102 GP practices were excluded, as influenza A(H1N1)pdm09 vaccination could not be assessed reliably in the electronic patient record. Of the remaining 122,876 persons, 1,430 had ILI between week 28 and start of follow-up (week 49) and were excluded as they were not at risk of H1N1 ILI anymore (assuming infection with H1N1). The final study population for the primary analysis included 121,446 patients with an average follow-up time of 75.8 days per person (SD 22.2) from week 49 onwards.

Nested case control: In total, 41 GP practices agreed to participate in the nested case control study. Two dropped out early due to time constraints.

Baseline characteristics cohort

The A(H1N1)pdm09 vaccinated and non-vaccinated persons differed regarding a number of baseline characteristics are presented in table 2.1.

Unvaccinated persons were younger and less likely to have received a seasonal influenza vaccine in 2008 and 2009. The majority of the cohort (73.5%) had at least one type of underlying disease that would qualify as indication for vaccination, thus including healthy people 60 years or older. With the exception of diabetes and respiratory disease, co-morbidities were more prevalent in vaccinated as compared to unvaccinated persons, most notably for cardiac disease and malignancies. The mean number of different drugs prescribed in the preceding year was higher in vaccinated persons, as was the number of GP contacts in the preceding year.

Table 2.1 Baseline characteristics cohort

		Exposed to first dose influenza A(H1N1)pdm09 vaccine[6]				
		unexposed		Exposed		
		51442		70004		
		n	(%)	n	(%)	p-value
Age[1]	Mean (st.dev)	49.8	(22.5)	63.6	(16.8)	<0.0001
	<=4	938	(1.8)	371	(0.5)	
	5-19	6717	(13.1)	2445	(3.5)	
	20-49	14266	(27.7)	7216	(10.3)	
	50-59	7223	(14.0)	7315	(10.4)	
	60-79	18809	(36.6)	43235	(61.8)	
	80+	3489	(6.8)	9422	(13.5)	
Gender	male	24720	(48.1)	32290	(46.1)	<0.0001
Seasonal influenza vaccination 09		12744	(24.8)	59965	(85.7)	<0.0001
Seasonal influenza vaccination 08		15153	(29.5)	51522	(73.6)	<0.0001
Pandemic H1N1 vaccine doses[1]	None	51442	(100)			
	1 dose			67048	(95.8)	
	2 doses			2956	(4.2)	
Days since first dose[1]	<7			11568	(16.5)	
	7-14			31420	(44.9)	
	≥14			35494	(50.7)	
Diabetes		16063	(31.2)	16269	(23.2)	<0.0001
Cardiac disease		12752	(24.8)	32781	(46,8)	<0.0001
Respiratory disease		12208	(23.7)	18840	(26,9)	<0.0001
Renal disease		884	(1.7)	2218	(3.2)	<0.0001
Malignancy		4929	(9.6)	10717	(15.3)	<0.0001
Immune compromised		95	(0.2)	199	(0.3)	<0.0001
Any chronic co-morbidity[3]		36334	(70.6)	53012	(75.7)	<0.0001
Mean number of different drugs prescribed[2,4]	Mean (st.dev)	3.69	(4.1)	6.0	(4.9)	<0.0001
Mean number of GP contacts[2]	Mean (st.dev)	11.0	(11.6)	17.3	(13.2)	<0.0001
Antiviral drugs use[5] prior 30-11-09		130	(0.3)	246	(0.4)	0.002
Antiviral drugs use[5] after 30-11-09		13	(0.0)	38	(0.1)	0.015

1 On 30-11-2009
2 Between 01-10-2008 and 01-10-2009
3 Includes respiratory, cardiovascular, diabetes and renal disease, persons with malignancies and immune compromised
4 Based on ATC (7 digits)
5 Antiviral drugs: Amantadine, rimantadine, oseltamivir, zanamivir which are all indicated for treatment of influenza infection; amantadine is also used in the treatment of parkinsons disease

Vaccination

Vaccine uptake was highest in persons 60 years and older (Figure 2.1).

By the end of the vaccination campaign, 88% of those having received a first dose also received a second dose of the influenza A(H1N1)pdm09 vaccine. At the start of follow-up, which was before the end of the vaccination campaign, point coverage for seasonal influenza vaccination in the cohort was 59.8%. For a single dose of influenza A(H1N1)pdm09 vaccine it was 57.6% and for two doses it was 4.2%. Fifty-one % of vaccinated persons had received a first dose at least 14 days before the start of the study. Only 16% had received their first dose less than 7 days before the start of the study.

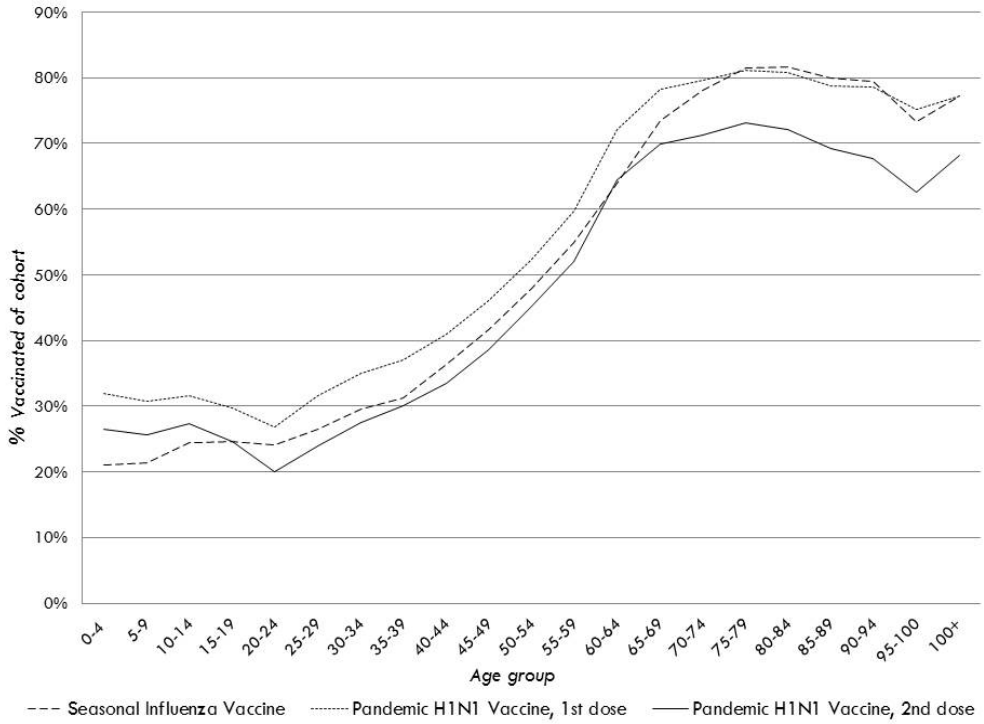


Figure 2.1 Vaccination coverage per age group for seasonal influenza vaccination and first and second doses of pandemic influenza vaccine in the cohort of patients that had an indication for pandemic influenza vaccination

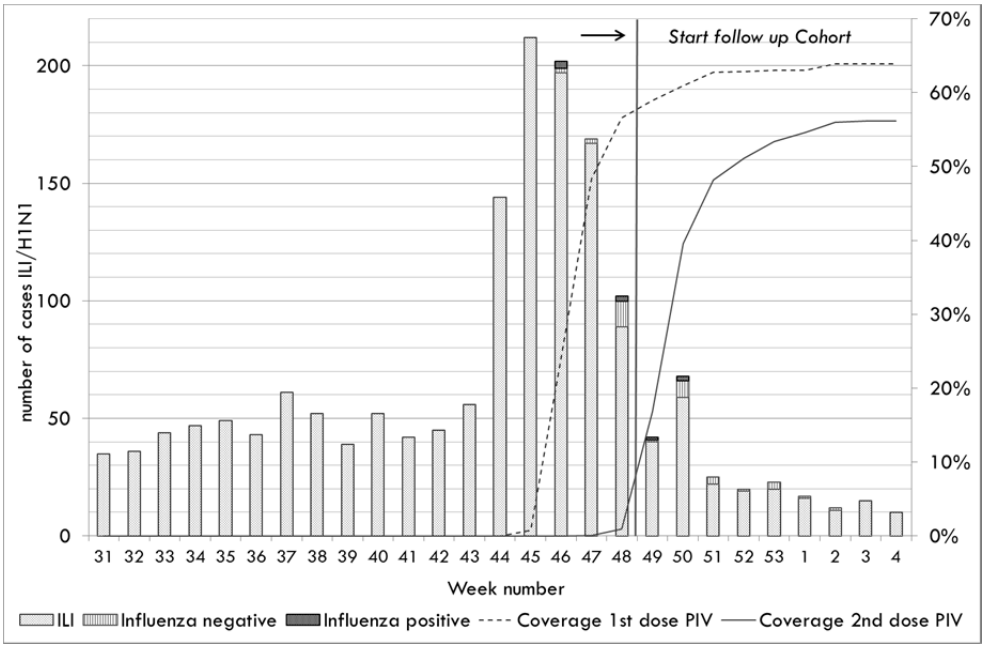


Figure 2.2 Coverage of the 1st and 2nd dose of the A(H1N1)pdm09 vaccine, ILI cases in the cohort, and influenza positive cases plus controls (influenza negative) from the nested case control study against calendar time

ILI & RT-PCR confirmed influenza

In the total cohort, 255 ILI cases were identified during follow up. The incidence rate of ILI during follow-up was age dependent, being highest in the youngest age group and slightly lower in subsequent age groups (Table 2.2). The overall incidence rate during follow-up was 10.1 per 1000 person years (95%CI: 8.9 - 11.4).

Table 2.2 Number and rate of ILI cases

	Age category					
	<=4	5-19	20-49	50-59	60-79	80+
Number of ILI cases (%)	4 (0.31%)	24 (0.26%)	59 (0.27%)	29 (0.20%)	112 (0.18%)	27 (0.21%)
Person Time[1]	268	1839	4319	2982	13106	2714
Incidence Rate (95%CI) [2]	14.9 (5.6 - 39.7)	13.1 (8.8- 19.5)	13.7 (10.6 - 17.6)	9.7 (6.8 – 14.0)	8.6 (7.1 - 10.3)	9.9 (6.8 - 14.5)

[1]In years
[2] per 1,000 person- years

Figure 2.2 shows the distribution of validated ILI cases, influenza positive and negative cases from the case control study over calendar time along with the coverage of the first and

second dose of pandemic influenza vaccination. Vaccination started around the peak of ILI incidence.

In the nested case control study 46 swabs were received for analysis. One swab could not be analysed. Of the remaining 45, 9 tested positive for influenza A, including 7 confirmed A(H1N1)pdm09 infections. The average age of cases was 33.4 years (SD: 22.3 years), controls were older with an average age of 55.4 years (SD 20.5 years).

Vaccine effectiveness

In the total cohort, we found a crude VE estimate against ILI of 17.3% (95%CI: -8.5% - 36.9%). Of the measured covariates, age, the number of different drugs prescribed in the preceding year and the number of GP contacts in the preceding year confounded the association between influenza A(H1N1)pdm09 vaccine and ILI with at least a 10% change in the point estimate. The adjusted VE against ILI was 20.8% (95%CI: -5.4% - 40.5%) (Table 2.3). The VE differed by age groups, with the highest adjusted VE in adults up to 50 years (59%, 95%CI: 23% - 78%).

Table 2.3 Crude and adjusted pandemic H1N1 vaccine effectiveness per age category (Primary analysis with baseline at 30-11-2009)

Age Category	Number of ILI cases (%)	Crude VE	95%CI	Adjusted VE*	95%CI
Overall		17.3%	-8.5% - 36.9%	20.8%	-5.4% - 40.5%
<=4 yrs	4 (0.31%)	-482.9%	-6988.3% - 52.1%	-505.8%	-8341.8% - 56.5%
5 – 19 yrs	24 (0.26%)	38.7%	-85.4% - 79.8%	50.9%	-51.0% - 84.0%
20 – 49 yrs	59 (0.27%)	42.2%	-7.1% - 68.8%	58.7%	22.7% - 77.9%
50- 59 yrs	29 (0.20%)	17.7%	-79.4% - 62.3%	20.9%	-76.1% - 64.5%
60 – 79 yrs	112 (0.18%)	-36%	-122% - 16%	-14,2%	-86.7% - 30.1%
80+ yrs	27 (0.21%)	12%	-114% - 64%	18,3%	-100.7% - 66.8%

* adjusted for number of different drugs prescribed and number of GP contacts in year before

Based on the 9 cases and 36 controls in the nested case control study, we estimated a crude VE for the influenza A(H1N1)pdm09 vaccine in preventing RT-PCR confirmed influenza was 73.3% (95%CI: 4.8% - 92.5%). The crude VE against RT-PCR confirmed influenza A(H1N1)pdm09 infection was 88% (95%CI: 25% - 98%). Due to the small sample size, no adjusted or matched analysis was performed.

Sensitivity analyses

In the primary analysis everyone who had received an influenza A(H1N1)pdm09 vaccine at the start of follow-up or at time of swabbing was considered exposed regardless of time since vaccination. As it takes 2 to 3 weeks to mount an immune response to seasonal influenza vaccines [10], in our primary analysis persons could have been considered exposed whilst they were not immunized. To address this potential misclassification we restricted the definition of exposure and only considered those as exposed who received a first dose more than 7 days before baseline, non-exposed were persons who were not vaccinated or vaccinated within 7 days. This decreased the crude VE against ILI to 13.3% (95%CI: -15.5% - 34.9%). Only considering as exposed those who received a first dose more than 14 days before baseline and as non-exposed those not vaccinated or vaccinated within 14 days prior to baseline decreased this estimate further to 5.1% (95%CI: -36.1% - 33.8%). Restricting the analysis of the nested case control study to swabs taken 14 days after the start of vaccination resulted in a crude VE against RT-PCR confirmed influenza A infection of 17% (95%CI -563% - 90%) and a crude VE against influenza A(H1N1)pdm09 infection of 75% (95%CI: -473% - 99%).

The baseline for the cohort study was chosen relatively late (figure 2) to allow for the majority of GP-practices to have administered at least the first dose of influenza A(H1N1)pdm09 vaccine plus 14 days for the vaccine to exert its effectiveness. When applying a start of follow-up two weeks earlier (week 47 instead of week 49) the crude overall VE increased to 23% (95%CI: 4% - 38%). Applying a cut-off two weeks later (week 51 instead of week 49) decreased the crude overall VE to -7.8% (95%CI: -48.0% - 22.4%).

In a post-hoc analysis we started follow-up in October 2009 and considered exposure to A(H1N1)pdm09 vaccination to be a time dependent variable. By doing so misclassification of exposure is limited. The most noticeable increase in number of cases was seen in the ≤ 4 year age group. Overall, the estimates move closer towards no effect (Table 2.4)

Table 2.4 Crude and adjusted pandemic H1N1 vaccine effectiveness per age category: Post hoc Time Dependent Analysis (Baseline = 01-10-2009).

Age Category	Number of ILI cases (%)	Crude VE	95%CI	Adjusted VE*	95%CI
Overall		9.0%	-19.2% - 30.5%	-17.4%	-54.9% - 11.0%
<=4 yrs	65 (4.6%)	-38.6%	-868.9% - 80.2%	-32.9%	-815.0% - 80.7%
5 – 19 yrs	289 (3.1%)	23.6%	-109.9% - 72.2%	34.6%	-79.8% - 76.2%
20 – 49 yrs	350 (1.5%)	21.4%	-40.2% - 55.9%	34.4%	-17.0% - 63.2%
50- 59 yrs	154 (1.0%)	3.3%	-109.1% - 55.3%	17.3%	-79.2% - 61.8%
60 – 79 yrs	307 (0.5%)	-57.0%	-161.2% - 5.6%	-28.5%	-114.3% - 22.9%
80+ yrs	73 (0.6%)	5.4%	-158.2% - 65.3%	16.3%	-130.4% - 69.6%

* adjusted for number of different drugs prescribed and number of GP contacts in year before

Discussion

In our retrospective cohort study we found an overall small non-significant protective effect of vaccination with an MF59™-adjuvanted influenza A(H1N1)pdm09 vaccine against ILI. The VE estimates against RT-PCR confirmed influenza and A(H1N1)pdm09 infection were substantially higher, however numbers were small estimates are relatively unstable and no adjusted analysis could be performed. Limited importance should be attached to this crude estimate as it may suffer from confounding.

The adjusted VE against ILI was highest in persons between the age of 20 and 49 years (59%; 95%CI: 20% - 78%) and in children between the age of 5 and 19 years (adjusted VE: 51%; 95% CI: -50% - 84%). We could not validly estimate the vaccine effectiveness in children ≤4 years as the group was very small and vaccinations could have been received through other routes than the GP. For persons between 50 and 59 years and persons between 60 and 79 years the adjusted VE was 21% (95% CI: -80% - 64%), and -15% (95% CI: -90% - 30%) respectively.

This is in line with findings from a large study by Castilla [5], who conducted a cohort study in children (1-17 years) and persons over 60 years, evaluating the VE of the MF59™-adjuvanted influenza A(H1N1)pdm09 vaccine against medically attended ILI. Similar to our findings, the VE in persons over 60 in their study was 25% (95%CI: -19% - 53%).

Immunosenescence resulting in reduced VE in older age groups is a known problem for seasonal inactivated influenza vaccines and adjuvants have been brought up as a possible solution [11]. As in the study by Castilla *et al* we found no evidence that the adjuvanted vaccine results into improved effectiveness against ILI in the elderly. A possible explanation of the absence of effectiveness against ILI in persons over 50 in our study is the lack of specificity of ILI for influenza, due to the presence of cross-reactive antibodies in older adults resulting from previous exposure to similar influenza strains [12]. These would protect against infection with influenza A(H1N1)pdm09 regardless of vaccination, whilst still being susceptible to a wide range of pathogens that could cause ILI. As a result ILI could be less specific for influenza in older people than in younger people who lack cross-reactive antibodies [12] leaving them vulnerable to influenza A(H1N1)pdm09 infection, hence a proportion of ILIs could be caused by influenza virus. Consequently, the specificity of ILI could not only change with time, as circulation of virus decreases, but also with age. These uncertainties underline the importance of including confirmed influenza infection as an endpoint to validate findings in the larger cohort. In our nested case control study we lacked the power to do this.

A test negative case control study evaluated the VE of the MF59™-adjuvanted influenza A(H1N1)pdm09 against laboratory confirmed influenza A(H1N1)pdm09 infection in a general population ≥ 10 years of age in Korea. Only14% had underlying disease. They found a VE of 73.4% (95%CI: 49.1% - 86.1%) against laboratory confirmed influenza A(H1N1)pdm09 infection [3], which did not vary significantly with age, supporting the theory that our findings are due to the lack of specificity of our endpoint rather than the vaccine. However, considering the differences in population ideally we would have validated this within our own cohort.

Several studies evaluated the effectiveness of ASO3-adjuvanted influenza A(H1N1)pdm09 vaccine against laboratory confirmed H1N1 in the general population [13-19], reporting VE estimates between 60% and 95%. The effectiveness of AS03-adjuvanted influenza A(H1N1)pdm09 vaccine was found to be lower in an at risk population under 65 in Denmark (49% against laboratory confirmed ILI, 44% against hospitalisation) [20]. Other studies for a mix of adjuvanted and non-adjuvanted vaccines against ILI, laboratory confirmed H1N1 and hospitalisations [21-25] reported combined VE estimates of 52% against ILI [21], 72% to 95% against lab confirmed ILI [22-25] and 90% to 100% against hospitalisations [22, 26] .

In our cohort, severity of underlying co-morbidity rather than its presence was a more important confounder, possibly as the majority of persons in the cohort had underlying medical conditions. The approximation used for determining severity of disease by number of different pharmaceutical compounds prescribed is a crude measure that should be further

refined and validated for future influenza vaccine effectiveness studies. Also, other methods of mapping severity of underlying co-morbidity remain to be evaluated. Given the large effect of disease severity, misclassification of this covariate can be an important source of residual confounding.

Being a study using observational data, misclassification and residual confounding are a potential concern. As the likelihood of being exposed increased when moving away from the epidemic peak, and the likelihood of ILI (and the specificity of ILI to represent influenza infection) decreased away from the peak we chose the start of follow-up where the majority of vaccinated persons had received at least one dose of influenza A(H1N1)pdm09 vaccine and there was still detectable influenza transmission in the community. This had two major consequences – it limited the power of the study, and misclassification of exposure was inevitable. We evaluated the effect of time since exposure by only considering those exposed who received a first dose more than 7 or 14 days before baseline. This did have a considerable impact on the estimate of VE, decreasing it from 17.3% to 13.3% (95%CI: -15.5% - 34.9%) and 5.1% (95%CI: -36.1% - 33.8%) respectively. The reduction in VE when including the time restriction to define exposed status illustrates how misclassification of exposure dilutes the estimate in our study. As the majority of vaccinated persons had received their first dose (table 2.1) at start of follow-up we hope to have minimized the consequences of exposure misclassification. This was further supported by the analysis in which exposure was considered as a time dependent variable. An increase in power and shift in effect estimate was seen in children under five years, however not in other age groups indicating only limited misclassification of exposure overall.

Misclassification of exposure also may have occurred since recording of influenza vaccinations in the patient record by the GP was not compulsory and vaccinations could have been obtained through other sources. To minimize such misclassification we excluded GPs with ambiguous vaccine registration in the electronic patient record. We did miss vaccinations in children below 5, and in health care workers who received vaccinations elsewhere than at the GP. This misclassification most likely would drive the VE toward no effect.

We varied the start of follow-up to evaluate the impact of calendar time on the study. The crude VE increased to 23% (95%CI: 4% - 38%) when applying an earlier start date (week 47), and decreased to -7.8% (95%CI: -48.0% - 22.4%) when applying a later start of follow-up (week 51), illustrating that the specificity of medically attended ILI changed during the epidemic.

False-negative misclassification of ILI is likely to have occurred since people were advised to stay at home and not contact the GP with flu symptoms. Differential misclassification may have arisen if people with more serious underlying disease were more likely than other

people to get the vaccination and to report ILI to their GP, leading to an underestimation of the VE.

Conclusion

With our study we demonstrated that the approach of combining a cohort study in a primary health care database with field sampling is a feasible option to monitor VE of influenza vaccines in the future. This approach had the benefit of reliably measuring the presence of a large number of potential confounding variables, including underlying comorbidities, severity of disease, health seeking behaviour, drug use patterns and vaccination history and evaluating their effect on VE estimates whilst validating the less specific outcomes that are measurable in the cohort, such as ILI, with more specific laboratory confirmed outcomes.

References

1. Ferguson NM, Cummings DA, Fraser C, Cajka JC, Cooley PC, Burke DS. Strategies for mitigating an influenza pandemic Nature 2006;442:448–52.

2. Johansen K, Nicoll A, Ciancio BC, Kramarz P. Pandemic influenza A(H1N1) 2009 vaccines in the European Union. Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin 2009;14:19361.

3. Song JY, Cheong HJ, Heo JY, et al. Effectiveness of the pandemic influenza A/H1N1 2009 monovalent vaccine in Korea. Vaccine 2011;29:1395-8.

4. Steens A, Wijnans E, Dieleman J, Sturkenboom M, van der Sande M, van der Hoek W. Effectiveness of a MF-59TM-adjuvanted pandemic influenza vaccine to prevent 2009 influenza A/H1N1-related hospitalisation; a matched case-control study. BMC Infect Dis 2011;11:196.

5. Castilla J, Morán J, Martínez-Artola V, et al. Effectiveness of the monovalent influenza A(H1N1)2009 vaccine in Navarre, Spain, 2009-2010: Cohort and case-control study. Vaccine 2011;29:5919-24.

6. Vlug A, van der Lei J, Mosseveld B, et al. Postmarketing surveillance based on electronic patient records: the IPCI project. Methods Inf Med 1999;38:339-44.

7. van der Lei J, Duisterhout JS, Westerhof HP, et al. The Introduction of Computer-based Patient Records in the Netherlands. Annals of Internal Medicine 1993;119:1036-41.

8. Voordouw BCG, Sturkenboom MCJM, Dieleman JP, Stijnen T, van der Lei J, Stricker BHC. Annual Influenza Vaccination in Community-Dwelling Elderly Individuals and the Risk of Lower Respiratory Tract Infections or Pneumonia. Arch Intern Med 2006;166:1980-5.

9. EC. Commission Decision of 30 April 2009 amending Decision 2002/253/EC laying down case definitions for reporting communicable diseases to the Community network under Decision No 2119/98/EC of the European Parliament and of the Council. . Official Journal of the European Union 1 May 2009;Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:110:0058:0059:EN:PDF

10. Core SPC for trivalent influenza vaccines. 2009. (Accessed 21-02, 2013, at www.hma.eu/fileadmin/dateien/Human_Medicines/CMD_h_/Product_Information/Core_SPC_PL/Influenza_vaccines/CMDh-128-2003-Rev3-Clean.pdf.)

11. Monto AS, Ansaldi F, Aspinall R, et al. Influenza control in the 21st century: Optimizing protection of older adults. Vaccine 2009;27:5043-53.

12. Hancock K, Veguilla V, Lu X, et al. Cross-Reactive Antibody Responses to the 2009 Pandemic H1N1 Influenza Virus The New England journal of medicine 2009;361:1945-52.

13. Örtqvist A, Berggren I, Insulander M, de Jong B, Svenungsson B. Effectiveness of an Adjuvanted Monovalent Vaccine Against the 2009 Pandemic Strain of Influenza A(H1N1)v in Stockholm County, Sweden. Clin Infect Dis 2011;52:1203-11.

14. Andrews NJ, Waight P, Yung CF, Miller E. Age-specific effectiveness of an oil-in-water adjuvanted pandemic (H1N1) 2009 vaccine against confirmed infection in high risk groups in England. J Infect Dis 2011;203:32-9.

15. Skowronski DM, Janjua NZ, De Serres G, et al. Effectiveness of AS03 adjuvanted pandemic H1N1 vaccine: case-control evaluation based on sentinel surveillance system in Canada, autumn 2009. Bmj 2011;342:c7297.

16. Wichmann O, Stocker P, Poggensee G, et al. Pandemic influenza A(H1N1) 2009 breakthrough infections and estimates of vaccine effectiveness in Germany 2009-2010. Euro Surveill 2010;15:pii: 19561.

17. Hardelid P, Fleming DM, McMenamin J, et al. Effectiveness of pandemic and seasonal influenza vaccine in preventing pandemic influenza A(H1N1)2009 infection in England and Scotland 2009-2010. Euro Surveill 2011;16:pii=19763.

18. Uphoff H, an der Heiden M, Schweiger B, et al. Effectiveness of the AS03-Adjuvanted Vaccine against Pandemic Influenza Virus A/(H1N1) 2009 â€“ A Comparison of Two Methods; Germany, 2009/10. PLoS ONE 2011;6:e19932.

19. Skowronski DM, Janjua NZ, De Serres G, et al. Effectiveness of AS03 adjuvanted pandemic H1N1 vaccine: case-control evaluation based on sentinel surveillance system in Canada, autumn 2009. BMJ 2011;342.

20. Emborg HD, Krause TG, Hviid A, Simonsen J, Molbak K. Effectivenss of vaccine against pandemic influenza A/H1N1 among people with underlying chronic diseases: cohort study. Denmark, 2009-2010. BMJ 2012;344:d7901.

21. Pelat C, Falchi A, Carrat F, et al. Field effectiveness of pandemic and 2009-2010 seasonal vaccines against 2009-2010 A(H1N1) Influenza: estimations from surveillance data in France. PLoS ONE 2011;6: e19621.

22. Simpson CR, Ritchie LD, Robertson C, Sheikh A, McMenamin J. Vaccine effectiveness in pandemic influenza - primary care reporting (VIPER): an observational study to assess the effectiveness of the pandemic influenza A (H1N1)v vaccine. Health Technol Assess 2010;14:313-46.

23. Valenciano M, Kissling E, Cohen JM, et al. Estimates of Pandemic Influenza Vaccine Effectiveness in Europe, 2009-2010: Results of Influenza Monitoring Vaccine Effectiveness in Europe (I-MOVE) Multicentre Case-Control Study. . PLoS Med 2011;8:e1000388.

24. Puig-Barberà J, Arnedo-Pena A, Pardo-Serrano F, et al. Surveillance and Vaccine Evaluation Group during the autumn 2009 H1N1 pandemic wave in Castellón, Spain. Effectiveness of seasonal 2008-2009, 2009-2010 and pandemic vaccines, to prevent influenza hospitalizations during the autumn 2009 influenza pandemic wave in Castellón, Spain. A test-negative, hospital-based, case-control study. . Vaccine 2010;28:7460-7.

25. Savulescu C, Jimenez-Jorge S, de Mateo S, et al. Using surveillance data to estimate pandemic vaccine effectiveness against laboratory confirmed influenza A(H1N1)2009 infection: two case-control studies, Spain, season 2009-2010. BMC Public Health 2011;11:899.

26. Puig-Barberà J, Arnedo-Pena A, Pardo-Serrano F, et al. Effectiveness of seasonal 2008-2009, 2009-2010 and pandemic vaccines, to prevent influenza hospitalizations during the autumn 2009 influenza pandemic wave in Castellón, Spain. A test-negative, hospital-based, case-control study. Vaccine 2010;28:7460-7.

Chapter 3. Effectiveness of a MF59™ adjuvanted pandemic influenza vaccine to prevent 2009 influenza A/H1N1-related hospitalisation: a matched case-control study

Anneke Steens

Leonoor Wijnans

Jeanne Dieleman

Miriam Sturkenboom

Marianne van der Sande

Wim van der Hoek

Published in: BMC infectious diseases. 2011; 11(1): 196.

Abstract

Background

During the 2009 influenza A(H1N1) pandemic, adjuvanted influenza vaccines were used for the first time on a large scale. Results on the effectiveness of the vaccines in preventing 2009 influenza A/H1N1-related hospitalisation are scanty and varying.

Methods

We conducted a matched case-control study in individuals with an indication for vaccination because of underlying medical conditions and/or age ≥60 years in the Netherlands. Cases were patients hospitalised with laboratory-confirmed influenza A(H1N1)pdm09 infection between November 16, 2009 and January 15, 2010. Controls were matched to cases on age, sex and type of underlying medical condition(s) and drawn from an extensive general practitioner network. Conditional logistic regression was used to estimate the vaccine effectiveness (VE=1–OR). Different sensitivity analyses were used to assess confounding by severity and the effect of different assumptions for missing dates of vaccination (35 of 68 vaccinees; 51%).

Results

One hundred and forty-nine cases and 28,238 matched controls were included. It was estimated that 22% of the cases and 28% of the controls received vaccination more than 7 days before the index date (symptom onset in cases). A significant number of breakthrough infections were observed. The VE was estimated at 19% (95%CI: -28-49). After restricting the analysis to cases with controls suffering from severe underlying medical conditions, the VE was 49% (95%CI: 16-69).

Conclusions

The number of breakthrough infections, resulting in modest VE estimates, suggests that the MF-59TM adjuvanted vaccine may have had only a limited impact on preventing influenza A(H1N1)pdm09-related hospitalisation in this setting. As the main aim of influenza vaccination programmes is to reduce severe influenza-related morbidity and mortality from influenza in persons at high risk of complications, a more effective vaccine, or additional preventive measures, is needed.

Background

Vaccination is the mainstay of preventing and mitigating the impact of influenza in spite of moderate vaccine effectiveness (VE). Lowering the burden of severe disease is one of the main aims of a vaccination program. As influenza can be a precipitating factor for the exacerbation of underlying medical conditions, an influenza vaccination strategy often targets specific risk groups. Unfortunately, VE is generally lower in individuals with a compromised immune system, such as the elderly (1).

Antigen supplies during a pandemic are expected to be limited. For that reason, in 2005 the WHO recommended adjuvanted vaccines in just such scenario (2). Inclusion of an adjuvant enhances immunogenicity of a vaccine(3), thereby reducing the amount of antigen required for equivalent immune responses. During the recent influenza A(H1N1) pandemic, adjuvanted influenza vaccines were used for the first time on a large scale in Europe. Five vaccines were authorised by the European Medicines Agency (EMA) for use in the European Union (4). Clinical trials reported high immunogenicity of the adjuvanted vaccines (5-10); post-marketing studies (11-16) showed an effectiveness on preventing confirmed influenza A(H1N1)pdm09 that was similar to that of seasonal influenza vaccines in well matched years (11, 17). So far, only limited and varying results have been reported on the effectiveness of the adjuvanted vaccines in preventing severe disease of influenza A(H1N1)pdm09 that required hospitalisation(13, 16, 18).

VE estimates with severe outcomes are important for guiding decisions on recommendations for complementary or alternative public health measures, and for communication to and preparedness of health care and the society. Studies estimating VE in specific risk groups are important, especially where new vaccines are used, as those groups are generally not included in clinical trials. Until now, none of the published studies on the effectiveness of the vaccines in preventing influenza A(H1N1)pdm09-related hospitalisation(13, 16, 18) focussed on a MF-59™ -adjuvanted vaccine. We investigated the effectiveness of a MF-59™-adjuvanted vaccine(19) in preventing influenza A(H1N1)pdm09-related hospitalisation in individuals with an indication for vaccination due to underlying medical conditions and/or age ≥60 years in the Netherlands. Such a study was enabled by the mandatory notification of influenza A(H1N1)pdm09 cases requiring hospitalisation. We combined the notification data with an extensive general practitioner (GP) network in the Netherlands (Integrated Primary Care Information (IPCI) database) (20, 21) employing a matched case-control design.

Methods

Study design and setting

We conducted a matched case-control study. As influenza A(H1N1)pdm09-related hospitalisation was notifiable, case-data were obtained through routine surveillance(22, 23). In the Netherlands, all suspected influenza A(H1N1)pdm09 hospitalised patients were swabbed and tested for influenza A(H1N1)pdm09 infection. The instructions were to perform a nose and a throat swab combined in one transport medium. Laboratory confirmation was done by real-time PCR for influenza virus type A and type A(H1N1) (24). After laboratory confirmation, the attending physician and the laboratory had the legal requirement to contact the Municipal Health Service. The Municipal Health Service notified the case by entering the reported data into the national password-secured web-based routine surveillance database. Reported data included information on underlying medical conditions (at aggregated level) and self-reported vaccination status for the seasonal and pandemic vaccines. Missing data were retrieved through the hospital physicians; before discharge the hospital physician would ask the patient directly, while after discharge, GPs were contacted in case the GP of the patient was known. Control-data were available anonymously in the IPCI database. Details about the database have been reported elsewhere (20). In short, the IPCI database is a longitudinal GP research database and contains electronic patient records of about 500 GPs from all over the Netherlands. This includes prescription data and specialists' letters. Currently there are over 750,000 active patients, representing approximately 5% of the Dutch population. As in the Netherlands, nearly all people are registered with a GP the patient population is representative of the Dutch population regarding sex and age, except for a slight under representation of the elderly population that is under care of medical practitioners in nursing homes.

The IPCI database complies with European Union guidelines on the use of medical data for medical research. The Scientific and Ethical Advisory Board of the IPCI project approved the study. Informed consent was not required.

Vaccination programme

In the Netherlands, different groups were eligible for a pandemic influenza vaccination in 2009: those with specified underlying medical conditions (pulmonary disease, cardiac disease, diabetes mellitus, chronic kidney failure, cancer, immunocomprised condition), pregnant women in their second and third trimester, institutionalised individuals, individuals aged ≥ 60 years, children aged 6 months to 4 years, health care workers with potential for direct patient contact, family members and caretakers of individuals with high risk for severe disease or death, and household members of children younger than 6 months.

The MF-59™ adjuvanted vaccine (19) was provided to individuals with underlying medical conditions and/or aged ≥60 years through the GP. Vaccination started in week 45 (November 2, 2009), though the majority of the GPs (99% of our control sample) provided

vaccination from week 46 (November 9, 2009) onwards. All persons were offered two doses, two weeks apart.

Study population

Cases were patients who had been hospitalised because of a laboratory confirmed influenza A(H1N1)pdm09 infection. Only cases with day of symptom-onset between November 16, 2009 and January 15, 2010 and with data on vaccination history were included (Figure 3.1). Pregnant women were not included in the analysis. The date of symptom onset was used as the index date.

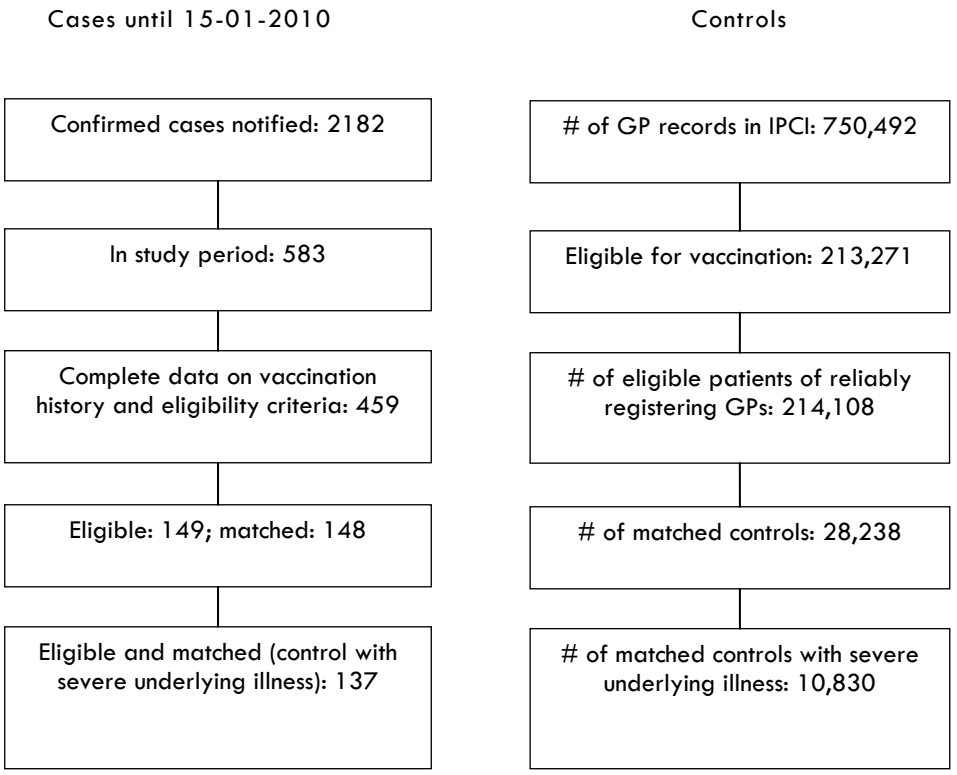


Figure 3.1 Flow diagram of cases and controls.

Patients from the IPCI database were eligible as controls when they had at least 1 year of valid database history available, they were eligible for vaccination by the GP due to underlying medical conditions and/or advanced age, and were registered with a GP that had consistent and complete registration of vaccinations (58%; see Figure 3.1). Underlying medical conditions were extracted from the IPCI database using International Classification of Primary Care (ICPC) codes as well as free text terms and were aggregated as pulmonary disease, cardiac disease, diabetes mellitus, chronic kidney failure, cancer and immunocompromised condition to equalise the level of information to the cases. Information on vaccination status and date of vaccination were extracted using algorithms based on ICPC code and open text fields including brands and batch-code. Consistent registration of vaccinations was defined as a coverage of the seasonal influenza vaccination, 1st dose of pandemic influenza vaccination and 2nd dose of pandemic vaccine of ≥50% in the GP practice population aged ≥60 years. This cut off was based on the national vaccination coverage estimate of the population aged ≥60 years in 2009 (respectively 76%, 77% and 69% (25)). From the selected eligible population, we sampled all possible controls matching a case on age (+/- 12 months), sex, underlying medical conditions (all conditions at aggregated level) and calendar date (e.g. were alive and present in the database for at least 1 year at the index date). One case did not have a matched control. Women with known confirmed pregnancy (ICPC codes; n=43) and individuals that had been hospitalised with confirmed or suspected influenza A(H1N1)pdm09 infection before the index date (open text fields; n=17) were excluded.

Exposure definition

We defined exposure (valid vaccination) using two different cut offs; having received at least one pandemic influenza vaccination more than 7 days (11, 12, 15, 16) or more than 14 days before the index date. If the exact date of vaccination was unavailable, we extrapolated the validity of vaccination from vaccinated cases with a known date of vaccination. We assumed that the availability of the date of vaccination was independent of the day of disease onset (see Figure 3.2). Therefore, we considered the percentage of invalid vaccinations among those with unknown vaccination date similar to the percentage of invalid vaccinations among vaccinated cases with known vaccination date. Furthermore, we assumed recent vaccination (i.e. invalid vaccination) to be more likely in the beginning of November. We calculated the percentage of invalid vaccinations among vaccinated cases with known vaccination date and determined the date on which such percentage of the vaccinees with unknown vaccination date had fell ill. For the cut off of respectively >7 and >14 days, we considered the pandemic influenza vaccination as valid if the date of symptom onset was on or after November 24, 2009 or November 28, 2009.

Data analysis

Vaccinees without available date of vaccination and symptom onset before the used cut off for validity were compared to those with symptom onset on or after the used cut off on age using a Wilcoxon-Mann-Whitney test, and on sex and underlying medical conditions using a Chi square test,

Vaccine effectiveness was computed as VE=1– odds ratio (OR) (26), with an exact 95% confidence interval (CI) around the point estimate. We used conditional logistic regression to calculate the OR. We used the VE estimate with the assumption on the validity of the vaccination based on the vaccinated cases with available date of vaccination (see exposure definition) as our primary analysis. Additionally, we conducted a sensitivity analysis using imputed delays between disease onset and date of vaccination for those with unknown date of vaccination. We used multiple imputation (n=10) and sampled from a uniform distribution with a lower bound of a delay of zero days and an upper bound of a delay of the sum of the number of days since the start of the study and 7 days. This upper bound is based on the start of the vaccination campaign relative to the start of this study. The overall estimates and confidence intervals were determined using the method described by Rubin (27). Additionally, we considered all vaccinations of vaccinated cases with unknown vaccination date to be invalid to yield the maximum VE.

Although we matched for underlying medical conditions, we were not able to match on the severity of these underlying medical conditions, as no such information was available for our cases. It could be reasoned that individuals hospitalised with influenza A(H1N1)pdm09 suffer from more severe underlying medical conditions than community controls with underlying medical conditions. Therefore, we performed a post hoc sensitivity analysis in which controls were sampled from the pool of controls who received five or more different active drug compounds prescribed in the year prior (above median number) and matched on the same criteria as described above. The number of active drug compounds was therefore considered as a proxy of disease severity.

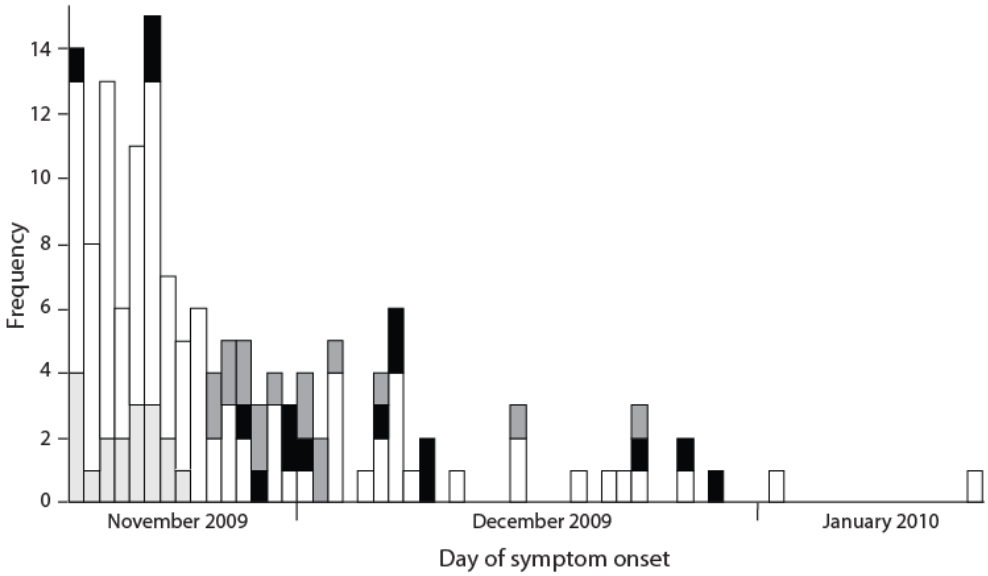
Data analysis was performed in SAS 9.2 (SAS Institute Inc. Cary, NC, USA).

Results

Sample characteristics

The 2009 influenza epidemic in the Netherlands started in week 41, peaked in week 46 with 190 cases per 100,000 inhabitants and ended in week 50 (28). Five-hundred eighty-three laboratory-confirmed influenza A(H1N1)pdm09 patients were hospitalised during the study period. From these, 459 (79%) had complete data on matching factors and history of vaccination. Only 149 were eligible for inclusion, as the majority of cases were not eligible

for pandemic influenza vaccination because of age (<6 months; 19%) and/or absence of an underlying medical condition (74%). The majority of the included cases fell ill in November 2009 (Figure 3.2), which coincided with the peak of the Dutch influenza epidemic (28).



* For 1 patient, day of symptom onset was unknown. For this patient we used 1 day before day of admission as day of symptom onset for this epidemic curve.

Figure 3.2 Epidemiological curve of included cases by exposure.

Cases whose vaccination was valid (black), or assumed valid (dark grey), and whose vaccination was assumed invalid (light grey) or who did not obtain a (valid) vaccination (white) are presented by day of symptom onset. The assumption on exposure was based on cases with known date of vaccination.

The median age of the included cases was 48 years (range 1-84), and 44% were male. Only 2% (n=3) of the included cases was previously healthy (aged ≥60 years; of which two had obtained vaccination that was assumed valid); all others suffered from underlying medical conditions. Seven included cases died from influenza A(H1N1)pdm09.

Forty-six percent (n=68) of the cases reported to have obtained at least one pandemic influenza vaccination (Table 3.1). Among the vaccinated cases, exact date of vaccination was available for only 49% (n=33). Vaccinees with or without available date of vaccination did not differ statistically on age (respectively 43 and 46 years old), sex (48% and 34% male) or the presence of underlying medical conditions (0/33 and 2/35). Furthermore, vaccinees without available date of vaccination and symptom onset before the used cut off

for validity of vaccination did not differ statistically from those with symptom onset on or after the used cut off on age (respectively 51 and 57 years old using the cut off of 7 days or 52 and 61 years for the cut off of 14 days), sex (22% and 47% male (7 days) or 29% and 45% male (14 days)), or the presence of underlying medical conditions (100% and 88% (7 days) or 100% and 82% (14 days)). Respectively 48% and 33% of the vaccinated cases with exact date of vaccination available had obtained their vaccination more than 7 or 14 days before symptom onset (Table 3.1). Based on the extrapolation from available dates of vaccination, we assumed that 22% of the cases had received vaccination more than 7 days before the index date and 15% more than 14 days before the index date. Of the seven fatal cases, four had obtained pandemic vaccination. For only one, date of vaccination was present. This case had obtained the vaccine five days before symptom onset which was therefore considered as an invalid vaccination on the index date. For two fatal cases (29%) pandemic influenza vaccination was assumed to be valid (disease onset at November 29, 2009 and December 15th, 2009).

Table 3.1 Vaccination history and completeness of data in cases, controls and controls with severe medical conditions.

	Used cut off	Cases	Controls	Controls with severe medical conditions
Crude vaccination coverage		46%	46%	53%
		(68/149)	(13012/28238)	(5752/10830)
Known vaccination date in vaccinees		49%	100%	100%
		(33/68)	(13012/13012)	(5752/5752)
Valid vaccination in vaccinees with known vaccination date	>7 days	48%	60%	58%
	> 14 days	(16/33)	(7798/13012)	(3320/5752)
		33%	19%	27%
		(11/33)	(3715/13012)	(1551/5752)
Assumed valid vaccination in vaccinees with unknown vaccination date	>7 days	49%	NA	NA
	> 14 days	(17/35)	NA	NA
		31%		
		(11/35)		
(Assumed) valid vaccination coverage	>7 days	22%	28%	31%
	> 14 days	(16+17/149)	(7798/28238)	(3320/10830)
		15%	13%	14%
		(11+11/149)	(3715/28238)	(1551/10830)

* NA = not applicable as for all controls, date of vaccination was available.

In controls, 46% had obtained at least one dose of pandemic influenza vaccination at the index date, of which 60% more than 7 days before the index date and 19% more than 14

days before the index date (Table 3.1). This resulted in a valid vaccination coverage of respectively 28% and 13% in controls. Because of the matching in which each stratum contains a single case, no direct comparison of vaccination coverage between cases and controls is possible.

Vaccine effectiveness

A considerable number of breakthrough infections was observed (Figure 3.2). The estimated VE was respectively 19% (95%CI: -28% - 49%) or < 0% (upper C.I. 30) when using a cut off of 7 or 14 days between date of vaccination and onset of disease (Table 3.2). The sensitivity analysis using imputed data yielded a VE of respectively 24% (95%CI: -139% - 76%) and 26% (95%CI: -188% - 81%). The maximum VE (all vaccinations of cases with unknown date of vaccination were assumed invalid) was estimated at respectively 74% (95%CI: 53% - 86%) or 61% (95%CI: 19% - 82%).

Table 3.2 Effectiveness of the pandemic influenza vaccine in preventing 2009 influenza A/H1N1-related hospitalisation

	VE (%) (95%CI)	
	Exposure valid > 7 days between vaccination and disease onset	Exposure valid > 14 days between vaccination and disease onset
VE*	19 (-28 - 49)	< 0 (upper C.I. 30)
VE imputed data$	24 (-139 - 76)	26 (-188 - 81)
Restricted VE	49 (16 - 69)	35 (-26 - 66)
Restr. VE imputed$	51 (-59 - 85)	59 (-78 - 91)
Maximum VE#	74 (53 - 86)	61 (19 - 82)

* For 36 cases vaccination date was missing. Respectively 48% of the vaccinated cases with unknown vaccination date were assumed to be exposed using 7 days or 14 days as cut off for the validity of vaccination (extrapolation from cases with known date of vaccination).
$ We used multiple imputation and sampled (n=10) from a uniform distribution with a lower bound of delay = 0 and an upper bound of delay = number of days since the start of the study + 7. This upper bound is based on the start of the vaccination campaign relative to the start of this study.
# For the maximum VE, vaccinations of all cases with unknown vaccination date were assumed invalid.

Sensitivity analysis restricted to controls with severe underlying medical conditions

After restricting our analysis to controls who were prescribed at least five different types of medications (proxy for more severe underlying medical conditions), the control population included 10830 individuals (38% of original control sample; see Figure 3.1). The number of different prescriptions in the restricted population of controls ranged from 5 to 52, with a median of 7 prescriptions. Thirty-one percent of this selection of controls had obtained vaccination more than 7 days before the index date and 14% more than 14 days before the index date (Table 3.1), which resulted in a restricted VE of respectively 49% (95%CI: 16% - 69%) or 35% (95%CI: -26% - 66%; Table 3.2). The sensitivity analysis using

imputed data yielded a restricted VE of respectively 51% (95%CI: -59% - 85%) and 59% (95%CI: -78% - 91%). The maximum VE using the restricted control population was estimated at respectively 84% (95%CI: 69% - 92%) or 81% (95%CI: 53% - 92%).

Discussion

This matched case-control study showed a considerable number of breakthrough infections, resulting in modest VE estimates. These results suggest that the MF-59™ adjuvanted vaccine may have had only a limited impact in preventing influenza A(H1N1)pdm09-related hospitalisation in risk groups. Because pandemic vaccination started around the peak of the influenza A(H1N1)pdm09 epidemic in the Netherlands, missing date of vaccination in hospitalised cases is a severe limitation to this study. Applying different scenarios partly overcame this limitation and provided a range of VE estimates, though residual confounding by time cannot be excluded.

As pandemics occur unexpectedly, and during pandemics available resources are heavily stretched, ideally routinely collected data should be used to provide estimates of VE against severe outcomes. We showed that using such data for VE estimates is feasible. However, observational studies to estimate the effectiveness of influenza vaccination are prone to bias (29-33). Our study is not immune to such potential bias and due to restricted available data we had only limited possibilities to adjust for potential confounding. The use of different types of routinely collected health care data and the consequent differences in the quality and level of information between cases and matched controls is a limitation of this study. In cases, vaccination status was self-reported whilst in controls vaccination was reported by the GP. GP registered data can be incomplete because of unreliable registration and because vaccination was also offered outside GP practices for certain individuals (i.e. those working in healthcare, those with children under the age of 6 months, children under the age of 5). By including controls who were eligible for vaccination by the GP and who were sampled from reliably-registering GPs we aimed to minimise potential underestimation of the vaccination coverage in controls. Recall bias in cases will have had only limited impact because of the short delay between the vaccination campaign and disease onset, and because of the substantial attention of the general population to the pandemic influenza vaccine.

Frailty selection, resulting in confounding by severity, can be important in VE studies focussing on severe disease or hospitalisation as outcome (30, 31). Because the majority of the study population was relatively young (median age 48 years), and suffered from one kind of underlying medical condition, frailty is likely to have been of lesser importance relative to studies on seasonal influenza. Moreover, by matching our cases with controls on underlying medical conditions, we decreased the probability of confounding. However, because our cases could have been suffering from more severe underlying medical conditions than our matched controls, but without preventing them to obtain the vaccine, we

performed a sensitivity analysis *post hoc* using controls with more severe underlying conditions. Using this restriction, the VE was estimated to be 35% (95%CI: -26% - 66%) or up to 59% (95%CI: -78% - 91%) depending on the cut off and method used to define validity of vaccination. However, it is known that several of our cases did not use any medication, suggesting only mild underlying medical conditions in those cases. These restricted VE estimates are therefore likely an overestimation of the actual VE.

The estimates of the effectiveness in preventing influenza A(H1N1)pdm09 related hospitalisation of the adjuvanted pandemic influenza vaccines used in Europe ranged from 45% (95%CI: 3% - 69%; UK using a ASO3 adjuvanted vaccine (16)) to 90% (95%CI: 48% - 100%; Spain using several vaccines (18)) or even 100% (95%CI: ∞ - 100%; Scotland (13)). Even our maximum VE estimate was not as high as the Spanish and Scottish estimates. To determine the maximum VE, we assumed for all 35 cases with unknown date of vaccination (51% of all vaccinees) that vaccination took place within 7 or 14 days of symptom onset. These are unlikely realistic assumptions and therefore the maximum VE estimates are likely overestimated. A possible explanation for the wide range of VE estimates in Europe is the difference in inclusion criteria, the control group and the used vaccine. In Spain (18), the vaccine was distributed to the usual influenza risk groups (including those with obesity), but all hospitalised patients were included in the study. The UK study (16) and our Dutch study focussed on the population most at risk for severe outcome of an influenza infection, and therefore only included individuals eligible for vaccination because of an underlying medical condition or advanced age. It is known that individuals with certain types of underlying medical condition or older age have a reduced response to vaccination (34, 35). A lower VE is therefore expected in this susceptible population. Furthermore, earlier published studies on VE against hospitalisation used the test-negative case-control design (16, 18). This design is susceptible to imperfect specificity and sensitivity of diagnoses and the vaccine coverage in test-negative hospital cases is possibly not representative for the general population. In addition, differential health care seeking behaviour between test-positives and test-negatives could result in biased estimates. As we used national data of notifications and data of an extensive GP network which are representative for the country, we expect that our cases and controls originate from the same, general population. However, our data have limited possibilities to refute the presence of potential bias which may have led to an underestimation of the VE.

The pandemic influenza vaccine used (19) contained half the amount of antigen relative to seasonal influenza vaccines plus an adjuvant to increase the immunogenicity. It is therefore not possible to make a direct comparison to seasonal influenza vaccines. However, the effectiveness of seasonal influenza vaccines in preventing influenza-related hospitalisations is also under debate. For seasonal influenza vaccination, a low VE estimate against hospitalisation (9-12%) was observed in those aged >50 years using a difference-in-

differences design (36). Additionally, a Cochrane review concluded that seasonal influenza vaccination had no effect on hospital admissions or complication rates (17). Taking into account the differences in study design and vaccines used, the effectiveness of influenza vaccines to prevent influenza-related hospitalisation appears lower than the effectiveness in preventing clinical disease (11-16). The fact that those most at risk of complications and hospitalisations due to influenza react less favourably to the vaccine may have contributed to this difference. Adding the adjuvant to the vaccine did not overcome this problem in our population.

Conclusion

In conclusion, the number of breakthrough infections resulting in a modest VE estimates suggests that the MF59™ adjuvanted vaccine may have had only a limited impact on preventing influenza A(H1N1)pdm09-related hospitalisation in this setting. As the main aim of influenza vaccination programmes is to reduce severe influenza-related morbidity and mortality from influenza in individuals at high risk of complications a more effective vaccine or additional preventive measures are needed. Furthermore, efforts should be made to put better real-time monitoring systems in place to study the effectiveness of influenza vaccines in preventing severe laboratory-confirmed influenza.

Acknowledgements

The authors thank Susan Hahné, Jane Whelan and Katie Greenland for their input and Andy Cherry for advice on use of English. We acknowledge Jan van de Kassteele for his help on imputation. Furthermore, we acknowledge all municipal health services and hospitals for providing data, and all general practitioners of the IPCI network for their participation.

References

1. Jefferson T, Di Pietrantonj C, Al Ansary LA, Ferroni E, Thorning S, Thomas RE. Vaccines for preventing influenza in the elderly. Cochrane Database Syst Rev 2010;CD004876.

2. World Health Organisation. Influenza preparedness and response. Report by the Secretariat. 2005.

3. Puig-Barbera J, Gonzalez VD. MF59-adjuvanted subunit influenza vaccine: an improved interpandemic influenza vaccine for vulnerable populations. Expert Rev Vaccines. 2007;6(659-665).

4. European Medicines Agency. Vaccines for pandemic influenza [cited 2015 07-02-2015]. Available from: www.ema.europa.eu/ema/index.jsp?curl=pages/special_topics/general/general_content_000462.jsp&mid=WC0b01ac058004b9ac.

5. Liang X-F, Wang H-Q, Wang J-Z, Fang H-H, Wu J, Zhu F-C, et al. Safety and immunogenicity of 2009 pandemic influenza A H1N1 vaccines in China: a multicentre, double-blind, randomised, placebo-controlled trial. The Lancet. 2010;375(9708):56-66.

6. Vajo Z, Tamas F, Sinka L, Jankovics I. Safety and immunogenicity of a 2009 pandemic influenza A H1N1 vaccine when administered alone or simultaneously with the seasonal influenza vaccine for the 2009-10 influenza season: a multicentre, randomised controlled trial. Lancet. 2010 Jan 2;375(9708):49-55. PubMed PMID: 20018367. Epub 2009/12/19. eng.

7. Plennevaux E, Sheldon E, Blatter M, Reeves-Hoché M-K, Denis M. Immune response after a single vaccination against 2009 influenza A H1N1 in USA: a preliminary report of two randomised controlled phase 2 trials. The Lancet. 2010;375(9708):41-8.

8. Greenberg ME, Lai MH, Hartel GF, Wichems CH, Gittleson C, Bennet J, et al. Response to a monovalent 2009 influenza A (H1N1) vaccine. The New England journal of medicine. 2009;361:2405-13.

9. Clark TW, Pareek M, Hoschler K, Dillon H, Nicholson KG, Groth N, et al. Trial of 2009 influenza A (H1N1) monovalent MF59-adjuvanted vaccine. The New England journal of medicine. 2009;361:2424-35.

10. Gasparini R, Schioppa F, Lattanzi M, Barone M, Casula D, Pellegrini M, et al. Impact of prior or concomitant seasonal influenza vaccination on MF59-adjuvanted H1N1v vaccine (Focetria) in adult and elderly subjects. Int J Clin Pract. 2010;64:432-8.

11. Valenciano M, Kissling E, Cohen JM, Oroszi B, Barret AS, Rizzo C, et al. Estimates of pandemic influenza vaccine effectiveness in Europe, 2009-2010: results of Influenza Monitoring Vaccine Effectiveness in Europe (I-MOVE) multicentre case-control study. PLoS Med. 2011 Jan;8(1):e1000388. PubMed PMID: 21379316. Pubmed Central PMCID: 3019108.

12. Wichmann O, Stocker P, Poggensee G, Altmann D, Walter D, Hellenbrand W, et al. Pandemic influenza A(H1N1) 2009 breakthrough infections and estimates of vaccine effectiveness in Germany 2009-2010. Euro Surveill. 2010;15(18):pii: 19561.

13. Simpson CR, Ritchie LD, Robertson C, Sheikh A, McMenamin J. Vaccine effectiveness in pandemic influenza - primary care reporting (VIPER): an observational study to assess the effectiveness of the pandemic influenza A (H1N1)v vaccine. Health Technol Assess. 2010;14(34):313-46.

14. Nicholson KG, Abrams KR, Batham S, Clark TW, Hoschler K, Lim WS, et al. Immunogenicity and safety of a two-dose schedule of whole-virion and AS03(A)-adjuvanted 2009 influenza A (H1N1) vaccines: a randomised, multicentre, age-stratified, head-to-head trial. . The Lancet infectious diseases. 2010;11(2):91-101.

15. Hardelid P, Fleming DM, McMenamin J, Andrews NJ, Robertson C, SebastianPillai P, et al. Effectiveness of pandemic and seasonal influenza vaccine in preventing pandemic influenza A(H1N1)2009 infection in England and Scotland 2009-2010. Euro Surveill 2011;16:pii=19763.

16. Andrews NJ, Waight P, Yung CF, Miller E. Age-specific effectiveness of an oil-in-water adjuvanted pandemic (H1N1) 2009 vaccine against confirmed infection in high risk groups in England. J Infect Dis. 2011;203(1):32-9.

17. Jefferson T, Di Pietrantonj C, Rivetti A, Bawazeer GA, Al Ansary LA, Ferroni E. Vaccines for preventing influenza in healthy adults. Cochrane Database Syst Rev 2010;7(CD001269).

18. Puig-Barberà J, Arnedo-Pena A, Pardo-Serrano F, Tirado-Balaguer MD, Pérez-Vilar S, Silvestre-Silvestre E, et al. Effectiveness of seasonal 2008-2009, 2009-2010 and pandemic vaccines, to prevent influenza hospitalizations during the autumn 2009 influenza pandemic wave in Castellón, Spain. A test-negative, hospital-based, case-control study. Vaccine. 2010;28(47):7460-7.

19. European Medicines Agency. Summary of Product Characteristics for Focetria, Influenza vaccine H1N1v (surface antigen, inactivated, adjuvanted). Last update on www.ema.europa.eu on 05/10/2010. . In: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000710/WC500023749.pdf, editor. 04-01-2011.

20. Vlug A, van der Lei J, Mosseveld B, van Wijk M, van der Linden P, Sturkenboom M, et al. Postmarketing surveillance based on electronic patient records: the IPCI project. Methods Inf Med. 1999;38(4-5):339-44.

21. Voordouw BCG, Sturkenboom MCJM, Dieleman JP, Stijnen T, van der Lei J, Stricker BHC. Annual Influenza Vaccination in Community-Dwelling Elderly Individuals and the Risk of Lower Respiratory Tract Infections or Pneumonia. Arch Intern Med. 2006 October 9, 2006;166(18):1980-5.

22. Hahné S, Donker T, Meijer A, Timen A, van Steenbergen J, Osterhaus A, et al. Epidemiology and control of influenza A(H1N1)v in the Netherlands: the first 115 cases. . Eurosurveillance. 2009;14:pii=19267-pii=71.

23. van 't Klooster TM, Wielders CC, Donker T, Isken L, Meijer A, van den Wijngaard CC, et al. Surveillance of hospitalisations for 2009 pandemic influenza A(H1N1) in the Netherlands, 5 June - 31 December 2009. Eurosurveillance. 2010;15:pii=19461.

24. Meijer A, Beerens A, Claas E, Hermans M, de Jong A, Molenkamp R, et al. Preparing the outbreak assistance laboratory network in the Netherlands for the detection of the influenza virus A(H1N1) variant. . J Clin Virol 2009;45:179-84.

25. Tacken M, Mulder J, Visscher S, Tiersma W, Donkers J, Verheij R, et al. Monitoring vaccinatiegraad nationaal programma griepppreventie 2009. [In Dutch]. 2010.

26. Orenstein WA, Bernier RH, Dondero TJ, Hinman AR, Marks JS, Bart KJ, et al. Field evaluation of vaccine efficacy. . Bull World Health Organ 1985;63:1055-68.

27. Rubin DB. Multiple Imputation for Nonresponse in Surveys. New York1987.

28. Donker GA. Continuous Morbidity Registration at Dutch Sentinel General Practice Network 2009. [In Dutch] 2010.

29. Orenstein EW, De Serres G, Haber MJ, Shay DK, Bridges CB, Gargiullo P, et al. Methodologic issues regarding the use of three observational study designs to assess influenza vaccine effectiveness. . Int J Epidemiol. 2007;36:623-31.

30. Simonsen L, Taylor RJ, Viboud C, Miller MA, Jackson LA. Mortality benefits of influenza vaccination in elderly people: an ongoing controversy. The Lancet infectious diseases. 2007;7:658-66.

31. Baxter R, Lee J, Fireman B. Evidence of bias in studies of influenza vaccine effectiveness in elderly patients. J Infect Dis. 2010;201:186-9.

32. Simonsen L. Commentary: Observational studies and the art of accurately measuring influenza vaccine benefits. Int J Epidemiol. 2007;36:631-2.

33. Hak E, Verheij TJ, Grobbee DE, Nichol KL, Hoes AW. Confounding by indication in non-experimental evaluation of vaccine effectiveness: the example of prevention of influenza complications. J Epidemiol Community Health. 2002 Dec;56(12):951-5. PubMed PMID: 12461118.

34. Cheong HJ, Song JY, Heo JY, Noh JY, W.S. C, Park DW, et al. Immunogenicity and safety of influenza A (H1N1) 2009 monovalent inactivated split vaccine in Korea. Vaccine. 2011;29(3):523-7.

35. Govaert TM, Thijs CT, Masurel N, Sprenger MJ, Dinant GJ, Knottnerus JA. The efficacy of influenza vaccination in elderly individuals. A randomized double-blind placebo-controlled trial. JAMA : the journal of the American Medical Association. 1994;272(21):1661-5. PubMed PMID: 7966893.

36. Baxter R, Ray GT, Fireman BH. Effect of influenza vaccination on hospitalizations in persons aged 50 years and older. Vaccine. 2010;28:7267-72.

Chapter 4. Safety of pandemic H1N1 vaccines in children and adolescents

Leonoor Wijnans

Sandra de Bie

Jeanne Dieleman

Jan Bonhoeffer

Miriam Sturkenboom

Published in: Vaccine 29 (2011) 7559– 7571

Abstract

During the 2009 influenza A (H1N1) pandemic several pandemic H1N1 vaccines were licensed using fast track procedures, with relatively limited data on the safety in children and adolescents. Different extensive safety monitoring efforts were put in place to ensure timely detection of adverse events following immunization. These combined efforts have generated large amounts of data on the safety of the different pandemic H1N1 vaccines, also in children and adolescents. In this review we summarize the safety experience with seasonal influenza vaccines as a background and focus on the clinical and post marketing safety data of the pandemic H1N1 vaccines in children.

We identified 25 different clinical studies including 10,505 children and adolescents, both healthy and with underlying medical conditions, between the ages of 6 months and 23 years. In addition, large monitoring efforts have resulted in large amounts of data, with almost 13,000 individual case reports in children and adolescents to the WHO. However, the diversity in methods and data presentation in clinical study publications and publications of spontaneous reports hampered the analysis of safety of the different vaccines.

As a result, relatively little has been learned on the comparative safety of these pandemic H1N1 vaccines – particularly in children. It should be a collective effort to give added value to the enormous work going into the individual studies by adhering to available guidelines for the collection, analysis, and presentation of vaccine safety data in clinical studies and to guidance for the clinical investigation of medicinal products in the paediatric population. Importantly the pandemic has brought us the beginning of an infrastructure for collaborative vaccine safety studies in the EU, USA and globally.

Introduction

In the course of the 2009 influenza A (H1N1) pandemic, different pandemic H1N1 vaccines were made available to children and adolescents across the world at an unprecedented scale and speed. Several pandemic H1N1 vaccines were developed to mitigate the impact of the pandemic. All were based on the same isolate influenza A/California/7/2009 (H1N1)v. However, vaccines differed in the methods for virus propagation, purification, and inactivation, as well as antigen preparation, the amount of antigen in the vaccine, the presence and type of adjuvants, and the presence of other excipients. Most pandemic H1N1 vaccines were found to elicit a sufficient immune response after one dose for (healthy) persons aged 10 years and above. For children between 6 months and three years of age a second dose was recommended, and for some vaccines a second dose was also recommended for children between three and nine years of age (1). Following official recommendations from the World Health Organization (WHO)(2), Centers for Disease Control (CDC) (3), the European Union (4) and national health authorities, children in most countries were amongst the target groups of pandemic H1N1 vaccination campaigns. In some countries only children with underlying co-morbidities were targeted, in other countries also healthy children with or without age restrictions were vaccinated (5).

Adjuvanted and non-adjuvanted monovalent pandemic H1N1 vaccines had been licensed in 2009 through fast track procedures in order to ensure availability (6, 7). Due to this fast track authorization process, only limited safety data was available prior to wide spread distribution. The non-adjuvanted H1N1 vaccines were expected to have a similar safety profile to the well-established non-adjuvanted seasonal influenza vaccines for all age groups (8, 9). However in many countries in Europe and in Canada pandemic H1N1 vaccines with oil-in-water adjuvants (AS03, MF59) were used, also in children. At the time vaccination campaigns started, clinical data on these pandemic H1N1 vaccines in children was very limited. The safety profile for these vaccines was mostly based on non-clinical testing and on data derived from clinical studies with avian influenza mock-up vaccines, in addition to experience with an MF59 adjuvanted vaccine for over a decade in elderly in Italy (6, 10, 11).

Due to the scale and scope of the pandemic H1N1 vaccination campaigns and the paucity of safety data, a stringent risk management plan including the capacity for early detection of adverse events was essential. Extensive monitoring of the safety of the vaccines was put in place through boosting of existing national and international passive surveillance systems (e.g. EudraVigilance, World Health Organization-Uppsala Monitoring Centre (WHO-UMC), U.S. Vaccine Adverse Events Reporting System (VAERS), Canadian Adverse Events Following Immunization Surveillance System (CAEFISS) and Immunization Monitoring Program ACTive (IMPACT), and the Australian Adverse Drug Reactions System (ADRS)) and through new active surveillance activities in the USA and European Union (12, 13). Adverse events of

special interest (AESIs), listed by the Committee for Human Medicinal Products (CHMP) in Europe and the Food and Drug Administration (FDA), included neuritis, convulsions, anaphylaxis, encephalitis, vasculitis, Guillain-Barré syndrome (GBS), Bell's palsy, demyelinating disorders, and laboratory-confirmed vaccination failure. In the USA, the FDA and Centers for Disease Control and Prevention (CDC) conducted active surveillance for the 2009 pandemic H1N1 vaccines through the newly established Post-Licensure Rapid Immunization Safety Monitoring (PRISM) project and the existing Vaccine Safety Datalink (VSD) (13). Under the auspices of WHO, a global study was initiated on the association between pandemic H1N1 vaccines and GBS. The European Centre for Disease Prevention and Control (ECDC) funded the Vaccine Adverse Event Surveillance & Communication (VAESCO) project to investigate the background rates of AESIs and the association between pandemic H1N1 vaccines and GBS in European countries (14).

The different passive and active surveillance efforts generated large amounts of information on the safety of the pandemic H1N1 vaccines, including in children. In this overview we summarize the safety experience with seasonal influenza vaccines as a background and focus on the clinical and post marketing safety data of the pandemic H1N1 vaccines in children. Although the monovalent pandemic H1N1 vaccines are not expected to be used again on a large scale any time soon, the safety of these vaccines may have wider implications for the use of (adjuvanted) influenza vaccines in children, and lessons can be learned for future safety monitoring efforts in mass vaccination campaigns.

To find data on the safety of pandemic H1N1 vaccines in children we searched PUBMED using the MESH term [influenza vaccines] which was subsequently limited to "All child (0-18 years)" and to articles published in the past three years. Further publications were derived from reference lists of identified articles and unpublished data was sought specifically to identify clinical studies, post marketing studies and case reports with data on the safety of pandemic H1N1 vaccines in children. In addition we searched websites of national health authorities and international health and regulatory organization(15-18).

Background experience with seasonal influenza vaccines in children

Existing evidence on traditional trivalent inactivated vaccines shows that these vaccines are generally well tolerated, with a minority of recipients reporting mild transient systemic reactions such as fever, malaise and myalgia (8, 19-26). Systemic symptoms mostly occur in young children (6 months – 3 years). This may be related to the first exposure to the viral antigens as part of the vaccine (23). In a review of the safety of trivalent inactivated vaccines in children under 2 years from VAERS, the most frequently reported adverse events were fever, rash, injection-site reactions and febrile seizures (27). A signal related to febrile

seizures in young children following trivalent inactivated vaccines as detected in VAERS was not confirmed in further studies (28).

Live attenuated influenza vaccines have been found to be equally safe as inactivated influenza vaccines in children. However, in infants and toddlers under the age of 2 years an increase in wheezing or reactive airway disease occurred in association with live attenuated influenza vaccines. Therefore, the use has been restricted to children over 2 years, and not recommended for children between 2 and 3 years with asthma or with recurrent wheezing (29-33).

Serious adverse reactions following seasonal influenza vaccination are rare (21, 25, 34) and include (febrile) seizures, anaphylaxis, exacerbation or new onset of asthma, GBS, oculo-respiratory syndrome (ORS) and Bell's palsy (25, 27, 35-38).

Safety of pandemic H1N1 vaccines in children: data from clinical studies

The safety data of inactivated influenza vaccines coming from clinical studies focus mostly on solicited and unsolicited local and systemic reactions, as other vaccine related adverse events are uncommon and clinical studies are generally too small to detect rare adverse events. Consequently, this section will mostly concentrate on the reactogenicity of the different vaccine formulations.

We identified 15 publications in peer reviewed journals regarding 13 clinical studies of different pandemic H1N1 vaccines which reported safety data in healthy children (39-54). An overview of these studies is given in Table 4.1.

Table 4.1 Overview of clinical studies evaluating pandemic H1N1 vaccines in children

Authors	Study type	Blinding	Random allocation	Registration	Country	Type of vaccine	Ages	N	Amount of antigen
Zhu et al. 2009 (43)	Placebo controlled	Double	Yes	NCT00975572	China	inactivated, split, AL adjuvanted	3y-17y	550	7.5 µg /15 µg /30 µg
						inactivated, split	3y-17y	440	15 µg / 30 µg
Arguedas et al. 2010 (48, 49)	Parallel intervention	Open	Yes	NCT00973700	Costa Rica	MF59 adjuvanted egg based	3y-17y	108	7.5 µg
						Inactivated subunit	3y-17y	279	15 µg / 30 µg
Carmona et al. 2010 (53)	Parallel intervention	Open	Yes	NCT00971321	Spain	AS03 adjuvanted	6m-35m	104	1.9µg
							6m-35m	53	3.75µg
Liang et al. 2010 (47)	Parallel intervention	Double	Yes	NCT00956111 NCT00975572*	China	inactivated, split	3y-18y	4572	7.5 µg /15 µg /30 µg
						inactivated, split AL adjuvanted	3y-18y	844	7.5 µg /15 µg /30 µg
Lu et al. 2010 (40)	Single intervention	Open	No	none	Taiwan	inactivated, split	1y-17y	180	7.5 µg / 15 µg
Mallory et al. 2010 (54)	Placebo controlled	Double	Yes	NCT00946101	US	Live Attenuated	2-17y	259	10 e7 FFU
Nolan et al. 2010 (44)	Parallel intervention	Single	Yes	NCT00940108	Australia	inactivated, split	6m-9y	369	15 µg / 30 µg
Oh et al. 2010 (39)	Single intervention	Open	No	none	Korea	inactivated, split	6m-18y	248	7.5 µg / 15 µg
Waddington *et al* 2010 (45, 46)	Parallel intervention	Open	Yes	NCT00980850	UK	Whole virion	6m-12y	466	7.5 µg
						AS03 adjuvanted	6m-12y	451	1.9µg
Yasuda et al. 2010 (50)	Parallel intervention	Single	Yes	NCT01000207	Japan	MF59 adjuvanted cell based	6m-19y	120	3.75µg / 7.5 µg
Garcia-Sicilia et al. 2011 (51)	Single intervention	Open	No	NCT00964158	Spain	AS03 adjuvanted	3y-17y	239	1.9µg
Garcia-Sicilia et al. 2011 (51)	Single intervention	Open	No	NCT00972517	Germany	AS03 adjuvanted	3y-17y	202	3.75µg
Plennevaux et al. 2011 (41, 42)	Placebo controlled	Single	Yes	NCT00952419	US	inactivated, split	6m-9y	423	7.5 µg / 15 µg
Scheifele et al. 2011 (52)	Parallel intervention	Single	Yes	NCT01000831	Canada	AS03 adjuvanted	6-35m	167	1.9µg

* of the 10 sites included 2 were registered at clinicaltrials.gov

Monovalent non-adjuvanted inactivated pandemic H1N1 vaccines

In total, nine publications on seven clinical studies were identified that reported data on the safety of monovalent inactivated pandemic H1N1 vaccines in healthy children (39-44, 47-49). These studies were conducted in Australia, China (n=2), Costa Rica, Korea, Taiwan and the United States. Each study evaluated two doses with varying amounts of antigen. In total, the identified studies included 6,511 children between 6 months and 18 years of age who were exposed to inactivated split or subunit pandemic H1N1 vaccines.

In none of the studies vaccine related serious adverse events, deaths or AESIs were reported.

All studies, except Liang et al, recorded events over a period of 7 days following vaccination and all studies examined two doses. There was, however, little consistency between studies regarding event definition and event types recorded. Lu et al. (40) is the only study reporting "nasal congestion" with inactivated vaccines and reported this as the most common systemic reaction in all age groups. Liang et al. considered a body temperature of 37.1°C as fever, whilst other studies reported fever as a body temperature ≥38°C, and Plennevaux et al. only reported fever above ≥39.5°C for children up to two years of age and ≥39.0°C for older children. Moreover, studies were inconsistent in the age categories used to present their results. Because of the methodological differences between studies and the heterogeneity of safety reporting, comparisons of the frequency of reactogenicity to vaccination can only reliably be made within studies.

Two of the identified studies were placebo controlled studies (41, 47). Plennevaux et al. did not detect differences in reactogenicity between the inactivated pandemic H1N1 vaccine and placebo. Conversely, Liang et al. observed increased reactogenicity associated with the vaccine as compared to the placebo. The absolute reactogenicity in this study was lower than in the study of Plennevaux et al. This may possibly be due to the shorter observation period (3 days compared to 7 days) in the study by Liang et al.

Two studies detected higher reactogenicity associated with the first dose (41, 47), three studies did not find noticeable differences between the first and second dose (39, 40, 44) and one study found an increased reactogenicity associated with the second dose (43).

The frequency of events per age group across studies ranged significantly. Local reactions were reported by 27% to 54% of children between 6 months and 3 years, by 15% to 61% of children between 3 and 12 years old and by 13% to 36% of children between 9 and 18 years old, dependent on the study. Systemic reactions following the first dose were reported in 31%-58%, 17%-35% and 16%-58% of children respectively. This may also be due to differences in study methodology and data presentation.

Not all studies reported all local or systemic reactions per age category (43, 44, 47). Zhu et al. (43) evaluated the effect of age on the safety of the vaccine, and found higher systemic reactogenicity for adolescents (12-17 years) as compared to children (3-11 years). However, they did not present numbers or types of reactions per age group. No clear effect of age was seen by Liang et al. (47). Other studies did not compare the reactogenicity between age groups.

None of the studies specifically evaluating the amount of antigen in the vaccine found either an increased or decreased reactogenicity with increasing amounts of antigen per dose (41, 43, 44). The results from Liang et al. do point towards an increase in reactions with antigen dose. Unfortunately this data was not reported separately for children. Lu et al. reported increased pain at injection site associated with the 15µg formulation as compared to the 7.5µg formulation. However, the 7.5µg was given to children at 1 to 2 years of age whilst only older children received the 15 µg formulation and the expression of pain is subject to age specific differences.

Considering the reactogenicity reported in the studies for inactivated non-adjuvanted pandemic H1N1 vaccines, no clear pattern of an age, dose or antigen related effect emerges. Overall, little can be concluded on the available data, besides that no alarming safety issues for the monovalent inactivated pandemic H1N1 vaccines have emerged from clinical studies. The limited comparability of pre-licensure vaccine safety data from published studies is a missed opportunity given that the issue is known, standardized case definitions and guidelines for data collection, analysis, and presentation are available for adverse events following immunization (AEFI) of interest related to influenza vaccines and their use is recommended by regulatory authorities (55-58).

Live attenuated pandemic H1N1 vaccines

One study was identified that evaluated a live attenuated pandemic H1N1 vaccine in children (54). Mallory et al randomized 326 children aged 2 to 17 years to either live attenuated pandemic H1N1 vaccine (n=261) or placebo (n=65), and did not see a significant difference in rates of solicited reactions or adverse events. Less solicited reactions and adverse events occurred following the second dose compared to the first. No vaccine related serious adverse events were reported.

Table 4.2 Results of clinical studies evaluating (oil-in-water) adjuvanted pandemic H1N1 vaccines in children

Reference	Vaccine	Age	N	Antigen content	Solicited local adverse events			Solicited systemic adverse events			
					Total % reported after 1st dose	Pain after 1st dose	Pain after 2nd dose	Total % reported after 1st dose	Fever definition	Fever after first dose	Fever after 2nd dose
MF59 Adjuvanted											
Yasuda et al. 2010 (50)	MF59 adj. cell based	6m-35m	10	3.75µg	NR	-	-	NR	≥38.0°C	10%	30%
	MF59 adj. cell based	6m-35m	11	7.5 µg	NR	-	-	NR	≥38.0°C	18%	18%
	MF59 adj. cell based	3y-19y	50	3.75µg	NR	64%	65%	NR	≥38.0°C	8%	2%
	MF59 adj. cell based	3y-19y	51	7.5 µg	NR	82%	63%	NR	≥38.0°C	10%	6%
Arguedas et al. 2010 (48, 49)	MF59 adj. egg based	3y-8y	55	7.5 µg	25%	21%	15%	24%	≥38.0°C	NR	NR
	Inactivated subunit	3y-8y	84	15 µg	31%	25%	27%	28%	≥38.0°C	NR	NR
	Inactivated subunit	3y-8y	54	30 µg	20%	17%	16%	17%	≥38.0°C	NR	NR
	MF59 adj. egg based	9y-17y	53	7.5 µg	37%	32%	27%	24%	≥38.0°C	NR	NR
	Inactivated subunit	9y-17y	84	15 µg	36%	30%	30%	36%	≥38.0°C	NR	NR
	Inactivated subunit	9y-17y	57	30 µg	29%	25%	26%	16%	≥38.0°C	NR	NR
AS03 Adjuvanted											
Carmona et al. 2010 (53)	AS03 adj.	6m-35m	104	1.9µg	45%	36%	41%	NR	≥37.5°C	20%	67%
		6m-35m	53	3.75µg	50%	NR	NR	NR	≥37.5°C	NR	NR
Garcia-Sicilia et al. 2011 (51)	AS03 adj.	3-5y	59	1.9µg	NR	66%	NR	NR	>37.5°C	NR	NR
		6-9y	63	1.9µg	NR	NR	NR	NR	>37.5°C	NR	NR
		10-17y	117	1.9µg	NR	NR	NR	NR	>37.5°C	NR	NR
		3-5y	53	3.75µg	NR	91%	NR	NR	>37.5°C	26%	50%
		6-9y	56	3.75µg	NR	NR	NR	NR	>37.5°C	NR	NR
		10-17y	93	3.75µg	NR	NR	NR	NR	>37.5°C	NR	NR
Scheifele et al. 2011 (52)	AS03 adj.	6-35m	167	1.9µg	47%	44%	32%	NR	≥38.5°C	4%	9%
Waddington et al. 2010 (45, 46)	AS03 adj.	6m-5y	278	1.9µg	2%¥	31%	39%	5%¥	≥38.0°C	9%	22%
	AS03 adj.	5-12y	181	1.9µg	7%¥	75%	71%	3%¥	≥38.0°C	8%	6%
	Whole virion cell culture	6m-5y	279	7.5 µg	0%¥	18%	17%	4.3%¥	≥38.0°C	9.3%	12.5%
	Whole virion cell culture	5-12y	187	7.5 µg	1%¥	40%	42%	1.7%¥	≥38.0°C	3.3%	2.9%

NR = not reported

¥ Only % with severe reactions reported

Monovalent adjuvanted pandemic H1N1 vaccines

Nine studies were identified with pandemic H1N1 vaccines with either oil-in-water adjuvants (MF59, AS03) or aluminum adjuvants (43, 45-53). Safety data were reported for 230 children exposed to MF59, 1,224 exposed to AS03 (255 to AS03A and 969 to AS03B), and 1,394 children exposed to aluminum adjuvanted vaccines. Local and systemic reactogenicity for the oil-in-water adjuvants is presented in table 4.2.

MF59

Two studies were identified reporting data on two different MF59 adjuvanted pandemic H1N1 vaccines (49, 50). Yasuda et al. compared a half dose versus a full dose of a cell culture-derived MF59 adjuvanted pandemic H1N1 vaccine in children aged 6 months to 19 years in Japan, given in two doses (50). They found that the full vaccine dose (7.5µg) was more reactogenic than the half dose, and that the frequency and severity of reactions did not increase after the second dose. There were five serious adverse events reported in this study, all of which were considered unrelated to the vaccine. These concerned one fracture and four instances of influenza: three children who contracted influenza A infections and one child an influenza B infection. One child developed influenza A (H1N1) six days after receiving the first dose of the 3.75µg vaccine and two children who developed influenza A seven and 25 days after the first dose of the 7.5µg vaccine.

Arguedas et al. evaluated the safety of an egg-based MF59 adjuvanted pandemic H1N1 vaccine and compared this to an inactivated split pandemic H1N1 vaccine in children aged 3 to 17 years (49). The adjuvanted vaccine was more reactogenic than the non-adjuvanted vaccine, which was most apparent in children between 9 and 17 years. Similar local reactogenicity was seen following the first and second dose whereas systemic reactogenicity was lower with the second dose. Children aged 9 to 17 years reported more systemic reactions compared to children aged 3 to 8 years. Although fever (≥38°C) was solicited as adverse event, no information on fever was reported (48, 49).

AS03

Five different clinical studies were identified that evaluated the safety of AS03-adjuvanted pandemic H1N1 vaccines in children. Two compared 1.9µg AS03B with 3.75µg AS03A-adjuvanted vaccines (51, 53), a non-comparative study evaluated the 1.9µg AS03B formulation (52) and one study compared the 1.9µg AS03B adjuvanted vaccine with a whole virion cell culture-derived vaccine (45, 46) in different age groups.

Carmona et al. (53) found that in children aged 6 months to 3 years local reactions increased with the 3.75µg AS03A-adjuvanted vaccine compared to the 1.9µg AS03B-adjuvanted vaccine, though not significantly. Similar observations were made by Garcia-

Scilia et al. in children aged 3 to 17 years, where a higher amount of antigen and adjuvant was associated with higher reactogenicity (51).

In the study by Carmona et al. mild, moderate and sever local and systemic reactions increased with the second dose, most notably for fever which was reported by approximately 20% following the first dose and around 70% following the second dose for both formulations (53). Garcia-Scilia et al. also saw an increase in systemic reactions following the second dose compared to the first dose, however this was mainly with the 3.75 µg AS03A formulation and, unlike Carmona et al. , was not significant for local reactions (not included in table 4.2 as information was not presented numerically) (51).

Scheifele et al. reported much lower fever rates of 3.6% after the first dose and 8.6% after the second, yet also showing a significant increase with the second dose in children aged 6months to 3 years (52). The lower rates could be the result of the difference in definitions of fever applied in the studies, although other factors might play a role as well. Waddington et al. also found the second dose to be more reactogenic than the first dose, most pronounced for fever in children between 6 months and 5 years (8.9% vs 22.4%). They did not see an increase in fever following the second dose in children aged 5 to 12 years (7.7% vs 6.3%) (46).

One AESI occurred in the study by Waddington et al. A child aged 11 months developed reactive arthritis following vaccination, which was judged possibly related to the vaccine, and resolved within 10 days (45, 46). No AESIs or vaccine related serious adverse events were reported by the three other studies.

ALUMINIUM

Two studies included aluminum adjuvanted pandemic H1N1 vaccines with different amounts of antigen in different age groups (43, 47). Solicited local adverse events following the first dose varied between 12% and 27.4%. There was no apparent relation between reactogenicity and amount of antigen or age for the aluminum adjuvanted formulations. Zhu et al. found that the adjuvant was associated with increased systemic reactogenicity. A similar trend can be seen in the data by Liang et al. , although this data was not presented for children separately.

Table 4.3 Overview clinical studies of pandemic H1N1 vaccines in children with underlying medical condition

Reference	Country	Vaccine	Antigen per dose	Number of doses	Number of children	Age (range / mean +- SD)	Patients
Bate et al. 2010 (59)	UK	AS03 adj.	1.9µg	2 doses	54	1y - 17y	Cancer
Busse et al. 2010 (60)	US	inactivated, split	15 µg	2 doses	NR	12y-79y	Asthma
			30 µg	2 doses	NR	12y-79y	Asthma
Esposito et al. 2010 (61)	Italy	MF59 adj.	7.5 µg	1 dose	31	17.8 y +- 8.7y	β-thalassemia major
				1 dose	28	17.6 y +- 7.0y	Healthy
Alghisi et al. 2011 (62)	Italy	MF59 adj.	7.5 µg	1 dose	48	8m-26y	Cystic Fibrosis
Altamirano-Diaz et al. 2011(63)	Canada	AS03 adj.	3.75µg	1 or 2 doses	5	6m-8y	Heart transplant
Esposito et al. 2011(64)	Italy	MF59 adj.	7.5 µg	2 doses	69	6m-23m	Preterm infants
					32	6m-23m	Healthy
Esposito et al. 2011 (65)	Italy	MF59 adj.	7.5 µg	1 dose	16	15.4 y +- 5.6y	Renal transplant
		MF59 adj.+seasonal	7.5 +3*15µg	1 dose	16	15.7 y +- 5.5y	Renal transplant
		MF59 adj.	7.5 µg	1 dose	16	15.6 y +- 5.5y	Healthy
		MF59 adj.+seasonal	7.5 +3*15µg	1 dose	16	15.4 y +- 5.7y	Healthy
Esposito et al. 2011 (66)	Italy	MF59 adj.	7.5 µg	1 dose	19	9y-20y	HIV infected
		MF59 adj.+seasonal	7.5 +3*15µg	1 dose	17	9y-20y	HIV infected
		MF59 adj.	7.5 µg	1 dose	19	9y-20y	Healthy
		MF59 adj.+seasonal	7.5 +3*15µg	1 dose	17	9y-20y	Healthy
Kelen et al. 2011 (67)	Hungary	aluminium phosphate gel adj.	6µg	1 dose	37	6y-23y	Renal transplant
Torii et al. 2011 (68)	Japan	Inactivated, subcutaneous	>15 µg	1 or 2 doses	13	1y-18y	Liver transplant
					31	1y-18y	hospital controls (cerebral palsy, malformation syndrome, muscular dystrophy)
Okike et al (69)	UK	AS03 adjuvanted	1.9µg/3.75 µg	2 doses	31	11.2 (median)	HIV infected

Whole virion pandemic H1N1 vaccines

The study by Waddington et al. evaluated a whole virion cell culture-derived pandemic H1N1 vaccine in comparison to an AS03 adjuvanted vaccine (46). The whole virion vaccine appears to be less reactogenic compared to the AS03 adjuvanted vaccine, as can be seen in table 4.2. For example, less severe local reactions were reported following the whole virion vaccine; 1.1% vs 7.2% after the first dose in children over 5 years. This was seen over almost the entire range of solicited adverse events. No clear difference between the first and second dose was seen in children less than 5 years of age. In children between 5 and 12 years of age the second dose was associated with a reduced rate of "feeling unwell" (15% vs 25% after the first dose), yet an increase in nausea and vomiting (10% vs 1% after the first dose) which was not seen for the AS03 adjuvanted vaccine.

Studies in children with underlying medical conditions

Ten studies were identified that evaluated different pandemic H1N1 vaccines in a total of 431 children and adolescents with underlying disease (59, 61-69). An additional study investigated pandemic H1N1 vaccination in 390 persons with asthma, including adolescents 12 years and above (60). An overview of all studies is presented in table 4.3. Most were single intervention, observational studies (59, 60, 62, 63, 67). Two studies included healthy controls (61, 64), and one study included hospital controls with different medical conditions (68). The two remaining studies evaluated the effect of simultaneous vs. sequential administration of seasonal and pandemic H1N1 vaccines in pediatric kidney patients (65) and HIV infected patients (66). None of the studies compared adjuvanted and non-adjuvanted vaccine formulations. Two studies did not report any safety outcomes (67, 69), and the study in five pediatric heart transplant patients was too small for meaningful observations (63). Torii et al only reported that there were no systemic reactions and no allograft rejections in a cohort of renal transplant patients (68), and Busse et al only reported safety findings by asthma severity in a cohort of asthma patients aged 12-79 years (60). The studies that included healthy controls did not observe any difference in reactogenicity or safety in children and adolescents with underlying medical conditions. The study in pediatric cancer patients had a relatively low fever rate, however all participants with fever had to be hospitalized and treated with intravenous antibiotics and two became neutropenic (59). No other significant adverse events were seen in any of the studies found.

Spontaneous reports, case reports, surveillance efforts

Several overviews on reported adverse events after administration of pandemic H1N1 vaccines have been published worldwide (15-18, 70-83). Reported events are mostly presented in stratified age-categories, often including paediatric groups. Only few

publications actually present age-specific reporting rates.(72-75) An overview of the number of reported events in children and adolescents reported by different sources is given in table 4.4. Importantly, many of the sources of spontaneous reports contain overlapping information as reports within national databases are also centrally collected by the WHO-UMC and, for European licensed vaccines in the EudraVigilance database. As a result of the overlap, comparisons between different publications are difficult to make.

Number of reports

The most complete overview of the number of spontaneous reports for children and adolescents after administration of pandemic H1N1 vaccines is published by the WHO-UMC (71). Up to February 2011, 34,256 individual case safety reports (ICSRs) on 110,883 suspected AEFI were received from 31 countries worldwide spanning 11 different vaccine brands. Of these, 12,900 ICSRs (37.6%) were reported for children and adolescents. The majority of the reports are in children aged 2-≤11 years (n=7,650; 59.3% of reports on children). The most frequently suspected vaccine was the AS03 adjuvanted vaccine (Pandemrix, n=6,260; 48.5%), followed by reports for vaccines with unknown brand (n=5,249; 40.7%). For the AS03 adjuvanted vaccine 46.1% of the reports were reported for the paediatric population, of which 0.3% were related to neonates <28 days, 29.5% to infants/children 28 days to 23 months, 57.5% for children age 2-≤11 years, and 12.7% for adolescents 12-≤17 years of age. For each vaccine the WHO-UMC presented the number of reported events stratified by system organ class (SOC) and the number of reported adverse events of special interest. These results were not stratified by age-category.

Reporting rates

We identified three studies that calculated reporting rates based on the number of reported adverse events and the number of vaccinated persons (72, 73, 75). An overview of the rates is given in table 4.5. These three studies were all based on spontaneous reporting. Reporting rates for serious adverse events ranged from 6.8 to 10.7 per 1,000,000 doses or vaccinated persons. The number of exposed persons per vaccine has not been published. Rates for non-serious events ranged from 82 to 120 per 100,000 doses or vaccinated persons. Reporting rates for serious events tend to be higher in the younger children compared to adolescents and adults. Vellozzi et al compared the reporting rates for the pandemic H1N1 vaccines with the events for seasonal vaccines in the previous seasons using the VAERS data. The rate for serious events was significantly higher for the pandemic H1N1 vaccines for all age groups except for children under the age of 5 years (73).

Table 4.4 Overview of post-marketing studies reporting the number of adverse event for pandemic H1N1 vaccines in children

Reports published on websites

Authors	Year published	Data lock point / last FUP	Source data	Type of data	Country	Type of vaccine	Age categories	Number of reports children included	Number of events	Remarks
WHO-UMC[220]	March 2011	Feb 2011	VigiBase	SR	Australia, Austria, Belgium, Brunei, Canada, Chile, Denmark, Estonia, Finland, Germany, Greece, Hungary, Iceland, Ireland, Italy, Malaysia, Malta, Mexico, Montenegro, Morocco, Netherlands, Norway, Peru, Saudi Arabia, Singapore, Slovenia, Spain, Sweden, Switzerland, United Kingdom and the United States.	Brand unknown	28d-23m	937		
							2y-11y	3,212		
							12y-17y	1,100		
						AS03-adjuvanted (Arepanrix)	2y-11y	2		
						AS03-adjuvanted (Arepanrix H1N1)	28d-23m	1		
						MF59-adjuvanted (Celtura)	2y-11y	2		
							12y-17y	3		
						Whole virion cell culture derived vaccine (Celvapan)	<28d	2		
							28d-23m	18		
							2y-11y	156		
							12y-17y	41		
						Whole virus, inactivated, aluminium adjuvated (Fluval P)	2y-11y	7		
							12y-17y	16		
						MF59-adjuvanted (Focetria)	<28d	1		
							28d-23m	97		
							2y-11y	391		
							12y-17y	136		
						Influenza A (H1N1) 2009 Pan. Mono. Vac. (non-adju.)	2y-11y	1		
						AS03-adjuvanted (Pandemrix)	<28d	20		
							28d-23m	1,845		
							2y-11y	3,601		
							12y-17y	794		
						Non-adjuvanted inactivated split (Panenza)	28d-23m	2		
						Non-adjuvanted inactivated split (Panvax)	<28d	1		
							28d-23m	184		
							2y-11y	278		
							12y-17y	52		

Table 4.4 Overview of post-marketing studies reporting the number of adverse event for pandemic H1N1 vaccines in children (Cont'd)

Reports published on websites (Cont'd)

Authors	Year published	Data lock point / last FUP	Source data	Type of data	Country	Type of vaccine	Age categories	Number of reports children included	Number of events	Remarks
MHRA[165]	01-04-2010	16-03-2010	MHRA	SR	UK	Whole virion cell culture derived vaccine; AS03-adjuvanted	0y-16y	440		4 fatal cases
Swedish Medical Products Agency [166]	02-06-2010	16-04-10	MPA	SR	Sweden	AS03-adjuvanted	Children			4 cases : lack of efficacy ((4-13 years of age)

Reports published in literature

Authors	Year published	Data lock point / last FUP	Source data	Type of data	Country	Type of vaccine	Age categories	Number of reports children included	Number of events	Remarks
Carvajal *et al.*[231]	2011			SR	Spain	MF59-adjuvanted	14y-<18y	7		
				FUP study	Spain	MF59-adjuvanted	14y-<18y			
Folkenberg *et al.*[221]	2011	31-03-10	Danish Pharmacovigilance database	SR	Denmark	AS03-adjuvanted	0y-4y		19	
							5y-14y		29	
							15y-64y		429	
Vellozzi *et al.*[222]	2010	31-01-10	VAERS	SR	United States	Inactivated; Live, attenuated, unknown	0.5y-4y	1,358		4 cases of GBS
							5y-24y	3,415		21 cases of GBS
Wu *et al.*[223]	2010		Diary cards; telephone interviews		China	Non adjuvated monovalent split-virion vaccine	4y-11y	42		9/42 vaccine-related
							12y-17y	69		28/69 vaccine-related

Table 4.4 Overview of post-marketing studies reporting the number of adverse event for pandemic H1N1 vaccines in children (Cont'd)

Reports published in literature (Cont'd)

Authors	Year published	Data lock point / last FUP	Source data	Type of data	Country	Type of vaccine	Age categories	Number of reports children included	Number of events	Remarks
Liang *et al.*[224]	2011	21-03-2011	Chinese CDS	SR	China	Nonadjuvant, split-virion vaccins	≤9y		1,669	137 serious
							10y-19y		3,436	293 serious 3 cases of GBS < 15 years
Kurz *et al.*[219]	2011	30-04-2010	EudraVigilance	SR	European Economic Area (98.2%)	Whole virion cell culture derived vaccine	< 3y	31		
							3y-8y	58		
							9y-17y	57		
						MF59-adjuvanted	< 3y	90		
							3y-8y	148		
							9y-17y	107		
						AS03-adjuvanted	< 3y	2,601		
							3y-8y	1,908		
							9y-17y	632		
Banzhoff *et al.*[226]	2011	Mar-2010	Novartis	SR (97%), trials (2.8%), lit. (0.3%)	Europe	MF59-adjuvanted	< 18y	805		
Parretta *et al.*[225]	2011	30-06-2010	Italian Pharmacovigilance Adverse Event Spontaneous Reporting System	SR	Italy	MF59-adjuvanted	0.5y-<2y	37		5 serious
							2y-11y	162		12 serious
							12y-17y	59		3 serious
Mahajan *et al.*[227]	2010	31-12-2009	Australian Adverse Drug Reactions System database	SR	Australia (New South Wales)	Unknown	< 7y	10		0 serious

FUP=Follow Up; SR=spontaneous reports; Lit=literature.

Table 4.5 Reporting rates of adverse events based on spontaneous reporting for pandemic H1N1 vaccines in children

Author	Country	Vaccine	Age	Serious adverse events	Non-serious adverse events	Total
Folkenberg et al.(72)	Denmark	Pandemrix	0-4 years			30.61 / 1,000,000 vaccinated persons
			5-14 years			27.02 / 1,000,000 vaccinated persons
			15-64 years			22.55 / 1,000,000 vaccinated persons
Vellozzi et al.(73)	US	Inactivated; Live, attenuated, unknown	0.5-4 years	8.1/1,000,000 vaccinated persons	113.2/1,000,000 vaccinated persons	
			5-24 years	6.8/1,000,000 vaccinated persons	120.4/1,000,000 vaccinated persons	
Liang et al.(75)	China	Non-adjuvant, split-virion vaccins	≤9 years	10.7/1,000,000 doses	119.9/1,000,000 doses	130.6 / 1,000,000 doses
			10-19 years	7.7/1,000,000 doses	82.4/1,000,000 doses	90.1 / 1,000,000 doses

Rates were transformed to 1/1,000,000 administered doses or vaccinated persons to enhance comparisons

Wu et al. (74) calculated reporting rates based on stimulated surveillance. This resulted in higher reporting rates: 0.77% (95%CI 0.54-1.01) for children aged 4 to 11 years and 0.28% (95%CI 0.22-0.35%) for adolescents aged 12 to 17 years.

Differences in reporting rates acquired through spontaneous reporting and those observed with active surveillance were also seen in the Spanish study by Carvajal et al (82). Comparing spontaneous reporting rates with rates based on a follow-up study it was estimated by the authors that the spontaneous reporting rates were 322-fold lower than the study rates. For serious events the rate of spontaneous reports was 37-fold lower.

Types of events

Parretta et al. presented the number of reported events on a SOC level for three pediatric age groups from Italy, all concerning the MF59 adjuvanted vaccine. For children aged 0.5-<2 years and 2-11 years, most reports were reported for the SOCs 'General disorders and administration site conditions' (59.5% resp. 63.6%), 'Skin and subcutaneous tissue disorders'

(24.3% resp. 18.5%) and 'Nervous system disorders' (24.3% resp. 25.3%). For adolescents aged 12-17 years the reports were within 'General disorders and administration site conditions' (66.1%), 'Nervous system disorders' (32.2%) and 'Respiratory, thoracic and mediastinal disorders' (8.5%).

For the pandemic H1N1 vaccines licensed centrally in Europe the most frequently reported adverse events in children and adolescents per vaccine were published by EMA (18). For the AS03 adjuvanted pandemic H1N1 vaccine produced in Canada (Arepanrix) these were anaphylactic reaction, cough, cyanosis, dyspnoea, angioedema, urticaria, throat tightness, pyrexia, nausea, erythema, rash, pallor, flushing, anaphylactic shock, hypersensitivity, depressed level of consciousness and wheezing. For AS03 adjuvanted pandemic H1N1 vaccine(Pandemrix) produced in Europe, the most frequently reported events were pyrexia, hyperpyrexia, vomiting, injection-site pain, headache, diarrhoea, cough, fatigue, rash, decreased appetite, nausea, abdominal pain, malaise, injection-site erythema, crying, somnolence, pallor, injection site swelling, listlessness, syncope, dyspnoea, pain in extremity, febrile convulsion, influenza-like illness, myalgia, urticaria, dizziness, erythema, tearfulness and erythema. The only difference between the two AS03 adjuvanted vaccines is the production site, they can otherwise be considered identical (84, 85). For the whole virion cell culture derived vaccine, dizziness, medication error, vomiting, nausea, pallor, pyrexia, headache, hypersensitivity, syncope, underdose, injection site pain, rash, fatigue, malaise, diarrhoea, vision blurred, feeling hot and wrong technique in drug usage process. For the MF59 adjuvanted pandemic H1N1 vaccine the following adverse events were most frequently reported in children: drug exposure during pregnancy, pyrexia, headache, premature baby, hyperpyrexia, vomiting, cough, small for dates baby, nausea, abdominal pain, diarrhoea, injection-site pain, myalgia, fatigue, influenza like illness, large for dates baby, dyspnoea, rash, malaise, urticaria, infection and convulsion.

Case reports

Six published case-reports were identified on children and adolescents experiencing adverse events after administration of a pandemic H1N1 vaccine. Two reports from Canada were identified. A 2-year old boy experienced bilateral optic neuritis and acute disseminated encephalomyelitits (ADEM) after two doses of AS03 adjuvanted pandemic H1N1 vaccine (86), and an 11-year old boy was diagnosed with GBS 13 days after vaccination with AS03 adjuvanted pandemic H1N1 vaccine (87). Two reports after vaccination with non-adjuvanted pandemic H1N1 vaccines were reported from China. A 17-year old Chinese girl experiencing bilateral sudden hearing loss 14 hours after vaccination (88) and a 13-year old boy diagnosed with acute transverse myelitis 5 days after vaccination (89). One report concerned a 9-year old boy from the United States with papular acrodermatitis of childhood, or Gianotti-Crosti syndrome following vaccination with

a live attenuated intranasal pandemic H1N1 vaccine (90). The final report concerned a 6-year old boy from France experiencing Cytophagic histiocytic panniculitis 1 week after the second injection of non-adjuvanted pandemic H1N1 vaccine (91).

Observational studies, active surveillance

Few observational studies evaluating potential adverse effects of pandemic H1N1 vaccination been published so far, although several are still ongoing. A German study compared adverse events following pandemic H1N1 vaccination in 72 children and adolescents (1 to 19 years) after liver transplantation with 27 vaccinated healthy siblings and 243 from a database (92). Most common adverse events reported were local symptoms. There was no significant difference in the frequency of adverse events between patients and controls, except for headache, diarrhea, fatigue and muscle pain which were reported at lower rates in the transplantation group. Another prospective cohort study, monitoring immunocompromised and immunocompetent children and adolescents either immunized with AS03 or MF59 adjuvanted pandemic H1N1 vaccine, or with confirmed influenza infection, found that adverse events increased with age and were more frequent following exposure to AS03 adjuvanted vaccine than to MF59 adjuvanted vaccine (93). In the Netherlands a study into the occurrence of fever following vaccination with the AS03 adjuvanted pandemic H1N1 vaccine in children between 6 months and 4 years of age was conducted (83). In this study, all parents or caregivers reporting fever to the Adverse drug reaction reporting database of the Netherlands Pharmacovigilance Center following the first dose of pandemic H1N1 vaccine were sent questionnaires. They found that 44% of children who experienced fever following the first dose did not develop fever following the second dose and that those with fever following the second dose experienced a less severe course. Unfortunately, this study did not consider those who did not develop fever following the first dose but did develop fever following the second dose. The EMA published a warning on their website concerning the risk of fever in young children after with the second dose of the AS03-adjuvanted pandemic H1N1 vaccine (94).

Following the experience with swine flu vaccination in 1976 in the US, much attention has gone out GBS in the active surveillance studies. The potential association with pandemic H1N1 vaccination has been evaluated in different studies in the US (95), Europe (96, 97) and Korea (80). All these studies included cases in children or adolescents. For the overall population no association between GBS and pandemic H1N1 vaccination was detected. Numbers were too low to draw conclusions for the pediatric population specifically. Preliminary results from a US based study indicate a small excess risk of 0.8 cases per 1 million vaccinations (95).

In August 2010 reports of a possible association between exposure to AS03 adjuvanted pandemic H1N1 vaccine and occurrence of narcolepsy-cataplexy in children and adolescents emerged first in Sweden and later in Finland, leading to the recommended discontinuation of this vaccine in these countries and a review of this vaccine within the EMA (98, 99). At the same time, France reported 6 cases, 5 following the AS03 adjuvanted vaccine and 1 following an inactivated split pandemic H1N1 vaccine (100). In November 2010 a publication appeared discussing 14 cases of narcolepsy after H1N1 vaccination, and 2 after H1N1 infection cases from 3 sleep centres in the US, Canada and France (101). Since then more cases have been identified, mostly in children and adolescents. A registry study in Finland, published in February 2011, found a 9-fold increase in narcolepsy in association with the AS03 adjuvanted pandemic H1N1 vaccine (102). Two Swedish studies also strengthened the signal by observing a relative risk of 4.19 (103) and 6.6 (104). A causal association between the onset of narcolepsy and exposure to a pandemic H1N1 vaccine has not been established. Alternative explanations accounting for the observed epidemiological association, have not been fully investigated so far. This includes effect modification by circulating pandemic virus, other circulating infections, or seasonal influenza vaccine. It also includes diagnostic bias by preferential shortening of the time to diagnosis in exposed cases due to increased awareness of narcolepsy and the potential relation with the vaccine. Currently, extended epidemiological assessments of the association between narcolepsy and pandemic H1N1 influenza vaccination are underway (105, 106). An investigation of narcolepsy following exposure to MF59 adjuvanted pandemic H1N1 vaccines did not identify any cases (107). In July 2010 the CHMP reviewed the European marketing authorization of the AS03-adjuvanted pandemic H1N1 vaccine and concluded that the vaccine should only be given to persons below the age of 20 if seasonal trivalent vaccines are absent (108).

Concluding remarks

Much has been published on the safety of pandemic H1N1 vaccines in children. There have been several studies with different vaccines spanning all age groups and several studies in children and adolescents with underlying medical conditions. In addition, large monitoring efforts have resulted in much data, with almost 13,000 individual case reports in children and adolescents to the WHO. However, both differences in study methodology and data presentation render meta-analytic safety analyses of the pandemic H1N1 vaccines in the different, relevant, age groups difficult. Especially the diversity in the clinical studies for inactivated non-adjuvanted pandemic H1N1 vaccines re-emphasizes the need for harmonization of study protocols and presentation of safety data in clinical study publications (58, 109). The added value of publications of spontaneous report analyses from overlapping source populations could be increased by crystallizing differences

between populations and age groups. With the currently published information this is impossible. Thus, although a large amount of data has been generated, relatively little has been learned on the comparative safety of these pandemic H1N1 vaccines – particularly in children. It should be a collective effort to give added value to the enormous work going into the individual studies by adhering to available guidelines for the collection, analysis, and presentation of vaccine safety data in clinical studies (56, 110) and to guidance for the clinical investigation of medicinal products in the pediatric population (111).

The 2009 H1N1 pandemic has shown that although spontaneous reporting of AEFI is necessary for the monitoring of vaccine safety, it is useful to enhance surveillance by methods and infrastructures to verify signals and test hypothesis. Observed over expected analyses to verify signals rely on accurate background incidence rates of disease within targeted age groups. Both in the USA and in Europe these rates were provided though coordinated action and use of health care databases. In Europe data were provided from 8 countries on a population of more than 50 million subjects. Several hypothesis testing studies were implemented to be able to assess the potential association of pandemic influenza vaccine with GBS and narcolepsy. All required multinational collaboration to meet the need. The pandemic has brought us the beginning of an infrastructure for collaborative vaccine safety studies in the EU, USA and globally.

References

1. Girard MP, Katz J, Pervikov Y, Palkonyay L, Kieny M-P. Report of the 6th meeting on the evaluation of pandemic influenza vaccines in clinical trials World Health Organization, Geneva, Switzerland, 17-18 February 2010. Vaccine. 2010;28(42):6811-20.

2. WHO. WHO Weekly Epidemiological Record: Strategic Advisory Group of Experts on Immunisation (SAGE) - report of the extraordinary meeting on the influenza A(H1N1) 2009 pandemic. . Available from: URL: http://www.who.int/wer/2009/wer8430.pdf2009. p. 301-8.

3. (US). C. Centers for Disease Control and Prevention: Novel H1N1 Vaccination Recommendations. . [Online]. Available from: URL: http://www.cdc.gov/h1n1flu/vaccination/acip.htm2009.

4. Mamelak M, Swick T, Emsellem H, Montplaisir J, Lai C, Black J. A 12-week open-label, multicenter study evaluating the safety and patient-reported efficacy of sodium oxybate in patients with narcolepsy and cataplexy. Sleep medicine. 2015 Jan;16(1):52-8. PubMed PMID: 25533539.

5. Hanquet G, Van Damme P, Brasseur D, De Cuyper X, Gregor S, Holmberg M, et al. Lessons learnt from pandemic A(H1N1) 2009 influenza vaccination. Highlights of a European workshop in Brussels (22 March 2010). Vaccine. 2011;29(3):370-7.

6. Johansen K, Nicoll A, Ciancio BC, Kramarz P. Pandemic influenza A(H1N1) 2009 vaccines in the European Union. Euro Surveill. 2009;14(41):19361.

7. Girard MP, Katz J, Pervikov Y, Palkonyay L, Kieny MP. Report of the 6th meeting on the evaluation of pandemic influenza vaccines in clinical trials World Health Organization, Geneva, Switzerland, 17-18 February 2010. Vaccine. 2010;28(42):Epub.

8. WHO. Influenza vaccines: WHO position paper. Wkly Epidemiol Rec. 2005;33.

9. CDC. National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention: Use of influenzaA(H1N1) 2009 monovalent vaccine. MMWR Recomm Rep. 2009;58(RR-10):1-8.

10. Vesikari T, Groth N, Karvonen A, Borkowski A, Pellegrini M. MF59®-adjuvanted influenza vaccine (FLUAD®) in children: Safety and immunogenicity following a second year seasonal vaccination. Vaccine. 2009;27(45):6291-5.

11. Vesikari T, Pellegrini M, Karvonen A, Groth N, Borkowski A, O'Hagan DT, et al. Enhanced immunogenicity of seasonal influenza vaccines in young children using MF59 adjuvant. The Pediatric infectious disease journal. 2009 Jul;28(7):563-71. PubMed PMID: 19561422. eng.

12. Bone A, Guthmann J-P, Nicolau J, Lévy-Bruhl D. Population and risk group uptake of H1N1 influenza vaccine in mainland France 2009-2010: Results of a national vaccination campaign. Vaccine. 2010;28(51):8157-61.

13. Salmon DA, Akhtar A, Mergler MJ, Vannice KS, Izurieta HS, Ball R, et al. Immunization-safety monitoring systems for the 2009 H1N1 monovalent influenza vaccination program. Paediatrics. 2011;127(Suppl 1:):S78-86.

14. Lundgren B. Narrating narcolepsy--centering a side effect. Medical anthropology. 2015;34(2):150-65. PubMed PMID: 25457625.

15. WHO-UMC. A/H1N1 Flu Vaccine Monitoring. 2011.

16. MHRA. MHRA PUBLIC ASSESSMENT REPORT: Swine flu vaccines and antiviral medicines: UK post-pandemic safety review. February 2011.

17. Final summary of adverse drug reaction reports in Sweden with Pandemrix through October 2009 – mid April 2010 June 2010. Available from: http://www.lakemedelsverket.se/upload/eng-mpa-se/Pandemrix%20ADRs%20in%20Sweden%2015%20april%202010.pdf.

18. European Medicines Agency: Twenty-second pandemic pharmacovigilance update 19 August 2010. EMA/527985/2010:[Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Report/2010/08/WC500095870.pdf.

19. Zangwill KM, Belshe RB. Safety and efficacy of trivalent inactivated influenza vaccine in young children: a summary for the new era of routine vaccination. The Pediatric infectious disease journal. 2004;23(3):189-97.

20. Ruben FL. Inactivated influenza virus vaccines in children. Clin Infect Dis. 2005;38(5):678-88.

21. Hambidge SJ, Glanz JM, France EK, McClure D, Xu S, Yamasaki K, et al. Safety of trivalent inactivated influenza vaccine in children 6 to 23 months old. JAMA : the journal of the American Medical Association. 2006 Oct 25;296(16):1990-7. PubMed PMID: 17062862. Epub 2006/10/26. eng.

22. Jefferson T, Rivetti A, Harnden AR, Di Pietrantonj C, Demicheli V. Vaccines for preventing influenza in healthy children. Cochrane Database of Systematic Reviews. 2008 (Issue 2):Art. No.: CD004879. .

23. Barry DW, Mayner RE, Hochstein HD, al. e. Comparative trial of influenza vaccines. II. Adverse reactions in children and adults. . Am J Epidemiol. 1976;104(47-59).

24. McMahon AW, Iskander J, Haber P, Chang S, Woo EJ, Braun MM, et al. Adverse events after inactivated influenza vaccination among children less than 2 years of age: analysis of reports from the vaccine adverse event reporting system, 1990-2003. Pediatrics. 2005 Feb;115(2):453-60. PubMed PMID: 15687455. eng.

25. Rosenberg M, Sparks R, McMahon A, Iskander J, Campbell JD, Edwards KM. Serious adverse events rarely reported after trivalent inactivated influenza vaccine (TIV) in children 6-23 months of age. Vaccine. 2009;27(32):4278-83.

26. Fiore AE, Shay D, Broder KR, Iskander JK, Uyeki T, Mootrey GT, et al. Prevention and Control of Seasonal Influenza with Vaccines: Recommendations of the Advisory Committee on Immunization Practices (ACIP), 2009. Morbidity and Mortality Weekly Report. 2009;Vol. 58 (RR-8).

27. McMahon AW, Iskander JK, Haber P, al. e. Inactivated influenza vaccine (IIV) in children <2 years of age: examination of selected adverse events reported to the Vaccine Adverse Event Reporting System (VAERS) after thimerosal-free or thimerosal-containing vaccine. . Vaccine. 2008;26:427-9.

28. Fiore AE, Shay DK, Broder K, Iskander JK, Uyeki TM, Mootrey G, et al. Prevention and control of seasonal influenza with vaccines: recommendations of the Advisory Committee on Immunization Practices (ACIP), 2009. MMWR Recomm Rep. 2009 Jul 31;58(RR-8):1-52. PubMed PMID: 19644442. eng.

29. Tennis P, Toback SL, Andrews E, McQuay LJ, Ambrose CS. A postmarketing evaluation of the frequency of use and safety of live attenuated influenza vaccine use in nonrecommended children younger than 5 years. Vaccine. 2011 Jul 12;29(31):4947-52. PubMed PMID: 21596087. eng.

30. Piedra PA, Yan L, Kotloff K, Zangwill K, Bernstein DI, King J, et al. Safety of the trivalent, cold-adapted influenza vaccine in preschool-aged children. Pediatrics. 2002 Oct;110(4):662-72. PubMed PMID: 12359778. eng.

31. Belshe RB, Mendelman PM. Safety and efficacy of live attenuated, cold-adapted, influenza vaccine-trivalent. Immunology and allergy clinics of North America. 2003 Nov;23(4):745-67. PubMed PMID: 14753390. eng.

32. Belshe RB, Nichol KL, Black SB, Shinefield H, Cordova J, Walker R, et al. Safety, efficacy, and effectiveness of live, attenuated, cold-adapted influenza vaccine in an indicated population aged 5-49 years. Clin Infect Dis. 2004 Oct 1;39(7):920-7. PubMed PMID: 15472840. eng.

33. Belshe RB, Ambrose CS, Yi T. Safety and efficacy of live attenuated influenza vaccine in children 2-7 years of age. Vaccine. 2008 Sep 12;26 Suppl 4:D10-6. PubMed PMID: 18611422. eng.

34. France EK, Glanz JM, Xu S, Davis RL, Black SB, Shinefield HR, et al. Safety of the trivalent inactivated influenza vaccine among children: a population-based study. Arch Pediatr Adolesc Med. 2004;158(11):1031-6

35. Izurieta HS, Haber P, Wise RP, Iskander J, Pratt D, Mink C, et al. Adverse events reported following live, cold-adapted, intranasal influenza vaccine. JAMA : the journal of the American Medical Association. 2005 Dec 7;294(21):2720-5. PubMed PMID: 16333007. eng.

36. Mutsch M, Zhou W, Rhodes P, Bopp M, Chen RT, Linder T, et al. Use of the Inactivated Intranasal Influenza Vaccine and the Risk of Bell's Palsy in Switzerland. The New England journal of medicine. 2004;350(9):896-903.

37. Muhammad RD, Haber P, Broder KR, Leroy Z, Ball R, Braun MM, et al. Adverse Events Following Trivalent Inactivated Influenza Vaccination in Children: Analysis of the Vaccine Adverse Event Reporting System. The Pediatric infectious disease journal. 2011 Oct 29. PubMed PMID: 21042229. Eng.

38. Skowronski DM, Bjornson G, Husain E, Metzger DL, Scheifele DW. Oculorespiratory syndrome after influenza immunization in children. The Pediatric infectious disease journal. 2005 Jan;24(1):63-9. PubMed PMID: 15665712. eng.

39. Oh C-E, Lee J, Kang J-H, Hong Y-J, Kim Y-K, Cheong H-J, et al. Safety and immunogenicity of an inactivated split-virus influenza A/H1N1 vaccine in healthy children from 6 months to <18 years of age: A prospective, open-label, multi-center trial. Vaccine. 2010;28(36):5857-63.

40. Lu C-Y, Shao P-L, Chang L-Y, Huang Y-C, Chiu C-H, Hsieh Y-C, et al. Immunogenicity and safety of a monovalent vaccine for the 2009 pandemic influenza virus A (H1N1) in children and adolescents. Vaccine. 2010;28(36):5864-70.

41. Plennevaux E, Blatter M, Cornish MJ, Go K, Kirby D, Wali M, et al. Influenza A (H1N1) 2009 two-dose immunization of US children: An observer-blinded, randomized, placebo-controlled trial. Vaccine. 2011;29(8):1569-75.

42. Plennevaux E, Sheldon E, Blatter M, Reeves-Hoché M-K, Denis M. Immune response after a single vaccination against 2009 influenza A H1N1 in USA: a preliminary report of two randomised controlled phase 2 trials. The Lancet. 2010;375(9708):41-8.

43. Zhu FC, Wang H, Fang HH, Yang JG, Lin XJ, Liang XF, et al. A novel influenza A (H1N1) vaccine in various age groups. The New England journal of medicine. 2009 Dec 17;361(25):2414-23. PubMed PMID: 19846844. eng.

44. Nolan T, McVernon J, Skeljo M, Richmond P, Wadia U, Lambert S, et al. Immunogenicity of a monovalent 2009 influenza A(H1N1) vaccine in infants and children: a randomized trial. JAMA : the journal of the American Medical Association. 2010;303(1):37-46.

45. Waddington C, Andrews N, Hoschler K, Walker W, Oeser C, Reiner A, et al. Open-label, randomised, parallel-group, multicentre study to evaluate the safety, tolerability and immunogenicity of an AS03(B)/oil-in-water emulsion-adjuvanted (AS03(B)) split-virion versus non-adjuvanted whole-virion H1N1 influenza vaccine in UK children 6 months to 12 years of age. Health technology assessment (Winchester, England). 2010 Oct;14(46):1-130. PubMed PMID: 20923610. eng.

46. Waddington CS, Walker WT, Oeser C, Reiner A, John T, Wilkins S, et al. Safety and immunogenicity of AS03B adjuvanted split virion versus non-adjuvanted whole virion H1N1 influenza vaccine in UK children aged 6 months-12 years: open label, randomised, parallel group, multicentre study. Bmj. 2010;340:c2649. PubMed PMID: 20508026. eng.

47. Liang X-F, Wang H-Q, Wang J-Z, Fang H-H, Wu J, Zhu F-C, et al. Safety and immunogenicity of 2009 pandemic influenza A H1N1 vaccines in China: a multicentre, double-blind, randomised, placebo-controlled trial. The Lancet. 2010;375(9708):56-66.

48. Arguedas A, Soley C, Abdelnour A, Sales V, Lindert K, Della Cioppa G, et al. Assessment of the safety, tolerability and kinetics of the immune response to A/H1N1v vaccine formulations with and without adjuvant in healthy pediatric subjects from 3 through 17 years of age. Human vaccines. 2010 Jan 1;7(1):58-66. PubMed PMID: 21285531. eng.

49. Arguedas A, Soley C, Lindert K. Responses to 2009 H1N1 vaccine in children 3 to 17 years of age. The New England journal of medicine. 2010 Jan 28;362(4):370-2. PubMed PMID: 20042746. eng.

50. Yasuda Y, Komatsu R, Matsushita K, Minami T, Suehiro Y, Sawata H, et al. Comparison of half and full doses of an MF59-adjuvanted cell culture-derived A/H1N1v vaccine in Japanese children. Advances in therapy. 2010 Jul;27(7):444-57. PubMed PMID: 20586002. eng.

51. Garcia-Sicilia J, Gillard P, Carmona A, Tejedor JC, Aristegui J, Merino JM, et al. Immunogenicity and safety of AS03-adjuvanted H1N1 pandemic vaccines in children and adolescents. Vaccine. 2011;29(26):4353-61.

52. Scheifele DW, Ward BJ, Dionne M, Vanderkooi O, Langley JM, Dobson S, et al. Evaluation of adjuvanted pandemic H1N1(2009) influenza vaccine after one and two doses in young children. The Pediatric infectious disease journal. 2011 May;30(5):402-7. PubMed PMID: 21178654. eng.

53. Carmona A, Omeñaca F, Tejedor JC, Merino JM, Vaman T, Dieussaert I, et al. Immunogenicity and safety of AS03-adjuvanted 2009 influenza A H1N1 vaccine in children 6-35 months. Vaccine. 2010;28(36):5837-44.

54. Mallory RM, Malkin E, Ambrose CS, Bellamy T, Shi L, Yi T, et al. Safety and immunogenicity following administration of a live, attenuated monovalent 2009 H1N1 influenza vaccine to children and adults in two randomized controlled trials. PLoS One. 2010;5(10):e13755. PubMed PMID: 21060780. eng.

55. Kohl KS, Gidudu J, Bonhoeffer J, Braun MM, Buettcher M, Chen RT, et al. The development of standardized case definitions and guidelines for adverse events following immunization. Vaccine. 2007;25(31):5671-4.

56. Bonhoeffer J, Bentsi-Enchill A, Chen RT, Fisher MC, Gold MS, Hartman K, et al. Guidelines for collection, analysis and presentation of vaccine safety data in pre- and post-licensure clinical studies. Vaccine. 2009;27(16):2282-8.

57. van der Heide A, Verduijn W, Haasnoot GW, Drabbels JJ, Lammers GJ, Claas FH. HLA dosage effect in narcolepsy with cataplexy. Immunogenetics. 2015 Jan;67(1):1-6. PubMed PMID: 25277311.

58. Bonhoeffer J, Zumbrunn B, Heininger U. Reporting of vaccine safety data in publications: systematic review. Pharmacoepidemiol Drug Saf. 2005;14(2):101-6.

59. Bate J, Yung CF, Hoschler K, Sheasby L, Morden J, Taj M, et al. Immunogenicity of pandemic (H1N1) 2009 vaccine in children with cancer in the United Kingdom. Clin Infect Dis. 2010 Dec 15;51(12):e95-104. PubMed PMID: 21067352. Epub 2010/11/12. eng.

60. Busse WW, Peters SP, Fenton MJ, Mitchell H, Bleecker ER, Castro M, et al. Vaccination of patients with mild and severe asthma with a 2009 pandemic H1N1 influenza virus vaccine. Journal of Allergy and Clinical Immunology. 2011;127(1):130-7.e3.

61. Esposito S, D'Angelo E, Daleno C, Peia F, Scala A, Serra D, et al. Immunogenicity, safety and tolerability of monovalent 2009 pandemic influenza A/H1N1 MF59-adjuvanted vaccine in patients with [beta]-thalassemia major. Vaccine. 2010;28(50):7825-8.

62. Alghisi F, Palma P, Montemitro E, Bernardi S, Pontrelli G, Rossi P, et al. Immunogenicity and safety profile of the monovalent A/H1N1 MF59-adjuvanted vaccine in patients affected by cystic fibrosis. Thorax. 2011 Mar;66(3):259-60. PubMed PMID: 21228426. eng.

63. Altamirano-Diaz L, West L, Humar A, Ely L, Urschel S, Gubbay J, et al. Early post-transplant vaccination with pandemic influenza A/H1N1 vaccine in pediatric heart transplant recipients. Pediatr Transplant. 2011 Mar;15(2):172-5. PubMed PMID: 21176017. Epub 2010/12/24. eng.

64. Esposito S, Pugni L, Daleno C, Ronchi A, Valzano A, Serra D, et al. Influenza A/H1N1 MF59-adjuvanted vaccine in preterm and term children aged 6 to 23 months. Pediatrics. 2011 May;127(5):e1161-8. PubMed PMID: 21464195. eng.

65. Esposito S, Meregalli E, Daleno C, Ghio L, Tagliabue C, Valzano A, et al. An open-label, randomized clinical trial assessing immunogenicity, safety and tolerability of pandemic influenza A/H1N1 MF59-adjuvanted vaccine administered sequentially or simultaneously with seasonal virosomal-adjuvanted influenza vaccine to paediatric kidney transplant recipients. Nephrol Dial Transplant. 2011 Jun;26(6):2018-24. PubMed PMID: 20974645. Epub 2010/10/27. eng.

66. Esposito S, Tagliaferri L, Daleno C, Valzano A, Picciolli I, Tel F, et al. Pandemic influenza A/H1N1 vaccine administered sequentially or simultaneously with seasonal influenza vaccine to HIV-infected children and adolescents. Vaccine. 2011;29(8):1677-82.

67. Kelen K, Ferenczi D, Jankovics I, Varga M, Molnar MZ, Sallay P, et al. H1N1 Vaccination in Pediatric Renal Transplant Patients. Transplantation Proceedings. 2011;43(4):1244-6.

68. Torii Y, Kimura H, Ochi N, Kaneko K, Ando H, Kiuchi T, et al. Immunogenicity of inactivated 2009 H1N1 influenza vaccine in pediatric liver transplant recipients. Vaccine. 2011;29(25):4187-9.

69. Okike OI, Yung C, Ladhania S, Oesera C, Bennett AM, Doerholt K, et al. The immunogenicity of a novel A (H1N1) vaccine in HIV-infected children. . Vaccine. In Press.

70. Kurz X, Domergue F, Slattery J, Segec A, Szmigiel A, Hidalgo-Simon A. Safety monitoring of Influenza A/H1N1 pandemic vaccines in EudraVigilance. Vaccine. 2011;29(26):4378-87.

71. WHO Collaborating Centre for International Drug Monitoring. UMC update on pandemic influenza vaccine safety monitoring March 2011. Available from: http://who-umc.org/graphics/24691.pdf.

72. Folkenberg M, Callréus T, Svanström H, Valentiner-Branth P, Hviid A. Spontaneous reporting of adverse events following immunisation against pandemic influenza in Denmark November 2009-March 2010. Vaccine. 2011;29(6):1180-4.

73. Vellozzi C, Broder KR, Haber P, Guh A, Nguyen M, Cano M, et al. Adverse events following influenza A (H1N1) 2009 monovalent vaccines reported to the Vaccine Adverse Event Reporting System, United States, October 1, 2009-January 31, 2010. Vaccine. 2010 Oct 21;28(45):7248-55. PubMed PMID: 20850534. eng.

74. Wu J, Xu F, Lu L, Lu M, Miao L, Gao T, et al. Safety and effectiveness of a 2009 H1N1 vaccine in Beijing. The New England journal of medicine. 2010 Dec 16;363(25):2416-23. PubMed PMID: 21158658. eng.

75. Liang X, Li L, Liu D, Li K, Wu W, Zhu B, et al. Safety of influenza A (H1N1) vaccine in postmarketing surveillance in China. The New England journal of medicine. 2011;364(7):638-47.

76. Parretta E, Ianniello B, Ferrazin F, Rossi F, Capuano A. Italian post-marketing surveillance for adverse event reports after MF59-adjuvanted H1N1v vaccination. Vaccine. 2011;29(20):3708-13.

77. Banzhoff A, Haertel S, Praus M. Passive surveillance of adverse events of an MF59-adjuvanted H1N1v vaccine during the pandemic mass vaccinations. Human vaccines. 2011 May 1;7(5). PubMed PMID: 21422814. Eng.

78. Mahajan D, Campbell-Lloyd S, Roomiani I, Menzies RI. NSW Annual Adverse Events Following Immunisation Report, 2009. New South Wales public health bulletin. 2010 Sep-Oct;21(9-10):224-33. PubMed PMID: 21211475. eng.

79. Cristiani C, Tuccori M, Pepe P, Sarteschi A, Maddalo F, Simonini G, et al. Safety of MF-59 adjuvanted vaccine for pandemic influenza: Results of the vaccination campaign in an Italian health district. Vaccine. 2011;29(18):3443-8.

80. Choe YJ, Cho H, Bae G-R, Lee J-K. Guillain-Barré syndrome following receipt of influenza A (H1N1) 2009 monovalent vaccine in Korea with an emphasis on Brighton Collaboration case definition. Vaccine. 2011;29(11):2066-70.

81. Fu C, Xu J, Tan H, Wang M. Serious Adverse Reactions Should be Further Monitored After Influenza A (H1N1) Vaccination Among Children: Postmarketing Experience With 570,000 Vaccinations Between October 2009 and August 2010 in Guangzhou, China. The Pediatric infectious disease journal. Jul;30(7):631-2. PubMed PMID: 21673552. eng.

82. Carvajal A, Ortega PG, Sainz M, Velasco V, Salado I, Arias LH, et al. Adverse events associated with pandemic influenza vaccines: comparison of the results of a follow-up study with those coming from spontaneous reporting. Vaccine. 2011 Jan 10;29(3):519-22. PubMed PMID: 21112311. eng.

83. Broos N, van Puijenbroek EP, van Grootheest K. Fever following immunization with influenza A (H1N1) vaccine in children: a survey-based study in the Netherlands. Drug Saf. 2010 Dec 1;33(12):1109-15. PubMed PMID: 21077701. eng.

84. EMA. Arepanrix : EPAR - Public assessment report

85. EMA. Pandemrix : EPAR - Public assessment report.

86. Lapphra K, Huh L, Scheifele DW. Adverse neurologic reactions after both doses of pandemic H1N1 influenza vaccine with optic neuritis and demyelination. The Pediatric infectious disease journal. Jan;30(1):84-6. PubMed PMID: 20686434. eng.

87. Tremblay ME, Closon A, D'Anjou G, Bussieres JF. Guillain-Barre syndrome following H1N1 immunization in a pediatric patient. The Annals of pharmacotherapy. 2010 Jul-Aug;44(7-8):1330-3. PubMed PMID: 20484170. eng.

88. Huang HH, Huang CC, Hsueh PY, Lee TJ. Bilateral sudden deafness following H1N1 vaccination. Otolaryngol Head Neck Surg. Dec;143(6):849-50. PubMed PMID: 21109091. eng.

89. McGuire-Wolfe C. Impact of organizational structure on vaccination of first responders: a case study. American journal of disaster medicine. Mar-Apr;6(2):71-2. PubMed PMID: 21678817. eng.

90. Kroeskop A, Lewis AB, Barril FA, Baribault KE. Gianotti-Crosti Syndrome After H1N1-INFLUENZA Vaccine. Pediatric dermatology. May 9. PubMed PMID: 21554384. Eng.

91. Pauwels C, Bulai Livideanu C, Maza A, Lamant L, Paul C. Cytophagic Histiocytic Panniculitis after H1N1 Vaccination: A Case Report and Review of the Cutaneous Side Effects of Influenza Vaccines. Dermatology (Basel, Switzerland).222(3):217-20. PubMed PMID: 21494027. eng.

92. Goldschmidt I, Pfister ED, Becker M, Häckl S, Bott OJ, Baumann U. Acceptance and Adverse Events of the 2009 H1N1 Vaccination in Immunosuppressed Pediatric Liver Transplant Recipients. The Journal of Pediatrics. 2011;158(2):329-33.

93. Meier S, Bel M, L'Huillier A, Crisinel P-A, Combescure C, Kaiser L, et al. Antibody responses to natural influenza A/H1N1/09 disease or following immunization with adjuvanted vaccines, in immunocompetent and immunocompromised children. Vaccine. 2011;29(19):3548-57.

94. EMA. European Medicines Agency advises of risk of fever in young children following vaccination with Pandemrix 4 December 2009. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Press_release/2009/12/WC500016894.pdf.

95. Prothro C, Kudish K, Fiellin M, Meek J, Tellman N, Milewski M, et al. Preliminary Results: Surveillance for Guillain-Barré Syndrome After Receipt of Influenza A (H1N1) 2009 Monovalent Vaccine --- United States, 2009--2010. MMWR Recomm Rep. 2010;June 2, 2010 / 59(Early Release);1-5.

96. Dieleman J, Romio S, Johansen K, Weibel D, Bonhoeffer J, Sturkenboom M. Guillain-Barre syndrome and adjuvanted pandemic influenza A (H1N1) 2009 vaccine: multinational case-control study in Europe. Bmj.343:d3908. PubMed PMID: 21750072. eng.

97. Grimaldi-Bensouda L, Alperovitch A, Besson G, Vial C, Cuisset JM, Papeix C, et al. Guillain-Barre Syndrome, Influenzalike Illnesses, and Influenza Vaccination During Seasons With and Without Circulating A/H1N1 Viruses. Am J Epidemiol. Jun 7. PubMed PMID: 21652600. Eng.

98. National Institute for Health and Welfare recommends discontinuation of Pandemrix vaccinations 25 Aug 2010 Available from: http://www.thl.fi/en_US/web/en/pressrelease?id=22930.

99. EMA. European Medicines Agency starts review of Pandemrix 27 August 2010. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Press_release/2010/08/WC500096005.pdf.

100. AFSSaPS. Cas de narcolepsie avec cataplexie notifiés en France à la suite d'une vaccination contre le virus de la grippe A/H1N1. Communiqué. . Available from: http://www.afssaps.fr/Infos-de-securite/Communiques-Points-presse/Cas-de-narcolepsie-avec-cataplexie-notifies-en-France-a-la-suite-d-une-vaccination-contre-le-virus-de-la-grippe-A-H1N1-Communique.

101. Dauvilliers Y, Montplaisir J, Cochen V, Desautels A, Einen M, Lin L, et al. Post-H1N1 narcolepsy-cataplexy. Sleep. 2010 Nov;33(11):1428-30. PubMed PMID: 21102981. Pubmed Central PMCID: 2954689.

102. THL. National Narcolepsy Task Force Interim Report. 31 January 2011.

103. MPA. A registry based comparative cohort study in four Swedish counties of the risk for narcolepsy after vaccination with Pandemrix - A first and preliminary report, by the Medical Products Agency 28 March 2011 Available from: http://www.lakemedelsverket.se/upload/nyheter/2011/PandemrixRegReport110328.pdf.

104. MPA. Occurrence of narcolepsy with cataplexy among children and adolescents in relation to the H1N1 pandemic and Pandemrix vaccinations - Results of a case inventory study by the MPA in Sweden during 2009-2010. 30 June 2011. Available from: http://www.lakemedelsverket.se/upload/nyheter/2011/Fallinventeringsrapport_pandermrix_110630.pdf.

105. Aita-Holmes C, Liacouras P, Wilson WO, Jr., Grant GT. Digital capture, design, and manufacturing of an extraoral device for a clarinet player with Bell's palsy. The Journal of prosthetic dentistry. 2015 May 16. PubMed PMID: 25985740.

106. PEI. Deutschlandweite Narkolepsie-Studie hat begonnen 11-05-2011. Available from: http://www.pei.de/DE/infos/fachkreise/pharmakovigilanz/narkolepsie-studie/narkolepsie-studie-inhalt.html.

107. Tsai TF, Crucitti A, Nacci P, Nicolay U, Cioppa GD, Ferguson J, et al. Explorations of clinical trials and pharmacovigilance databases of MF59((R))-adjuvanted influenza vaccines for associated cases of narcolepsy. Scand J Infect Dis. 2011 May 2. PubMed PMID: 21534891. Epub 2011/05/04. Eng.

108. EMA. Press release: European Medicines Agency recommends restricting use of Pandemrix 2011. Available from: http://www.ema.europa.eu/ema/index.jsp?curl=pages/news_and_events/news/2011/07/news_detail_001312.jsp&murl=menus/news_and_events/news_and_events.jsp&mid=WC0b01ac058004d5c1&jsenabled=true.

109. Poland GA. Methodologic reporting requirements for clinical trials: Advancing the science today and tomorrow. Vaccine. 2011;29(32):5087-9.

110. CHMP. Guideline on clinical evaluation of new vaccines 2006. Available from: www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003870.pdf.

111. ICH. International Conference On Harmonisation Of Technical Requirements For Registration Of Pharmaceuticals For Human Use. Clinical investigation of medicinal products in the pediatric population E11. 2000.

Chapter 5. Bell's palsy after influenza A(H1N1)pdm09 containing vaccines: a self-controlled case series

Leonoor Wijnans,

Caitlin N. Dodd,

Daniel Weibel,

Miriam Sturkenboom

In preparation

Abstract

Background

An association between AS03 adjuvanted pandemic influenza vaccine and the occurrence of Bell's palsy was found in a population based cohort study in Stockholm, Sweden. To evaluate this association in a different population we conducted a self-controlled case series in a primary health care database, THIN, in the United Kingdom. The aim of this study was to determine whether there was an increased risk of Bell's palsy following vaccination with any influenza vaccine containing A/California/7/2009 (H1N1)-like viral strains. Secondly, we looked whether risks were different following pandemic influenza A(H1N1)pdm09 vaccines and seasonal influenza vaccines containing the influenza A(H1N1)pdm09 strain.

Methods

The study population comprised all incident Bell's palsy cases between 1 June 2009 and 30 June 2013 identified in THIN. We determined the relative incidence (RI) of Bell's palsy during the 6 weeks following vaccination with either pandemic or seasonal influenza vaccine. All analyses were adjusted for seasonality and identified confounding variables.

Results

We found an incidence rate of Bell's palsy of 38.7 per 100,000 person years. Both acute respiratory infection (ARI) consultations and pregnancy were found to be confounders. When adjusted for seasonality, ARI consultations and pregnancies, the RI during the 42 days after vaccination with an influenza vaccine was 0.77 (95% CI: 0.65 – 0.91). The RI was not significantly different during the 42 days following seasonal vaccine (0.70, 95%CI: 0.58-0.84) or pandemic vaccine (0.67, 95%CI: 0.44-1.03). When stratified by vaccine and age it seems that the reduced RI is driven by the effect in persons in the age group between 45 and 65 and persons over 65 years old. Cases plotted relative to vaccination do not show a clear pattern of clustering of cases outside the risk period, or indeed evident troughs during the risk period.

Conclusion

In conclusion, we found no evidence for an increased incidence of Bell's palsy following seasonal influenza vaccination overall, nor for monovalent pandemic influenza vaccine in 2009. Conversely, a significantly reduced RI for Bell's palsy during the six weeks after vaccination with any influenza vaccine was found. It is unclear what could explain this reduced incidence.

Introduction

Bell's palsy is an idiopathic peripheral-nerve palsy affecting the cranial nerve and the most common cause for facial paralysis (1). It is characterized by an acute onset, unilateral facial paralysis, numbness or pain around the ear, a reduction in taste and hypersensitivity to sounds. The diagnosis is made after excluding other possible causes for facial paralysis, including congenital, genetic and acquired causes. Standard diagnostic criteria are not available (2). Bell's palsy resolves spontaneously without treatment in most patients within 6 months. Some patients experience long-term sequelae with incomplete return of facial motor function and synkinesis (1).

Bell's palsy has an incidence between 15 to 50 cases per 100,000 people per year (1, 3, 4). Men and women are affected equally. Bell's palsy can occur at any age, with the lowest incidence reported in children. The incidence has been reported to be highest in older people over the age of 70-75 (1, 3, 5) or in persons between the ages of 15 and 45 (6).

The exact cause of Bell's palsy is unknown. Inflammation is thought to play an important role in the aetiology of Bell's palsy (1) and an auto-immune aetiology has also been suggested (7). Known risk-factors for Bell's palsy include diabetes, a weakened immune system and pregnancy (1, 6).

Bell's palsy has been reported following inactivated influenza vaccines (8) and live attenuated influenza vaccine (9-11). An increased risk of Bell's palsy has been associated with an intranasal inactivated influenza vaccine which contained Escherichia coli heat-labile toxin as a mucosal adjuvant, resulting in the discontinuation of this vaccine (12). A signal of possible association between inactivated trivalent influenza vaccines and an increased risk of Bell's palsy was detected in the Vaccine Adverse Event Reporting System in the US (13). A large population based study in the UK did not find a relationship between inactivated influenza vaccines and Bell's palsy (14), as did a more recent study in the US which was limited to children (15). Due to the earlier associations and the unknown aetiology, Bell's palsy remains an adverse event of interest following influenza vaccination.

Following the 2009/2010 influenza A(H1N1) pandemic an association with Bell's palsy was found with an AS03 adjuvanted pandemic influenza vaccine, Pandemrix, in a population based cohort study in Stockholm, Sweden with a hazard ratio (HR) of 1.25, 95% CI 1.06 to 1.48 (16). The risk was highest during the first 6 weeks following vaccination (HR: 1.60, 1.25 to 2.05) and particularly present in those vaccinated early on in the campaign (HR: 1.74, 95% CI 1.16 to 2.59), which were those with more (severe) underlying co-morbidity. Similarly, a signal was detected for monovalent pandemic influenza vaccines used in the Vaccine Safety Datalink (VSD) Project in the US in adults over the age of 25 years with a relative risk of 1.6 (17). This last signal was not confirmed in a case centred analysis where an odds ratio was found of 1.1 (95% CI: 0.93 – 1.57). Finally, through passive surveillance

an increased risk of Bell's palsy during the 42 days after vaccination with pandemic (H1N1) 2009 vaccine was detected in Taiwan (18).

In order to evaluate the potential association of Bell's palsy following influenza A(H1N1)pdm09 vaccination in a different population, we conducted a self-controlled case series study. The aim of this study was to determine whether there was an increased risk of Bell's palsy following vaccination with any influenza vaccine containing A/California/7/2009 (H1N1)-like viral strains. Secondly, we looked whether risks were different following pandemic influenza A(H1N1)pdm09 vaccines and seasonal influenza vaccines containing the influenza A(H1N1)pdm09 strain.

Methods

We used a self-controlled case series (19, 20) design in The Health Improvement Network (THIN) database. THIN includes data from 562 general practices across the UK that have elected to participate. The population covered by THIN is representative of the UK population. The data in THIN have been validated for pharmacoepidemiology studies (21, 22).

Study population, study period and outcome

The study population comprised all incident Bell's palsy cases between 1 June 2009 and 30 June 2013 identified in THIN. A Bell's palsy case was defined as a person who had a consultation with a READ diagnosis code for Bell's palsy (see Appendix). Multiple cases per person were allowed. If diagnosis dates were more than 6 months apart, they constituted two separate cases. Considering the high predictive value of READ diagnosis codes for Bell's palsy (14) no validation on identified cases was performed.

Exposures

Influenza vaccination was identified through relevant codes (see Appendix) and recorded by year and vaccine type (seasonal or pandemic), including seasonal influenza vaccination for 2009/2010, pandemic influenza vaccination, seasonal influenza vaccination for 2010/2011, seasonal influenza vaccination for 2011/2012 and seasonal influenza vaccination for 2012/2013. In the UK both Pandemrix and Celvapan were used during the 2009-2010 influenza A(H1N1) pandemic, and information on brand was retrieved if available. Moreover, during the 2009-2010 season, persons could have received both a seasonal vaccine and a pandemic influenza vaccine. In theory these could have been given on the same day or close together making it difficult to attribute the risk to either. Considering the study by Stowe *et al* (14) no increased risk was expected for the seasonal vaccine, therefore this was disregarded in the primary analysis.

As each person serves as its own control, stable confounders such as gender, genetics, socio-economic status, frailty and severity of underlying disease are controlled for. Considering the short observation period no age effect was expected. Covariates that were considered as potential confounders were calendar time, occurrence of acute respiratory infections, influenza diagnoses, and pregnancy. ARI episodes and influenza diagnoses were identified by relevant READ codes (see Appendix). Consultations for ARI or influenza occurring within 28 days of a previous consultation were excluded because they were considered to be likely related to the same episode. Pregnancies were identified by the date of delivery (see Appendix for codes). The risk period was the 270 days (9 months) before the date of delivery.

Analysis

We described the population using means and standard deviations for all continuous variables, counts and percentages for categorical variables.. Descriptive statistics were compared between vaccinated cases (anytime) and unvaccinated cases using t-tests for continuous variables and chi-squared tests for categorical variables. Associations between pregnancy, ARI consultations, influenza diagnoses and Bell's palsy or influenza vaccination were determined.

We determined the RI of Bell's palsy during the 6 weeks following vaccination with either pandemic or seasonal influenza vaccine by using a conditional Poisson regression. The risk period of interest was from D1 to D42, as this was the period with the highest risk found by Bardage et al (16). As vaccination could be delayed following an episode of Bell's palsy, the 14 days prior to vaccination were treated as a separate risk period in the analysis. D0 was also regarded as a separate risk period as opportunistic recording would hamper establishing a temporal relation. The remaining periods served as the reference period. All univariate associations with risk factors that were significant, were included for adjustment in addition to calendar time (by quarter).

Relative incidences were calculated separately for pandemic and for seasonal influenza vaccines, and for each season (vaccination period). During the 2009/2010 influenza A(H1N1) pandemic two brands of pandemic vaccines were used in the UK, Celvapan and Pandemrix. As less than 0.1% of vaccinated persons received Celvapan during the 2009/2010 pandemic (23), we consider the findings with pandemic vaccines in our study to be applicable to Pandemrix. Age and sex specific relative incidences of Bell's palsy within 6 weeks of influenza vaccination were calculated.

To further account for the risk of deferral of vaccination after receiving a diagnosis of Bell´s palsy, we performed sensitivity analyses in which only the observation time after vaccination was considered. All analyses were conducted using SAS 9.2.

Results

We identified 6381 Bell's palsy cases in 6288 persons with a median follow up of 1489 days. Of these cases, 6198 persons had a single diagnosis code for Bell's palsy, 87 persons had two episodes and three persons had three episodes of Bell's palsy during the study period. The crude incidence rate was 38.7 per 100,000 person years. The distribution of Bell's palsy dates relative to vaccination dates is presented in figure 5.1. Of cases, 14% received the monovalent pandemic influenza vaccine whilst seasonal vaccines were received by 24 to 28% of cases dependent on the year.

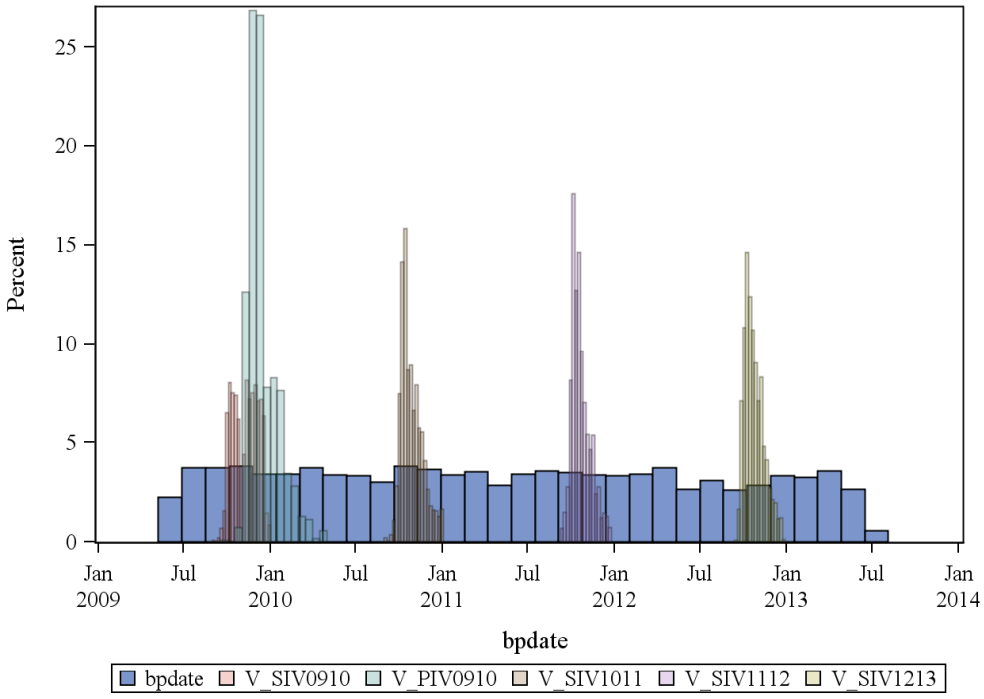


Figure 5.1 Bell's palsy cases by month relative to vaccination dates for the study period.

The characteristics of the cases by vaccination status (seasonal and pandemic) are presented in table 5.1. Those who received at least one seasonal influenza vaccine tended to be older and were more likely to be female. Those who received pandemic influenza vaccine were also more likely to be older. Males and females were equally likely to be exposed to pandemic influenza vaccine (Table 5.1). Thirty-five percent (2232 persons) experienced at least one episode of ARI during follow-up, whereas only 3.5% (220 cases) had a recorded influenza diagnosis, 155 women had one pregnancy (4.85%) – three women had two pregnancies (0.09%).

Pregnancy was associated with Bell's palsy (Relative Risk (RR) 1.75, 95% CI 1.19 – 2.57) and with influenza vaccination (RR 5.1, 95% CI 3.25 – 7.82). ARI was strongly associated with Bell's palsy on the day of consultation (RR 6.99, 95% CI: 4.39- 11.13), but also in the 7 days following a consultation for ARI (RR 2.44, 95% CI 1.81 – 3.30). In addition, an episode of ARI was also associated with vaccination - with an increased risk of vaccination on the day of consultation for ARI (RR 2.93, 95% CI 1.58 – 5.46) and a reduced risk of vaccination during the week following a consultation for ARI (RR 0.50, 95% CI 0.28 – 0.89). The distribution of ARI dates relative to vaccination dates over calendar time is given in figure 5.2.

Table 5.1 Main characteristics of Bell's palsy cases by vaccination status during follow-up (each case appears in both vaccine type groups)

	Received seasonal vaccine					Received pandemic vaccine				
	Yes		No			Yes		No		
	n=2408		n= 3880		*p-value*	n=901		n= 5387		*p-value*
Demographics										
Female (n (%))	1313	(54.53)	1881	(48.48)	<.0001	454	(50.86)	2740	(50.39)	0.79
Mean age (SD)[1]	58.59	(18.07)	36.56	(16.51)	<.0001	56.75	(19.64)	43.03	(19.61)	<.0001
Age (n (%))										
<45 yrs	532	(22.09)	2685	(69.20)		212	(23.53)	3005	(55.78)	
45 – 65 yrs	897	(37.25)	1052	(27.11)		357	(39.62)	1592	(29.55)	
>65 yrs	979	(40.66)	143	(3.69)	<.0001	332	(36.85)	790	(14.66)	<.0001

[1] At start follow up

There was no statistical evidence of an association between Bell's palsy and a consultation for influenza (RR 1.69, 95% CI 0.54 – 5.32). Although the relative incidence of Bell's palsy was raised in the 7 days following an influenza consultation, this was not significant (RR 2.41, 95% CI 0.76 - 7.58, 257 cases).

The crude RI of Bell's palsy during the 42 days after vaccination with an influenza vaccine was 0.88 (95% CI: 0.74 – 1.04). On the day of vaccination the relative incidence was 2.15 (95% CI: 1.12 – 4.14). The RI was slightly reduced in the fourteen days prior to vaccination, 0.70 (95% CI: 0.51 – 0.96).

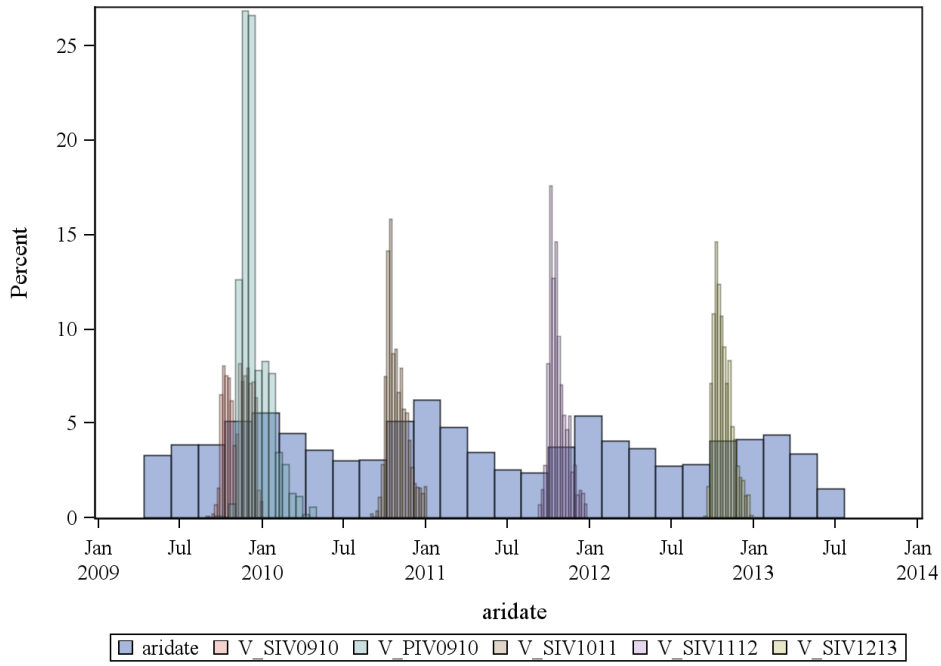


Figure 5.2 ARI cases by month relative to vaccination dates for the study period

When adjusted for seasonality, episodes of ARI and pregnancies, the RI during the 42 days after vaccination with an influenza vaccine was reduced from a crude of 0.88 to 0.77 (95% CI: 0.65 – 0.91). At the date of vaccination the RI reduced from the crude of 2.15 to 1.88 (95% CI: 0.98 - 3.62), during the 14 days preceding vaccination the RI reduced from a crude of 0.70 to 0.63 (95% CI: 0.46 - 0.86). When considering the type of vaccine (i.e. seasonal vs pandemic) the RI was not significantly different during the 42 days following seasonal vaccine (0.70, 95%CI: 0.58-0.84) or pandemic vaccine (0.67, 95%CI: 0.44-1.03). Restricting the analysis to cases without any ARI episodes, showed that the adjusted RI was 0.85 (95% CI: 0.57 – 1.24). The RI during the 42 days following influenza vaccination (any) was not significantly different in women (0.79, 95% CI: 0.62 – 1.01) compared to men (0.58, 95% CI: 0.46 – 0.74).

The relative incidence of Bell's palsy within 42 days of influenza vaccine stratified by vaccine and age can be found in table 5.2. A significantly reduced RI following vaccination is seen with the 2010-2011 and 2011-2012 vaccine. When considering the type of vaccine (i.e. seasonal vs pandemic) the RI was not significantly different during the 42 days following seasonal vaccine (0.70, 95%CI: 0.58-0.84) or pandemic vaccine (0.67, 95%CI: 0.44-1.03).

Table 5.2 Age and season specific relative incidences (95% CI) of Bell's palsy within 42 days of influenza vaccination

Risk Period	All age groups			Age <45			Age 45 to 64			Age 65 +		
	N	RI	95% CI	N	RI	95% CI	N	RR	95% CI	N	RR	95% CI
Any vaccine												
Day -14 to -1	41	0.63	0.46-0.86	7	0.72	0.34-1.54	15	0.19	0.12-0.32	19	0.44	0.28-0.70
Day 0	9	1.88	0.98-3.62	2	2.77	0.69-11.14	2	0.33	0.08-1.31	5	1.52	0.63-3.66
Day 1 to 42	154	0.77	0.65-0.91	25	0.86	0.57-1.30	45	0.18	0.13-0.24	84	0.60	0.48-0.76
2009 Pandemic												
Day -14 to -1	2	0.15	0.04-0.61	1	0.32	0.04-2.34	1	0.14	0.02-1.04	0	NA	NA
Day 0	0	NA	NA	0	NA	NA	0	NA	NA	0	NA	NA
Day 1 to 42	24	0.67	0.44-1.03	3	0.36	0.11-1.17	9	0.52	0.25-1.05	12	0.85	0.45-1.59
Season 2010-2011												
Day -14 to -1	7	0.23	0.11-0.49	0	NA	NA	1	0.05	0.01-0.38	6	0.22	0.10-0.50
Day 0	2	0.86	0.21-3.44	0	NA	NA	1	0.67	0.10-4.80	1	0.49	0.07-3.50
Day 1 to 42	53	0.54	0.40-0.72	11	1.42	0.65-3.08	16	0.25	0.15-0.41	26	0.32	0.21-0.48
Season 2011-2012												
Day -14 to -1	17	0.50	0.31-0.82	3	1.02	0.29-3.58	7	0.33	0.15-0.70	7	0.24	0.11-0.50
Day 0	1	0.37	0.05-2.62	1	4.31	0.57-32.80	0	NA	NA	0	NA	NA
Day 1 to 42	45	0.39	0.28-0.53	5	0.48	0.18-1.31	14	0.20	0.12-0.35	26	0.27	0.18-0.40
Season 2012-2013												
Day -14 to -1	8	0.51	0.25-1.06	3	1.31	0.37-4.67	2	0.11	0.03-0.46	3	0.32	1.10-1.04
Day 0	3	2.35	0.74-7.43	1	4.97	0.65-38.01	1	0.57	0.08-4.09	1	1.30	0.18-9.42
Day 1 to 42	44	0.75	0.52-1.06	9	0.98	0.43-2.24	9	0.12	0.06-0.23	26	0.72	0.45-1.17

Adjusted for seasonality by quarter, ARI consultations, and pregnancy in strata that contained pregnant cases

In all age groups, a reduced RI is observed the 14 days prior to vaccination. In persons between the ages of 45 and 65 years a significantly reduced RI is observed during the 42 days after vaccination.

As we detected a significantly reduced RI following vaccination, we considered the age stratified RIs per vaccine. The results are also presented in table 5.2. When stratified by vaccine and age it seems that the reduced RI is driven by the effect in persons in the age group between 45 and 65 and persons over 65 years old. To better understand this reduced RI we further stratified by sex for the vaccines and age-groups for which we detected an overall reduced RI in an exploratory analysis. This analysis suggested that in persons aged between 45 and 65 years the reduced risk is significant in women only. In persons over 65 it is significant in both men and women.

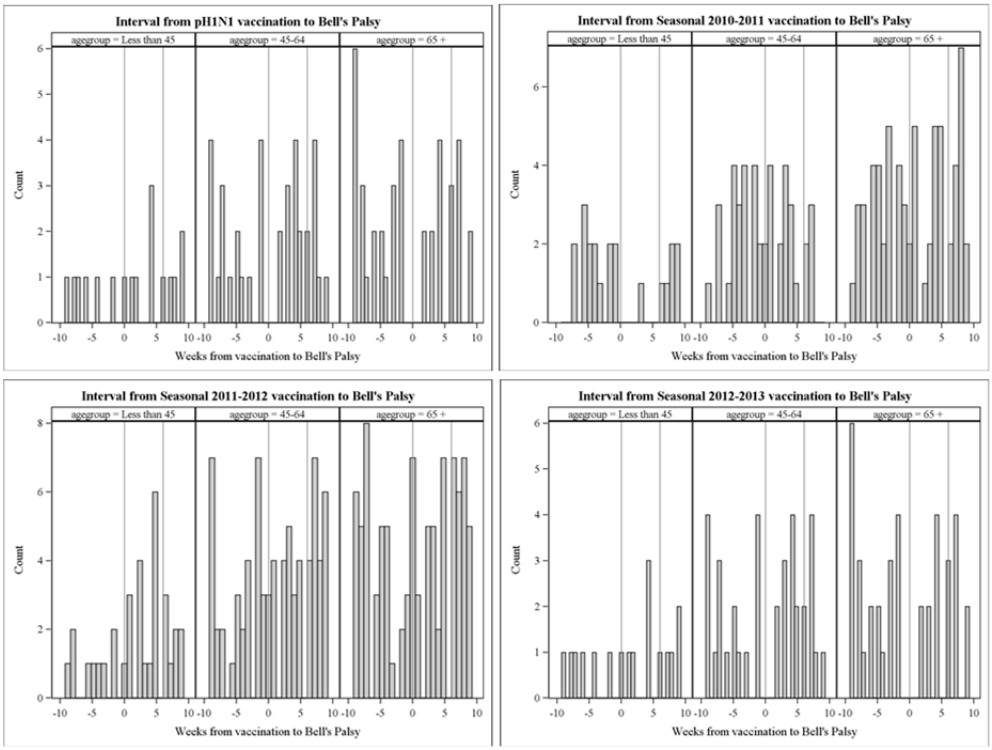


Figure 5.2 Bell's palsy consultations relative to vaccination for the 2009-2010 pandemic vaccines (a) and the 2010-2011 (b), 2011-2012 (c) and 2012-2013 (d) seasonal influenza vaccines per age group in 1 week intervals.

A case plot showing cases relative to vaccination in the three age strata is shown in figure 5.3. From the graphs there is no clear pattern of troughs of cases during the 6 weeks after vaccination, or clusters of cases outside this period.

In the analysis where only observation time after vaccination was included, exposure to any vaccine with control for ARI and seasonality produced a RI of 0.87 (95% CI: 0.71, 1.08).

Discussion

We found no evidence for an increased incidence of Bell's palsy consultations following seasonal influenza vaccination overall, nor for monovalent pandemic influenza vaccine in 2009. Therefore our study does not confirm the results identified by Bardage et al (16) in Sweden. Conversely, we found a significantly reduced RI for Bell's palsy consultations during the six weeks after vaccination with any influenza vaccine. When adjusted for seasonality, episodes of ARI and pregnancies, the RI during the 42 days after vaccination with an influenza vaccine was 0.77 (95% CI: 0.65 – 0.91). In further stratifications it became clear that this was driven by women aged 45-64 years of age and persons aged 65 years or more (table 5.3). Bell's palsy is a syndrome for which the exact cause is unclear. As a result it could have multiple triggers, of which – considering the hypothetical autoimmune aetiology – influenza and influenza vaccination could possibly be one. Clusters of Bell's palsy cases have been reported following influenza vaccination in the past. An association was reported for Bell's palsy and Pandemrix, an AS03 adjuvanted pandemic influenza vaccine in Sweden (16), and a signal was reported from Taiwan (18). In this study, we evaluated the risk of Bell's palsy following vaccination with influenza vaccines containing A/California/7/2009 (H1N1)-like viral strains, including pandemic vaccines, in the UK.

It is unclear what could explain this reduced incidence. The cases plotted relative to vaccination do not show a pattern of clustering of cases outside the risk period, or indeed evident troughs during the risk period. Nonetheless, the reduced RI is consistently seen in persons aged 45 to 64 years following all seasonal influenza vaccines and for all seasonal vaccines except the 2012-2013 vaccine for persons aged 65 and older. We cannot exclude a potential protective effect of influenza vaccination on Bell´s palsy. Such a protective effect could be in line with the increased RI of Bell's palsy observed during the 7 days after a consultation for ARI. However, it is not evident why this would not affect persons under the age of 45 similarly as those over the age of 45. Other potential explanations include an unmeasured time-varying confounder, for example health seeking behaviour which changes after vaccination. We found no evidence that health care seeking behaviour might change following vaccination from other vaccine safety studies reported in the literature. This would be interesting to follow up in future studies seeing that if health seeking behaviour did change following vaccination in certain age/sex strata this could have an impact on vaccine safety studies.

One of the more restrictive assumptions of the SCCS method is that the distribution of exposure after a certain time must be independent of the event history prior to that time (19). Bell's palsy is not a contra-indication for influenza vaccination. Nonetheless, it is

possible that people will delay vaccination after Bell's palsy, which can represent a violation of the assumption of the SCCS. Generally, this delay in vaccination would bias RI estimates upward by producing a scarcity of cases in control intervals. In our main analysis we fixed the 14 days prior to vaccination as a separate risk period. The reduced incidence found during this risk period demonstrates that persons will delay vaccination when diagnosed with Bell's palsy. We assumed that a 14 day period would be sufficient to exclude any bias resulting from this delay. As evidenced by the sensitivity analysis which only considered observation time after vaccination and produced estimates very similar to those produced with a 14-day low risk period, this 14-day period was sufficient to control for a potential healthy vaccinee effect.

A second restrictive assumption of the SCCS method is that events are either recurrent and independent within individuals or not-recurrent and uncommon (19). Bell's palsy can recur, however this is rare (1) and is reported to do after a latency period of approximately 10 years (7, 14). In our study we considered any second consultation of Bell's palsy within 6 months to belong to a single episode. Still, we found that 1.4% of persons had more than one episode within our relatively short observation period. As recurrent events are rare the bias is negligible. Only considering first episodes, the RI is 0.78 (95% CI: 0.65 - 0.92) compared to 0.77 (95% CI: 0.65 - 0.91) when recurrent episodes are also considered.

Our study has limitations that could impact the observed results.

Whilst the SCCS inherently deals with measured and unmeasured fixed confounding variates, time varying confounders will still need to be measured and adjusted for. We adjusted for seasonality by quarter, consultations for ARI and pregnancies, as these factors were identified as confounders in our study. We could not adjust for time varying factors that were not measured. An increased risk of Bell's palsy is seen on the day of influenza vaccination, which is in line with the findings of Stowe et al (14), and is most likely related to opportunistic recording of cases, hence D0 was excluded from the risk interval.

Finally, not all persons who develop Bell's palsy will consult their GP therefore incomplete reporting of cases is likely in our study. If reporting was differential by vaccination status, thus if persons who develop Bell's palsy shortly following vaccination were more likely or less likely to consult their GP compared to persons who develop Bell's palsy at other time points, this would have introduced bias in this study.

In conclusion, our study did not provide evidence of an increased risk of Bell's palsy following vaccination with any influenza vaccine containing A/California/7/2009 (H1N1)-like viral strains, either pandemic or seasonal vaccines.

References

1. Gilden DH. Clinical practice. Bell's Palsy. The New England journal of medicine. 2004 Sep 23;351(13):1323-31. PubMed PMID: 15385659. Epub 2004/09/24. eng.

2. Rath B, Linder T, Cornblath D, Hudson M, Fernandopulle R, Hartmann K, et al. All that palsies is not Bell's -the need to define Bell's palsy as an adverse event following immunization. Vaccine. 2007 Dec 21;26(1):1-14. PubMed PMID: 18037542.

3. Rowlands S, Hooper R, Hughes R, Burney P. The epidemiology and treatment of Bell's palsy in the UK. Eur J Neurol. 2002 Jan;9(1):63-7. PubMed PMID: 11784378. Epub 2002/01/11. eng.

4. Rowhani-Rahbar A, Baxter R, Rasgon B, Ray P, Black S, Klein JO, et al. Epidemiologic and clinical features of Bell's palsy among children in Northern California. Neuroepidemiology. 2012;38(4):252-8. PubMed PMID: 22678408.

5. Morales DR, Donnan PT, Daly F, Staa TV, Sullivan FM. Impact of clinical trial findings on Bell's palsy management in general practice in the UK 2001-2012: interrupted time series regression analysis. BMJ open. 2013;3(7). PubMed PMID: 23864211. Pubmed Central PMCID: 3717449.

6. Holland NJ, Weiner GM. Recent developments in Bell's palsy. BMJ. 2004 Sep 4;329(7465):553-7. PubMed PMID: 15345630. Pubmed Central PMCID: 516110. Epub 2004/09/04. eng.

7. Greco A, Gallo A, Fusconi M, Marinelli C, Macri GF, de Vincentiis M. Bell's palsy and autoimmunity. Autoimmunity reviews. 2012 Dec;12(2):323-8. PubMed PMID: 22684016.

8. Chou CH, Liou WP, Hu KI, Loh CH, Chou CC, Chen YH. Bell's palsy associated with influenza vaccination: two case reports. Vaccine. 2007 Apr 12;25(15):2839-41. PubMed PMID: 17084492. Epub 2006/11/07. eng.

9. Baxter R, Toback SL, Sifakis F, Hansen J, Bartlett J, Aukes L, et al. A postmarketing evaluation of the safety of Ann Arbor strain live attenuated influenza vaccine in adults 18-49 years of age. Vaccine. 2012 Apr 26;30(20):3053-60. PubMed PMID: 22425787.

10. Baxter R, Toback SL, Sifakis F, Hansen J, Bartlett J, Aukes L, et al. A postmarketing evaluation of the safety of Ann Arbor strain live attenuated influenza vaccine in children 5 through 17 years of age. Vaccine. 2012 Apr 19;30(19):2989-98. PubMed PMID: 22386746.

11. Izurieta HS, Haber P, Wise RP, Iskander J, Pratt D, Mink C, et al. Adverse events reported following live, cold-adapted, intranasal influenza vaccine. JAMA : the journal of the American Medical Association. 2005 Dec 7;294(21):2720-5. PubMed PMID: 16333007. eng.

12. Mutsch M, Zhou W, Rhodes P, Bopp M, Chen RT, Linder T, et al. Use of the inactivated intranasal influenza vaccine and the risk of Bell's palsy in Switzerland. The New England journal of medicine. 2004 Feb 26;350(9):896-903. PubMed PMID: 14985487.

13. Zhou W, Pool V, DeStefano F, Iskander JK, Haber P, Chen RT, et al. A potential signal of Bell's palsy after parenteral inactivated influenza vaccines: reports to the Vaccine Adverse Event Reporting System (VAERS)--United States, 1991-2001. Pharmacoepidemiology and drug safety. 2004 Aug;13(8):505-10. PubMed PMID: 15317028.

14. Stowe J, Andrews N, Wise L, Miller E. Bell's palsy and parenteral inactivated influenza vaccine. Hum Vaccin. 2006 May-Jun;2(3):110-2. PubMed PMID: 17012908. Epub 2006/10/03. eng.

15. Rowhani-Rahbar A, Klein NP, Lewis N, Fireman B, Ray P, Rasgon B, et al. Immunization and Bell's palsy in children: a case-centered analysis. American journal of epidemiology. 2012 May 1;175(9):878-85. PubMed PMID: 22411861.

16. Bardage C, Persson I, Ortqvist A, Bergman U, Ludvigsson JF, Granath F. Neurological and autoimmune disorders after vaccination against pandemic influenza A (H1N1) with a monovalent adjuvanted vaccine: population based cohort study in Stockholm, Sweden. BMJ. 2011;343:d5956. PubMed PMID: 21994316. Pubmed Central PMCID: 3192001. Epub 2011/10/14. eng.

17. Lee GM, Greene SK, Weintraub ES, Baggs J, Kulldorff M, Fireman BH, et al. H1N1 and seasonal influenza vaccine safety in the vaccine safety datalink project. American journal of preventive medicine. 2011 Aug;41(2):121-8. PubMed PMID: 21767718.

18. Huang WT, Huang WI, Huang YW, Hsu CW, Chuang JH. The reporting completeness of a passive safety surveillance system for pandemic (H1N1) 2009 vaccines: a capture-recapture analysis. Vaccine. 2012 Mar 9;30(12):2168-72. PubMed PMID: 22265861.

19. Whitaker HJ, Farrington CP, Spiessens B, Musonda P. Tutorial in biostatistics: the self-controlled case series method. Statistics in medicine. 2006 May 30;25(10):1768-97. PubMed PMID: 16220518.

20. Farrington CP. Relative incidence estimation from case series for vaccine safety evaluation. Biometrics. 1995;51:228-35.

21. Blak BT, Thompson M, Dattani H, Bourke A. Generalisability of The Health Improvement Network (THIN) database: demographics, chronic disease prevalence and mortality rates. Informatics in primary care. 2011;19(4):251-5. PubMed PMID: 22828580.

22. Lewis JD, Schinnar R, Bilker WB, Wang X, Strom BL. Validation studies of the health improvement network (THIN) database for pharmacoepidemiology research. Pharmacoepidemiology and drug safety. 2007 Apr;16(4):393-401. PubMed PMID: 17066486.

23. Department of Health. Pandemic H1N1 (swine) influenza vaccine uptake amongst patient groups in primary care in England 2009/10. In: DH/HPA28, editor. Oct 2010.

Chapter 6. The incidence of narcolepsy in Europe: before, during, and after the influenza A(H1N1)pdm09 pandemic and vaccination campaigns.

Leonoor Wijnans, Coralie Lecomte, Corinne de Vries, Daniel Weibel, Cormac Sammon, Anders Hviid, Henrik Svanström, Ditte Mølgaard-Nielsen, Harald Heijbel, Lisen Arnheim Dahlström, Jonas Hallgren, Par Sparen, Poul Jennum, Mees Mosseveld, Martijn Schuemie, Nicoline van der Maas, Markku Partinen, Silvana Romio, Francesco Trotta, Carmela Santucci, Angelo Menna, Giuseppe Plazzi, Keivan Kaveh, Moghadam, Salvatore Ferro, Gert Jan Lammers, Sebastiaan Overeem, Kari Johansen, Piotr Kramarz, Jan Bonhoeffer, Miriam CJM Sturkenboom

Published in: Vaccine 31 (2013) 1246-54.

Abstract

Background

In August 2010 reports of a possible association between exposure to AS03 adjuvanted pandemic A(H1N1)pdm09 vaccine and occurrence of narcolepsy in children and adolescents emerged in Sweden and Finland. In response to this signal, the background rates of narcolepsy in Europe were assessed to rapidly provide information for signal verification.

Methods

We used a dynamic retrospective cohort study to assess the narcolepsy diagnosis rates during the period 2000-2010 using large linked automated health care databases in six countries: Denmark, Finland, Italy, the Netherlands, Sweden and the United Kingdom.

Results

Overall, 2,608 narcolepsy cases were identified in almost 280 million person years (PY) of follow up. The pooled incidence rate was 0.93 (95% CI: 0.90 – 0.97) per 100,000 PY. There were peaks between 15-30 year of age (women>men) and around 60 years of age. In the age group 5-19 years olds rates were increased after the start of pandemic vaccination compared to the period before the start of campaigns, with rate ratios of 1.9 (95% CI: 1.1 -3.1) in Denmark , 6.4 (95% CI: 4.2 - 9.7) in Finland (RR:) and 7.5 (95% CI: 5.2 - 10.7) in Sweden. Cases verification in the Netherlands had a significant effect on the pattern of incidence over time.

Conclusions

The results of this incidence study provided useful information for signal verification on a population level. The safety signal of increased narcolepsy diagnoses following the start of the pandemic vaccination campaign as observed in Sweden and Finland could be observed with this approach. An increase in narcolepsy diagnoses was not observed in other countries, where vaccination coverage was low in the affected age group, or did not follow influenza A(H1N1)pdm09 vaccination. Patient level analyses in these countries are being conducted to verify the signal in more detail.

Introduction

Narcolepsy

Narcolepsy is a disabling chronic sleep disorder that interferes severely with normal daily activities, interpersonal relations, education, and career opportunities (1, 2). The classic clinical syndrome consists of excessive daytime sleepiness (EDS), cataplexy, sleep paralysis, hypnagogic hallucinations and disrupted nocturnal sleep. While cataplexy is considered pathognomonic for narcolepsy, it is not always part of the clinical presentation. In contrast to narcolepsy with cataplexy, narcolepsy without cataplexy is probably not a single disease entity. Regardless of the variety and extent of symptoms at presentation, further laboratory investigations to confirm the diagnosis are state of the art today. Nocturnal polysomnography and multiple sleep latency test (MSLT) are performed most frequently. However, the specificity of these investigations is limited (3). Determining very low or undetectable levels of the neuropeptide hypocretin-1 (also called orexin A) in the cerebrospinal fluid levels is a novel diagnostic approach with high specificity for the diagnosis of narcolepsy with and without cataplexy, and high sensitivity for narcolepsy with cataplexy although less so for narcolepsy without cataplexy (4). Hypocretin-1 testing is a recent development, however not yet standardized and not widely available. Furthermore, HLA DQB1*0602 is strongly associated with but not specific for the narcolepsy diagnosis, as it is present in 12-38% of the normal European population, depending on the genetic origin(5). As a consequence, many patients are diagnosed long after onset of symptoms, with delays ranging from 1 to 60 years (6, 7).

The narcolepsy diagnosis is particularly challenging in children due to a wide range of daytime sleep requirements, which are often considered normal in this age group. In addition, cataplexy in children may present with atypical features (i.e. absence of triggering emotions, a semi-permanent state of facial muscle weakness on which partial or complete cataplectic attacks are superimposed), first described in 2008 (8) Moreover, there is currently a lack of objective diagnostic criteria specific to the paediatric phenotype (1). As a result, narcolepsy in childhood is possibly an underdiagnosed disease (9).

The estimated prevalence of narcolepsy in Western countries is 20–50 per 100,000 (2, 10, 11). It is thought to affect men and women equally, although a male predominance has been found in some studies (12). More than 50% of narcolepsy cases appear to exhibit symptom onset before 18 years of age, beginning typically in adolescence (13-15). Bimodal peaks of onset have been reported, with one peak around 15 years of age (range 10-19 years) and another around 35 years(13). In the past, diagnoses were typically not established prior to early adulthood in the majority of cases (6).

Little is known about the aetiology of narcolepsy. The strong association between hypocretin-1 deficiency and the presence of the Human Leukocyte Antigen (HLA) subtype DQB1*0602(16) has led to the hypothesis that an autoimmune process may lead to loss of hypocretin producing neurons. However, as only very few carriers of this allele develop narcolepsy, other factors must contribute to its development. Given the age at onset of symptoms it is thought that an exposure which could trigger narcolepsy would occur during or before adolescence (11). Some studies have focused specifically on environmental factors and disease inducing or promoting health events preceding clinical manifestation of narcolepsy. These types of studies are typically hampered by the considerable uncertainties around aetiology and pathogenesis of narcolepsy as well as the associated methodological difficulties, such as under-diagnosis and recall-bias (11). Recently, streptococcal infection markers and antibodies against the protein Tribbles homolog 2 have been found to be associated with narcolepsy (17-19). In addition, a study from China reported an increase in narcolepsy in children following influenza A(H1N1)pdm09 infection (20). To the best of our knowledge, no association between vaccination and narcolepsy has been described prior to 2010.

Influenza A(H1N1)pdm09 vaccination campaigns

Eight influenza A(H1N1)pdm09 vaccines [Cantgrip (Cantacuzino), Celvapan (Baxter), Celltura (Novartis), Fluval P (Omnivest), Focetria (Novartis), Pandemrix (GSK), Panenza (Sanofi Pasteur) and PanvaxH1N1 (CSL)] were licensed within the EU/EEA area during the 2009 pandemic. International recommendations on which groups should be offered vaccination and in what order came from the Strategic Advisory Group of Experts (SAGE) Committee of the World Health Organization (WHO) and the EU Health Security Committee, which were taken into account in national decisions on priority groups. AS03-adjuvanted Pandemrix® was the most used vaccine in Europe (21).

Two EU Member States participating in this study (Finland and Sweden) recommended vaccines to their entire population while other Member States (Denmark, Italy, the Netherlands and the United Kingdom) recommended vaccines only to selected risk groups, notably individuals with chronic disorders and in case of the Netherlands also children younger than five years of age (21).

Influenza A(H1N1)pdm09 vaccine safety signal

In August 2010 reports of a possible association between exposure to AS03 adjuvanted influenza A(H1N1)pdm09 vaccine and occurrence of narcolepsy commonly with cataplexy in children and adolescents emerged in both Sweden and Finland. This led to the discontinuation of the general recommendation of this vaccine in these countries and a review of the vaccine by the European Medicines Agency (EMA). Subsequently, cases

following influenza A(H1N1)pdm09 vaccination and infection were reported from France, Germany (22) and Norway (23) Canada and the US (24, 25). Since then more cases have been identified, mostly in children and adolescents in the initial two signalling countries. A registry study in Finland detected a 12.7-fold increase in narcolepsy in association with the AS03 adjuvanted influenza A(H1N1)pdm09 vaccine (26, 27). Two Swedish studies have detected a relative risks of 4.2 in a rapid cohort study (28) and 6.6 in a case series study (29) . More recently, a 13-fold increase in risk of narcolepsy was found associated with Pandemrix vaccination in a cohort study in Ireland (30) and a case control study in France found an increased risk of 4.6 (31). The Irish and French studies both also identified an increased risk in persons over 19 years of age, while this has not been reported from Sweden and Finland. As of August 2012 more than 600 cases of narcolepsy following influenza A(H1N1)pdm09 vaccination have been reported to the Eudravigilance database, of which more than 100 are reported to have occurred in adults (32).

In response to the signal from Sweden and Finland, a study into the background rates of narcolepsy in Europe was started by the VAESCO (Vaccine Adverse Events Surveillance and Communication) consortium on request of and in collaboration with the European Centre for Disease Prevention and Control (ECDC). The aim of the study was to provide information to ECDC, the EMA and national agencies for signal verification and public health decision making. Here we present the main findings of this study.

Materials and Methods

Design and setting

A dynamic retrospective cohort study was used to assess the rates of narcolepsy diagnosis during the period 2000-2010 in six countries with large linked automated health care databases. Participating countries were: Denmark, Finland, Italy (Tuscany and Emilia Romagna regions), the Netherlands (NL), Sweden, and the United Kingdom (UK). Although Norway provided data, insufficient data history was available to exclude prevalent cases. Therefore incidence rates could not be calculated and the Norwegian data could not be considered for this study.

Databases could be included if they comprised electronic health records for a defined population, the observation time of each captured individual could be determined, the occurrence and date of narcolepsy diagnosis could be identified during the observation time, and the outcomes file could be linked to the respective population file. Table 6.1 describes the key characteristics of the databases which can be broadly categorized into two types: population-based medical record databases from general practitioners (GPs) (the Netherlands, the United Kingdom) (33) and regional or national administrative databases (Denmark, Finland, Emilia Romagna and Tuscany regions in Italy, and Sweden).

Table 6.1 Overview of databases and codes used for the calculation of incidence rates of narcolepsy diagnosis

Data source		Type of data	Disease coding schemes	Codes used
Medical record databases				
United Kingdom (GPRD) (34)	3.5 million	population based medical records (GP & specialist diagnoses)	READ	F27.00, F270.00, F271.00, F27z.00
Netherlands (IPCI) (33)	1 million	population based medical records (GP & specialist diagnoses)	narratives	GP/spec./hosp. diagnoses (text & validation)
Administrative databases national				
Denmark(35)	5.5 million	inpatient and outpatient diagnoses	ICD10	G47.4 (primary)
Sweden(36)	9 million	inpatient and outpatient diagnoses	ICD10	G47.4 (primary)
Finland	5 million	inpatient and outpatient diagnoses	ICD10	G47.4 (primary/secondary)
Administrative databases regional				
Italy Emilia Romagna	3 million	inpatient diagnoses	ICD9-CM	347.00, 347.01, 347.10, 347.11
Italy Tuscany	3 million	inpatient diagnoses	ICD9-CM	347.00, 347.01, 347.10, 347.11

Typically the medical record databases comprise the longitudinal patient records of a well-defined population, which is registered with a GP in the Netherlands and the United Kingdom. The recorded information is a combination of administrative and medical data entered directly by the GP and includes symptoms, signs, drug prescriptions, laboratory examinations, diagnoses and referrals. Information from specialists and hospitals is entered into these records when the GP is notified by the specialist/hospital. Both participating medical record databases (UK-GPRD, NL-IPCI) (33) have been proven valid for pharmacoepidemiology studies. National administrative databases were available in the Nordic countries (Denmark, Finland, Sweden) (35, 37), where population registries can be linked with outpatient and inpatient records through a unique identifier. Administrative databases in Italy were regional and captured population and hospital discharge diagnoses. Here outpatient diagnoses were unavailable. Individual record linkage was not

possible at the time of the study in Finland but numerators and denominators were obtained independently of each other in an aggregated format.

Each health care database provider obtained local ethical and governance approval to use data for this study.

Study population

The study population comprised all individuals registered within one of the databases during the study period. The observation time began on the date of first registration of individuals in the database or the start date of data collection, whichever was the latest. The observation time ended on the date of death, the date an individual was transferred out of the study population, or the end of data collection, whichever was the earliest.

Case Identification

The primary outcome of interest was narcolepsy with or without cataplexy and independent of age. Harmonization of database queries followed an iterative process with six stages using the processes established in the EU-ADR project (38): 1) event definition using clinical criteria established by the Brighton Collaboration (39); 2) identification of Unified Medical Language System® (UMLS®) concepts corresponding to the event; 3) discussion among database license holders regarding relevance and applicability of the UMLS concepts identified; 4) translation of the concepts into the terminology of each individual database (i.e. ICD-9 CM, ICD-10, READ and ICPC) 5) extraction of data; and 6) creation of input files for Jerboa© (see below) and verification of output.

Verification of cases identified in the NL based IPCI database was conducted in a two-step procedure: a) two medical doctors (NvdM, JvB) reviewed the electronic medical records, b) additional clinical case information was obtained for possible narcolepsy cases via standardized questionnaires from GPs and specialist letters. The totality of information was used for verification of the cases by two narcolepsy experts (GJL, SO) using the Brighton Collaboration case definition (39). Rates were not verified for other participating databases.

Data handling

A distributed network approach was used for the collaborative work and for data sharing across databases and national borders. This approach is based on the principle that database owners should be involved in and be responsible for the elaboration of data, as they best understand the context within which the data are recorded (40). Local elaboration of data and sharing of aggregated de-identified data complies with all privacy constraints regarding secondary use of health care data and sharing data across country borders within the European Union.

In our study, database holders created two pre-specified common input files: a population file and an event file. The population file contained a single record for each individual in the entire database, including a personal identifier (i.e. linking-key across the files), date of birth, sex, start and end date of observation time in the population. The event file was comprised of a single record for each event occurring in the entire population during the observation period, including a personal identifier, event type and start date. These two input files were subsequently processed locally by Jerboa©, a purpose-built JAVA based software (40). Jerboa© generated fully encrypted aggregated counts of diseases and person time (age-, sex-, calendar year and calendar month-specific counts) locally, which were transmitted to the central database for pooling and further analysis.

Analysis

Incidence rates were calculated by dividing the number of narcolepsy diagnoses by the accumulated person time. Jerboa© software for incidence calculation has been validated against standard SAS code.

Incidence rates of narcolepsy diagnosis were calculated by age, sex, calendar year and month for each centre separately and for the pooled overall population. 95% confidence intervals were calculated assuming a normal binomial distribution. Age standardized rates were calculated based on the age distribution of the world population as presented by the WHO (40). Additionally, incidence rates were reported for the pre-pandemic influenza period (January 2000-March 2009), the pandemic influenza but pre-vaccination period (April 2009- September 2009), and the period after the start of influenza vaccination programs with overlapping pandemic activity (October 2009-June 2010). Rate ratios with 95% confidence intervals were calculated to estimate the relative change in rates before the start of vaccination campaigns (i.e. until September 2009) and after.

Results

The seven participating databases from six countries provided just under 280 million person years (PY) of observation time. Denmark, Emilia Romagna region in Italy and Sweden provided data up until December 2010. Finland provided data up until December 2009 and age specific counts for 2010. The Tuscany region in Italy, the NL-IPCI database and the UK-GPRD database provided data up until June, July, and October 2010, respectively. The cumulative amount of person-time by centre and calendar year is shown in Figure 6.1.

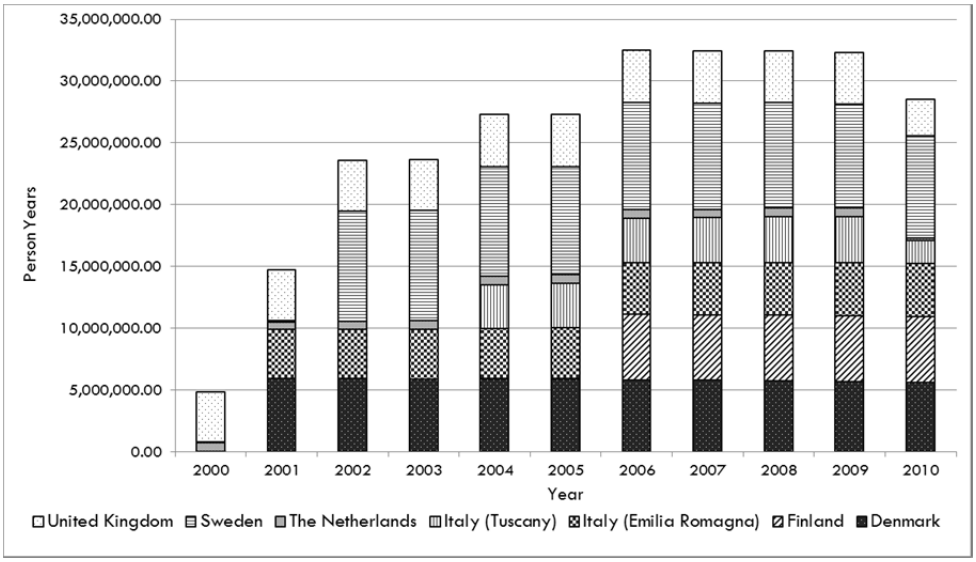


Figure 6.1 Cumulative person-time contributions to narcolepsy incidence rates by database and calendar year

In total, 2,608 narcolepsy cases were identified. The overall pooled incidence rate of narcolepsy diagnosis was 0.93 (95% CI: 0. 90 – 0.97) per 100,000 PY. The age-standardized incidence rate was 0.90 (95%CI: 0.84 – 0.97) per 100,000 PY. Pooled and country-specific incidence rates of narcolepsy diagnosis per age category are presented in Table 6.2.

Table 6.2 Incidence rate of narcolepsy by country and age group (2000-2010)

	Under 5 years		*5-19 years*		*20-59 years*		*Over 60 years*		*Group Total*	
	IR	95%CI	IR	95%CI	IR	95%CI	IR	95%CI	IR	95%CI
Pooled*	0.13	(0.07-0.20)	0.83	(0.75-0.91)	1.06	(1.01-1.11)	0.88	(0.81-0.95)	0.93	(0.90-0.97)
Denmark	0.17	(0.07-0.35)	0.90	(0.74-1.09)	1.91	(1.76-2.07)	1.36	(1.16-1.58)	1.42	(1.33-1.52)
Finland	0.00	-	2.12	(1.73-2.56)	1.30	(1.12-1.49)	0.81	(0.61-1.06)	1.25	(1.12-1.39)
Italy *(Tuscany & Emilia Romagna)*	0.10	(0.03-0.28)	0.18	(0.10-0.29)	0.29	(0.23-0.35)	0.30	(0.23-0.28)	0.27	(0.23-0.31)
Sweden	0.22	(0.07-0.52)	0.85	(0.71-1.01)	1.07	(0.98-1.18)	1.15	(1.00-1.31)	1.03	(0.96-1.10)
United Kingdom (GPRD)	0.12	(0.03-0.31)	1.22	(1.09-1.37)	1.02	(0.93-1.11)	1.05	(0.86-1.27)	1.02	(0.93-1.11)
The Netherlands (IPCI) (unverified)	0.00	-	0.93	(0.51-1.57)	1.65	(1.28-2.09)	0.86	(0.46-1.49)	1.26	(1.02-1.55)
The Netherlands (IPCI) (verified)	0.00	-	0.31	(0.10-0.74)	0.24	(0.12-0.43)	0.00	-	0.19	(0.11-0.32)

*All countries, including Finland for 2010 and validated NL-IPCI rates.

Incidence rates in the NL-IPCI database as in Italian regions were lower than those calculated for Denmark, Finland, Sweden and the UK-GPRD database. In the NL-IPCI database 86 cases were identified through a text search followed by a review of the electronic medical record to discard obvious false positives. After verification of these 86 cases by narcolepsy experts according to the Brighton Collaboration criteria (39) we found a positive predictive value (PPV) for 'text searches' of 17.4%. Of the 86 cases, 43 had other diagnoses, for 28 cases insufficient clinical or laboratory information was available and two cases were diagnosed correctly but diagnosed prior to the defined observation time (i.e., prevalent cases). The PPV for children aged 5-19 years was 33%. Figure 6.2 depicts the substantial change of the incidence rate pattern over time after verification.

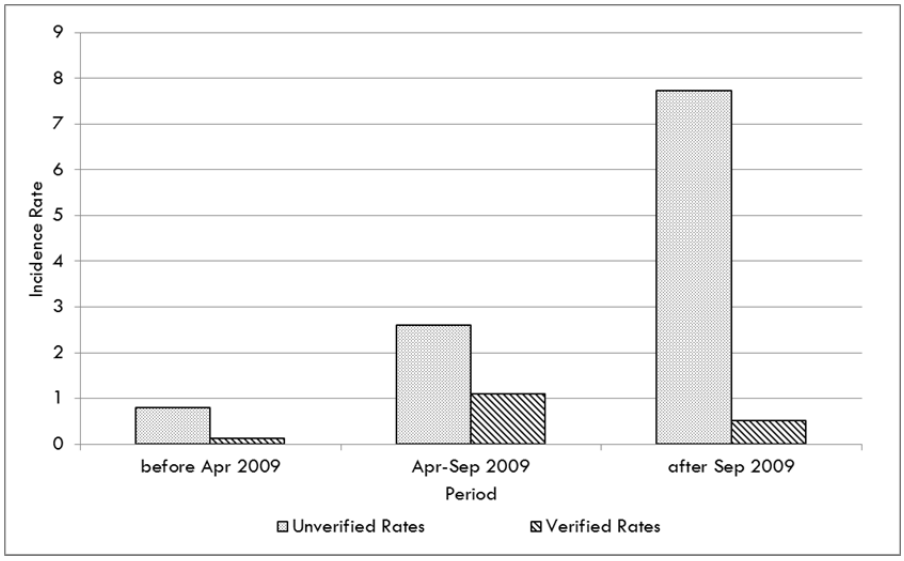


Figure 6.2 Effect of case verification on incidence rates by time period in the NL-IPCI database

Incidence rates by age and sex

The incidence rate of narcolepsy diagnosis was age dependent. The overall lowest background incidence rate was seen in children under five years of age, at 0.13 per 100,000 PY (95% CI: 0.07 – 0.20). The highest rate of narcolepsy diagnosis was observed in the age group 20-59 years (1.06 per 100,000 PY, 95% CI: 1.01 – 1.11). Figure 6.3 demonstrates a major peak between 15 to 30 years of age and a less pronounced, peak around 60 years of age.

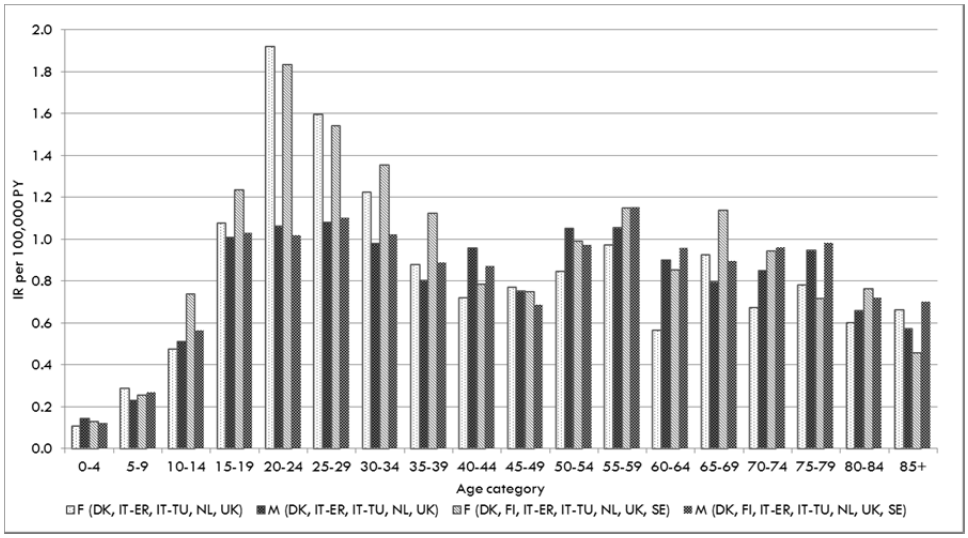


Figure 6.3 Pooled incidence of narcolepsy diagnosis (2000-2010) by age and sex for all non-signalling countries (DK, IT-Emilia Romagna, IT-Tuscany, NL, UK) and for all countries including signalling countries (FI, SE).

The incidence rates were very similar for men and women over 40 years of age (Figure 6.3). The age and sex dependent pattern is driven by data prior to the pandemic period (2137 cases vs. 471 cases after September 2009), which is also evident when considering the pattern without data from the signalling countries (Figure 6.3). At younger ages, women had higher rates - most markedly between 15 and 40 years of age. The overall pooled incidence rate was slightly higher in women as compared to men (0.97 (95% CI: 0.92-1.02) vs. 0.85(95% CI: 0.80-0.90) per 100,000 PY). In the Italian regions and the UK-GPRD database, incidence rates were slightly lower in women as compared to men. They were 0.23 vs. 0.26 in the Tuscany region and 0.23 vs. 0.34 in Emilia Romagna region, in the UK-GPRD 0.96 vs. 1.07 per 100,000 PY.

Figure 6.4 depicts the pooled monthly incidence rates of narcolepsy for the period 2000-2010. The diagnosis rates exhibit a seasonal pattern and appear highest around January with a marked decrease in July.

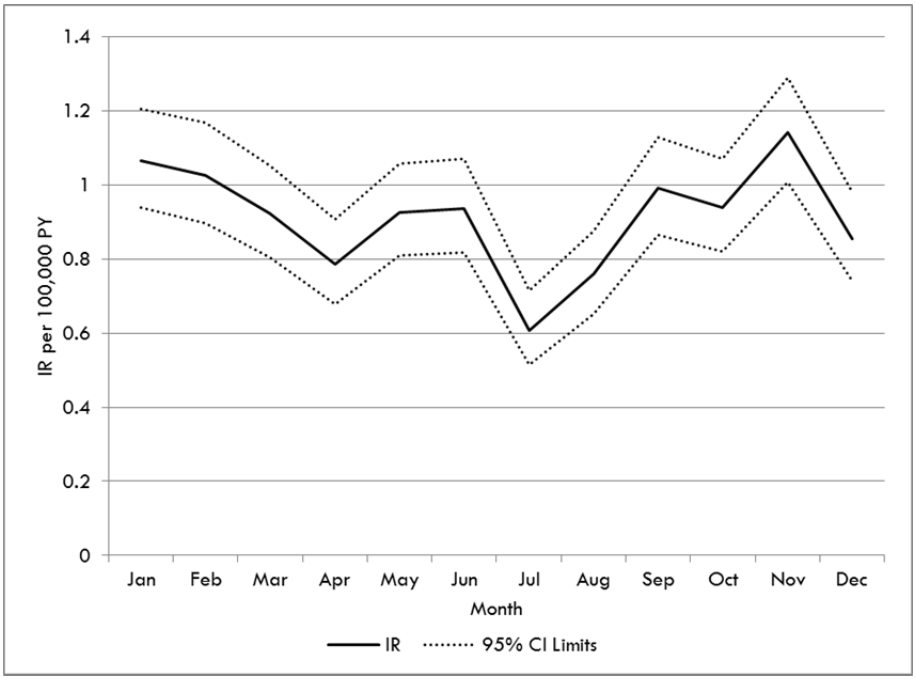


Figure 6.4 Incidence of narcolepsy diagnosis by month pooled for 2000-2010 and for all countries (except 2010 data from Finland)

In Figure 6.5 the pooled incidence rate of narcolepsy diagnosis is shown to vary around 1 per 100,000 PY. Although confidence intervals are wide, no considerable changes are seen until the second half of 2010 when there was a marked increase in the incidence rate of narcolepsy diagnosis. This overall increase was attributable to the increases observed in Sweden and Denmark. Finnish data for 2010 were not included in this figure as they were not available per month.

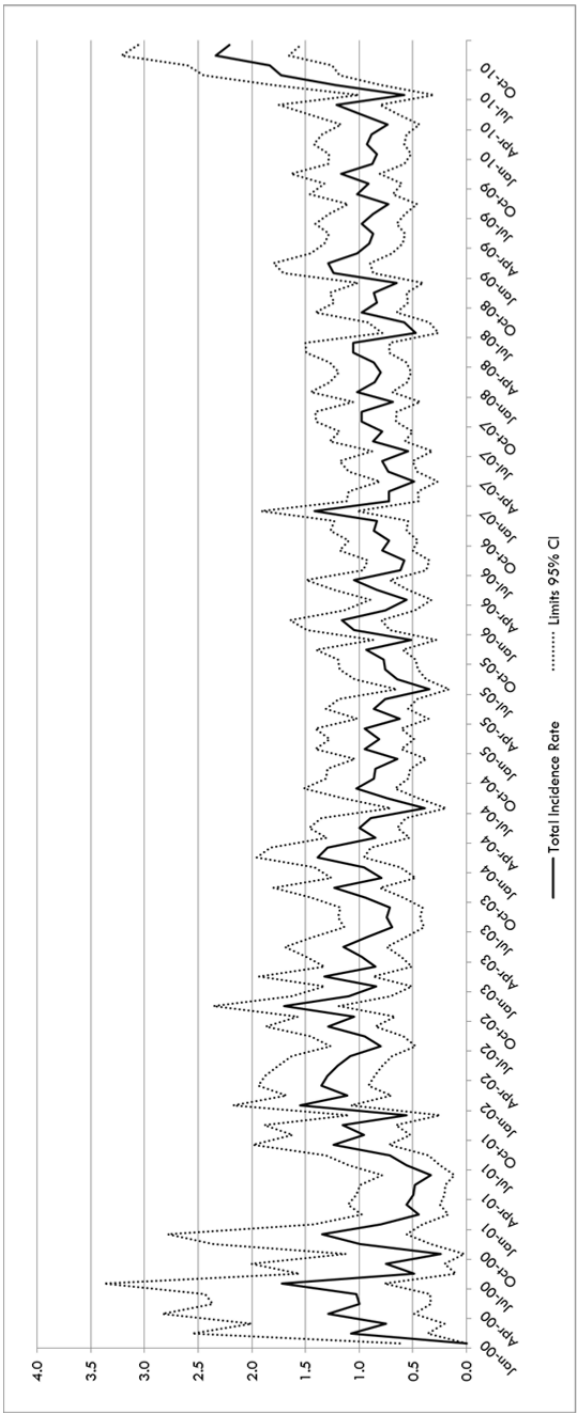


Figure 6.5 Pooled incidence of narcolepsy diagnosis over time for all countries (except data for Finland from 2010).

Table 6.3 Incidence rate (per 100,000 person year) of narcolepsy diagnosis by age, period and country (2000-2010)

		Age group							
		<5 years		5-19 years		20-59 years		60+ years	
	Time period	Events	IR	Events	IR	Events	IR	Events	IR
All periods & countries		16	0.13	389	0.83	1607	1.06	596	0.88
All countries per time period	before Apr 2009	13	0.12	214	0.56	1273	1.03	492	0.91
	Apr-Sep 2009	0	0.00	21	0.79	96	1.10	28	0.67
	Till Sep 2009	13	0.11	235	0.57	1369	1.04	520	0.89
	after Sep 2009	3	0.22	154	2.61	238	1.21	76	0.79
	IR after/IR before=RR		1.9 (0.5-6.6)		**4.6 (3.7-5.6)**		**1.16 (1.01-1.33)**		0.89 (0.70-1.13)
Signaling									
Sweden	before Apr 2009	4	0.22	60	0.50	368	1.07	195	1.26
	Apr-Sep 2009	0		1	0.14	20	0.86	6	0.52
	Till Sep 2009	4	0.22	61	0.48	388	1.06	201	1.21
	after Sep 2009	0		60	3.62	69	1.18	23	0.79
	IR after/IR before=RR		NA		**7.5 (5.2-10.7)**		1.12 (0.87-1.45)		0.65 (0.42-1.01)
Finland	before Apr 2009	0	0.00	25	0.82	125	1.34	28	0.71
	Apr-Sep 2009	0	0.00	7	1.51	14	0.99	2	0.31
	Till Sep 2009	0	0.00	32	0.91	139	1.29	30	0.66
	after Sep 2009	0	0.00	67	5.80	46	1.31	20	1.24
	IR after/IR before=RR		NA		**6.4 (4.2-9.7)**		1.01 (0.72-1.41)		**1.90 (1.07-3.33)**
Non-signaling									
Denmark	before Apr 2009	4	0.14	70	0.75	430	1.62	127	1.32
	Apr-Sep 2009	0	0.00	10	1.88	35	2.31	11	1.72
	Till Sep 2009	4	0.13	80	0.82	465	1.66	138	1.35
	after Sep 2009	2	0.48	20	1.53	95	2.55	23	1.43
	IR after/IR before=RR		3.8 (0.7-20.5)		**1.9 (1.1-3.1)**		**1.54 (1.23-1.92)**		1.05 (0.68-1.64)

Table 6.3 Incidence rate (per 100,000 person year) of narcolepsy diagnosis by age, period and country (2000-2010) (Cont'd)

		Age group							
		<5 years		5-19 years		20-59 years		60+ years	
	Time period	Events	IR	Events	IR	Events	IR	Events	IR
Non-signaling									
Netherlands-IPCI (verified)[1]	before Apr 2009	0	0.00	2	0.17	5	0.15	0	0.00
	Apr-Sep 2009	0	0.00	1	1.48	3	1.49	0	0.00
	Till Sep 2009	0	0.00	3	0.25	8	0.22	0	0.00
	after Sep 2009	0	0.00	1	1.40	1	0.47	0	0.00
	IR after/IR before=RR		NA		5.7 (0.6-54)		2.13 (0.27-17)		NA
United Kingdom-GPRD[2]	before Apr 2009	2	0.09	46	0.67	268	1.28	92	1.08
	Apr-Sep 2009	0	0.00	1	0.27	16	1.44	5	1.04
	Till Sep 2009	2	0.09	47	0.65	284	1.29	97	1.08
	after Sep 2009	1	0.43	4	0.58	11	0.52	7	0.76
	IR after/IR before=RR		5.0 (0.5-55.4)		0.9 (0.3-2.5)		0.41 (0.22-0.74)		0.71 (0.33-1.52)
Italy (Emilia Romagna/Tuscany[3])	before Apr 2009	3	0.13	11	0.18	77	0.27	50	0.32
	Apr-Sep 2009	0	0.00	1	0.20	8	0.37	4	0.33
	Till Sep 2009	3	0.12	12	0.18	85	0.28	54	0.32
	after Sep 2009	0	0.00	2	0.20	16	0.37	3	0.12
	IR after/IR before=RR		NA		1.1 (0.2-4.9)		1.33 (0.78-2.28)		0.38 (0.12-1.22)

The data is also presented by pandemic influenza vaccination period (pre-pandemic influenza, pandemic influenza/pre-vaccination, and pandemic influenza/post-vaccination). In Table 6.3 pooled and country specific rates are shown by age and influenza/vaccination period with rate ratios estimating the relative change in pre-vaccination and post-vaccination diagnosis rates. Considering the pooled data, a significant increase in narcolepsy diagnosis is seen in the 5-19 and the 20-59 year age group between the pre and post vaccination periods. The country specific rate ratios show significant increases in the 5–19 year age group in Finland (RR: 6.4 (4.2 – 9.7)), Sweden (RR: 7.5 (5.2 – 10.7)) and Denmark (RR: 1.9 (1.1-3.1)). In Finland an increase was also observed in the age group over 60 years of age (RR: 1.9 (1.1 – 3.3)), and in Denmark in the age group 20-59 years of age (RR: 1.5 (1.2 – 1.9)). In the UK-GPRD a significant decrease in narcolepsy diagnoses between the pre- and post-vaccination periods was observed in the 20-59 year age group (RR: 0.41 (0.22 – 0.74)).

Discussion

To our knowledge, this is the largest published study on narcolepsy epidemiology to date. It was designed as a proof of principle study utilizing the described approach to inform public health decision-making during an emerging public vaccine safety concern.

Incidence rate data of narcolepsy were useful to quantify background diagnostic rates and to provide insight into changing epidemiologic patterns of diagnoses over time, by age, sex, and country (41). We found the incidence of narcolepsy diagnosis to be stable over time around 1 per 100,000 PY. Incidence rates were age dependent with a peak between 15-30 years of age in women especially, and a smaller peak at around 60 years of age. Overall we found a slightly higher incidence rate in women than men. We observed significant increases in the diagnosis of narcolepsy in the 5–19 year age group in Finland and Sweden following the start of the influenza A(H1N1)pdm09 vaccination campaigns, in line with the reported signal. Increases were also seen in the 5-19 and 20-59 year age groups in Denmark and in the over 60 year age group in Finland after September 2009. These increases occurred in spite of relatively low influenza A(H1N1)pdm09 vaccination coverage in the Danish population.

A common protocol, common infrastructure for data sharing, standardized data elaboration and central data analysis were employed to avoid heterogeneity due to differences in study methods beyond the local data collection. However, differences in narcolepsy incidence rates were observed between countries. This may be explained by differences between national healthcare databases (e.g., in- and outpatient claims vs. primary care medical record databases), or by country specific changes in referral and diagnostic patterns over time. Nevertheless, the resulting variability in incidence over time and across countries was within a narrow range. Therefore, not only country specific data but also

pooled data were presented to describe patterns of narcolepsy diagnosis incidence more clearly.

Very few estimates of the incidence of narcolepsy or narcolepsy diagnosis have been published. A US-based study by Silber *et al* reported an incidence of narcolepsy with cataplexy of 0.74 (95%CI: 0.47 – 1.16) per 100,000 PY, and of narcolepsy with or without cataplexy of 1.37 (95%CI: 0.95 – 1.90) per 100,000 PY over a 30 year period (42). In their study the incidence rate was higher in men than in women (1.72 vs. 1.05) and all except one case occurred between the ages of 10 and 29 years. Pooled incidence rates of narcolepsy diagnosis from our study are in the same magnitude. As in the study by Silber *et al* the highest background incidence rate of narcolepsy diagnosis was also seen between 15 and 30 years of age. However, incidence rates outside this age range were relatively high in our study compared to Silber *et al*. As our rates reflect diagnoses rather than onset of narcolepsy, which is reported by Silber *et al*, this could indicate a long lag time between onset of disease and diagnosis. In contrast to the study by Silber *et al*, we detected an overall slightly higher incidence rate in women. This was most marked between the ages of 15 and 30 years, coinciding broadly with the reproductive age. We cannot determine whether this peak is a result of biological mechanisms or due to determinants of diagnosis.

In a recent study in China, Han *et al* found a seasonal pattern for narcolepsy, with onset of narcolepsy being least frequent in November and most frequent in April (20). While our data also indicate a seasonal effect on incidence rates, our peaks and troughs are not during the same months. However, we considered diagnoses of narcolepsy as the index date, while Han *et al*. used onset of disease. The lower rates we observed in July are unlikely to be a function of disease, but rather reflective of a lower diagnosis rate of a chronic condition during the major holiday period in Europe.

Over 50% of initially identified cases in the NL-IPCI database were excluded during case verification as narcolepsy had been ruled out in these cases. In a quarter, data were insufficient to confirm the clinical diagnosis. Consequently, a rate increase initially observed in 2009 and 2010 disappeared upon verification and that the age, time, and gender specific patterns changed. Case verification has not been performed for the other participating databases, for which case detection methods vary substantially. The effect of systematic case verification on the epidemiologic pattern in these countries remains to be elucidated.

In Finland, we observed an increase in the incidence rate of narcolepsy diagnosis after September 2009 in children and adolescents aged between 5-19 years of age, but not in young children, adults aged 20-64 and to a lesser extent in older adults. An increase in the age group of 5-19 years of age after September 2009 was also observed in Sweden. This is in line with the signal reported in these countries for the influenza A(H1N1)pdm09 vaccine.

Both countries had a high coverage of influenza A(H1N1)pdm09 vaccination in most age-groups, including those in which the increase in diagnosis of narcolepsy was observed. In the NL-IPCI data, no increase in incidence was seen in the influenza A(H1N1)pdm09 vaccine targeted age groups (persons over 60 years, children under 5 years in addition to high risk groups) after the A(H1N1)pdm09 vaccination campaign started. However, vaccination coverage was low in the 5 – 19 year age group. Also in the Italian regions and the UK-GPRD no increase was seen in influenza A(H1N1)pdm09 vaccine targeted age groups. Here too vaccination coverage was low in the 5-19 year age group. Note that for the Netherlands, IT-Tuscany and the UK, data was not available for the whole of 2010, therefore comparisons with countries that do have complete data for 2010 should be interpreted with caution. In Denmark, an increase in the incidence of narcolepsy was observed after September 2009 (Table 6.3). In Denmark risk-groups were targeted for A(H1N1)pdm09 vaccination as well as health care workers (43). The vaccination coverage in risk groups was 20%. Therefore, coverage is expected to have been low in the overall population. Consequently, influenza A(H1N1)pdm09 vaccination is unlikely to form an explanation for the observed increase in narcolepsy diagnosis in the 5-19 and 20-59 year age group.

Population based background rate data cannot provide conclusive evidence on a potential causal association between influenza A(H1N1)pdm09 vaccination and narcolepsy diagnosis. However, it can be used for a rapid assessment of public health impact on a population level. The signal reported in Finland and Sweden was also detected in the background rate data collected for this study. The mismatch of changes in age specific narcolepsy diagnosis rates with the underlying influenza A(H1N1)pdm09 vaccine coverage rates in certain age groups provides some indication that other factors than influenza A(H1N1)pdm09 vaccination may also be associated with increasing incidence rates of narcolepsy diagnosis. Thus, additional factors that could explain an increase in incidence of diagnosis of narcolepsy should be considered for formal hypothesis testing.

This study is unique as it is of unprecedented size, covering six countries and a 10 year period employing a standardized approach. Unfortunately, case status could not be verified for all participating countries, and further verification of identified cases could impact on the magnitude and patterns of narcolepsy diagnosis rates. Nonetheless, the data provide a useful insight into patterns of diagnosis of narcolepsy over time, by age and by sex. The most striking observations are the higher rate of narcolepsy diagnosis in women of reproductive age and especially the increases in diagnosis rates observed towards the end of 2009 and 2010 in Finland, Sweden and Denmark, that in the case of Denmark do not coincide with a high influenza A(H1N1)pdm09 vaccine coverage.

Acknowledgements

Erasmus MC, Rotterdam, NL: A. Vanrolleghem. Nico van Blijderveen. RIVM, Bilthoven, NL: H. de Melker. Danish Center for Sleep Medicine, Copenhagen, DK: S. Knudsen. Karolinska Institutet, Stockholm, SE: J. Pege. National Public Health Institute, Helsinki, FI: H. Nohynek, J. Jokinen, T. Kilpi. Norwegian Institute of Public Health, Oslo, NO: J. Storsaeter. University Hospital, Oslo, NO: K. Bronder. HPA, London, UK: N. Andrews. Brighton Collaboration, Basel, Switzerland: S. Faisst.

This study is based in part on data from the Full Feature General Practice Research Database obtained under licence from the UK Medicines and Healthcare products Regulatory Agency and covers the data collection time period up to 31 October 2010. However, the interpretation and conclusions contained in this report are those of the authors alone.

References

1. ICSD, editor. International Classification of Sleep Disorders. Westchester, IL: American Academy of Sleep Medicine; 2005.

2. Overeem S, Black Jr, Lammers G. Narcolepsy: immunological aspects. Sleep Med Rev. 2008;12:95-107.

3. Littner M, Kushida C, Wise M, Davila D, Morgenthaler T, Lee-Chiong T, et al. Standards of Practice Committee of the American Academy of Sleep Medicine. Practice parameters for clinical use of the multiple sleep latency test and the maintenance of wakefulness test. . Sleep. 2005;28(1):113-21.

4. Cao M. Advances in narcolepsy. Med Clin North Am. 2010;94:541-55.

5. Guilleminault C, Abad V. Narcolepsy. In: Chokroverty S, editor. Sleep Disorders Medicine Basic Science, Technical Considerations, and Clinical Aspects: Elsevier Inc; 2009. p. p. 377-96.

6. Chakravorty S, Rye D. Narcolepsy in the older adult: epidemiology, diagnosis and management. Drugs Aging. 2003;20(5):361-76.

7. Morrish E, King M, Smith I, Shneerson J. Factors associated with a delay in the diagnosis of narcolepsy. Sleep Med Rev. 2004;5:37-41.

8. Plazzi G, Pizza F, Palaia V, Franceschini C, Poli F, Moghadam K. Complex movement disorders at disease onset in childhood narcolepsy with cataplexy. . Brain. 2011;134:3480-92.

9. Nevsimalova S. Narcolepsy in childhood. Sleep Medicine Reviews. 2009;13(2):169-80.

10. Ahmed I, Thorpy M. Clinical features, diagnosis and treatment of narcolepsy. Clin Chest Med. 2010;31(2):371-81.

11. Longstreth WJ, Koepsell T, Ton T, Hendrickson A, van Belle G. The epidemiology of narcolepsy. Sleep. 2007;30(1):13-26.

12. Overeem s, Mignot E, van Dijk J, Lammers G. Narcolepsy: clinical features, new pathophysiologic insights, and future perspectives. J Clin Neurophysiol 2001;18: 78–105.

13. Dauvilliers Y, Montplaisir J, Molinari N, Carlander B, Ondze B, Besset A, et al. Age at onset of narcolepsy in two large populations of patients in France and Quebec. Neurology. 2001;57:2029-33.

14. Chakravorty SS, Rye DB. Narcolepsy in the older adult: epidemiology, diagnosis and management. Drugs & aging. 2003;20(5):361-76. PubMed PMID: 12696996.

15. Yoss R, Daly D. Narcolepsy in children. Pediatrics. 1960;25:1025-33.

16. Fronczek R, Baumann C, Lammers G, Bassetti C, Overeem S. Hypocretin/orexin disturbances in neurological disorders. Sleep Med Rev. 2009;13:9-22.

17. Aran A, Lin L, Nevsimalova S, Plazzi G, Hong S, Weiner K, et al. Elevated anti-streptococcal antibodies in patients with recent narcolepsy onset. . Sleep. 2009;32:979-83.

18. Cvetkovic-Lopes V, Bayer L, Dorsaz S, Maret S, Pradervand S, Dauvilliers Y, et al. Elevated Tribbles homolog 2-specific antibody levels in narcolepsy patients. J Clin Invest. 2010;120(3):713-9.

19. Kornum B, Faraco J, Mignot E. Narcolepsy with hypocretin/orexin deficiency, infections and autoimmunity of the brain. Curr Opin Neurobiol. 2011;21:897-903.

20. Han F, Lin L, Warby S, Faraco J, Li J, Dong S, et al. Narcolepsy onset is seasonal and increased following the 2009 H1N1 pandemic in China. Ann Neurol 2011;70(3):410-7.

21. Vaccine European New Integrated Collaboration Effort (VENICE). Pandemic A(H1N1)2009 influenza vacciantion survey, influenza season 2009/2010. VENICE-II consortium. 2011.

22. Paul Ehrlich Institute (PEI). Aktuelle Informationen zu Narkolepsie im zeitlichen Zusammenhang mit A/H1N1 Impfung 2011. Available from: http://www.pei.de/cln_320/nn_2299076/DE/arzneimittelsicherheit-vigilanz/archiv-sicherheitsinformationen/2012/ablage2012/2012-09-27-narkolepsie-studien-europa.html?utm_medium=twitter&utm_source=dlvr.it.

23. Folkehelseinstitutt. Pandemrix og narkolepsi. Available from: http://www.fhi.no/eway/default.aspx?pid=233&trg=MainLeft_5565&MainArea_5661=5565:0:15,5001:1:0:0:::0:0&MainLeft_5565=5544:91109::1:5569:8:::0:0.

24. AFSSAPS. Cases of narcolepsy in adolescents after vaccination with Pandemrix® in France. http://laegemiddelstyrelsendk/en/topics/side-effects-and-trials/side-effects/news/cases-of-narcolepsy-in-adolescents-after---in-france. 2011.

25. Dauvilliers Y, Montplaisir J, Cochen V, Desautels A, Einen M, Lin L, et al. Post-H1N1 narcolepsy-cataplexy. Sleep. 2010;33:1428-30.

26. Nohynek H, Jokinen J, Partinen M, Vaarala O, Kirjavinen T, Sundman J, et al. AS03 Adjuvanted AH1N1 Vaccine associated with an abrupt increase in the incidence of childhood narcolepsy in Finland. PLoS One. 2012;7(e33536-33723).

27. National Narcolepsy Task Force. Interim Report, 31 January 2011, National Institute for Health and welfare (THL), www.thl.fi.

28. Medical Products Agency. A registry based comparative cohort study in four Swedish counties of the risk for narcolepsy after vaccination with Pandemrix - A first and preliminary report, by the 2011 [March 28,2011]. Available from: www.mpa.se

29. MPA Sweden. Occurrence of narcolepsy with cataplexy among children and adolescents in relation to the H1N1 pandemic and Pandemrix vaccinations - Results of a case inventory study by the MPA in Sweden during 2009-2010. June 30, 2011.

30. O 'Flanagan D, Bonner C, Crowe C, Lynch B, Sweeney B, Gilvarry J, et al. Investigation of an increase in the incidence of narcolepsy in children and adolescents in 2009 and 2010. Final Report of National Narcolepsy Study Steering Committee. Department of Health, Ireland. 2012.

31. Dauvilliers Y, Arnulf I, Lecendreux M, Monaca Charley C, Franco P, Drouot X, et al. Increased risk of narcolepsy in children and adults after pandemic H1N1 vaccination in France. Brain : a journal of neurology. 2013 Aug;136(Pt 8):2486-96. PubMed PMID: 23884811.

32. European Centre for Disease Prevention and Control. Narcolepsy in association with pandemic influenza vaccination (a multi-country European epidemiological investigation). Stockholm: September 2012.

33. Vlug A, van der Lei J, Mosseveld B, van Wijk M, van der Linden P, Sturkenboom M, et al. Postmarketing surveillance based on electronic patient records: the IPCI project. Methods Inf Med. 1999;38:339-44.

34. Wood L, Martinez C. The general practice research database: role in pharmacovigilance. Drug Saf. 2004;27(12):871-81.

35. Andersen T, Madsen M, Jorgensen J, Mellemkjoer L, Olsen J. The Danish National Hospital Register. A valuable source of data for modern health sciences. Dan Med Bull. 1999;46:3.

36. Ludvigsson J, Otterblad-Olausson P, Pettersson B, Ekbom A. The Swedish personal identity number: possibilities and pitfalls in healthcare and medical research. . Eur J Epidemiol. 2009;24:659-66.

37. Malig C. The Civil Registration System in Denmark. IIVRS Technical Paper. Report. Bethesda, Maryland: International Institute for Vital Registration and Statistics, 1996 66.

38. Avillach P, Mougin F, Joubert M, Thiessard F, Pariente A, Dufour J, et al. A semantic approach for the homogeneous identification of events in eight patient databases: a contribution to the European eu-ADR project. Stud Health Technol Inform. 2009;150:190-4.

39. Poli Fea. Narcolepsy as a possible adverse event following immunization: case definition and guidelines for data collection, analysis and presentation. Vaccine to be submitted. 2012.

40. Coloma P, Schuemie M, Trifirò G, Gini R, Herings R, Hippisley-Cox J, et al. Combining electronic healthcare databases in Europe to allow for large-scale drug safety monitoring: the EU-ADR Project. Pharmacoepidemiol Drug Saf 2011;20:1-11.

41. Black S, Eskola J, Siegrist CA, Halsey N, Macdonald N, Law B, et al. Importance of background rates of disease in assessment of vaccine safety during mass immunisation with pandemic H1N1 influenza vaccines. Lancet. 2009 Dec 19;374(9707):2115-22. PubMed PMID: 19880172. Pubmed Central PMCID: 2861912.

42. Silber M, Krahn L, Olson E, Pankratz V. The epidemiology of narcolepsy in Olmsted County, Minnesota: a population-based study. Sleep. 2002;25:197-202.

43. Mereckiene J, Cotter S, Weber J, Nicoll A, D'Ancona F, Lopalco P, et al. Influenza A(H1N1)pdm09 vaccination policies and coverage in Europe. Euro Surveill 2012:pii: 20064.

Chapter 7. Pandemic influenza vaccine & narcolepsy: simulations on the potential impact of bias

Caitlin Dodd*

Leonoor Wijnans*

Maria de Ridder

Silvana Romio

Daniel Weibel

Jan Bonhoeffer

Sebastiaan Overeem

Gert-Jan Lammers

Steve Black

Miriam Sturkenboom

* *the first two authors contributed equally to this work*

Submitted

Abstract

Background

Several studies conducted during a period of heightened attention identified an association between Pandemrix™, an AS03 adjuvanted pandemic influenza A(H1N1) vaccine, and narcolepsy, a rare and under-diagnosed sleep disorder with a median onset-to-diagnosis interval of ten years. Using the example of narcolepsy and Pandemrix, this paper studies potential sources of bias in order to provide methodological recommendations for assessment of vaccine safety signals.

Methods

We simulated the effects of two key potential sources of bias on the association between Pandemrix and narcolepsy: 1) Detection bias, defined as accelerated diagnosis of narcolepsy in vaccinated children upon awareness of the association and 2) Differential misclassification bias defined as misattribution of date of narcolepsy onset to the period following vaccination. The simulated data sets were analyzed using cohort and case-control methods.

Results

The relative risks of narcolepsy in vaccinated versus non-vaccinated children could become as high as 28.4 in the presence of the two simulated sources of bias. They had less impact when vaccine coverage was higher, the underlying interval from onset to diagnosis was shorter, or the case capture period was extended. The case-control design was less influenced by these biases than the cohort design.

Conclusions

Our simulation study showed that in the absence of a real association between the vaccine and the outcome, presence of detection bias and differential exposure misclassification could account for elevated RRs for vaccine exposure in association studies. These may play a major role, particularly in alert situations when observation times are limited, the disease is rare, and the disease has a long latency period. Overall, these simulations provide useful insights for the design and interpretation of future studies.

Background

In August 2010, case reports linking the occurrence of narcolepsy in children aged 5 to 19 years to an AS03 adjuvanted H1N1pdm09 (pH1N1) vaccine, Pandemrix™ (GlaxoSmithKline, Middlesex, United Kingdom) were published in Finland and Sweden (1, 2). In the European Union, Pandemrix was widely used, with over 30 million doses administered. Coverage was particularly high in the Nordic countries (3). Following reports from Sweden and Finland, the European Medicines Agency initiated a review procedure (4) which eventually led to the restriction of indication for Pandemrix (5).

Narcolepsy is a chronic sleep disorder that is severely debilitating. The dysregulation of the sleep-wake cycle is caused by the destruction of hypocretin forming neurons in the hypothalamus, which is thought to result from an auto-immune process (6). Symptoms include excessive daytime sleepiness (EDS) and cataplexy (7). Symptoms usually emerge gradually and can initially be non-specific. Consequently, symptoms can be attributed to other diagnoses resulting in a delay of narcolepsy diagnosis and treatment (8-11). Despite significant improvements in the speed and accuracy of narcolepsy diagnosis (10-12), a recent study found that the median delay between onset and diagnosis remains approximately 10 years (9).

As of May 2015, eight epidemiological studies testing the association between Pandemrix and clusters of narcolepsy cases (13-21) have been published reporting risk estimates ranging from 1.6 to 14.4. Generally, published studies were meticulous in their methods and applied sensitivity analyses to evaluate the presence of biases. Nonetheless, studies were inevitably observational and, as studies were mostly initiated rapidly after the signal emerged, they had limited time for case capture. Combined with the often nonspecific symptoms and onset of narcolepsy resulting in delayed diagnosis these studies are particularly prone to bias. Five years after the original signal emerged it remains unclear if and how potential sources of bias affected the estimates from the association studies. Consequently it is still unknown what the exact association between Pandemrix and narcolepsy is (22, 23).

It is not unthinkable that a similar scenario could unfold in the future, i.e. a safety signal involving a difficult to diagnose condition with a delayed onset is linked to exposure with a new vaccine. Indeed, a similar situation has occurred in the past, when clusters of cases of Guillain-Barré syndrome were detected after the introduction of a new swine flu vaccine (24). Using the example of narcolepsy and Pandemrix, we explore the potential impact of two sources of bias that are likely to occur in similar scenarios.

Detection bias. The first source of bias is a type of selection bias. Awareness of a potential association between narcolepsy and vaccination amongst physicians and the general public could result in earlier diagnosis for vaccinated cases compared to unvaccinated cases,

making vaccinated cases more likely to be included in observational studies with limited observation time (15). We refer to this as 'detection bias'.

Differential exposure misclassification. A second source of bias we consider is a form of recall bias, in which the onset of symptoms is misattributed, resulting in misclassification of onset dates to the period following vaccination. As narcolepsy symptoms often develop gradually and onset of symptoms is not always clearly identifiable, studies into narcolepsy are particularly prone to recall bias. We hypothesize that recalling onset of EDS with knowledge of a putative association between vaccination and narcolepsy could lead a patient to recall that symptoms started after vaccination (25). We refer to this as 'differential exposure misclassification'.

Methods

We considered the impact of detection bias and differential exposure misclassification as defined above on the association measure between Pandemrix and narcolepsy.

Simulation

We simulated a population of 100,000 subjects < 19 years of age on April 1st 2009 to mimic the signal-generating population. We subsequently simulated dates of birth and death (based upon average lifespans in western Europe) to create a simulated lifetime for each subject. EDS onset dates were assigned over the lifespan of subjects based upon the reported age and gender specific incidence rates of narcolepsy with cataplexy onset (26). Given these EDS onset dates, initial narcolepsy diagnosis dates were assigned using a random value drawn from a distribution of narcolepsy onset-to-diagnosis intervals which was assumed to have a gamma distribution chosen to mimic the distribution of onset-to-diagnosis intervals reported in the literature: a median of 10 years with a range of 0 to 40 years (11). Additionally, since the underlying onset-to-diagnosis interval in children is potentially shorter (10), alternate gamma distributions with medians of 3 (range 0-13) and 7 (range 0-27) years were also used. All onset-to-diagnosis intervals were simulated to be at least 40 days long.

Overall vaccination coverage in this population was simulated at 25, 50 and 75%. Vaccination dates were assigned independent of the age of a subject using a beta distribution of administration times mimicking real-life Pandemrix administration dates between October 12, 2009 and February 12, 2010 (27).

A null association (RR=1) was assumed for the actual relation between vaccine exposure and outcome.

Detection bias: Reduction in the EDS onset-to narcolepsy diagnosis interval was applied only to vaccinated cases for whom diagnosis occurred after the date of media attention (simulated to be August 15, 2010). If EDS onset occurred before August 15, 2010, the reduction was applied only to the interval from August 15, 2010 to the date of diagnosis. The date of narcolepsy diagnosis was reset in this way with 30, 60, and 90% reductions of the interval with the restriction that the interval should be at least 40 days. Data with no reduction in the interval was also simulated (Figure 7.1).

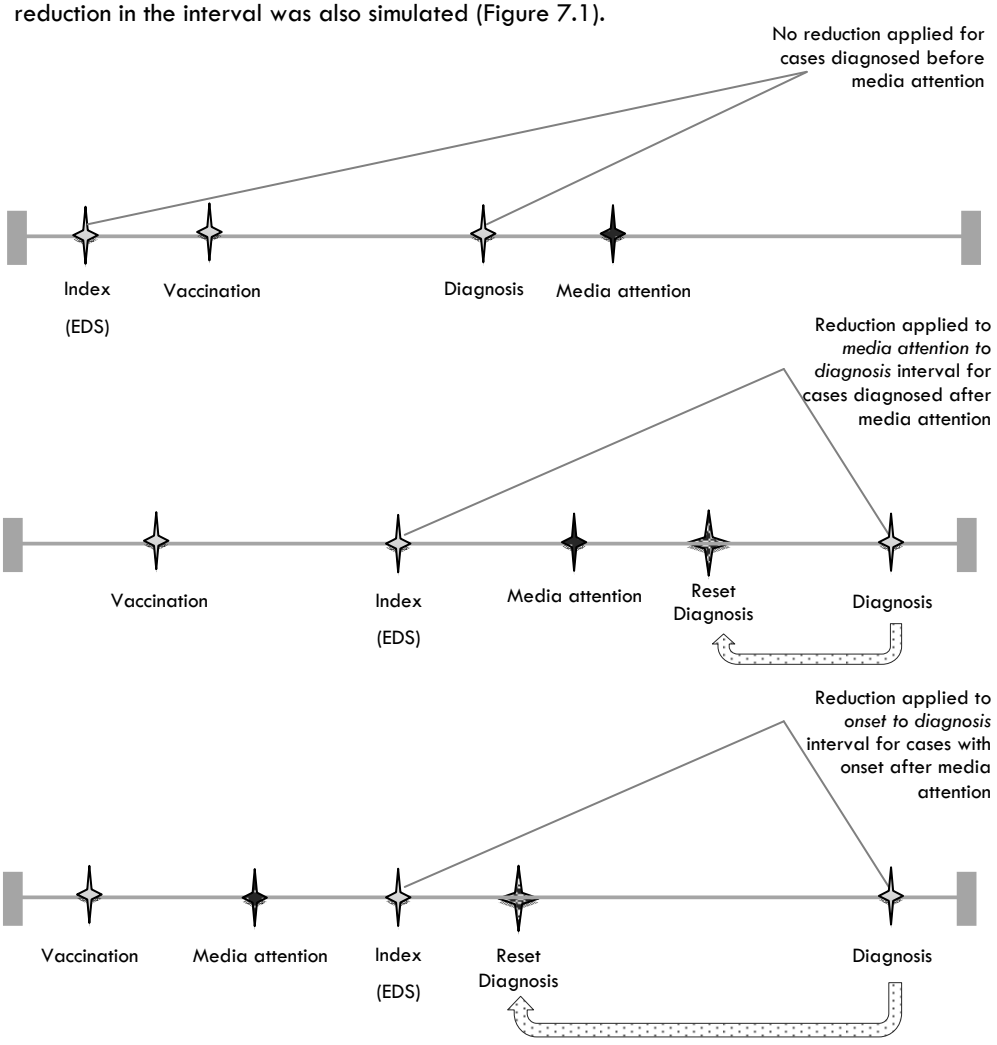


Figure 7.1 Application of Reduction in the Interval from Onset to Diagnosis (Detection Bias)

Differential exposure misclassification: Misattribution of EDS onset dates to the period following vaccination was applied with probability 30, and 60% to subjects who were diagnosed with narcolepsy after vaccination and after the start of media attention. In this

case the onset date was reset to a random date between the date of vaccination and the minimum of diagnosis date or vaccination date plus 180 days, based upon the six month risk period used by Miller *et al.* in their self-controlled case series analysis (19). Data with no misattribution of onset dates was also simulated (Figure 7.2).

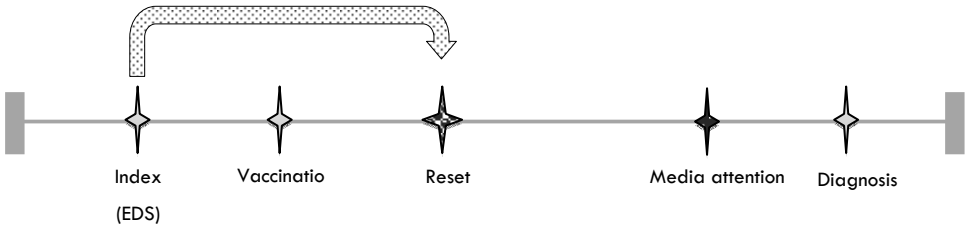


Figure 7.2 Application of misattribution of onset dates for cases with onset prior to vaccination (differential exposure misclassification bias)

We simulated 9 combinations of the underlying population settings: gamma scale (baseline onset to diagnosis interval, 3 different values) and vaccination coverage (3 values), to which we applied 12 combinations of the simulated sources of bias: detection bias (4 values), and differential exposure misclassification parameters (3 values) for a total of 108 combinations of simulation parameters

Analysis

The association between vaccination and narcolepsy in children aged 4 - <19 years during the study period was analyzed using dynamic cohort and case-control designs. In the primary analyses a case capture (study period) of April 1, 2009 to December 1, 2010 was used in line with several published studies. We calculated absolute incidence rates in 6-month periods and calculated case counts during exposed and unexposed person time. In the comparative cohort analysis, the incidence rate of narcolepsy was compared between dynamic cohorts of vaccinated and non-vaccinated persons. All person time after the date of vaccination was considered exposed, whereas the entire case-capture period of non-vaccinated persons as well as the pre-vaccination time in vaccinated subjects contributed to non-exposed person time. Rate ratios were calculated based on Poisson regression. In the case-control analysis, cases were matched to 10 controls on sex, age in years and onset date. Odds ratios were calculated using conditional logistic regression.

We conducted several analyses to investigate the effects of different design choices and ways to mitigate bias. All sensitivity analyses were conducted using vaccination coverage of 50% and the baseline onset-to-diagnosis interval distribution described in literature with median 10 years, range 0-40 years. To study the effect of the length of case capture

period, analyses with observation periods as long as 50 years were conducted. To study the effect of exclusion of cases possibly affected by awareness of a putative association, in one of the settings we excluded the cases with onset dates and diagnosis dates after August 15, 2010.

For each set of simulation parameters, 500 replications were analyzed, each producing an estimate and 95% confidence interval. Reported results are the exponentiated median of these 500 estimates calculated on the log scale and medians of the lower and upper confidence limits. All analyses were conducted using SAS 9·2.

Results

Application of onset-to-diagnosis interval reduction (detection bias) and differential exposure misclassification over three coverage rates and three baseline onset-to-diagnosis intervals increased the number of narcolepsy onset dates observed in the study period. Figure 7.3 for exposed and unexposed children, the number of onset dates associated with narcolepsy diagnosed cases in scenarios with different percentages of differential exposure misclassification (columns), vaccination coverage (rows) and levels of detection bias (X-axis in each plot), using a baseline onset-to-diagnosis interval with a median of 10 (range 0-40) years. The number of observed narcolepsy onset dates increases at approximately the same rate in exposed and unexposed person time with an increasing detection bias in the absence of differential exposure misclassification (within columns Figure 7.3). except when vaccine coverage is 25% in which case no onset dates are observed in exposed person time. With the introduction of differential exposure misclassification in exposed subjects, new narcolepsy diagnoses occur more often in post-vaccination person time. Figure 7.4 shows the effects of reduction of EDS onset-to-diagnosis date on the shape of incidence rates in this cohort of 0-19 year olds in 2009. With a reduction of 60 to 90% in lag time, a clear peak in incidence of narcolepsy diagnoses occurs after media attention.

Table 7.1 shows the results of cohort and case-control analyses of all 108 different parameter settings.

Because a 10 year onset-to-diagnosis interval has been reported in the literature, we have chosen to illustrate our results using underlying populations with this onset-to-diagnosis interval and the intermediate vaccine coverage of 50%.

Using a cohort analysis on this underlying population to which the maximum reduction of time from EDS onset-to-diagnosis (90%) has been applied in the absence of differential exposure misclassification produced a median RR of 2.24 (95% CI: 1.39, 3.62). In case-control analysis, the same simulation parameter settings produced an OR of 2.99 (95% CI: 1.79, 5.00) (Table 7.1).

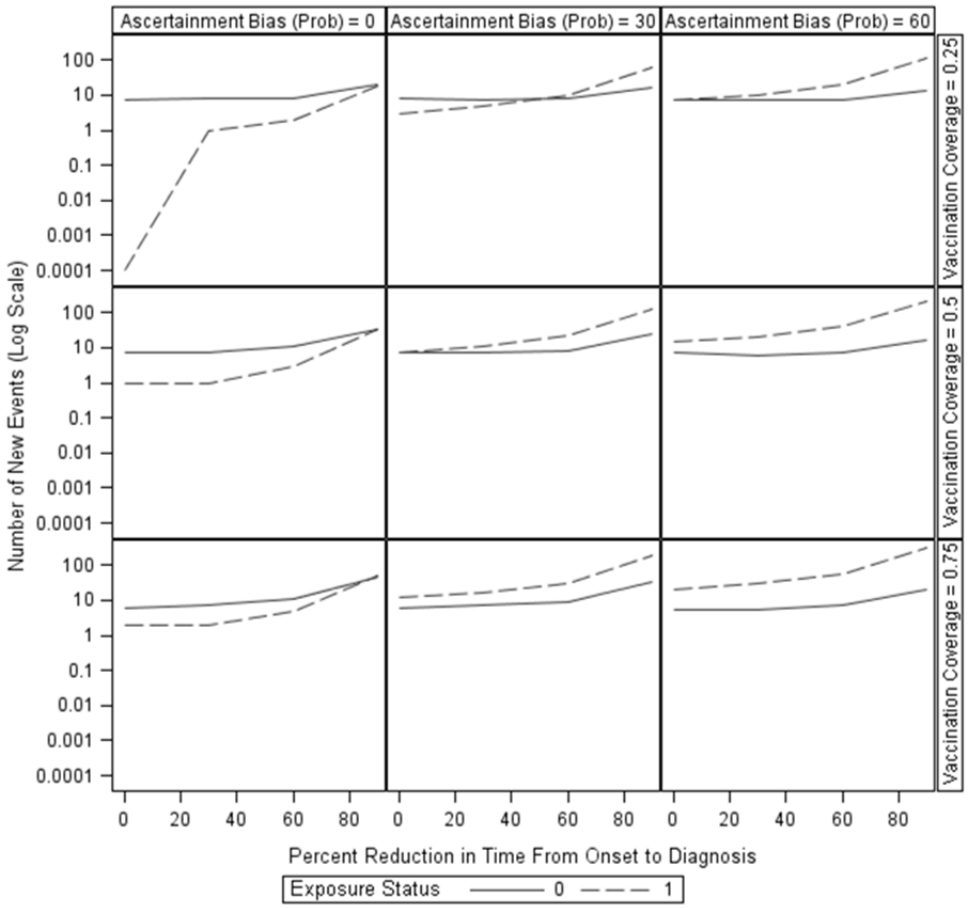


Figure 7.3 Number of EDS onset dates in exposed and unexposed person time during follow up with different probabilities of ascertainment bias (columns), reductions in time from onset to diagnosis (X-axis) and vaccination coverages (rows)

In the absence of a reduction in the EDS onset-to-diagnosis interval, differential exposure misclassification resulted in a RR of 4.31 (95% CI: 1.68, 10.74) when vaccination coverage was 50% and the EDS date was attributed to the post-vaccination period with a median probability of 60% for vaccinated cases. In the case-control analysis, the same simulation parameter settings produced an OR of 4.16 (95% CI: 1.54, 11.17) (Table 7.1).

Table 7.1 Relative Risks and Odds Ratios in primary cohort and case control analyses

Baseline interval (O-D)	DEMB	DB	Coverage = 25%		Coverage = 50%		Coverage = 75%	
			Cohort RR (95% CI)	Case Control OR (95%CI)	Cohort RR (95%CI)	Case Control OR (95%CI)	Cohort RR (95%CI)	Case Control OR (95%CI)
Median: 3 years. Range 0-13 years (γ - 2)	0	0	0.42 (0.16-1.12)	0.97 (0.34-2.77)	0.38 (0.19-0.77)	0.99 (0.47-2.11)	0.31 (0.17-0.57)	1.02 (0.53-1.94)
		30	0.57 (0.25-1.32)	1.31 (0.54 -3.13)	0.50 (0.27-0.93)	1.19 (0.61 - 2.31)	0.42 (0.25-0.71)	1.14 (0.65-1.98)
		60	1.05 (0.57-1.92)	2.06 (1.06 -4.04)	0.82 (0.52-1.31)	1.69 (1.03 - 2.76)	0.61 (0.41-0.91)	1.33 (0.87-2.04)
		90	3.61 (2.62-5.02)	5.03 (3.50 -7.17)	2.15 (1.68-2.76)	2.83 (2.18-3.69)	1.32 (1.07-1.63)	1.43 (1.19, 1.68)
	30	0	1.09 (0.57-2.08)	2.10 (1.02 -4.21)	0.99 (0.60-1.62)	1.74 (1.02-2.93)	0.88 (0.56-1.4)	1.43 (0.88-2.31)
		30	1.51 (0.88-2.60)	2.60 (1.37 -4.87)	1.40 (0.89-2.18)	2.08 (1.29-3.31)	1.11 (0.74-1.66)	1.58 (1.04-2.42)
		60	2.75 (1.76-4.27)	3.92 (2.42 -6.34)	2.27 (1.59-3.27)	2.73 (1.85-4.01)	1.8 (1.29-2.52)	1.89 (1.34-2.67)
		90	8.09 (6.11-10.73)	8.11 (5.97-11.05)	5.27 (4.18-6.63)	4.57 (3.58-5.83)	3.39 (2.76-4.17)	2.55 (2.06-3.17)
	60	0	1.81 (1.07-3.09)	2.93 (1.63 -5.15)	1.64 (1.06-2.55)	2.20 (1.37 -3.51)	1.54 (1.02-2.31)	1.71 (1.12-2.64)
		30	2.61 (1.63-4.23)	3.62 (2.15 -6.08)	2.34 (1.58-3.48)	2.69 (1.79-4.07)	2.07 (1.42-3.02)	1.95 (1.32-2.91)
		60	4.71 (3.2-6.9)	5.41 (3.55 -8.28)	4.10 (2.94-5.75)	3.76 (2.64-5.35)	3.43 (2.48-4.79)	2.54 (1.80-3.57)
		90	14.36 (10.9-18.9)	12.49 (9.24-16.84)	10.39 (8.13-13.29)	7.48 (5.78-9.69)	7.18 (5.71-9.04)	4.19 (3.30-5.35)
Median 7 years. Range 0-27 years (γ = 4)	0	0	0.35 (0.05-2.92)	0.9 (0.11-9.73)	0.31 (0.07-1.38)	0.97 (0.22-4.5)	0.26 (0.08-0.83)	1.03 (0.31-3.47)
		30	0.53 (0.12-3.11)	1.16 (0.19-7.65)	0.43 (0.13-1.41)	1.29 (0.36-4.33)	0.37 (0.14-0.98)	1.16 (0.41-3.32)
		60	0.93 (0.31-2.95)	2.18 (0.63-7.85)	0.75 (0.31-1.75)	1.74 (0.72-4.22)	0.58 (0.28-1.19)	1.36 (0.63-2.93)
		90	4.13 (2.58-6.67)	5.71 (3.37-9.75)	2.24 (1.57-3.22)	2.99 (2.03-4.38)	1.27 (0.93-1.72)	1.74 (1.26-2.40)
	30	0	1.88 (0.75-4.78)	3.1 (1.10-9.02)	1.73 (0.80-3.74)	2.35 (1.02-5.38)	1.5 (0.72-3.11)	1.76 (0.83-3.75)
		30	2.71 (1.19-6.25)	3.89 (1.56-9.89)	2.16 (1.11-4.27)	2.75 (1.31-5.71)	1.89 (0.98-3.59)	2.01 (1.02-3.94)
		60	4.73 (2.47-9.05)	5.57 (2.75-11.61)	3.79 (2.16-6.73)	3.74 (2.06-6.81)	3.06 (1.78-5.26)	2.39 (1.38-4.14)
		90	14.96 (9.84-22.58)	12.7 (8.19-19.61)	8.43 (6.05-11.7)	6.44 (4.57-9.12)	5.12 (3.83-6.85)	3.28 (2.44-4.44)
	60	0	3.45 (1.57-7.59)	4.42 (1.82-10.78)	3.29 (1.66-6.5)	3.24 (1.59-6.66)	3.01 (1.54-5.83)	2.37 (1.19-4.75)
		30	5.12 (2.56-10.3)	5.77 (2.62-12.43)	4.42 (2.41-8.24)	3.83 (2.06 -7.45)	3.94 (2.14-7.31)	2.89 (1.51 -5.52)
		60	8.97 (4.97-16.34)	8.55 (4.48 -16.2)	8.44 (4.84-14.56)	5.79 (3.27-10.28)	6.63 (3.85-11.22)	3.74 (2.14 -6.64)
		90	32.43 (21.0-49.3)	22.68 (14.6-35.3)	20.09 (13.9-29.0)	12.95 (8.84-19.04)	12.69 (9.1-17.65)	6.52 (4.44-8.81)

γ = gamma scale parameter, DEMB= Differential Exposure Misclassification Bias, DB= Detection Bias

Table 7.1 Relative Risks and Odds Ratios in primary cohort and case control analyses (C'td)

Baseline interval (O-D)	DE MB	DB	Coverage = 25%		Coverage = 50%		Coverage = 75%	
			Cohort RR (95% CI)	Case Control OR (95%CI)	Cohort RR (95%CI)	Case Control OR (95%CI)	Cohort RR (95%CI)	Case Control OR (95%CI)
Median 10 years. Range 0-40 years (γ = 6)	0	0	0 (0-NA)	0 (0-NA)	0.31 (0.04-4.43)	1.03 (0.16-12.32)	0.22 (0.03-1.83)	1.15 (0.18-6.7)
		30	0.48 (0.06-9.09)	1.12 (0.11-61.51)	0.36 (0.05-2.98)	1.15 (0.2 -8.23)	0.34 (0.08-1.55)	1.27 (0.26 -5.98)
		60	1.03 (0.2-6.25)	2.1 (0.34-15.68)	0.72 (0.21-2.65)	1.83 (0.5 -6.71)	0.55 (0.19-1.57)	1.43 (0.45-4.46)
		90	4.37 (2.35- 8.25)	6.08 (2.93-12.35)	2.24 (1.39-3.62)	2.99 (1.79 -5)	1.26 (0.84-1.88)	1.73 (1.14 -2.62)
	30	0	2.34 (0.67- 8.67)	3.76 (0.90-16.51)	2.29 (0.8-6.82)	2.91 (0.93 -8.74)	2.02 (0.76-5.43)	1.95 (0.70 -5.61)
		30	3.82 (1.22-11.13)	4.96 (1.49-16.85)	2.87 (1.13-7.32)	3.41 (1.25 -9.25)	2.77 (1.11-6.53)	2.19 (0.87 -5.58)
		60	6.19 (2.48-15.72)	7.47 (2.67-20.57)	5.65 (2.56-12.36)	4.52 (2 -10.46)	4.08 (1.92-8.38)	2.76 (1.26-5.85)
		90	19.32 (11.4-33.15)	15.55 (8.85-27.5)	10.36 (6.83-15.73)	7.55 (4.86-11.72)	6.11 (4.24-8.82)	3.76 (2.56-5.47)
	60	0	4.76 (1.66-13.52)	5.78 (1.81-19.46)	4.31 (1.68-10.74)	4.16 (1.54-11.17)	4.54 (1.69-11.83)	2.85 (1.05-7.6)
		30	6.82 (2.71-17.06)	7.24 (2.54-21.47)	6.44 (2.67-15.22)	5.22 (2.04-12.88)	5.71 (2.34-13.40)	3.17 (1.32-7.97)
		60	12.92 (5.70-29.72)	11.30 (4.53-27.27)	10.63 (5.08-22.61)	7.73 (3.44-16.83)	8.65 (4.14-18.89)	4.83 (2.18-10.74)
		90	46.02 (25.69-82.4)	32.1 (17.8-58.5)	28.4 (17.1-47.1)	16.98 (10.15-28.1)	17.29 (11.1-27.0)	7.74 (4.98-12.12)

γ = gamma scale parameter, DEMB= Differential Exposure Misclassification Bias, DB= Detection Bias

When combining the effect of detection bias and differential exposure misclassification , the estimates were higher in cohort analyses than in case-control analyses as the biases became more pronounced. In the most extreme scenario, with a median 90% reduction in the onset-to-diagnosis interval in vaccinated cases and a median probability of differential misclassification equal to 60% in vaccinated cases, we found a RR of 28.4 in the cohort analysis (95% CI: 17.13, 47.12). The same parameter settings produced an OR of 16.98 (95% CI: 10.15, 43.85) in the case-control analysis (Table 1). In the absence of either source of bias, median RR estimates from the cohort analysis for all scenarios were less than one when observation time was limited. However, with extension of observation time up to 25 years, the RR was estimated to equal to the simulated RR of one.

Results from case-control analyses were less inflated when detection bias and differential exposure misclassification were present. For both case-control and cohort designs, increased vaccination coverage and a shorter baseline onset-to-diagnosis interval lead to RR estimates closer to the true rate of one when biases are present (Table 7.1).

When combining the effect of detection bias and differential exposure misclassification , the estimates were higher in cohort analyses than in case-control analyses as the biases became more pronounced. In the most extreme scenario, with a median90% reduction in the onset-to-

diagnosis interval in vaccinated cases and a median probability of differential misclassification bias equal to 60% in vaccinated cases, we found a RR of 28.4 in the cohort analysis (95% CI: 17.13 - 47.12). The same parameter settings produced an OR of 16.98 (95% CI: 10.15 - 43.85) in the case-control analysis (Table 7.1). Increased vaccination coverage and a shorter baseline onset-to-diagnosis interval each led to reduced impact of bias.

Results from case-control analyses were less inflated when detection bias and differential exposure misclassification were present. For both case-control and cohort designs, increased vaccination coverage leads to RR estimates closer to the true rate of one when biases are present (Table 7.1).

Extension of the case capture period reduces the bias (Figure 7.4). With each extension, the rate of narcolepsy in vaccinated subjects converges toward the background rate.

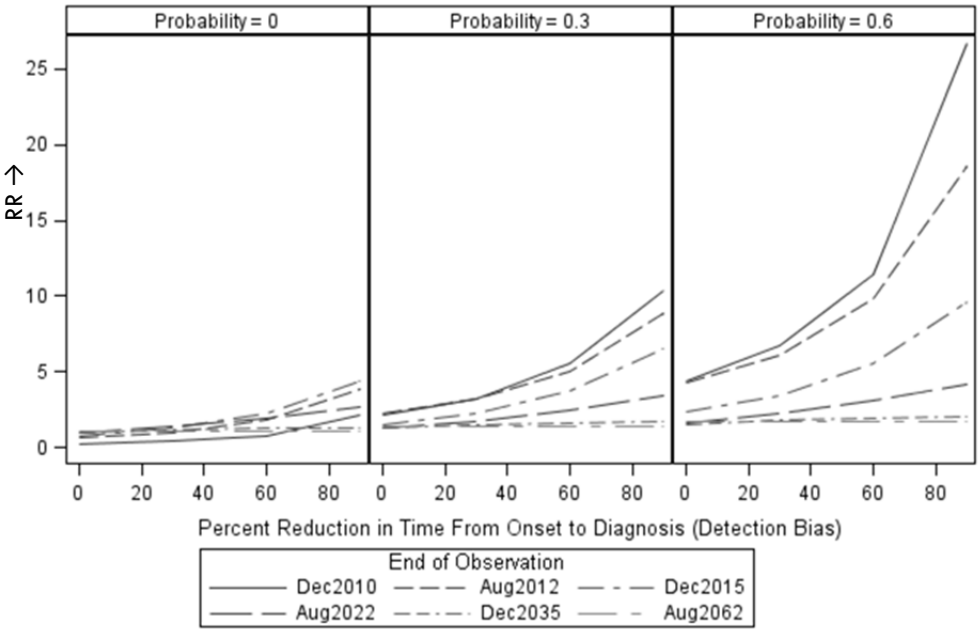


Figure 7.4 Relative rates of narcolepsy by probability of differential exposure misclassification with extended observation periods, cohort analysis, 50% vaccination coverage, start observation 1 April 2009

As illustrated in Figure 7.5 reduction in time from onset to diagnoses leads to incidences greater than the background rate in the period following awareness of the association in vaccinated cases, followed by reduction in the incidence rate to levels below the background rate

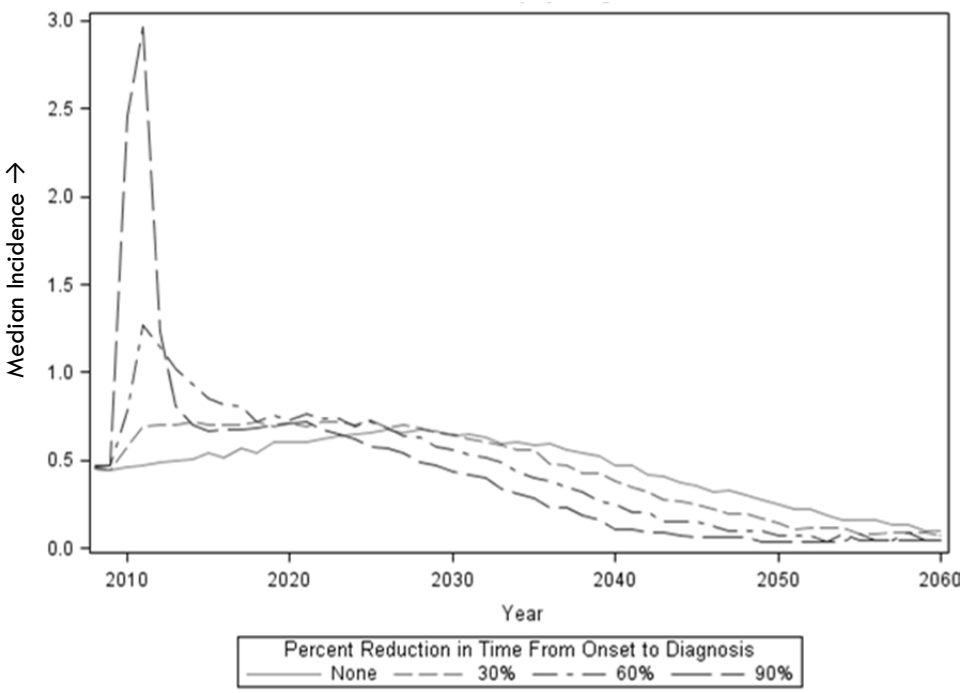


Figure 7.5 Incidence of new narcolepsy diagnoses over time

When cases with an onset date after August 15, 2010 were excluded, the RR was 1.87 (95% CI: 1.15, 3.05) in cohort analyses for the extreme setting of detection bias in the absence of differential exposure misclassification. Similarly, the RR was 4.45 (1.76, 11.67) with exclusion of cases with onset after August 15, 2010 at the most extreme setting of differential exposure misclassification in the absence of detection bias. Exclusion of cases with EDS onset dates after media attention, with a 90% reduction in the onset-to-diagnosis interval and a 60% probability of differential exposure misclassification, produced an RR of 27.10 (95% CI: 16.52, 44.11) while the estimate was 28.4 (95% CI: 17.13, 47.12) when these cases were not excluded. Exclusion of all cases with a diagnosis of narcolepsy after media attention resulted in estimates less than one and confidence intervals including one for all parameter settings. Excluding these cases nullified the effect of differential misclassification bias because only those cases diagnosed after media attention were simulated to misattribute their date of EDS onset to the period following vaccination.

Discussion

Our results indicate that, in the absence of a real association between Pandemrix and narcolepsy, the presence of detection bias or differential exposure misclassification elevates risk estimates.

In the absence of either source of bias, median RR estimates from the cohort analysis for all scenarios were less than the expected value of one. Our explanation for this observation is as follows. The study observation period is limited and the interval between onset and diagnosis can be longer than the study observation time, therefore, as diagnosis is the criteria for case inclusion, a number of cases with onset within the observation period will not be included as case. However, exposed and unexposed person time within the cohort is fixed. When we analyzed all cases with onset within the observation period regardless of their diagnosis date, the RR was equal to one. In the absence of either bias, using diagnosis dates for case capture, an observation period as long as 25 years would be necessary to obtain the true RR of one.

We found that biased attribution of EDS onset (differential exposure misclassification) has a greater impact on the estimates than a reduction in the EDS onset-to-diagnosis interval (detection bias) both in the cohort and case-control designs. While detection bias increases the relative risk estimates, the effect is not discernible until the onset-to-diagnosis interval is so reduced that many additional cases can be detected in a short observation period. The simultaneous presence of detection bias and differential exposure misclassification increases RRs more rapidly than could be expected by the effect size of each bias in isolation.

In an attempt to exclude detection bias, several published studies limited their primary observation period for EDS onset to the period before media attention (15, 18) or included sensitivity analyses using such a reduced study period (28). Additionally, studies used primary index dates that were thought to be less susceptible to such a bias including onset of symptoms (14, 19), first contact with health care (18, 28) or referral to specialist care (15, 20). In line with observations from our simulations, limiting analysis to subjects with an onset date prior to media attention will not eliminate the effect of detection bias, since all patients need to be diagnosed to be included, which is where the bias arises. To illustrate this, when limiting cases to those with an EDS date before media attention, Nohynek et al. found that the RR increased from 11·4 to 12·7 (18) and O'Flanagan et al. found that the RR increased from 13·0 to 14·5 (21). Since only diagnosed subjects can be included as cases, detection bias will be unavoidable if the onset-to-diagnosis interval is shorter in vaccinated individuals. The only way to circumvent the combined effects of detection bias and differential exposure misclassification would be to select only patients diagnosed before media attention. This will result in limited observation time and limited case inclusion as illustrated by our simulations and as was shown in the VAESCO study (13). We are not aware of any existing statistical

methods to control for detection bias although quantitative bias analysis could adjust for hypothesized biases (29).

With limited observation time, we found that, in the presence of detection bias and differential exposure misclassification, estimates from the case-control design are less inflated than those from the cohort design. The resilience of the case-control in this scenario has several reasons: the outcome is rare and the pool of controls, matched only by sex and age at onset, is large; also, the invariability of exposed person time, which is limited by observation time and vaccine coverage in the cohort approach, is avoided. Additionally, in this simulated scenario, we were able to sample controls from the same population as the cases and to assess their exposure without error, thereby avoiding the most problematic sources of bias in case-control studies. The only study to date in which data were analyzed using both a case-control and a cohort design found lower estimates in the case-control than in the cohort design (16).

Increased vaccination coverage reduced the bias in cohort and case-control analyses. In cohort analyses, this is explained by an increase in the person time denominator for vaccinated cases with a smaller increase in events and, simultaneously, a decrease in the person time denominator for unvaccinated cases with a smaller decrease in the number of events. In case-control analyses, this could be attributed to a greater probability of matching to vaccinated controls as vaccination coverage increases.

When a shorter interval from onset to diagnosis was assumed, the impact of simulated biases was less pronounced. This is due to the fact that, with a shorter onset-to-diagnosis interval, more cases, whether vaccinated or not, are being captured during the study period.

We chose to simulate only those sources of bias for which data in the absence of a vaccine safety signal exists and for which simulated variables could be modified to mimic the bias. Our simulations therefore do not reflect all of the biases that could potentially affect estimates of an association between Pandemrix and narcolepsy. By focusing on biases that could be evaluated without making untenable assumptions, these simulations provide insights that can improve rapid evaluation of vaccine safety signals by decision makers. There were several uncertainties, including the true background rate of narcolepsy and the true interval between onset of symptoms and diagnosis, for which we made assumptions in order to conduct our simulations. The validity of these assumptions will ultimately determine the robustness of our simulations.

The introduction of a new vaccine, or an existing vaccine in new populations, requires the assessment of vaccine safety. Large numbers of people can be exposed in a relatively short period providing a challenge to real-time safety surveillance. In such situations, as illustrated by the experience with Pandemrix and narcolepsy during the 2009/2010 H1N1 pandemic,

it can be difficult to determine if a safety finding is a true association or not. Despite these challenges, the timely and accurate assessment of potential associations between adverse events and vaccination are crucial to ensure vaccine safety and maintain the public's confidence. We believe that our simulations provide useful insights for the design and interpretation of future studies. Importantly, our results illustrate that in future analyses of safety signals for diseases with long latency periods for which observation times are limited the effect of limited case capture together with fixed person time denominators should be recognized. Similarly, the changes in exposed and unexposed person time denominators with changing vaccination coverage should be also taken into account. As we have shown, the case-control design provides less biased estimates in these circumstances as it does not require the calculation of person time. Moreover, our simulations illustrate the importance of not only understanding background rates of adverse events of special interest prior to vaccination campaigns, but also having insight in the background onset-to-diagnosis interval.

To conclude, our results indicate that, in the absence of a real association between the vaccine and narcolepsy, presence of detection bias and differential exposure misclassification could account for elevated RRs in vaccinees in association studies. While this does not exclude a real increased risk of narcolepsy following Pandemrix, it is possible that the levels of increased risk observed were at least partially due to bias.

Acknowledgements:

The authors would like to thank Dr. Hector Izurieta for assistance with conception of the project and editing of the final manuscript. All work was conducted at Erasmus University Medical Centre with no external funding.

References

1. National Institute for Health and Welfare (THL). National Institute for Health and Welfare recommends discontinuation of Pandemrix vaccinations 25 Aug 2010 Available from: http://www.thl.fi/en_US/web/en/pressrelease?id=22930.

2. Medical Products Agency. The MPA investigates reports of narcolepsy in patients vaccinated with Pandemrix 2010 [cited 2013 09/10/2013]. Available from: http://www.lakemedelsverket.se/english/All-news/NYHETER-2010/The-MPA-investigates-reports-of-narcolepsy-in-patients-vaccinated-with-Pandemrix/.

3. European Medicines Agency. Committee for Medicinal Products for Human Use (CHMP) Assessment report - Pandemrix Influenza vaccine (H1N1) (split virion, inactivated, adjuvanted) A/California/7/2009 (H1N1)v like strain (x-179a) - Procedure No.: EMEA/H/C/000832/A20/0045. 21 July 2011.

4. European Medicines Agency. European Medicines Agency starts review of Pandemrix. 27 August 2010 [cited 2012 22/03/2012]. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Press_release/2010/08/WC500096005.pdf.

5. European Medicines Agency. Press release: European Medicines Agency recommends restricting use of Pandemrix 21 July 2011. Available from: http://www.ema.europa.eu/ema/index.jsp?curl=pages/news_and_events/news/2011/07/news_detail_001312.jsp&murl=menus/news_and_events/news_and_events.jsp&mid=WC0b01ac058004d5c1&jsenabled=true.

6. Kornum B, Faraco J, Mignot E. Narcolepsy with hypocretin/orexin deficiency, infections and autoimmunity of the brain. Curr Opin Neurobiol. 2011;21:897-903.

7. Nishino S, Ripley B, Overeem S, Lammers GJ, Mignot E. Hypocretin (orexin) deficiency in human narcolepsy. Lancet. 2000 Jan 1;355(9197):39-40. PubMed PMID: 10615891.

8. Overeem S, Black JL, 3rd, Lammers GJ. Narcolepsy: immunological aspects. Sleep medicine reviews. 2008 Apr;12(2):95-107. PubMed PMID: 18291691. Pubmed Central PMCID: 2366136.

9. Luca G, Haba-Rubio J, Dauvilliers Y, Lammers GJ, Overeem S, Donjacour CE, et al. Clinical, polysomnographic and genome-wide association analyses of narcolepsy with cataplexy: a European Narcolepsy Network study. Journal of sleep research. 2013 Oct;22(5):482-95. PubMed PMID: 23496005.

10. Thorpy MJ, Krieger AC. Delayed diagnosis of narcolepsy: characterization and impact. Sleep medicine. 2014 May;15(5):502-7. PubMed PMID: 24780133.

11. Morrish E, King MA, Smith IE, Shneerson JM. Factors associated with a delay in the diagnosis of narcolepsy. Sleep medicine. 2004 Jan;5(1):37-41. PubMed PMID: 14725825. Epub 2004/01/17. eng.

12. Singh AK, Mahlios J, Mignot E. Genetic association, seasonal infections and autoimmune basis of narcolepsy. Journal of autoimmunity. 2013 Jun;43:26-31. PubMed PMID: 23497937.

13. Medical Products Agency. Narcolepsy – Case inventory study in Sweden 2009-2010 2011. Available from: http://www.lakemedelsverket.se/english/All-news/NYHETER-2011/Report-from-an-epidemiological-study-in-Sweden-on-vaccination-with-Pandemrix-and-narcolepsy-/.

14. Medical Products Agency. A registry based comparative cohort study in four Swedish counties of the risk for narcolepsy after vaccination with Pandemrix - A first and preliminary report, by the 2011 [March 28,2011]. Available from: www.mpa.se

15. European Centre for Disease Prevention and Control. Narcolepsy in association with pandemic influenza vaccination (a multi-country European epidemiological investigation). Stockholm: September 2012.

16. Montplaisir J, Petit D, Quinn MJ, Ouakki M, Deceuninck G, Desautels A, et al. Risk of Narcolepsy Associated with Inactivated Adjuvanted (AS03) A/H1N1 (2009) Pandemic Influenza Vaccine in Quebec. PloS one. 2014;9(9):e108489. PubMed PMID: 25264897. Pubmed Central PMCID: 4180737.

17. Persson I, Granath F, Askling J, Ludvigsson JF, Olsson T, Feltelius N. Risks of neurological and immune-related diseases, including narcolepsy, after vaccination with Pandemrix: a population- and registry-based cohort study with over 2 years of follow-up. Journal of internal medicine. 2013 Oct 17. PubMed PMID: 24134219.

18. Nohynek H, Jokinen J, Partinen M, Vaarala O, Kirjavainen T, Sundman J, et al. AS03 adjuvanted AH1N1 vaccine associated with an abrupt increase in the incidence of childhood narcolepsy in Finland. PloS one. 2012;7(3):e33536. PubMed PMID: 22470453. Pubmed Central PMCID: 3314666.

19. Miller E, Andrews N, Stellitano L, Stowe J, Winstone AM, Shneerson J, et al. Risk of narcolepsy in children and young people receiving AS03 adjuvanted pandemic A/H1N1 2009 influenza vaccine: retrospective analysis. BMJ. 2013;346:f794. PubMed PMID: 23444425. Epub 2013/02/28. eng.

20. Dauvilliers Y, Arnulf I, Lecendreux M, Monaca Charley C, Franco P, Drouot X, et al. Increased risk of narcolepsy in children and adults after pandemic H1N1 vaccination in France. Brain : a journal of neurology. 2013 Aug;136(Pt 8):2486-96. PubMed PMID: 23884811.

21. O'Flanagan D, Barret A, Foley M, Cotter S, Bonner C, Crowe C, et al. Investigation of an association between onset of narcolepsy and vaccination with pandemic influenza vaccine, Ireland April 2009-December 2010. Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin. 2014;19(17). PubMed PMID: 24821121.

22. Barker CI, Snape MD. Pandemic influenza A H1N1 vaccines and narcolepsy: vaccine safety surveillance in action. The Lancet Infectious diseases. 2014 Mar;14(3):227-38. PubMed PMID: 24360892.

23. Sturkenboom MC. The narcolepsy-pandemic influenza story: Can the truth ever be unraveled? Vaccine. 2015 Jun 8;33 Suppl 2:B6-B13. PubMed PMID: 26022571.

24. Schonberger LB, Bregman DJ, Sullivan-Bolyai JZ, Keenlyside RA, Ziegler DW, Retailliau HF, et al. Guillain-Barre syndrome following vaccination in the National Influenza Immunization Program, United States, 1976--1977. American journal of epidemiology. 1979 Aug;110(2):105-23. PubMed PMID: 463869.

25. Andrews N, Miller E, Taylor B, Lingam R, Simmons A, Stowe J, et al. Recall bias, MMR, and autism. Archives of disease in childhood. 2002 Dec;87(6):493-4. PubMed PMID: 12456546. Pubmed Central PMCID: 1755823.

26. Silber MH, Krahn LE, Olson EJ, Pankratz VS. The epidemiology of narcolepsy in Olmsted County, Minnesota: a population-based study. Sleep. 2002 Mar 15;25(2):197-202. PubMed PMID: 11902429.

27. Mereckiene J, Cotter S, Weber JT, Nicoll A, D'Ancona F, Lopalco PL, et al. Influenza A(H1N1)pdm09 vaccination policies and coverage in Europe. Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin. 2012;17(4). PubMed PMID: 22297139. Epub 2012/02/03. eng.

28. O'Flanagan D, Bonner C, Crowe C, Lynch B, Sweeney B, Gilvarry J, et al. Investigation of an increase in the incidence of narcolepsy in children and adolescents in 2009 and 2010. Final Report of National Narcolepsy Study Steering Committee. Department of Health, Ireland. 2012.

29. Lash TL, Fox MP, MacLehose RF, Maldonado G, McCandless LC, Greenland S. Good practices for quantitative bias analysis. International journal of epidemiology. 2014 Dec;43(6):1969-85. PubMed PMID: 25080530. Epub 2014/08/01. eng.

Chapter 8. Adjuvanted versus non-adjuvanted influenza vaccines in young children: comparing results from recent clinical trials

Leonoor Wijnans

Daniel Weibel

Miriam Sturkenboom

Published in: Clinical Investigation (2012) 3(4): 395-408

Abstract

A relatively high burden of influenza is experienced by young children. In order to successfully tackle the burden of influenza in children effective vaccines are necessary. Accumulated evidence on the efficacy and effectiveness of traditional inactivated split or subunit TIVs points towards no significant protection in the youngest children, who are largely unprimed. Adjuvanted influenza vaccines have been developed to improve the immune response and could possibly overcome limitations of traditional influenza vaccines in the youngest age groups.

In this review we consider evidence from recent clinical trials of adjuvanted vs non-adjuvanted influenza vaccines in children younger than three years of age. We highlight important findings from identified studies and discuss ongoing challenges concerning the use of adjuvanted influenza vaccine in young children.

Background

The Burden of Influenza in Children

A relatively high burden of influenza is experienced by young children (1-4). Young children often have not been exposed to influenza viral antigens, meaning that they have not been vaccinated or infected by influenza, thus are immunologically naïve. As a result they have limited protection against infection with influenza viruses. Serological evidence suggests that around the age of six most children will have encountered at least one type of influenza virus (5). As a result they will have built up some immunity through the presence of cross reacting antibodies against drifted strains. With increasing age it is primarily children with underlying cardiopulmonary, neurological or immunological disorders that are most vulnerable to the consequences of influenza infection (6). As influenza viruses drift, individuals could be at a renewed risk of infection as pre-exiting antibodies no longer confer protection. In order to overcome this, vaccines would ideally offer broad protection against heterotypic strains during influenza epidemics and pandemics.

Dependent on the predominant circulating strain there is large variability in actual morbidity by year, region and age group. The burden is a combination of morbidity and mortality resulting from influenza infection, but also of indirect effects in those with underlying chronic conditions. For example, influenza infection is associated with exacerbations of wheezing and asthma (7). In addition, the impact on primary and secondary health care resources, parental absenteeism from work and child absenteeism from school should also be considered. The latter can be considerable, as demonstrated in a recent study in Hong Kong (8). This diversity and variability of the influenza burden should be kept in mind when considering estimates expressing different aspects of the burden.

An estimated 20-30% of the paediatric population is affected during annual influenza outbreaks (2). This translates into considerable use of health care resources, caused by outpatient visits, prescription of antibiotics and anti pyretics and hospitalizations (9). Rates of influenza related hospitalisation have been reported ranging from 10 to over 100 per 10,000 children under the age of one. Although less hospitalisation occurs in older children, influenza remains one of the most important causes for hospitalisation (2). Influenza infection can lead to complications in children such as encephalitis, acute encephalopathy, Guillain-Barré syndrome, myocarditis and cardiac failure (10, 11). Global mortality due to influenza in children under five in 2008 was estimated at between 28.000 and 111.500 deaths (12), most of which occurred in developing countries. Noteworthy, it has been found that about half of influenza deaths in children occur in previously healthy children (13). With effective vaccination all these deaths could have been prevented.

Need for better influenza vaccines

Vaccination is the mainstay in the prevention of influenza and in order to successfully tackle the burden of influenza in children effective vaccines are necessary. Split or subunit trivalent inactivated vaccines (TIVs) are available for seasonal influenza vaccination programs in most countries. It has long been known that these vaccines induce a limited immune response in immunologically naïve persons (14). Now, accumulated evidence on the efficacy and effectiveness of traditional inactivated split or subunit TIVs points towards no significant protection in the youngest children, who are largely immunologically unprimed (15, 16). This could indicate that inactivated split or subunit vaccines do not adequately prime immune naïve persons. Priming is the activation and expansion of antigen specific T-cells which are able to establish memory and exert effector functions (17) and is essential for a lasting protective immune response. The possible inability to prime can have large implications if these vaccines were relied on in a pandemic scenario in which there is limited or no cross-reactive antibody present in the population. In children between two to six years there is evidence of protection albeit being moderate (15, 16, 18).

A range of inactivated split or subunit vaccines has been licensed around the world, several of which are also licensed for use in children. Special paediatric formulations exist which consist of half the adult dose. As pointed out, the evidence to support the use of split or subunit TIVs in young children is limited and does not point towards a clear benefit (15, 16, 18). The evidence to support use of a half dose in this group is more limited. A recent study showed that the full dose provided superior immunogenicity compared to the half dose in infants and toddlers (6 to 23 months) without increased reactogenicity (19), bringing the existence of half dose recommendations in to question. It is generally recommended that young children who have not been previously vaccinated with influenza vaccine, and are likely to be unprimed, receive two doses. Some studies have shown that two doses could result in effective protection in young children (20-24), yet evidence is limited. Another option for improving the response to TIVs is increasing the presentation of antigens to antigen-presenting-cells such as dendritic cells. This can be achieved through intradermal vaccination. Intradermal influenza vaccines are licensed for use in adults and elderly in the US and in Europe, but not for use in children (25, 26). There is only limited data in children, but studies have demonstrated that intradermal influenza vaccine increases the immune response compared to intramuscular vaccination in primed children over the age of three and in children aged 6 to 12 months old (27, 28).

In addition to inactivated influenza vaccines, live attenuated influenza vaccines (LAIVs) are available in several parts of the world. LAIVs have been found to be more effective than inactivated influenza vaccines in children (29, 30). However LAIV cannot be given to children

under the age of two as its use has been associated with increased rates of medically attended wheezing and hospitalisation (30-33).

Clearly, the current situation of influenza vaccination is poignant. For the age group with the highest attack rates there is a lack of effective vaccines. Adjuvanted influenza vaccines have been developed to improve the immune response and could possibly overcome limitations of traditional influenza vaccines in the youngest age groups.

Adjuvanted Influenza Vaccines

Adjuvants are components included in vaccine formulations in order to potentiate the immune response. Experience with adjuvanted influenza vaccines goes back to the 1950-s, when mineral-in-oil adjuvanted influenza vaccines were used on a large scale. These were abandoned as their use was associated with severe local reactions including cysts and abscess forming (34). Other adjuvanting systems have been studied however it wasn't until the end of the 20th century that the first adjuvanted influenza vaccines were licensed. In 1997 an oil-in-water (MF59™) adjuvanted seasonal influenza vaccine was licensed for use in older adults in Europe (35). In that same year a virosomal adjuvanted influenza vaccine was licensed for use in all age groups (36). Towards the end of the 20th century increased awareness of potential pandemic threats in a world with only limited production capacity for influenza vaccines made way for the development and licensing of new adjuvanted pandemic influenza vaccines (37). These were eventually used on a very large scale during the 2009/2010 pandemic, also in children (38).

A variety of different formulations of adjuvanted influenza vaccines have been studied over the past decades (39). Several pre-pandemic, pandemic and seasonal adjuvanted influenza vaccines are licensed including aluminium adjuvanted, oil-in-water adjuvanted and virosomal adjuvanted vaccines (40, 41). It has been demonstrated that oil-in-water adjuvants can potentiate the immune response to influenza vaccine thereby reducing the amount of HA needed (40). Studies have also shown that the MF59 adjuvant induces a broader immune response providing protection against drifted strains, and increase the diversity and affinity of antibodies (39, 42, 43). No two adjuvants are the same, and the interaction between the virosomal antigens and adjuvants can be different for different antigens. Therefore safety and efficacy of each adjuvanted vaccine needs to be considered separately. An overview of adjuvanted influenza vaccines and recommended dosage for children aged 6 to 36 months is given in Table 8.1.

Table 8.1 Overview of adjuvanted influenza vaccines with recommended dosage for children aged 6-35 months.

Brand name	Culture medium	Product description	HA amount (0.5 ml)	Adjuvanting system	Dosage (6-35 months)
Adjuvanted seasonal influenza vaccines					
Fluad®	Egg	Trivalent influenza vaccine (surface antigen, inactivated, MF59-adjuvanted)	3x 15 µg	MF59C.1 adjuvant	1 dose of 0.25 ml. For children who have not previously been vaccinated, a 2nd dose should be given after an interval of at least 4 weeks.
Inflexal® V	Egg	Trivalent influenza vaccine (surface antigen, inactivated, virosome)	3x 15 µg	Virosomes	Dosages of 0.25 or 0.5 ml may be given. For children who have not previously been vaccinated, a 2nd dose should be given after an interval of at least 4 weeks.
Adjuvanted H1N1pdm09 vaccines					
Fluval® P	Egg	Inactivated, whole reassortant virus A/California/7/2009 (H1N1)v-like strain	6 µg	Aluminium phosphate gel	1 dose of 0.25 ml
Arepanrix®[2]	Egg	Inactivated, split-influenza, reassortant, A/California/7/2009 (H1N1)v-like strain	3.75 µg	AS03 adjuvant	1 dose of 0.25 ml [1]
Pandemrix®	Egg	Inactivated, split-influenza, reassortant, A/California/7/2009 (H1N1)v-like strain	3.75 µg	AS03 adjuvant	1 dose of 0.25 ml [1]
Humenza®[2]	Egg	A/California/7/2009 (H1N1)-like strain (NYMC X-179A)	3.8 µg	AF03 adjuvant	1 dose of 0.25 ml [1]
Focetria®	Egg	Inactivated, surface-influenza antigens (haemagglutinin and neuraminidase), reassortant, A/California/7/2009 (H1N1)v-like strain	7.5 µg	MF59C.1 adjuvant	1 dose of 0.5 ml at an elected date. A 2nd dose of vaccine should be given after an interval of at least 3 weeks.
Celtura®	MDCK[3] cells	A/California/7/2009 (H1N1)-like strain (X-179A)	3.75 µg	MF59C.1 adjuvant	1 dose of 0.25 ml at an elected date. A 2nd dose of vaccine should be given after an interval of at least 3 weeks.

[1]The Summary of Product Characteristics indicates that the immune response is increased with a second dose, and this can be considered taking into account the increased reactogenicity seen with a second dose.
[2]Withdrawn from the market
[3]Madin Darby Canine Kidney

In the present paper we review evidence from recent clinical trials of adjuvanted influenza vaccines versus non-adjuvanted influenza vaccines in the younger, unprimed, children (up to 3 years old) in order to evaluate whether adjuvanted vaccines might be able to address the limitations of current inactivated non-adjuvanted inactivated split or subunit vaccines. Data on comparative immunogenicity, efficacy and safety will be brought together to form a picture whether adjuvanted vaccines form a safe and efficacious option for protecting the youngest children against influenza and which existing gaps would need to be addressed.

We conducted a search of electronic databases (PubMed, EMBASE) in order to identify relevant studies comparing adjuvanted with non-adjuvanted influenza vaccines in infants and children. The medical subject heading terms 'influenza vaccine', 'adjuvants, immunologic' were combined. The search was limited to articles concerning infants and pre-school aged children up to 3 years of age. Publications up to November 2012 were included. Pertinent articles were retrieved and reference lists were scanned to identify any further publications. Furthermore, electronic public assessment reports (EPARs) on the website of the European Medicines Agency (EMA) were consulted for data on pandemic, pre-pandemic and seasonal inactivated adjuvanted influenza vaccines.

Immunogenicity of adjuvanted vs non-adjuvanted vaccines

The haemagglutination inhibition (HI) assay and virus neutralisation (VN) or microneutralisation (MN) assay are bioassays widely used to measure the immune response to the influenza virus or vaccine in the serum. A limitation of both assays is the large intra-laboratory variability, which results from poor standardization (44, 45). Comparisons across studies are therefore not reliable, and only head-to-head comparative studies should be considered.

Based upon findings from a challenge study in healthy adults with attenuated strains (46), a cut-off value of HI-titre ≥ 1:40 is commonly used as a predictor for 50% protection in adults and elderly. Although no HI based correlate for children has ever been defined, the cut-off of HI≥ 1:40 or HI≥ 1:32 is also widely used as a measure of seroprotection to express the immune response against influenza vaccination in children. A recent study in children however found that an HI titre ≥1:110 correlated to 50% protection in children aged 6 to 72 months, and that the cut-off of 1:40 correlated with a mere 22% protection (47). It has not been established whether these findings can be extrapolated to other situations, i.e. influenza seasons, virus strains and populations. A VN or MN based correlate for protection has not been validated. Due to above named limitations, measures of seroprotection or seroconversion can be misleading and are likely to hamper a proper assessment of the benefits of a vaccine in the youngest age groups. Rather, the focus should be on more qualitative comparisons of the immune response such as geometric mean titres and ratios (post-immunization compared to pre-immunisation). In any case, without standardization of

assays and the availability of a validated correlate of protection for children it is not possible to translate immunogenicity findings from different studies into actual effects on protection against infection or disease offered by the vaccine.

Aluminium adjuvanted vaccines

Aluminium salts do not appear to potentiate the immune response to influenza antigens (40). In one study the addition of aluminium salts actually was found to decrease the immune response (48). In Hungary, an inactivated whole virus trivalent aluminium phosphate gel (ALPO4) adjuvanted influenza vaccine is licensed (49), of which also an H5N1 and an H1N1pdm09 variant exist (50, 51). No comparative immunogenicity, safety or efficacy data in young children could be found therefore it is not clear whether there is a benefit of ALPO4 compared to a whole virus vaccine.

One safety and immunogenicity study with the whole virion H5N1 aluminium phosphate vaccine (6 µg + ALPO4) in 12 children aged 9 to 17 years was identified (41). As there was no non-adjuvanted comparator it is unclear what the added benefit of the aluminium adjuvant for this vaccine is. Only limited safety data in children < 36 months could be found (52). Nolan et al. report on the immunogenicity and safety of two formulations of aluminium phosphate adjuvanted H5N1 vaccines (two doses of 30 µg HA + ALPO4 per or 45µg HA + ALPO4 given 20 days apart) in children aged 6 months to 9 years (53). Here too no non-adjuvanted control arm was included. Finally, one study was found in which aluminium adjuvanted whole virion influenza H5N1 vaccine was compared to non-adjuvanted influenza vaccine in children aged 6 months to 17 years (54). Children received two injections of vaccine containing either 30 µg HA + AL or 7.5 µg HA without adjuvant. Of the children in the 30 µg HA + Al vaccine arm, 79% achieved an HI titre ≥ 1:32, whilst 46% in the 7.5 µg HA arm achieved a titre ≥1:32. From a design perspective it is surprising that the HA content of the adjuvanted vaccine is higher than that of the non-adjuvanted comparator. The finding that the children who received 30 µg HA + AL adjuvant had higher immune responses than those receiving 7.5 µg HA cannot be attributed to the adjuvant as it could simply be a result of the higher HA content.

Virosomes

Kanra et al. (2004) reported on an open label randomised controlled trial in which the safety and immune response to a virosomal adjuvanted vaccine and a non-adjuvanted split influenza vaccine were compared in 454 children aged 6 to 71 months (55). Those previously vaccinated were considered primed and received a single dose. Unprimed children received a second dose after four weeks. Children up to 36 months of age received a half dose. The immunogenicity was assessed with an HI assay prior to vaccination, and 4 weeks after the last dose received. Although point estimates were higher for the virosomal

adjuvanted vaccine, differences were small and not statistically significant. No statistically significant difference between the adjuvanted and unadjuvanted vaccine in increase in GMTs was found. Seroconversion and seroprotection (percentage with HI ≥ 1:40) were also broadly similar between the two vaccines. A recently published study showed that a single adult dose (15µg) of virosomal adjuvanted influenza vaccine elicited a similar immune response as two half doses (7.5µg) given 4 weeks apart in children aged 6 to 36 months (56).

Oil-in-water adjuvanted vaccines

AF03

During the 2009/2010 H1N1 pandemic, an inactivated split virion H1N1pdm09 AF03 adjuvanted vaccine was licensed in Europe. This vaccine was not used. A study in 401 children aged 6 to 35 months looked at immunogenicity and safety of different dosages of this vaccine (57, 58). Children received either two doses of 1.9 µg HA + ½ AF03, 3.8 µg HA + ½ AF03, 3.8 µg HA + AF03 or 7.5 µg HA. The response following the first dose was modest, and improved with a second dose. Antibody titers were 5 to 7 times higher following adjuvanted vaccine compared to the non-adjuvanted vaccine. This also translated into better persistence of antibodies.

AS03

Two publications were identified that reported the immunogenicity and tolerability or safety of AS03 adjuvanted H1N1pdm09 vaccine (Pandemrix™) compared to a non-adjuvanted influenza vaccine (59, 60) in children aged 6 to 36 months.

Langley et al. (2012) randomized 323 children aged 6 months to <9 years of age to receive two doses of non-adjuvanted influenza A(H1N1)pdm09 vaccine (15 µg or 7.5 µg HA) or AS03 adjuvanted influenza A(H1N1)pdm09 vaccine (3.75 µg HA/AS03A or 1.9 µg HA/AS03B), 21 days apart. The immune response was measured as HI antibody response and as MN response and evaluated according to European regulatory (CHMP) criteria. Overall, immune responses were improved with the adjuvanted vaccine compared to the non-adjuvanted vaccine. CHMP criteria were met for all vaccine groups expect the half dose unadjuvanted group, however this carries little meaning as explained above.

In children aged 6 to 11 months antibody titres were 5 to 10 times higher with the adjuvanted compared to the non-adjuvanted vaccines following the first dose. A second dose further increased antibody titres in for all vaccine groups, resulting in titres 3 to 22 times higher in the adjuvanted compared to the non-adjuvanted groups. In children aged 12 to 35 months a similar pattern was seen, with higher responses in the adjuvanted vs the non-adjuvanted vaccine groups. Notably, the response to the half-dose unadjuvanted vaccine was higher in this age group compared with younger children. After 6 months, antibody

levels remained higher for the adjuvanted vaccine groups compared to the non-adjuvanted vaccine groups. Note that as only a modest number of young children was included (5 to 25 per vaccine group) there is limited power to detect differences and confidence intervals overlap.

In the study by Waddington et al. (2010) the immunogenicity and safety of a two dose regimen of AS03 adjuvanted split virion H1N1pdm09 vaccine was compared to a whole virion cell culture-derived H1N1pdm09 vaccine (Celvapan, Baxter) in children aged 6 months to 12 years. A single dose of the AS03 adjuvanted vaccine contained 1.9 µg HA whilst a single dose of the whole virion vaccine contained 7.5 µg. In all children the AS03 adjuvanted vaccine elicited higher antibody titres than the whole virion vaccine. In children aged 6 to 36 months the GMT after a second dose of AS03 adjuvanted vaccine was 461.0 compared to 44.0 for the whole virion vaccine. The fold rise in haemagglutination inhibition titre from baseline was also higher for the AS03 adjuvanted vaccine (107.4 vs 9.5). In line with the increase in GMTs, seroconversion rates were also consistently higher for the adjuvanted vaccine compared to the whole virion vaccine. Note that a whole virion is not comparable to a split or subunit vaccine. Whole virion vaccines not only contain surface proteins but also matrix proteins and genomic RNA. It has been suggested that whole virion vaccines have a "built-in adjuvant" through the remaining RNA in the vaccine (40). In an extension of this study the T-cell responses were evaluated 1 year after vaccination with the AS03 adjuvanted split virion and the non-adjuvanted whole virion H1N1pdm09 vaccines. An important observation in this study was that children who received an AS03 adjuvanted split virion H1N1pdm09 vaccine had higher T-cell responses to internal influenza antigens 1 year after vaccination compared to children who received a whole virion non-adjuvanted H1N1pdm09 vaccine (61).

MF59

A dose finding study by Block et al.. clearly demonstrated that MF59 enhances the immune response in children aged 6 to 36 months (62). In their study, 654 healthy children 6 to <36 months of age were randomised to receive two half doses of MF59-adjuvanted vaccine (3.75µg HA + ½ MF59); two half doses of non-adjuvanted vaccine (7.5µg HA); two full doses with half the amount of MF59 adjuvant (7.5µg HA + ½ MF59) or two full doses of non-adjuvanted vaccine (15 µg HA). Antibody responses were measured by the HI assay. On day 22, 3 weeks after the first dose, seroprotection rate (HI titre ≥ 1:40) was 79% (95% CI: 71% –86%) and 86% (95% CI: 79%–91%) in half dose and full dose adjuvanted group. The response was lower for non-adjuvanted vaccines, 37% (95% CI: 29%–46%) and 50% (95% CI: 41% – 59%) for the half and full dose respectively. Three weeks after the second dose the response increased to 100% (95% CI: 97% – 100%) in both adjuvanted groups and to 70% (95% CI: 61% – 78%) and 81% (95% CI: 74% – 88%) for the half and full dose non-adjuvanted group. The geometric mean ratio was also higher for both

adjuvanted vaccines. An important observation in this study was that after 6 months children immunized with the MF59-adjuvanted vaccine formulations had persisting antibodies, whilst this was not the case for children in the non-adjuvanted arms.

An indication that MF59-adjuvanted influenza vaccine also enhances cross-reactivity comes from the study by Vesikari et al (2009) in which not only HI titres against vaccine strains were measured but also against mismatched strains (63). In their study they randomised 281 unprimed children aged 6-36 months to receive either two doses of an MF59 adjuvanted inactivated split vaccine (7.5 µg HA per strain) or a non-adjuvanted inactivated split vaccine (7.5 µg HA per strain). After one year subjects received a repeat vaccination. Antibody titres were measured with the HI assay. GMTs and fold increase was significantly higher after MF59-adjuvanted vaccination compared to non-adjuvanted vaccination for all three vaccine strains. And although titres decreased over the following year, they remained significantly higher for the MF59 adjuvanted vaccine. The booster response was also stronger in those receiving the adjuvanted vaccine. When tested against mismatched strains, post vaccination titres and fold increase was significantly higher with the adjuvanted vaccine 3 weeks after the second dose for all three strains (A/H1N1, A/H3N2 and B). In an extension to this study, 89 children were re-vaccinated in the following season. Children who had received an adjuvanted vaccine in the previous season had higher pre-vaccination HI antibody titres than those who received a non-adjuvanted vaccine. Three weeks after being revaccinated, the immune responses were significantly higher following the adjuvanted vaccine compared to the non-adjuvanted vaccine (64).

Efficacy of adjuvanted vs non-adjuvanted vaccines

Only one single study was identified in which the efficacy of an adjuvanted influenza vaccine was compared to the efficacy of a non-adjuvanted influenza vaccine, and control vaccine, in young children. This large randomised controlled trial evaluated the protective efficacy of an MF59-adjuvanted seasonal influenza vaccine compared to that of a non-adjuvanted seasonal influenza vaccine and a non-influenza vaccine control in unprimed children aged 6 to 72 months (65). The study was conducted over two seasons. In the first season 654 children were randomised to receive adjuvanted influenza, non-adjuvanted subunit influenza or control (meningococcal) vaccine in a 2:1:1 ratio. In the second season 4,053 children were randomised to receive adjuvanted influenza, non-adjuvanted split influenza or control (meningococcal) vaccine in a 2:2:1 ratio. Efficacy was determined against influenza illness confirmed by reverse transcription polymerase-chain-reaction (RT-PCR) assay. Children up to 36 months received two half doses, older children received full doses. In the first season there were insufficient cases of influenza to determine vaccine efficacy. In the second year the vaccine efficacy (VE) against all strains was 86% (95% CI: 73% – 92%) for the MF59-adjuvanted vaccine. The VE for the non-adjuvanted split vaccine

was 40% (95% CI: 11% - 60%). For the subgroup aged 6 to 36 months this was 79% (95%CI: 55-90) vs 40% (95%CI:-6% - 66%) for the adjuvanted and non-adjuvanted vaccine respectively. Note that for children under two years of age there was no significant VE for the two half dose of non-adjuvanted vaccine (VE: 11%, 95%CI: -89% – 58%) whilst the VE for the adjuvanted vaccine remained relatively high at 77% (95% CI: 37% – 92%). The VE against matched strains was slightly higher.

Although the publication states that the study was conducted according to Good Clinical Practice (GCP) guidelines, during an inspection it was found that the study was not compliant with GCP standards. Several critical issues are discussed in the CHMP withdrawal assessment report (66) pertaining to the validity and adequacy of the PCR used, but also the reliability of recorded adverse events and suspected influenza cases (67). In response, the authors re-analysed all samples using validated methods, but also reanalysed the efficacy excluding one critical investigational site. This re-analysis yielded a higher VE for the adjuvanted vaccine and similar results concerning the exclusion of the questioned site. The authors pointed out that the VE for the non-adjuvanted vaccine is similar to those reported in the same age groups in published studies, providing external validation. In addition, serological findings from this study are in line with the efficacy findings. Nonetheless, as the GCP issues have not been resolved and there is no full insight into the extent to which the different issues affect the validity of the findings, uncertainty surrounding the findings of this trial will remain.

Safety of adjuvanted vs non-adjuvanted vaccines

Data from clinical trials

Clinical trials are useful for describing and comparing the reactogenicity of vaccine formulations, but are usually too small to detect and evaluate (rare) vaccine related adverse events as these are fortunately uncommon. This section therefore focusses on the comparative tolerability or reactogenicity of adjuvanted vs non-adjuvanted influenza vaccines. The collection and presentation of safety data varies between studies and publications. Although calls have been made to harmonize study protocols and the presentation of safety data in clinical study publications, this is not yet reality (68-70). Comparisons of safety between different types of vaccines should ideally come from head-to-head trials.

Non-adjuvanted split and subunit influenza vaccines have a long track record of safe use, also in the youngest age groups. Accumulated evidence shows that these vaccines are well tolerated with only a small minority of children reporting mild transient systemic reactions including malaise, fever and myalgia. Systemic symptoms are most prominent in children younger than 36 months of age, possibly as these are unprimed to the viral antigens. Most

frequently reported adverse events include fever, rash, injection-site reactions and febrile seizures (71-76).

Little can be concluded on the relative safety of aluminium-adjuvanted influenza vaccines compared to non-adjuvanted inactivated split or subunit vaccines in children <36 months of age. Results from head to head comparisons were not reported in the published studies (41, 53), or the non-adjuvanted comparator vaccine had a different HA content (54). This prevents any conclusions regarding the added risk resulting from the addition of an aluminium adjuvant to influenza vaccines.

Based upon available data from one clinical study in young children, the tolerability profile of the virosomal adjuvanted vaccines is quite similar compared to that of a non-adjuvanted split influenza vaccine. With regards to solicited adverse events, no difference between the two vaccines was found. The virosomal adjuvanted vaccine has a lower content of ovalbumin and is therefore expected to induce less allergic reactions (36). Although the vaccine has been safely administered to children with egg-allergy (77), large post-marketing safety studies would be needed to support such a claim.

The overall picture for the oil-in-water adjuvanted vaccines is that there is an increase in reactogenicity compared to the non-adjuvanted vaccines. Although there are no head to head comparisons between the different types of adjuvanted influenza vaccines, there are clearly differences. For both AS03- as AF03-adjuvanted vaccines, fever appears to increase with the second dose. This was especially evident in a study with the AS03-adjuvanted H1N1pdm09 vaccine (78). Here, fever defined as a temperature ≥37.5°C was reported by 20% of children aged 6 to 35 months following the first dose. Following the second dose 67% reported a temperature ≥37.5°C. In a study with the AF03-adjuvanted H1N1pdm09 vaccine, 8% of children aged 6 to 11 months reported fever (≥38.0°C) following the first dose. This increased to 33% following the second dose (58). This was not seen in children aged 12 to 35 months. For the MF59-adjuvanted vaccine there is an increase in local reactions, but no apparent increase in systemic reactions. In the largest randomised controlled trial by Vesikari et al. sufficient children were included to evaluate less frequent adverse events. Febrile convulsions were reported in five children (out of 993) who received non-adjuvanted split influenza vaccine and in five children (out of 1099) who received adjuvanted influenza vaccine, indicating no increased risk.

Safety of adjuvanted influenza vaccines – lessons learned during the 2009/2010 Pandemic

Both MF59 and AS03 adjuvanted influenza vaccines were used on a large scale during the 2009/2010 H1N1 pandemic. Children belonged to one of the main target groups for vaccination, including children younger than 3 years. Considering that in Europe alone over

37 million people had been vaccinated by April 2010 (79), and for the countries which reported Pandemrix™ was used by 74% (38) – it can be assumed that the exposure to adjuvanted influenza vaccines in the youngest age group was substantial. An in depth review on the safety of influenza A(H1N1)pdm09 vaccines, including an evaluation of post marketing data for adjuvanted and non-adjuvanted vaccines, is presented elsewhere and is not the focus of the present article (70). Yet, when discussing the use of adjuvanted influenza vaccines in young children we think it is important to highlight the experience with adjuvanted influenza vaccines during the 2009/2010 H1N1 pandemic.

As there was only limited safety data prior to the start of vaccination campaigns, especially in children, active monitoring of safety took place with focus on adverse events of special interest (AESI's) including neuritis, convulsions, anaphylaxis, encephalitis, vasculitis, Guillain–Barré syndrome (GBS), Bell's palsy, demyelinating disorders, and laboratory-confirmed vaccination failure (79). Evaluations of background rates of these AESIs were performed in order to be able to use observed to expected analyses for rapid signal detection (80). Following the observed increased risk of GBS associated with swine flu vaccination in 1976 in the US, studies were started around the world to prospectively evaluate the risk of GBS following vaccination (81-86). Largely, studies pointed out that there was no increased risk of AESIs following vaccination with adjuvanted influenza vaccines (79, 81, 82, 87-90). However, a cohort study in Sweden found a small increased risk of Bell's Palsy, paraesthesia, and inflammatory bowel disease associated with AS03 adjuvanted influenza vaccine (91) and a small but significant increase in the risk of GBS was seen in Quebec, Canada following vaccination with AS03 adjuvanted influenza vaccine (86).

In August 2010, a signal of narcolepsy associated with Pandemrix appeared in Sweden and Finland in children and adolescents aged 5 to 19. Epidemiological investigations have since then confirmed the signal (92-96), and more European countries have reported an increase in narcolepsy associated with the use of Pandemrix (97, 98). Two years further it remains unclear what the exact explanation is for the increased incidence of narcolepsy associated with Pandemrix and much work needs to be done before we can fully understand what happened. The absolute risk is small (about 1 in 20,000 vaccinations), yet considering the severity of the disease and the ages it affects a small risk can have a considerable impact. With suitable alternatives available the EMA restricted the use of Pandemrix in children in 2011 (99). No association between MF59-adjuvanted influenza vaccines and narcolepsy has been seen (100) although absolute exposure of the affected age groups is expected to be lower than is the case for Pandemrix. Moreover, a study in China found no association with non-adjuvanted influenza vaccines but did find an association between onset of narcolepsy and infection with influenza A(H1N1)pdm09 virus (101).

Discussion

It has long been known that traditional split and subunit influenza vaccines do not perform well in younger, immunologically unprimed, children. This has been confirmed by few studies showing that the efficacy of non-adjuvanted split or subunit influenza vaccines in this group is limited (15, 16, 18, 65). Fortunately, so are the safety concerns. These vaccines have proven to be well tolerated with adverse events reported in a small minority. Several clinical trials comparing different adjuvanted influenza vaccines with non-adjuvanted influenza vaccines in young children years were identified. No strong evidence was found that either aluminium salts or virosomes significantly enhance the immune response in this age group. However, identified studies clearly demonstrated that oil-in-water adjuvants improve the immune response to influenza vaccines, leading to higher antibody titres when measured with either the HI or VN assay. Not only were titres greater directly after vaccination, antibodies persisted for longer and showed better response against heterologous or drifted strains. These are potentially important benefits for the youngest children, but also for other age groups as this could mean that annual revaccination against influenza would not be necessary. Yet, we do not know what an increase in the antibody response means in terms of protection against infection or disease as there is no validated correlate of protection. This uncertainty makes it difficult to weigh benefits against identified risks. For a proper benefit-risk analysis studies evaluating the efficacy against relevant clinical outcomes are needed. Only one such study is known (65).

In this study it was found that the increased immune response does translate into improved efficacy. The largest gain was for children younger than 24 months, where there was no apparent efficacy of the non-adjuvanted split vaccine (VE: 11%, 95%CI −89 to 58) whilst the efficacy of the MF59 adjuvanted vaccine, Fluad, remained high (VE: 77%, 95%CI 37 to 92). This forms an indication that where non-adjuvanted vaccines are failing to adequately prime, adjuvanted vaccines do achieve this. However, as this study was not performed according to GCP guidelines some uncertainty on these findings remains. Clearly, much more work could be done on the evaluation of adjuvanted influenza vaccines and more large scale studies evaluating the efficacy against clinically relevant outcomes in immunologically naïve children would be welcomed. The finding that adjuvanted influenza vaccines confer some degree of cross-protection against drifted strains opens up the possibility of alternative vaccination approaches, i.e. annual re-vaccination might no longer be needed.

The gains in immunogenicity and efficacy provided by the different oil-in-water adjuvants evaluated do come at a price. With the MF59-adjuvanted seasonal and pandemic vaccines this cost appears to be limited to a small increase in local reactogenicity compared to the non-adjuvanted vaccines. With the AS03 adjuvant an increase in febrile reactions is seen following the second dose in several clinical studies, and in 2009 this led to a warning from

EMA (102). A similar trend was seen in the limited data available for AF03 adjuvanted influenza A(H1N1)pdm09 vaccine, with an increase in febrile reactions in children aged 6 to 12 months.

For adjuvanted influenza vaccines ideally more work would be done to investigate the most optimal schedule and antigen/adjuvant balance. Especially where a second dose is associated with increases in febrile reactions, careful consideration of the need for a second dose with an adjuvanted vaccine would be needed. Although influenza infection in young children does lead to complications, hospitalisations, and even death, in most children the disease is self-limiting. Therefore the tolerability and safety of the vaccine should be optimal. The dose recommendations for the AS03 and AF03 adjuvanted pandemic influenza vaccines for children for example state that there is a further immune response to a second dose of 0.25 ml administered after an interval of three weeks, but that the use of a second dose should take the increased reactogenicity into consideration (103-105). This advice should be improved if these vaccines were to be used in the future.

As highlighted earlier, there is some evidence indicating that two doses of unadjuvanted traditional split or subunit influenza vaccines could be effective in protecting young children against influenza (20-24). This underlines the necessity of proper evaluation of different dosing regimens for the traditional, non-adjuvanted, split or subunit influenza vaccines in unprimed children before disregarding these vaccines as an option for protecting young children.

Unfortunately, dose finding trials are naturally limited to immunogenicity studies and it is not known what the gains of an increased antibody titre translate to in terms of protection against infection and disease. Thus the benefit of a full versus half dose or two versus one dose is not fully understood. Considering the shortcomings of current serological studies, we need to press for collaborative efforts to increase understanding into immune markers, their correlation to protection, and to overcome limitations of existing assays to measure these markers.

The finding that the AS03-adjuvanted influenza A(H1N1)pdm09 vaccine was associated with an increase in the incidence in narcolepsy in children aged 5 to 19 has led to the restriction of its use in Europe. Although this influenza A(H1N1)pdm09 vaccine will unlikely be used in the future, the association between Pandemrix and narcolepsy has undoubtedly cast a shadow over the use and development of adjuvanted influenza vaccines in children. Narcolepsy is a serious debilitating chronic condition, and it is imperative that the role of Pandemrix as a potential trigger is fully investigated and understood. The epidemiological studies so far have probably led to more questions than answers, and investigations are expanding globally in order to gain more insight in countries that did use Pandemrix but did not have the same media coverage on the association with narcolepsy as was the case in

many European countries (106). Moreover, studies that can shed light on potential mechanisms are needed to start understanding how narcolepsy is triggered and what role Pandemrix could have played.

It is clear that improved vaccines for young children are needed, and oil-in-water adjuvanted vaccines are an effective alternative which could address an urgent need in the youngest, immunological naïve children. The limited studies available point towards greatly improved immunogenicity, both quantitative as qualitative, but also improved efficacy. There is a cost in the tolerability which needs to be carefully considered for each vaccine separately when determining the optimal dosage and schedule. What should be underlined above all is that the uncertainties regarding rare but serious adverse events, such as the association between Pandemrix and narcolepsy, need to be addressed and fully investigated if we are to move forward with these vaccines for young children. Until we fully understand how these adjuvants work in children with immature, developing, immune systems basic research to increase our understanding is needed. At the same time, other options to increase the immune response and efficacy in young, unprimed, children including higher dosages of traditional inactivated split or subunit influenza vaccines and intradermal vaccination should be further considered as these could also form effective alternatives yet data to substantiate this is limited.

References

1. Poehling KA, Edwards KM, Weinberg GA, Szilagyi P, Staat MA, Iwane MK, et al. The underrecognized burden of influenza in young children. The New England journal of medicine. 2006 Jul 6;355(1):31-40. PubMed PMID: 16822994. Epub 2006/07/11. eng.

2. Fraaij PL, Heikkinen T. Seasonal influenza: the burden of disease in children. Vaccine. 2011 Oct 6;29(43):7524-8. PubMed PMID: 21820476. Epub 2011/08/09. eng.

3. Steinhoff MC. Influenza in young children: burden, immunisation, and policy. The Lancet infectious diseases. 2011 Jan;11(1):2-3. PubMed PMID: 21106444. Epub 2010/11/26. eng.

4. Paget WJ, Balderston C, Casas I, Donker G, Edelman L, Fleming D, et al. Assessing the burden of paediatric influenza in Europe: the European Paediatric Influenza Analysis (EPIA) project. Eur J Pediatr. 2010 Aug;169(8):997-1008. PubMed PMID: 20229049. Pubmed Central PMCID: 2890072. Epub 2010/03/17. eng.

5. Bodewes R, de Mutsert G, van der Klis FR, Ventresca M, Wilks S, Smith DJ, et al. Prevalence of antibodies against seasonal influenza A and B viruses in children in Netherlands. Clin Vaccine Immunol. 2011 Mar;18(3):469-76. PubMed PMID: 21209157. Pubmed Central PMCID: 3067385. Epub 2011/01/07. eng.

6. Patria MF, Tenconi R, Esposito S. Efficacy and safety of influenza vaccination in children with asthma. Expert Rev Vaccines. 2012 Apr;11(4):461-8. PubMed PMID: 22551031. Epub 2012/05/04. eng.

7. Neuzil KM, Wright PF, Mitchel EF, Jr., Griffin MR. The burden of influenza illness in children with asthma and other chronic medical conditions. J Pediatr. 2000 Dec;137(6):856-64. PubMed PMID: 11113844. Epub 2000/12/13. eng.

8. Chiu SS, Chan K-H, So LY, Chen R, Chan ELY, Peiris JSM. The population based socioeconomic burden of pediatric influenza-associated hospitalization in Hong Kong. Vaccine. 2012;30(10):1895-900.

9. Antonova EN, Rycroft CE, Ambrose CS, Heikkinen T, Principi N. Burden of paediatric influenza in Western Europe: a systematic review. BMC Public Health. 2012;12(968):doi:10.1186/471-2458-12-968.

10. Peltola V, Ziegler T, Ruuskanen O. Influenza A and B virus infections in children. Clin Infect Dis. 2003 Feb 1;36(3):299-305. PubMed PMID: 12539071. Epub 2003/01/23. eng.

11. Steininger C, Popow-Kraupp T, Laferl H, Seiser A, Godl I, Djamshidian S, et al. Acute encephalopathy associated with influenza A virus infection. Clin Infect Dis. 2003 Mar 1;36(5):567-74. PubMed PMID: 12594636. Epub 2003/02/21. eng.

12. Nair H, Brooks WA, Katz M, Roca A, Berkley JA, Madhi SA, et al. Global burden of respiratory infections due to seasonal influenza in young children: a systematic review and meta-analysis. Lancet. 2011 Dec 3;378(9807):1917-30. PubMed PMID: 22078723. Epub 2011/11/15. eng.

13. Bhat N, Wright JG, Broder KR, Murray EL, Greenberg ME, Glover MJ, et al. Influenza-associated deaths among children in the United States, 2003-2004. The New England journal of medicine. 2005 Dec 15;353(24):2559-67. PubMed PMID: 16354892. Epub 2005/12/16. eng.

14. Hobson D. The potential role of immunological adjuvants in influenza vaccines. Postgrad Med J. 1973 Mar;49(569):180-84. PubMed PMID: 4802631. Pubmed Central PMCID: 2495378. Epub 1973/03/01. eng.

15. Osterholm MT, Kelley NS, Sommer A, Belongia EA. Efficacy and effectiveness of influenza vaccines: a systematic review and meta-analysis. The Lancet infectious diseases. 2012 Jan;12(1):36-44. PubMed PMID: 22032844. Epub 2011/10/29. eng.

16. Jefferson T, Rivetti A, Di Pietrantonj C, Demicheli V, Ferroni E. Vaccines for preventing influenza in healthy children. Cochrane Database Syst Rev. 2012;8:CD004879. PubMed PMID: 22895945. Epub 2012/08/17. eng.

17. Zhou L, Chong MM, Littman DR. Plasticity of CD4+ T cell lineage differentiation. Immunity. 2009 May;30(5):646-55. PubMed PMID: 19464987. Epub 2009/05/26. eng.

18. Vaccines against influenza WHO position paper - November 2012. Wkly Epidemiol Rec. 2012 Nov 23;87(47):461-76. PubMed PMID: 23210147. Epub 2012/12/06. eng
fre.

19. Skowronski DM, Hottes TS, Chong M, De Serres G, Scheifele DW, Ward BJ, et al. Randomized controlled trial of dose response to influenza vaccine in children aged 6 to 23 months. Pediatrics. 2011 Aug;128(2):e276-89. PubMed PMID: 21768314. Epub 2011/07/20. eng.

20. Englund JA, Walter EB, Gbadebo A, Monto AS, Zhu Y, Neuzil KM. Immunization with trivalent inactivated influenza vaccine in partially immunized toddlers. Pediatrics. 2006 Sep;118(3):e579-85. PubMed PMID: 16950949. Epub 2006/09/05. eng.

21. Neuzil KM, Englund JA. Influenza vaccine for young children: two doses are better than one. J Pediatr. 2006 Dec;149(6):737-8. PubMed PMID: 17137882. Epub 2006/12/02. eng.

22. Allison MA, Daley MF, Crane LA, Barrow J, Beaty BL, Allred N, et al. Influenza vaccine effectiveness in healthy 6- to 21-month-old children during the 2003-2004 season. J Pediatr. 2006 Dec;149(6):755-62. PubMed PMID: 17137887. Epub 2006/12/02. eng.

23. Ritzwoller DP, Bridges CB, Shetterly S, Yamasaki K, Kolczak M, France EK. Effectiveness of the 2003-2004 influenza vaccine among children 6 months to 8 years of age, with 1 vs 2 doses. Pediatrics. 2005 Jul;116(1):153-9. PubMed PMID: 15995046. Epub 2005/07/05. eng.

24. Shuler CM, Iwamoto M, Bridges CB, Marin M, Neeman R, Gargiullo P, et al. Vaccine effectiveness against medically attended, laboratory-confirmed influenza among children aged 6 to 59 months, 2003-2004. Pediatrics. 2007 Mar;119(3):e587-95. PubMed PMID: 17332179. Epub 2007/03/03. eng.

25. FDA. Fluzone, Fluzone High-Dose and Fluzone Intradermal. Available from: www.fda.gov/BiologicsBloodVaccines/Vacciens/ApprovedProducts/ucm112854.htm.

26. European Medicines Agency. Intanza influenza vaccine (split virion, inactivated). Available from: www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/000957/human_med_000842.jsp&mid=WC0b01ac058001d124.

27. Esposito S, Daleno C, Picciolli I, Tagliaferri L, Scala A, Prunotto G, et al. Immunogenicity and safety of intradermal influenza vaccine in children. Vaccine. 2011;29:7606-10.

28. Siugimura T, Ito Y, Tananari Y, Ozaki Y, Maeno Y, Yamaoka T, et al. Improved antibody responses in infants less than 1 year old using intradermal influenza vaccination. Vaccine. 2008;26:2700-5.

29. Ambrose CS, Levin MJ, Belshe RB. The relative efficacy of trivalent live attenuated and inactivated influenza vaccines in children and adults. Influenza Other Respi Viruses. 2011 Mar;5(2):67-75. PubMed PMID: 21306569. Pubmed Central PMCID: 3151550. Epub 2011/02/11. eng.

30. Belshe RB, Edwards KM, Vesikari T, Black SV, Walker RE, Hultquist M, et al. Live attenuated versus inactivated influenza vaccine in infants and young children. The New England journal of medicine. 2007 Feb 15;356(7):685-96. PubMed PMID: 17301299. Epub 2007/02/16. eng.

31. MedImmune. FluMist®, Influenza Virus Vaccine Live, Intranasal. Highlights of prescribing information Gaithersburg, MD2007. Available from: http://www.medimmune.com/pdf/products/flumist_pi.pdf.

32. Belshe RB, Ambrose CS, Yi T. Safety and efficacy of live attenuated influenza vaccine in children 2–7 years of age. Vaccine. 2008;26, Supplement 4(0):D10-D6.

33. European Medicines Agency. European Public assessment report – Fluenz 2011 [cited 2013 05-01]. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/001101/WC500103711.pdf.

34. Stuart-Harris CH. Adjuvant influenza vaccines. Bull World Health Organ. 1969;41(3):617-21. PubMed PMID: 5309486. Pubmed Central PMCID: 2427695. Epub 1969/01/01. eng.

35. O'Hagan DT, Ott GS, Nest GV, Rappuoli R, Giudice GD. The history of MF59((R)) adjuvant: a phoenix that arose from the ashes. Expert Rev Vaccines. 2013 Jan;12(1):13-30. PubMed PMID: 23256736. Epub 2012/12/22. eng.

36. Herzog C, Hartmann K, Kunzi V, Kursteiner O, Mischler R, Lazar H, et al. Eleven years of Inflexal V-a virosomal adjuvanted influenza vaccine. Vaccine. 2009 Jul 16;27(33):4381-7. PubMed PMID: 19450630. Epub 2009/05/20. eng.

37. Kieny MP, Costa A, Homback J, Carrasco P, Pervikov Y, Salisbury D, et al. A global pandemic influenza vaccine action plan. Vaccine. 2006;24(40-41):6367-70.

38. Mereckiene J, Cotter S, Weber JT, Nicholl A, D'Ancona F, Lopalco PL, et al. Influenza A(H1N1)pdm09 vaccination policies and coverage in Europe. Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin. 2012;17(4):pii=20064.

39. Durando P, Iudici R, Alicino C, Alberti M, de Florentis D, Ansaldi F, et al. Adjuvants and alternative routes of administration towards the development of the ideal influenza vaccine. Hum Vaccin. 2011 Jan-Feb;7 Suppl:29-40. PubMed PMID: 21245655. Epub 2011/01/20. eng.

40. Tetsutani K, Ishii KJ. Adjuvants in influenza vaccines. Vaccine. 2012;30(52):7658-61.

41. Vajo Z, Kosa L, Szilvasy I, Pauliny Z, Bartha K, Visontay I, et al. Safety and immunogenicity of a prepandemic influenza A (H5N1) vaccine in children. Pediatr Infect Dis J. 2008 Dec;27(12):1052-6. PubMed PMID: 18978514. Epub 2008/11/04. eng.

42. Durando P, Icardi G, Ansaldi F. MF59-adjuvanted vaccine: a safe and useful tool to enhance and broaden protection against seasonal influenza viruses in subjects at risk. Expert Opin Biol Ther. 2010 Apr;10(4):639-51. PubMed PMID: 20218923. Epub 2010/03/12. eng.

43. Khurana S, Verma N, Yewdell JW, Hilbert AK, Castellino F, Lattanzi M, et al. MF59 adjuvant enhances diversity and affinity of antibody-mediated immune response to pandemic influenza vaccines. Sci Transl Med. 2011 Jun 1;3(85):85ra48. PubMed PMID: 21632986. Epub 2011/06/03. eng.

44. Wagner R, Gopfert C, Hammann J, Neumann B, Wood J, Newman R, et al. Enhancing the reproducibility of serological methods used to evaluate immunogenicity of pandemic H1N1 influenza vaccines-an effective EU regulatory approach. Vaccine. 2012 Jun 8;30(27):4113-22. PubMed PMID: 22446639. Epub 2012/03/27. eng.

45. Wood JM, Major D, Heath A, Newman RW, Hoschler K, Stephenson I, et al. Reproducibility of serology assays for pandemic influenza H1N1: collaborative study to evaluate a candidate WHO International Standard. Vaccine. 2012 Jan 5;30(2):210-7. PubMed PMID: 22100887. Epub 2011/11/22. eng.

46. Hobson DF, Curry RL, Beare AS, Ward-Gardner A. The role of serum haemagglutination-inhibiting antibody in protection against challenge infection with influenza A2 and B viruses. J Hyg. 1972 19730315 DCOM-19730315(0022-1724 (Print)). eng.

47. Black S, Nicolay U, Vesikari T, Knuf M, Del Giudice G, Della Cioppa G, et al. Hemagglutination inhibition antibody titers as a correlate of protection for inactivated influenza vaccines in children. Pediatr Infect Dis J. 2011 Dec;30(12):1081-5. PubMed PMID: 21983214. Epub 2011/10/11. eng.

48. Yin DP, Zhu BP, Wang HQ, Cao L, Wu WD, Jiang KY, et al. Effect of Aluminum Hydroxide Adjuvant on the Immunogenicity of the 2009 Pandemic Influenza A/H1N1 Vaccine: Multi-level Modeling of Data with Repeated Measures. Biomedical and Environmental Sciences. 2011;24(6):624-9.

49. Vajo Z, Kosa L, Szilvasy I, Pauliny Z, Bartha K, Visontay I, et al. Yearly licensing studies from 1997 to 2007 of the inactivated whole virus seasonal influenza vaccine fluval--a useful approach to pandemic vaccine development even in less well developed countries? Influenza Other Respi Viruses. 2008 Nov;2(6):221-8. PubMed PMID: 19453398. Epub 2009/05/21. eng.

50. Vajo Z. Influenza A (H5N1) pandemic prototype vaccine Fluval. Expert Rev Vaccines. 2009 May;8(5):619-24. PubMed PMID: 19397418. Epub 2009/04/29. eng.

51. Vajo Z, Tamas F, Sinka L, Jankovics I. Safety and immunogenicity of a 2009 pandemic influenza A H1N1 vaccine when administered alone or simultaneously with the seasonal influenza vaccine for the 2009-10 influenza season: a multicentre, randomised controlled trial. Lancet. 2010 Jan 2;375(9708):49-55. PubMed PMID: 20018367. Epub 2009/12/19. eng.

52. National Institute of Pharmacy Hungary O. Public Assessment Report: Fluval P Suspension for injection November 2009 [cited 2012 31-12]. Available from: http://www.ogyi.hu/dynamic/Fluval%20P_PublicAR.pdf.

53. Nolan T, Richmond PC, Formica NT, Hoschler K, Skeljo MV, Stoney T, et al. Safety and immunogenicity of a prototype adjuvanted inactivated split-virus influenza A (H5N1) vaccine in infants and children. Vaccine. 2008 Nov 25;26(50):6383-91. PubMed PMID: 18801398. Epub 2008/09/20. eng.

54. Chotpitayasunondh T, Thisyakorn U, Pancharoen C, Pepin S, Nougarede N. Safety, humoral and cell mediated immune responses to two formulations of an inactivated, split-virion influenza A/H5N1 vaccine in children. PloS one. 2008;3(12):e4028. PubMed PMID: 19112513. Pubmed Central PMCID: 2605261. Epub 2008/12/30. eng.

55. Kanra G, Marchisio P, Feiterna-Sperling C, Gaedicke G, Lazar H, Durrer P, et al. Comparison of immunogenicity and tolerability of a virosome-adjuvanted and a split influenza vaccine in children. Pediatric Infectious Disease Journal. 2004;23(4):300-6.

56. Esposito S, Marchisio P, Montinaro V, Bianchini S, Weverling GJ, Pariani E, et al. The immunogenicity and safety of a single 0.5 ml dose of virosomal subunit influenza vaccine administered to unprimed chidlren aged ≥6 to ≤36 months: data from a randomized, phase III study. Vaccine. 2012;30:7005-12.

57. Vesikari T, Pepin S, Kusters I, Hoffenbach A, Denis M. Assessment of squalene adjuvanted and non-adjuvanted vaccines against pandemic H1N1 influenza in children 6 months to 17 years of age. Hum Vaccin Immunother. 2012 Sep 1;8(9):1283-92. PubMed PMID: 22906943. Epub 2012/08/22. eng.

58. European Medicines Agency E. European Public assessment report – Humenza 2010 [cited 2012 31-12]. Available from: www.ema.europa.eu/humandocs/Humans/EPAR/humenza/humenza.htm

59. Waddington CS, Walker WT, Oeser C, Reiner A, John T, Wilkins S, et al. Safety and immunogenicity of AS03B adjuvanted split virion versus non-adjuvanted whole virion H1N1 influenza vaccine in UK children aged 6 months-12 years: open label, randomised, parallel group, multicentre study. BMJ. 2010;340:c2649. PubMed PMID: 20508026. Pubmed Central PMCID: 2877808. Epub 2010/05/29. eng.

60. Langley JM, Reich D, Aggarwal N, Connor D, Lebel MH, Gupta A, et al. Randomized, multicenter trial of a single dose of AS03-adjuvanted or unadjuvanted H1N1 2009 pandemic influenza vaccine in children 6 months to <9 years of age: safety and immunogenicity. Pediatr Infect Dis J. 2012;31(8):848-58.

61. Lambe T, Spencer AJ, Mullarkey CE, Antrobus RD, Yu LM, de Whalley P, et al. T-cell responses in children to internal influenza antigens, 1 year after immunization with pandemic H1N1 influenza vaccine, and response to revaccination with seasonal trivalent-inactivated influenza vaccine. Pediatr Infect Dis J. 2012 Jun;31(6):e86-91. PubMed PMID: 22466328. Epub 2012/04/03. eng.

62. Block SL, Ruiz-Palacios GM, Guerrero ML, Beygo J, Sales V, Holmes SJ. Dose-range study of MF59-adjuvanted versus nonadjuvanted monovalent A/H1N1 pandemic influenza vaccine in six- to less than thirty-six-month-old children. Pediatr Infect Dis J. 2012 Jul;31(7):e92-8. PubMed PMID: 22481427. Epub 2012/04/07. eng.

63. Vesikari T, Pellegrini M, Karvonen A, Groth N, Borkowski A, O'Hagan DT, et al. Enhanced immunogenicity of seasonal influenza vaccines in young children using MF59 adjuvant. Pediatr Infect Dis J. 2009 Jul;28(7):563-71. PubMed PMID: 19561422. Epub 2009/06/30. eng.

64. Vesikari T, Groth N, Karvonen A, Borkowski A, Pellegrini M. MF59®-adjuvanted influenza vaccine (FLUAD®) in children: Safety and immunogenicity following a second year seasonal vaccination. Vaccine. 2009;27(45):6291-5.

65. Vesikari T, Knuf M, Wutzler P, Karvonen A, Kieninger-Baum D, Schmitt HJ, et al. Oil-in-water emulsion adjuvant with influenza vaccine in young children. New England Journal of Medicine. 2011;365(15):1406-16.

66. European Medicines Agency E. Withdrawal assessment report: Fluad pediatric 2012 [cited 2012 31-12]. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Application_withdrawal_assessment_report/2012/04/WC500126030.pdf.

67. Sancho A, Melchiorri D, Abadie E. More on influenza vaccine in young children. The New England journal of medicine. 2012 Jun 28;366(26):2528; author reply -9. PubMed PMID: 22738111. Epub 2012/06/29. eng.

68. Bonhoeffer J, Zumbrunn B, Heininger U. Reporting of vaccine safety data in publications: systematic review. Pharmacoepidemiol Drug Saf. 2005;14(2):101-6.

69. Poland GA. Methodologic reporting requirements for clinical trials: Advancing the science today and tomorrow. Vaccine. 2011;29(32):5087-9.

70. Wijnans L, de Bie S, Dieleman J, Bonhoeffer J, Sturkenboom M. Safety of pandemic H1N1 vaccines in children and adolescents. Vaccine. 2011 Oct 6;29(43):7559-71. PubMed PMID: 21821086. Epub 2011/08/09. eng.

71. Barry DW, Mayner RE, Hochstein HD, al. e. Comparative trial of influenza vaccines. II. Adverse reactions in children and adults. . Am J Epidemiol. 1976;104(47-59).

72. Zangwill KM, Belshe RB. Safety and efficacy of trivalent inactivated influenza vaccine in young children: a summary for the new era of routine vaccination. Pediatr Infect Dis J. 2004 Mar;23(3):189-97. PubMed PMID: 15014289.

73. Ruben FL. Inactivated influenza virus vaccines in children. Clin Infect Dis. 2005;38(5):678-88.

74. McMahon AW, Iskander J, Haber P, Chang S, Woo EJ, Braun MM, et al. Adverse events after inactivated influenza vaccination among children less than 2 years of age: analysis of reports from the vaccine adverse event reporting system, 1990-2003. Pediatrics. 2005 Feb;115(2):453-60. PubMed PMID: 15687455. eng.

75. McMahon AW, Iskander JK, Haber P, al. e. Inactivated influenza vaccine (IIV) in children <2 years of age: examination of selected adverse events reported to the Vaccine Adverse Event Reporting System (VAERS) after thimerosal-free or thimerosal-containing vaccine. . Vaccine. 2008;26:427-9.

76. Rosenberg M, Sparks R, McMahon A, Iskander J, Campbell JD, Edwards KM. Serious adverse events rarely reported after trivalent inactivated influenza vaccine (TIV) in children 6-23 months of age. Vaccine. 2009;27(32):4278-83.

77. Esposito S, Gasparini C, Martelli A, Zenga A, Tremolati E, Varin E, et al. Safe administration of an inactivated virosomal adjuvanted influenza vaccine in asthmatic children with egg allergy. Vaccine. 2008 Aug 26;26(36):4664-8. PubMed PMID: 18639601. Epub 2008/07/22. eng.

78. Carmona A, Omeñaca F, Tejedor JC, Merino JM, Vaman T, Dieussaert I, et al. Immunogenicity and safety of AS03-adjuvanted 2009 influenza A H1N1 vaccine in children 6-35 months. Vaccine. 2010;28(36):5837-44.

79. Kurz X, Domergue F, Slattery J, Segec A, Szmigiel A, Hidalgo-Simon A. Safety monitoring of Influenza A/H1N1 pandemic vaccines in EudraVigilance. Vaccine. 2011;29(26):4378-87.

80. VAESCO. Background Rates [cited 2013 04-01]. Available from: http://vaesco.net/vaesco/results/BGR-2010.html.

81. Dieleman J, Romio S, Johansen K, Weibel D, Bonhoeffer J, Sturkenboom M. Guillain-Barre syndrome and adjuvanted pandemic influenza A (H1N1) 2009 vaccine: multinational case-control study in Europe. BMJ. 2011;343:d3908. PubMed PMID: 21750072. Pubmed Central PMCID: 3134565. Epub 2011/07/14. eng.

82. Deeks SL, Lim GH, Simpson MA, Rosella L, Mackie CO, Achonu C, et al. Estimating background rates of Guillain-Barre Syndrome in Ontario in order to respond to safety concerns during pandemic H1N1/09 immunization campaign. BMC Public Health.11:329. PubMed PMID: 21586163. eng.

83. Prothro C, Kudish K, Fiellin M, Meek J, Tellman N, Milewski M, et al. Preliminary Results: Surveillance for Guillain-Barré Syndrome After Receipt of Influenza A (H1N1) 2009 Monovalent Vaccine --- United States, 2009--2010. MMWR Recomm Rep. 2010;June 2, 2010 / 59(Early Release);1-5.

84. Grimaldi-Bensouda L, Alperovitch A, Besson G, Vial C, Cuisset JM, Papeix C, et al. Guillain-Barre syndrome, influenzalike illnesses, and influenza vaccination during seasons with and without circulating A/H1N1 viruses. Am J Epidemiol. 2011 Aug 1;174(3):326-35. PubMed PMID: 21652600. Epub 2011/06/10. eng.

85. Choe YJ, Cho H, Bae G-R, Lee J-K. Guillain-Barré syndrome following receipt of influenza A (H1N1) 2009 monovalent vaccine in Korea with an emphasis on Brighton Collaboration case definition. Vaccine. 2011;29(11):2066-70.

86. De Wals P DGTE, et al. Risk of guillain-barré syndrome following h1n1 influenza vaccination in quebec. JAMA : the journal of the American Medical Association. 2012;308(2):175-81.

87. Isai A, Durand J, Le Meur S, Hidalgo-Simon A, Kurz X. Autoimmune disorders after immunisation with Influenza A/H1N1 vaccines with and without adjuvant: EudraVigilance data and literature review. Vaccine. 2012;30(49):7123-9.

88. Deeks SL, Lim GH, Simpson MA, Rosella L, Mackie CO, Achonu C, et al. Estimating background rates of Guillain-Barre Syndrome in Ontario in order to respond to safety concerns during pandemic H1N1/09 immunization campaign. BMC Public Health. 2011;11:329. PubMed PMID: 21586163. eng.

89. Candela S, Pergolizzi S, Ragni P, Cavuto S, Nobilio L, Di Mario S, et al. An early (3–6 weeks) active surveillance study to assess the safety of pandemic influenza vaccine Focetria® in a province of Emilia-Romagna region, Italy – Part one. Vaccine. 2012;[Epub ahead of print](0).

90. Banzhoff A, Haertel S, Praus M. Passive surveillance of adverse events of an MF59-adjuvanted H1N1v vaccine during the pandemic mass vaccinations. Human Vaccines. 2011;7(5):539-48.

91. Bardage C, Persson I, Ortqvist A, Bergman U, Ludvigsson JF, Granath F. Neurological and autoimmune disorders after vaccination against pandemic influenza A (H1N1) with a monovalent adjuvanted vaccine: population based cohort study in Stockholm, Sweden. BMJ. 2011;343:d5956. PubMed PMID: 21994316. Pubmed Central PMCID: 3192001. Epub 2011/10/14. eng.

92. Partinen M, Saarenpaa-Heikkila O, Ilveskoski I, Hublin C, Linna M, Olsen P, et al. Increased incidence and clinical picture of childhood narcolepsy following the 2009 H1N1 pandemic vaccination campaign in Finland. PloS one. 2012;7(3):e33723. PubMed PMID: 22470463. Pubmed Central PMCID: 3314680. Epub 2012/04/04. eng.

93. Nohynek H, Jokinen J, Partinen M, Vaarala O, Kirjavainen T, Sundman J, et al. AS03 adjuvanted AH1N1 vaccine associated with an abrupt increase in the incidence of childhood narcolepsy in Finland. PloS one. 2012;7(3):e33536. PubMed PMID: 22470453. Pubmed Central PMCID: 3314666.

94. European Centre for Disease Prevention and Control. Narcolepsy in association with pandemic influenza vaccination. A multi-country European epidemiological investigation. Stockholm: September 2012.

95. Final summary of adverse drug reaction reports in Sweden with Pandemrix through October 2009 – mid April 2010 June 2010. Available from: http://www.lakemedelsverket.se/upload/eng-mpa-se/Pandemrix%20ADRs%20in%20Sweden%2015%20april%202010.pdf.

96. Wijnans L, Lecomte C, de Vries C, Weibel D, Sammon C, Hviid A, et al. The incidence of narcolepsy in Europe: Before, during, and after the influenza A(H1N1)pdm09 pandemic and vaccination campaigns. Vaccine. 2012 Dec 13. PubMed PMID: 23246544. Epub 2012/12/19. Eng.

97. O 'Flanagan D, Bonner C, Crowe C, Lynch B, Sweeney B, Gilvarry J, et al. Investigation of an increase in the incidence of narcolepsy in children and adolescents in 2009 and 2010. Final Report of National Narcolepsy Study Steering Committee. Department of Health, Ireland. 2012.

98. L'Agence nationale de sécurité du médicament et des produits de santé (ANSM) Service de pharmacologie (INSERM CIC-P 0005 Pharmaco-Epidémiologie) UBSCdB. Etude NarcoFlu-VF (NarcoFlu VAESCO-France) : Grippe, vaccination antigrippale et narcolepsie : contribution française à l'étude cas-témoins européenne. . 2012.

99. EMA. Press release: European Medicines Agency recommends restricting use of Pandemrix 21 July 2011. Available from: http://www.ema.europa.eu/ema/index.jsp?curl=pages/news_and_events/news/2011/07/news_detail_001312.jsp&murl=menus/news_and_events/news_and_events.jsp&mid=WC0b01ac058004d5c1&jsenabled=true.

100. Tsai TF, Crucitti A, Nacci P, Nicolay U, Cioppa GD, Ferguson J, et al. Explorations of clinical trials and pharmacovigilance databases of MF59((R))-adjuvanted influenza vaccines for associated cases of narcolepsy. Scand J Infect Dis. 2011 May 2. PubMed PMID: 21534891. Epub 2011/05/04. Eng.

101. Han F, Lin L, Warby SC, Faraco J, Li J, Dong SX, et al. Narcolepsy onset is seasonal and increased following the 2009 H1N1 pandemic in china. Annals of Neurology. 2011;70(3):410-7.

102. European Medicines Agency. European Medicines Agency advises of risk of fever in young chidlren following vaccination with Pandemrix (03-12-2009) 2009 [cited 2013 04-02]. Available from: http://www.ema.europa.eu/ema/index.jsp?curl=pages/news_and_events/news/2009/12/news_detail_000584.jsp&mid=WC0b01ac058004d5c1.

103. European Medicines Agency. European Public assessment report – Humenza 2010 [cited 2012 31-12]. Available from: www.ema.europa.eu/humandocs/Humans/EPAR/humenza/humenza.htm

104. European Medicines Agency. Pandemrix : EPAR - Public assessment report.

105. European Medicines Agency. Arepanrix : EPAR - Public assessment report

106. ECDC. Narcolepsy and Pandemrix Vaccine – an association in adults as well as children 24-09-2012 [cited 2012 31-12]. Available from: http://ecdc.europa.eu/en/activities/sciadvice/Lists/ECDC%20Reviews/ECDC_DispForm.aspx?List=512ff74f%2D77d4%2D4ad8%2Db6d6%2Dbf0f23083f30&ID=1289. .

Chapter 9. A review of the changes to the licensing of influenza vaccines in Europe

Leonoor Wijnans

Bettie Voordouw

Accepted for publication in Influenza and other Respiratory Viruses

Abstract

In 2014 the European Committee for Human Medicinal Products (CHMP) published a draft regulatory guideline for the evaluation of influenza vaccines. Following a public consultation round, the final guidance will be published in the near future. Here we highlight the main changes in the clinical section this guideline, discuss the background to these changes and whether the new consolidated guidance document can be expected to achieve a better understanding of the performance of seasonal, zoonotic and pandemic influenza vaccines during the regulatory licensing process.

The new influenza guideline reflects a changed approach to the regulatory assessment of influenza vaccines, resulting in the abolition of serological criteria, known as the CHMP criteria, which have been the mainstay for evaluating the influenza vaccine immunogenicity for several decades. The new guideline adopts a more diversified approach to the measurement and reporting of the immune response to influenza vaccines and sets a requirement to conduct clinical outcome trials in young children. Importantly, more emphasis is placed on the post licensure monitoring of the benefit-risk of influenza vaccines, including a request for continuous monitoring of efficacy and enhanced safety surveillance.

Despite the improvements these new requirements will expectedly bring to the regulatory assessment of influenza vaccines, major challenges remain which cannot be overcome by new guidance alone. Ongoing initiatives in which academia, manufacturers, public health institutes and regulators work together to address these challenges are central to the development of robust tools to evaluate and monitor performance of influenza vaccines in the future.

Introduction

In 2014 the European Committee for Medicinal Products for Human Use (CHMP) published a draft regulatory guideline for the evaluation of influenza vaccines (1). This guideline is intended to update the multitude of guidance documents in Europe which cover quality, non-clinical and clinical regulatory requirements for seasonal, zoonotic or pandemic vaccines into a consolidated guidance document for the development of new influenza vaccines.

As outlined in a concept note published in 2011 (2) the use of influenza vaccines is in certain aspects based upon "long-standing practices rather than rigorous scientific appraisal". The experience gained from the influenza A(H1N1)2009 pandemic brought into question the validity of several aspects of the existing regulatory guidelines. In particular the assessment of the immune response, which focuses on haemagglutination inhibition (HI) and single radial haemolysis (SRH) assays, was considered to be in need of revision (3). Furthermore, a lack of understanding of the effect of vaccination in certain subpopulations, such as young children, called for improvements to existing guidelines (3, 4).

In this article we highlight the main changes in the clinical section of the new influenza guideline and their scientific background, and discuss whether this consolidated guidance document can be expected to achieve a better understanding of the performance of seasonal, zoonotic and pandemic influenza vaccines during the regulatory licensing process. We consider the evidence and current understanding surrounding the evaluation of the immune response, efficacy and safety of influenza vaccines and how the proposed guideline might improve the understanding on the effect of influenza vaccines in different subpopulations.

European Regulatory Framework

In Europe influenza vaccines are either licensed on a Europe-wide scale where all Member States are involved, referred to as a 'central procedure; via procedures in which selected Member States are involved, referred to as 'decentralized procedures' or 'mutual recognition procedures'; or on a national level. Whilst this provides a diverse regulatory landscape, in general all Member States adhere to scientific and regulatory guidance as set out by the CHMP. The CHMP is the committee of the European Medicines Agency (EMA), responsible for preparing the Agency's scientific opinion regarding the licensing of human medicinal products, including vaccines. Regulatory guidelines, such as the new influenza guideline, inform industry on the minimum requirements for licensing of new medicinal products. They reflect the information needed to determine the benefit-risk balance of a product, and to adequately describe the characteristics of the product to ensure safe and effective use. In the drafting of these guidelines the CHMP is supported by several expert groups, such as the Vaccine Working Party and the Biologics Working Party. The

Pharmacovigilance Risk Assessment Committee (PRAC) is the committee of the EMA responsible for the assessment and monitoring of safety issues that arise post licensure.

The newly revised influenza guideline distinguishes three types of influenza vaccines: those aimed at protecting individuals against seasonal, annually recurring influenza; zoonotic vaccines that contain an influenza virus strain of animal origin and which were previously referred to as pre-pandemic vaccines; and pandemic influenza vaccines which are intended for use in a pandemic and which include pandemic preparedness vaccines, formerly referred to as pandemic mock-up vaccines. The revised guideline integrates recommendations for new influenza vaccines however it clearly indicates that it does not intend to cover novel constructs, for example vaccines targeted at epitopes other than those on the haemagglutinin stalk.

Serological correlates of protection: moving away from the existing paradigm for establishing efficacy of influenza vaccines.

Traditionally, efficacy of inactivated influenza vaccines for regulatory assessment in Europe has been estimated through the determination of immunogenicity with serological assays. This assessment focused primarily on the HI assay for which seroprotection was defined as a cut off of HI≥1:40, or the SRH assay for which a zone area of 25 mm^2 is defined as a protective threshold. These cut-offs stem from limited data from challenge studies conducted decades ago, demonstrating a relationship between HI titres and infection rates. These studies found that a pre-challenge serum HI titre of 18-36 (5) measured by HI assays or 42-44 (6) measured by SRH assay correlated with 50% protection against infection. The serological response would be assessed by applying a set of criteria commonly referred to as the CHMP criteria (Table 9.1). For the annual variation of influenza strains in seasonal inactivated vaccines one or more of the CHMP criteria had to be met. For pandemic vaccines all three of the criteria had to be met.

Table 9.1. European CHMP criteria for evaluation of influenza vaccine immunogenicity

	Adults	Older adults (>60 years)
GMT increase	2.5	2
Seroconversion /significant increase*	40%	30%
Seroprotection*	70%	60%

* In HI tests seroconversion corresponds to: negative prevaccination serum (HI<1:10), postvaccination serum HI≥1:40; Prevaccination serum >1:10, significant increase: at least a fourfold increase in titre. Seroprotection corresponds to the % with serum HI≥1:40. Alternative criteria have been defined for the SRH assay.

There has been a growing recognition that relying on a single serological cut-off for determining the benefit of different influenza vaccines for different subgroups and different vaccine constructs is not the most informative approach (7) and that the appropriateness of the defined correlate of HI≥1:40 can be questioned (3, 8-10).

Challenge studies on which the protective thresholds are based were performed in healthy adults with attenuated strains (5). However, influenza vaccines are not only intended to protect healthy adults but also to protect vulnerable children, older adults and adults with underlying comorbidities against consequences of natural infections with virulent influenza strains. Whether the correlates established in these challenge studies (5) can be transferred to these situations has not been established. For example, one study identified that in children an HI titre >1:110 would predict 50% of clinical protection and a titre of 1:330 would predict 80% of protection (11). A second study could not consistently predict protection with HI titres in healthy adults (8) and in older adults it has been suggested that cell-mediated immunity (CMI) rather than humoral immunity would be associated with protection (12). Serological assays are not an appropriate measure for the assessment of immunity against live attenuated influenza vaccines (9).

Nonetheless, for decades the regulatory assessment of vaccines has relied on these criteria and correlates of protection even though their suitability to the situations for which they have been applied has not been established. The use of these correlates has arguably resulted in a loss of opportunity to gain knowledge and understanding of the functioning of influenza vaccines. Moreover, presenting and communicating study results against these criteria may have led to a false sense of security from the impression that a vaccine will convey a level of protection in the target population, when in fact this has not been established. The abolishing of these criteria marks a major shift in regulatory thinking, and paves the way to a more evidence-based approach for the assessment of vaccine performance.

A potentially more pressing problem arising from reliance on serological assays is the lack of standardization (13, 14). An international collaborative study which evaluated assay reproducibility for pandemic influenza H1N1 found the inter-laboratory variation in the HI and virus neutralization (VN) assay to be up to 6- and 7-fold respectively (15) whilst inter-laboratory variation has been found up to 80-fold for HI assays and 109-fold for VN assays (16). This forms a clear impediment to reliance on these serological assays for the determination of efficacy. Comparisons of vaccine performance between different studies, including those performed in different seasons, cannot be made, limiting the accrual of understanding in the performance of different vaccines.

In response to these issues the new guideline firstly requests a more diversified characterisation of the immune response and secondly the guideline no longer relies on serological assays with a predefined protective threshold to establish benefit.

Requirements on Immunogenicity

The guideline requests a more comprehensive package on the immunogenicity which includes - next to quantifying the HA antibody response - quantifying functional antibodies by determining neutralizing antibody titres with VN assays and assessing the CMI in a subset of trial participants, in particular in older adults.

All these assays come with limitations. The VN assay is considered a suitable alternative to HA based assays (3), however the optimal protocol for this assay is yet to be identified.

Although the assessment of CMI is regarded as an integral part of the characterisation of the immune response to influenza vaccination and should therefore be performed for every new vaccine (17),the difficulty is in deciding what to measure, when to measure and how to measure and it is here that the guideline lacks specificity. Here too, a clear correlation with protection has not been established and the interpretation of results will be challenging. As the scientific understanding of the mechanisms through which CMI conveys protection evolves so will the ability to set clear requirements and to determine what aspects of CMI can best be used to characterise the immune response and bring understanding to the level of protection that vaccines can elicit in different target groups. Until such time, regulators, manufacturers and scientists will need to maintain a dialogue to improve the characterisation of influenza vaccines.

The guideline additionally states that the neuraminidase antibody (NA) response to vaccination should be determined where appropriate. NA has been found to play a role in the prevention of clinical disease whereas HA inhibits infection and viral replication (18-22). As, ultimately, influenza vaccination aims at preventing clinical disease, insight in the NA response for new influenza vaccines could be an important step in achieving a better characterisation of the clinical characteristics of influenza vaccines. However the amount of NA is not standardized in current influenza vaccines. Therefore, for these vaccines, it does not make sense to determine the NA response, however should be considered in the development of future influenza vaccines.

The challenges regarding assay standardization apply to all these assays mentioned. Certain measures are proposed to minimize the impact, e.g. using a single centralized laboratory, employing validated assays and international standards where available, and using in-house controls and unified protocols. Although some of these may prove logistically challenging, the variability in assays necessitate these steps. It would be impossible for example to rely on different laboratories to analyses samples from a single study. Ongoing research and collaboration between public health institutes, regulators, manufacturers and academia focussing on the standardisation and development of assays can be expected to result in improved assays and assay reproducibility (23).

A consequence of abandoning the CHMP criteria is a change to the requirements in the presentation of immunogenicity data. Data from the SRH, HI and VN assays should be presented according to geometric mean titres (GMTs) and reverse cumulative distribution curves (RCDCs). In addition, seroconversion rates should be given. Since there is no set definition for seroconversion several definitions could be applied when presenting the data. GMTs are a summary measure which can be useful in comparing responses between two groups. The RCDCs will allow the visualization of the immune response across the population. These changes will allow for a more comprehensive assessment of the vaccine induced immune response than under the former guideline, which often resulted in the simple conclusion ´The CHMP criteria were met´.

How to establish clinical efficacy in the post CHMP criteria era?

As stated earlier, serological data alone will no longer be sufficient to conclude whether a vaccine is protective in the target population. The new approach for seasonal and for zoonotic and pandemic vaccines is outlined below.

Seasonal vaccines

For persons over 18 years of age, the proposed guideline states that efficacy of seasonal, non-adjuvanted inactivated vaccines can be determined either in a direct head to head comparison with a licensed vaccine with a similar construct for which there is "at least some data to support effectiveness". If the immune response of the new vaccine is non-inferior it is thought reasonable to assume the protective efficacy would at least be comparable.

For children younger than three years, there is inconsistent evidence on the efficacy and effectiveness of seasonal inactivated vaccines (24, 25). Efficacy in this age group cannot be assumed for existing vaccines and cannot therefore be deduced from comparative immunogenicity studies. Hence the proposed guideline requires applicants to conduct randomized controlled trials with clinical endpoints in order to conclude efficacy for children aged six months to three years. For children between the ages of three to six years, there is some evidence to support efficacy of inactivated influenza vaccines, albeit being moderate (24-26). Yet the proposed guideline states that as the proportion of children up to the age of approximately nine years who are immunologically primed is thought to be variable, efficacy can be deduced from demonstrating a non-inferior immune response to the youngest children for whom efficacy against clinical endpoints should have been demonstrated. For children over the age of nine the approach taken in the proposed guideline is similar to the approach in adults.

Zoonotic vaccines and pandemic vaccines

Zoonotic and pandemic vaccines pose a regulatory challenge. Prior to licensure it is not possible to obtain efficacy data, and the clinical package will be limited to immunogenicity and safety data. Moreover, ethical considerations of testing vaccines in human subjects when there is no direct benefit to the recipient, as there is no immediate threat of a circulating virus, certainly have an impact on regulatory expectations. No firm requirements are set for children, it is merely stated that immunogenicity and safety data in this age group should be obtained "as far as may be possible".

Requirements regarding annual changes in seasonal inactivated vaccines

For seasonal influenza vaccines the annual change in composition has always posed a unique challenge i.e. how to determine the impact of the change in viral strains on the clinical characteristics of the vaccine in a short timeframe between production and epidemic. There has been a substantial shift in the proposed guideline. Previously, the CHMP required manufacturers of inactivated influenza vaccines to conduct small clinical trials in 100 adults, including 50 subjects aged ≥60 years, to demonstrate that immunogenicity and reactogenicity was not affected by the strain change.

These trials are not able to detect changes in the clinical characteristics of influenza vaccines (27). More importantly however, it is unlikely that a change in vaccine strains as a result of antigenic drift will affect the clinical characteristics of these vaccines to such a degree that the benefit risk balance is radically altered. Consequently, these trials are no longer required. The proposed guideline and an earlier published annex to this guideline (28) instead move towards closer monitoring of seasonal influenza vaccine performance.

Moving towards sustainable monitoring of vaccine performance

Effectiveness

For all seasonal influenza vaccines licensed in Europe a Risk Management Plan (RMP) will be required which should include the monitoring of influenza vaccine effectiveness (IVE).

From a regulatory perspective, the monitoring of IVE would fit into the life-cycle approach of medicines. It will inform the evolution of the benefit risk balance, allow the detection of potential issues with effectiveness, and provide data on the benefits to balance potential safety issues. In addition, once well-established, these routine studies could provide a platform to address questions surrounding the performance of new influenza vaccines that are difficult to address pre-licensure, and to measure product-specific effectiveness in a pandemic.

Observational studies into IVE are notoriously subject to bias (29) and the success of this measure will depend on the robustness of the study protocols and implementation thereof. Moreover, studies should ideally be capable of reporting effectiveness estimates in a timely manner and provide brand-specific estimates, potentially challenging the feasibility of this exercise.

The proposed guideline builds upon experience already gained in the field through initiatives such as the European I-MOVE collaboration (30) and encourages manufacturers to tap into this experience and use existing networks. It refers to protocols developed by the European Centre for Disease Control (ECDC). These include (test negative) case control studies, cohort studies and screening studies. Influenza cases have to be laboratory confirmed via either RT-PCR or culture, although within the cohort design non-specific endpoints such as medically attended influenza like illness, all-cause deaths, intensive care admissions and hospitalisations for all respiratory conditions are considered endpoints of interest. When conducting a cohort study the guideline requires a nested (test-negative) case-control study to confirm the effectiveness against laboratory confirmed influenza, ensuring a specific measure of effectiveness is available. For details on most aspects the guideline refers back to the ECDC protocols.

The measurement of IVE is a challenging undertaking and it should not be the expectation that requested studies will provide clear answers during the first few years. The landscape of vaccination in Europe is diverse, and although this diversity can be an advantage when evaluating vaccines it will prove a challenge when implementing IVE monitoring. Not only will the epidemiology differ between regions, vaccination policies vary between countries as does the uptake of vaccines and vaccines used. Moreover, vaccination registries are not operational in all countries and regions within the EU (31). Where they are in place it is not always possible to link these to outcome data such as electronic health care data. This will certainly limit the initial ability to conduct larger scale studies that could provide product-specific estimates in selected target groups.

It is important to realise that IVE is not only a consequence of the product used but of a range of determinants such as the vaccination programme and viral epidemiology which play an important role. Any estimates obtained will have to be placed within the context of the myriad determinants of IVE, many of which are poorly understood. This underlines the shared responsibilities between manufacturers, public health institutes and regulators in evaluating and assessing vaccine effectiveness.

Safety

The monitoring of safety is central to the monitoring of vaccine performance. In Europe, routine pharmacovigilance activities are currently the main source for the identification of

potentially serious but rare adverse events following influenza vaccination, and they rely heavily on passive reporting. This comes with limitations as it does not allow for estimation of the incidence of specific adverse events or the association with vaccination. Although the safety of inactivated influenza vaccines that have been used over recent decades is well characterized (26, 32, 33) there is always the possibility of serious adverse events occurring following manufacturing changes, contamination of batches, or through the introduction of new pandemic influenza strains. Moreover, the introduction of new influenza vaccines would necessitate intensive surveillance of their safety as clinical trials are insufficient to detect rare but serious adverse events.

Whilst some European countries have the infrastructure in place to rapidly evaluate safety signals, this capacity is fragmented. Moreover, countries are often too small, or vaccine use too limited, to properly evaluate rare safety signals. As mentioned earlier, vaccination registries do not exist in all countries and regions of Europe, and it is not always possible to link vaccination data to outcome data (31). With an increasing need for rapid evaluation of safety signals in order to provide timely guidance to policy makers and address public concerns, there is a clear need to invest further in European systems to monitor and evaluate the safety of vaccines.

The proposed guideline requires that the RMP includes plans for enhanced surveillance of vaccine safety, as detailed in an Annex to the guideline (28). The aim of this enhanced surveillance is to rapidly detect a significant increase in reactogenicity that would signal potential serious risks following annual strain changes. Adverse events of interest include typical local and systemic reactions to vaccination such as rash, injection site reactions, myalgia, fever, nausea and headache. In order to achieve this, defined cohorts of children and adults, including a minimum total of 500 persons - 100 per age stratum, should be followed after vaccination for the occurrence of several adverse events of interest. Rates of adverse events will have to be compared to rates in previous years. Alternatively, enhanced passive surveillance could be employed in which the reporting of adverse events is facilitated to obtain reporting rates which can function as a surrogate for the adverse events of interest. Furthermore, data mining of electronic health record data can be also employed. However such mining has the clear limitation of the near impossibility of gathering information on vaccine reactogenicity from electronic health care databases.

Although the increased attention to the monitoring of influenza vaccine safety is welcomed, it is questionable whether the proposed enhanced surveillance is the most efficient means to achieve the goal; the rapid identification of safety signals and have the ability to thoroughly evaluate the association between the signal and vaccination. It would seem more sensible to further invest in the creation of vaccine registries in Europe, improve the registration of vaccination data in existing registries, facilitate the linkage of these registries

to electronic health care databases, limit the data lag for registries and databases and invest in the capacity to implement rapid signal detection and evaluation. Such an infrastructure would permit continuous monitoring and evaluating the safety of influenza vaccines, also after annual strain changes. It is unlikely that data on vaccine reactogenicity in 500 persons will be predictive of any serious but rare adverse events and whether the studies will be able to discriminate relevant changes from year to year that could predict adverse events which could alter the BR balance of the vaccines.

The 2014/2015 influenza season was the first season for which the enhanced surveillance should have been up and running and time will tell how suitable these studies are in detecting potential safety signals associated with the updating of influenza strains in seasonal vaccines.

Final considerations

Lessons learned during the influenza A(H1N1)2009 pandemic together with advances in the scientific understanding of influenza and the immune response to influenza viruses and vaccines have resulted in the revision of existing regulatory guidelines for the licensing of influenza vaccines in Europe. Following a public consultation round, it is expected that the final guidance will be published in the near future.

The proposed guideline reflects a changed approach to the regulatory assessment of influenza vaccines. This has resulted in the abolition of the CHMP criteria, the introduction of more diversified requirements for measuring and reporting the immune response to influenza vaccines, and the requirement for all new influenza vaccines to conduct trials with clinical outcomes in children aged 6 to 36 months. Furthermore, immunogenicity data is no longer requested to support annual strain changes. Importantly, more emphasis is placed on the post licensure monitoring of the benefit risk of influenza vaccines, including a request for continuous monitoring of efficacy and enhanced safety surveillance.

Presently several gaps remain in the understanding of the performance of seasonal influenza vaccines. It is expected that the changes made to the influenza guideline will improve the characterisation of clinical characteristics of new and existing influenza vaccines. The new requirements will certainly improve our knowledge on the functioning of influenza vaccines in children and can be expected to provide a better insight into the immune response overall. The move towards sustained monitoring of the benefits and risks of influenza vaccines underlines regulation does not stop at licensure, and will undoubtedly lead to more accurate data on the benefits and risks to address public concerns should these arise.

Major challenges however remain, such as the absence of standardized serological assays and the absence of a correlate of protection to facilitate vaccine evaluation. Moreover, the

limited availability of an infrastructure in Europe which would allow timely and consistent evaluation of the effectiveness and safety of vaccines currently impedes adequate benefit risk monitoring of new influenza vaccines. New guidance cannot overcome these challenges, and regulators can merely encourage investment in improved methods.

Manufacturers are responsible for their products, regulators guard those products, and public health institutes are responsible for the programmes in which the vaccines are used. Improving the evaluation of vaccines is therefore a shared responsibility between manufacturers, regulators and public health institutes – all of which are dependent on academia for scientific input. This recognition, in addition to the identified need for improved methods and collaboration for vaccine evaluation, has resulted in EU-wide collaboration between public health institutes, industry, regulators and academia which aims to improve the benefit risk monitoring of vaccines (34) and serological assays for evaluating influenza vaccines (23). Collaborative initiatives like these will ultimately result in improved vaccines a better understanding of their immunology and clinical performance, but also more robust tools to monitor performance of influenza vaccines in the future.

References

1. European Medicines Agency. Guideline on influenza vaccines. Non-clinical and clinical module. EMA/CHMP/VWP/457259/2014. 2014.

2. European Medicines Agency. Concept paper on the revision of guidelines for influenza vaccines. EMA/CHMP/VWP/734330/2011. 2011.

3. Pfleiderer M, Trouvin JH, Brasseur D, Granstrom M, Shivji R, Mura M, et al. Summary of knowledge gaps related to quality and efficacy of current influenza vaccines. Vaccine. 2014 Jul 31;32(35):4586-91. PubMed PMID: 24954696.

4. Granstrom M, Voordouw AC. Registration of influenza vaccines for children in Europe. Vaccine. 2011 Oct 6;29(43):7572-5. PubMed PMID: 21820474.

5. Hobson D, Curry RL, Beare AS, Ward-Gardner A. The role of serum haemagglutination-inhibiting antibody in protection against challenge infection with influenza A2 and B viruses. J Hyg (Lond). 1972;70(4):767-77. PubMed PMID: 4509641.

6. Al-Khayatt R, Jennings R, Potter CW. Interpretation of responses and protective levels of antibody against attenuated influenza A viruses using single radial haemolysis. J Hyg (Lond). 1984;93(2):301-12. PubMed PMID: 6389697.

7. Plotkin SA. Complex correlates of protection after vaccination. Clin Infect Dis. 2013 May;56(10):1458-65. PubMed PMID: 23386629.

8. Ohmit SE, Petrie JG, Cross RT, Johnson E, Monto AS. Influenza hemagglutination-inhibition antibody titer as a correlate of vaccine-induced protection. The Journal of Infectious Diseases. 2011;204:1879-85.

9. Belshe RB, Gruber WC, Mendelman PM, Mehta HB, Mahmood K, Reisinger K, et al. Correlates of immune protection induced by live, attenuated, cold-adapted, trivalent, intranasal influenza virus vaccine. J Infect Dis. 2000 Mar;181(3):1133-7. PubMed PMID: 10720541.

10. Treanor J, Wright PF. Immune correlates of protection against influenza in the human challenge model. Dev Biol (Basel). 2003;115:97-104. PubMed PMID: 15088780.

11. Black S, Nicolay U, Vesikari T, Knuf M, Del Giudice G, Della Cioppa G, et al. Hemagglutination inhibition antibody titers as a correlate of protection for inactivated influenza vaccines in children. Pediatr Infect Dis J. 2011 Dec;30(12):1081-5. PubMed PMID: 21983214. Epub 2011/10/11. eng.

12. McElhaney JE, Xie D, Hager WD, Barry MB, Wang Y, Kleppinger A, et al. T cell responses are better correlates of vaccine protection in the elderly. J Immunol. 2006 May 15;176(10):6333-9. PubMed PMID: 16670345.

13. Wagner R, Pfleiderer M. Reproducibility of assays for influenza vaccine immunogenicity determination: progress towards consistency. Expert Rev Vaccines. 2012 Aug;11(8):881-3. PubMed PMID: 23002967.

14. Wood JM, Gaines-Das RE, Taylor J, Chakraverty P. Comparison of influenza serological techniques by international collaborative study. Vaccine. 1994 Feb;12(2):167-74. PubMed PMID: 8147099.

15. Wagner R, Gopfert C, Hammann J, Neumann B, Wood J, Newman R, et al. Enhancing the reproducibility of serological methods used to evaluate immunogenicity of pandemic H1N1 influenza vaccines-an effective EU regulatory approach. Vaccine. 2012 Jun 8;30(27):4113-22. PubMed PMID: 22446639. Epub 2012/03/27. eng.

16. Wood JM, Major D, Heath A, Newman RW, Hoschler K, Stephenson I, et al. Reproducibility of serology assays for pandemic influenza H1N1: collaborative study to evaluate a candidate WHO International Standard. Vaccine. 2012 Jan 5;30(2):210-7. PubMed PMID: 22100887.

17. European Medicines Agency. Clinical evaluation of new vaccines. EMEA/CHMP/VWP/164653/05. 2006.

18. Murphy BR, Kasel JA, Chanock RM. Association of Serum Anti-Neuraminidase Antibody with Resistance to Influenza in Man. New England Journal of Medicine. 1972;286(25):1329-32. PubMed PMID: 5027388.

19. Clements ML, Betts RF, Tierney EL, Murphy BR. Serum and nasal wash antibodies associated with resistance to experimental challenge with influenza A wild-type virus. J Clin Microbiol. 1986 Jul;24(1):157-60. PubMed PMID: 3722363. Pubmed Central PMCID: 268857.

20. Monto AS, Kendal AP. Effect of neuraminidase antibody on Hong Kong influenza. Lancet. 1973 Mar 24;1(7804):623-5. PubMed PMID: 4121842.

21. Rockman S, Brown LE, Barr IG, Gilbertson B, Lowther S, Kachurin A, et al. Neuraminidase-inhibiting antibody is a correlate of cross-protection against lethal H5N1 influenza virus in ferrets immunized with seasonal influenza vaccine. J Virol. 2013 Mar;87(6):3053-61. PubMed PMID: 23283953. Pubmed Central PMCID: 3592172.

22. Marcelin G, Sandbulte MR, Webby RJ. Contribution of antibody production against neuraminidase to the protection afforded by influenza vaccines. Reviews in medical virology. 2012 Jul;22(4):267-79. PubMed PMID: 22438243. Pubmed Central PMCID: 3389592.

23. FLUCOP project gets underway 2015 [cited 2015 9 June]. Available from: http://www.imi.europa.eu/content/newsletter-archive#4.

24. Osterholm MT, Kelley NS, Sommer A, Belongia EA. Efficacy and effectiveness of influenza vaccines: a systematic review and meta-analysis. The Lancet infectious diseases. 2012 Jan;12(1):36-44. PubMed PMID: 22032844. Epub 2011/10/29. eng.

25. Jefferson T, Rivetti A, Di Pietrantonj C, Demicheli V, Ferroni E. Vaccines for preventing influenza in healthy children. Cochrane Database Syst Rev. 2012;8:CD004879. PubMed PMID: 22895945. Epub 2012/08/17. eng.

26. World Health Organisation. Vaccines against influenza WHO position paper - November 2012. Wkly Epidemiol Rec. 2012 Nov 23;87(47):461-76. PubMed PMID: 23210147.

27. Voordouw AC, Beyer WE, Smith DJ, Sturkenboom MC, Stricker BH. Evaluation of serological trials submitted for annual re-licensure of influenza vaccines to regulatory authorities between 1992 and 2002. Vaccine. 2009 Dec 11;28(2):392-7. PubMed PMID: 19878750.

28. European Medicines Agency. Interim guidance on enhanced safety surveillance for seasonal influenza vaccines in the EU, EMA/PRAC/222346/2014. 2014.

29. Nelson JC, Jackson ML, Weiss NS, Jackson LA. New strategies are needed to improve the accuracy of influenza vaccine effectiveness estimates among seniors. Journal of clinical epidemiology. 2009 Jul;62(7):687-94. PubMed PMID: 19124221.

30. Valenciano M, Ciancio B, team IMs. I-MOVE: a European network to measure the effectiveness of influenza vaccines. Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin. 2012;17(39). PubMed PMID: 23041022.

31. Pebody R. Vaccine registers--experiences from Europe and elsewhere. Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin. 2012;17(17). PubMed PMID: 22551493.

32. Vellozzi C, Burwen DR, Dobardzic A, Ball R, Walton K, Haber P. Safety of trivalent inactivated influenza vaccines in adults: background for pandemic influenza vaccine safety monitoring. Vaccine. 2009 Mar 26;27(15):2114-20. PubMed PMID: 19356614.

33. Hambidge SJ, Glanz JM, France EK, McClure D, Xu S, Yamasaki K, et al. Safety of trivalent inactivated influenza vaccine in children 6 to 23 months old. JAMA : the journal of the American Medical Association. 2006 Oct 25;296(16):1990-7. PubMed PMID: 17062862.

34. the ADVANCE Consortium. ADVANCE – Accelerated development of vaccine benefit-risk collaboration in Europe [cited 2015 27 06]. Available from: http://www.advance-vaccines.eu/.

Chapter 10. General discussion

General Discussion

In September 2009 several pandemic influenza vaccines were approved by the Committee for Human Medicinal Products (CHMP) in Europe in response to the evolving 2009/2010 influenza A(H1N1) pandemic. The vaccines were based on mock-up vaccines with an H5N1 strain, for which limited immunogenicity and safety data had been available pre-licensure. Some supplementary immunogenicity and safety data was collected with the H1N1pdm09 vaccines prior to licensing. Following the licensing, vaccination programmes were rapidly implemented across Europe and millions of people were vaccinated in relatively short time frame.

Vaccination programmes were accompanied by extensive monitoring of safety in order to rapidly identify potential adverse events and establish the association with vaccination. Moreover, a range of post licensure studies were conducted in order to evaluate the benefits of the vaccines. The work described in this thesis was conducted in the wake of the 2009/2010 H1N1 pandemic and consists of several studies that evaluate the benefits and risks of pandemic influenza vaccines used across Europe. The experience with the H1N1pdm09 vaccines is collated and lessons drawn to improve the monitoring of the benefits and risks of pandemic influenza vaccines.

Benefits: findings and methods

'*Benefit evaluation is the measurement of the reduction in risk of morbidity and mortality from the infection in the vaccinated population. This is dependent on - amongst others - the efficacy of the vaccine used.*'

The first section of this thesis includes studies evaluating the benefits of Focetria, an MF59™ adjuvanted influenza A(H1N1)pdm09 vaccine (1). The main findings and considerations on the methods used are discussed here.

Medically attended influenza

Chapter 2 describes a cohort with a nested case-control designed to estimate the effectiveness of Focetria against medically attended influenza-like illness (ILI) and RT-PCR confirmed influenza in the at-risk population and persons over 60 in the Netherlands. The study was performed in the primary health care database, Integrated Primary Care Information (IPCI). The cohort included 121,446 patients with an average follow-up time of 75.8 days per person. Prospectively collected data on a wide range of disease outcomes and drug use was available as well as information on health care seeking behaviour, enabling a thorough evaluation of potential confounding variables. Whilst generally the presence of co-morbidities are considered important confounders in influenza vaccine effectiveness (VE) studies, in this study the severity of co-morbidity as approximated by the

number of different pharmaceutical compounds that were used formed an important confounder. A possible explanation for this finding is that the study included risk groups and elderly therefore the majority had underlying medical conditions. The approximation used for determining severity of disease by number of different pharmaceutical compounds prescribed is a crude measure that should be further refined and validated for future influenza VE studies. In addition to severity of co-morbidities, it was found that health care seeking behaviour was a confounder.

The specificity of medically attended ILI for influenza infection can vary with age and change during the influenza season. Therefore it is critical to include laboratory confirmed influenza infection as an endpoint in influenza VE studies to validate findings (2). Large primary health care databases, such as the IPCI database used for this study, do not systematically include information on laboratory confirmed endpoints as laboratory testing in patients presenting with flu symptoms is not routinely performed. As a result the most practical design for influenza VE studies are case-control studies which include persons presenting themselves at the primary health care level with flu symptoms and subsequently test for the presence of influenza virus. To overcome confounding by health care seeking behaviour, a test negative controlled design is now often deployed where those who test negative for influenza form the controls (3-6). This method has become the standard to determine influenza VE (7).

The effectiveness of influenza vaccines is determined by a complex interplay of myriad factors which are not fully elucidated. Moreover, there is much interest in evaluating the performance of influenza vaccines in smaller subgroups, such as pregnant women and frail elderly, or by product. Case-control studies are limited by available funds for laboratory confirmation of samples (8) and, often, by the ability to obtain prospectively collected data on a wide range of health outcomes which could potentially confound the relation between vaccination and influenza related disease. The value of large cohort studies in primary health care databases which contain this information and have sufficient power to obtain reliable estimates of VE in smaller strata should therefore not be overlooked. The information in these databases can be linked to laboratory confirmed cases and test-negative controls from a random selection of ILI cases through a nested case-control design. Chapter 2 illustrates that this approach is a feasible option to monitor VE of influenza vaccines in future. As vaccination in the Netherlands started around the peak of the pandemic (see figure 2.2) the study had insufficient power to fully test this principle. Overall a small non-significant protective effect of vaccination against medically attended ILI was detected: 20.8% (95%CI: -5.4% - 40.5%). Although the – uncontrolled - VE estimates against RT-PCR confirmed influenza and A(H1N1)pdm09 infection detected in the nested case-control study were substantially higher, 73.3% (95%CI: 4.8% - 92.5%) and 88%

(95%CI: 25% - 98%) respectively, numbers were small and estimates were unstable therefore no adjusted analysis were performed.

Hospitalisation

Influenza vaccines are not only used to prevent self-limiting influenza but are particularly employed to prevent the more severe outcomes in the at-risk population. Chapter 3 considered the effectiveness of Focetria against hospitalisation with influenza A(H1N1)pdm09 in individuals with an indication for vaccination in the Netherlands. This study was enabled by the mandatory notification of influenza A(H1N1)pdm09 cases requiring hospitalisation in the Netherlands. The notification data supplied cases which were matched with controls selected from the cohort also described in chapter 2.

This study illustrates the difficulties faced when conducting observational studies during a pandemic. As pandemics remain unpredictable and as available resources during pandemics are heavily stretched, routinely collected data is ideally used to provide estimates of VE against severe outcomes. The study described in chapter 3 shows that this is feasible however comes with limitations. Data regarding the notified hospitalised influenza A(H1N1)pdm09 cases was not collected for the purpose of evaluating VE. Consequently the quality of data on vaccination was imperfect. Information on vaccination was available for 79% of cases. The exact date of vaccination was available for only 49% of vaccinated cases. This limitation was addressed by imputing the vaccination date in those with missing data through different approaches, which result in a range of VE estimates from <0% to 74%. The more realistic scenario, where the vaccination status for persons for whom this was not known is based upon the cases which had perfect registration of vaccination resulted in a VE of 19% (95% CI: -28 – 49). Although not ideal, this approach provides an insight in the protection afforded by the vaccine against more severe outcomes. There were numerous breakthrough infections leading to hospitalisation in persons who were vaccinated (see figure 3.2) which suggest that a vaccine capable of protecting against influenza infection (9) might not be equally successful at protecting at-risk groups against severe outcomes. This study does underline the need to use the inter-pandemic period to improve systems for routine data collection so that it can be used to evaluate the performance of pandemic influenza vaccines in future pandemics. As health care providers are primarily concerned with providing the best care for patients and not less so with recording vaccination status, other systems to accurately record vaccination status should be considered. Ideally, linkage of such a database with accurate vaccination data to disease notification systems or other data-sources with outcome data would be possible on an individual level.

Recommendations:

1. The importance and value of cohort studies in primary health care as a tool to better understand the myriad factors predicting vaccine effectiveness and vaccine failure should not be overlooked.
2. Not only should effectiveness of influenza vaccines against influenza be monitored but also against severe outcomes, including hospitalisations.

Risks: findings and methods

'*Risk evaluation comprises the identification of adverse events after vaccination, the determination of the association between vaccination and these events and the understanding of the mechanisms behind this association.*'

In the second section of this thesis the risks of the pandemic influenza vaccines are considered.

Due to the uncertainties regarding the risks of the pandemic influenza A(H1N1)pdm09 vaccines at the time of licensure, extensive monitoring of the safety of the vaccines was put in place through boosting of existing national and international passive surveillance systems (e.g. EudraVigilance, World Health Organization-Uppsala Monitoring Centre (WHO-UMC), U.S. Vaccine Adverse Events Reporting System (VAERS), Canadian Adverse Events Following Immunization Surveillance System (CAEFISS) and Immunization Monitoring Program ACTive (IMPACT) and the Australian Adverse Drug Reactions System (ADRS)) and through new active surveillance activities in the USA and European Union (10, 11).

Chapter 4 brings together the evidence generated from pre-licensure clinical trials and from post-licensure monitoring programmes on the safety of the pandemic influenza vaccines used in children and adolescents during the 2009/2010 influenza A(H1N1) pandemic.

Clinical studies were identified that evaluate a variety of pandemic influenza vaccines, including monovalent non-adjuvanted inactivated influenza A(H1N1)pdm09 vaccines, monovalent (MF59-, AS03-, and AL-) adjuvanted influenza A(H1N1)pdm09 vaccines live attenuated influenza A(H1N1)pdm09 vaccines and whole virion pandemic H1N1 vaccines. Studies included 10,505 children and adolescents, both healthy and with underlying medical conditions, between the ages of 6 months and 23 years. In addition, the safety monitoring efforts resulted in large amounts of data, with almost 13,000 individual case reports in children and adolescents to the WHO.

However, the diversity in methods and data presentation in clinical study publications and in publications of spontaneous reports hampered the analysis of safety of the different vaccines. As a result, relatively little has been learned on the comparative safety of these influenza A(H1N1)pdm09 vaccines – particularly in children. A collective effort needs to be made to give added value to the enormous work going into individual studies by adhering to available guidelines for the collection, analysis and presentation of vaccine safety data in clinical studies and to guidance for the clinical investigation of medicinal products in the paediatric population.

Recommendations:

3. Investigators should make an increased effort to adhere to available guidelines for the collection, analysis and presentation of vaccine safety data in clinical trials in order to give added value to the enormous volume of work going into the individual vaccine safety studies.

In the months following the 2009/2010 influenza A (H1N1) pandemic two safety signals emerged: an increased incidence of Bell's palsy was reported in persons who received an AS03 adjuvanted influenza A(H1N1)pdm09 vaccine, Pandemrix, in Sweden (12) and increased cases of narcolepsy were seen in children and adolescents following exposure to that same vaccine in Sweden and Finland (13, 14). Pandemrix (15) is an inactivated split virion AS03 adjuvanted H1N1pdm09 vaccine which was used in 38 countries worldwide during the 2009/2010 influenza A(H1N1) pandemic (16). In the European Union Pandemrix was the predominant vaccine used with over 30 million doses administered and very high coverage, especially in the Nordic countries (17).

Bell's palsy

In chapter 5 the signal of Bell's palsy following vaccination with Pandemrix in Sweden (12) is evaluated through a self-controlled case series (SCCS) study. The study was set in The Health Improvement Network (THIN) database, a UK based primary health care database. The aim was to determine whether there was an increased risk of Bell's palsy following vaccination with any influenza vaccine containing A/California/7/2009 (H1N1)-like viral strains and whether risks would be different following pandemic influenza A(H1N1)pdm09 vaccines and seasonal influenza vaccines containing this influenza A(H1N1)pdm09 strain.

The SCCS is a case centred method which uses only information from persons who developed an adverse event. The method estimates the relative incidence of an event in a pre-defined risk period after vaccination compared to pre-defined control periods. As the analysis is

within individual cases all fixed confounding variables, such as sex, are implicitly controlled for. The presence of time varying confounding variables, such as age, will need to be measured and, if necessary, controlled for (18, 19). There are three restrictive assumptions made by the SCCS method which need to be accounted for: 1) events are either recurrent and independent or unique and uncommon over the observation periods; 2) the occurrence of an event must not alter the probability of exposure and 3) the occurrence of an event must not alter the observation period (19).

The relative incidence of Bell's palsy from day 1 to day 42 after vaccination was determined for persons who received an influenza A(H1N1)pdm09 vaccine or for seasonal influenza vaccines for the 2010/2011, 2011/2012 and 2012/2013 periods, based upon the elevated risk during that period reported by Bardage et al (12). The day of vaccination, was included as a separate risk period as an earlier study looking at the risk of Bell's palsy after influenza vaccination found an increased risk of Bell's palsy on the day of vaccination, most likely related to opportunistic recording of a diagnosis (20). Secondly, as vaccination may be delayed following an episode of Bell's palsy – violating the second assumption for a SCCS – a 14 day period prior to vaccination was included as a separate risk period. Important time varying confounding variables identified in this study were episodes of acute respiratory infections and pregnancies. All analyses were adjusted for seasonality.

This study provided no evidence for an increased incidence of Bell's palsy following vaccination with influenza A(H1N1)pdm09 containing vaccines and did not confirm the signal identified by Bardage et al (12) in Sweden. Conversely, a significantly reduced RI for Bell's palsy during the six weeks after vaccination was found. The relative incidence of Bell's palsy during the 42 days after vaccination with any influenza A(H1N1)pdm09 containing vaccine was 0.77 (95% CI: 0.65 – 0.91).

The SCCS was originally developed to evaluate adverse events following vaccination. As the design inherently controls for (unmeasured) fixed confounders it limits any bias that is normally present in observational vaccine studies due to differences between vaccinated and unvaccinated persons, such as underlying co-morbidities and differences health seeking behaviour – as long as these remain unchanged over the observation period. It is important to remain aware of time varying variables which could introduce bias.

The SCCS is an efficient and relatively robust design which can be used to evaluate the association between relatively rare outcomes and vaccination. Importantly, sufficient observation time has to have been accumulated after exposure to be able to include adequate risk and control periods. This limits the usefulness of this study design for urgent situations. Moreover, the definition of a risk window requires some understanding of the mechanism underlying the association between vaccine and adverse event. However, when a

signal emerges it is expectedly unknown whether the adverse event in question is related to and triggered by vaccination. When evaluating a new safety signal, it is unlikely risk periods can be defined a-priori on more than an informed guess based upon likely mechanisms and hypothesised associations. An option would be to define multiple risk periods – which would be similar to using different mesh sizes when fishing as you are not certain what size fish you are after. This can be a valid and useful approach as long as the definition of a "significant finding" is adapted accordingly.

Narcolepsy

The most important safety issue that emerged in the wake of the 2009/2010 influenza A(H1N1) pandemic was undoubtedly the occurrence of narcolepsy in children and adolescents following exposure to Pandemrix. In August 2010 the first reports of a possible association between exposure to this vaccine and narcolepsy-cataplexy in children and adolescents emerged first in Sweden and later in Finland. This resulted in the recommended discontinuation of Pandemrix in these countries (21, 22). The European Medicines Agency (EMA) initiated a review procedure (23) which eventually led to the restriction of indication for Pandemrix (24). In Chapter 6 and 7 this signal was further evaluated.

Chapter 6 is a multinational study looking into the incidence rates of narcolepsy diagnoses before, during and shortly after the 2009/2010 influenza A(H1N1) pandemic in different European countries with different vaccine use and coverage.

Narcolepsy diagnosis rates were assessed through a retrospective cohort study for the period 2000-2010 using large linked automated health care databases in six countries: Denmark, Finland, Italy, the Netherlands, Sweden and the United Kingdom. Overall, 2608 narcolepsy cases were identified in almost 280 million person years (PY) of follow up. The pooled incidence rate was 0.93 (95% CI: 0. 90-0.97) per 100,000 PY. In the age group 5-19 years olds rates were increased after the start of pandemic vaccination compared to the period before the start of campaigns, with rate ratios (RR) of 1.9 (95% CI: 1.1-3.1) in Denmark, 6.4 (95% CI: 4.2-9.7) in Finland and 7.5 (95% CI: 5.2-10.7) in Sweden.

Population based background rate data cannot provide evidence of a potential causal association between influenza A(H1N1)pdm09 vaccination and narcolepsy diagnosis. It can be used for a rapid assessment of public health impact on a population level. The signal reported in Finland and Sweden was also detected in the background rate data collected for this study. The mismatch of changes in age specific narcolepsy diagnosis rates with the underlying influenza A(H1N1)pdm09 vaccine coverage rates in certain age groups provides some indication that factors other than influenza A(H1N1)pdm09 vaccination may also be associated with increasing incidence rates of narcolepsy diagnosis. Thus, additional factors

that could explain an increase in incidence of diagnosis of narcolepsy should be considered for formal hypothesis testing.

Since the narcolepsy signal emerged, eight epidemiological studies have tested the association between Pandemrix and clusters of narcolepsy cases (25-33). The outcomes of these studies vary: whilst most confirmed an association between Pandemrix and narcolepsy, the strength of the association ranges from 1.6 to 14.4. Moreover, whilst early studies detected an association in children and adolescents only (26, 27, 33, 34), later studies also detected associations in older persons (29, 32, 35, 36). The strength of the association found in certain studies and the consistent detection of an increased risk of narcolepsy after Pandemrix vaccination in different countries has given credence amongst public health officials, regulators and policy makers to the causality of the association (17, 37, 38). When relying on epidemiological studies for evaluating the association between a vaccine and a specific event, before concluding on causality, all possible explanations for the event should be considered (39). For the association between Pandemrix and narcolepsy alternative explanations deserve further exploration.

Narcolepsy is a disorder that is inherently difficult to diagnose (40). Median delays from onset to diagnosis of 10 years are the norm (41, 42). Symptoms include excessive daytime sleepiness, disrupted nocturnal sleep, cataplexy and sleep paralysis (43). The presentation of symptoms can vary (44). Although cataplexy is specific to narcolepsy and relatively easy to recognise, the onset of cataplexy is delayed in approximately half of all diagnosed cases (45, 46). This gradual development of symptoms makes it difficult to recognise and to accurately recall a date of onset. Due to the non-specificity of symptoms and the low prevalence of narcolepsy health care providers and patients may be unfamiliar with narcolepsy such that symptoms may initially be attributed to other conditions. Although symptoms of narcolepsy typically start in childhood and young adults, diagnosis for many does not occur until much later in life. The corollary being that narcolepsy begins much earlier than clinically recognized in most cases (44, 47). As a result, at any time a substantial proportion of cases are undiagnosed. Although the diagnosis is thought to have improved in the previous decade due to better understanding of the disease and discovery of the role of hypocretin (45, 48), a recent study found that the median delay between onset and diagnosis is still around 10 years (46).

Epidemiological studies initiated rapidly after the signal emerged in Europe were forcibly observational and retrospective, had limited follow-up time and were conducted in an alert situation in which knowledge of the association increased. The under-diagnosis of narcolepsy – particularly in children – combined with these methodological limitations of association testing studies are likely to have introduced bias (49, 50).

In chapter 7 the potential impact of two such biases is explored through simulations.

The first source of bias is a selection bias labelled 'detection bias'. Suspicion of a potential association between narcolepsy and Pandemrix was already spreading amongst healthcare professionals in Finland from as early as February 2010 (51, 52) and was general knowledge after August 2010 when regulatory agencies published on the association which was picked up by the media (14, 53). Knowledge on the association with vaccination may have resulted in a reduction of the onset to diagnosis interval in vaccinated individuals, whereas this would not happen to the same extent in non-vaccinated subjects (27). The second source of bias is a form of recall bias labelled 'differential exposure misclassification', where the onset of symptoms is misattributed to vaccination resulting into misclassification of onset dates to after vaccination. Knowledge of a putative association between vaccination and a specific event could result in a patient placing symptom onset after vaccination (54). Moreover, using 'diagnosis date' or 'date of first health-care contact' as an index date could result in misclassification of exposure as the start date of a case is brought forward. If vaccinated persons seek healthcare earlier than unvaccinated persons this will similarly result in differential exposure misclassification.

The simulations demonstrated that risk estimates can be inflated in the presence of these biases. They demonstrated that biased attribution of excessive daytime sleepiness onset to after vaccination (differential exposure misclassification) has a greater impact on risk estimates than a reduction in the onset-to-diagnosis interval (detection bias) both in the cohort and case-control designs. Moreover, the simultaneous presence of detection bias and differential exposure misclassification increases RRs more rapidly than could be expected by the effect size of each bias in isolation.

Notably, without any bias present risk estimates were reduced in the cohort analysis whilst they were around one in the case-control analysis. Within the cohort analysis, with limited observation time, cases with an onset within the observation time will not all be diagnosed within the observation period and therefore not included as cases in the analysis. Exposed and unexposed person time within the cohort however remain constant. As a consequence, with no real risk present, a detected risk estimate is reduced.

Finally, in the simulations, the case-control design appeared more robust to the influence of these biases than the cohort design. Whilst in this simulated scenario we were able to sample controls from the same population as the cases and to assess their exposure without error, avoiding the most problematic sources of bias in case-control studies, this is not possible in real-life situations. Therefore the trade-off between the potential bias introduced by control selection and the increased resilience against potential detection bias and differential exposure misclassification will need to be considered when considering study designs to evaluate vaccine safety signals.

Despite the limitations of these simulations – their overly simplistic portrayal of the complexities of the real world – they do illustrate that in the absence of a real association between the vaccine and narcolepsy, presence of detection bias and differential exposure misclassification could account for the elevated risks detected in different association studies.

The sensitivity analyses performed by the different studies published insufficiently address the presence of these biases. Several studies limited their primary observation period for EDS onset to the period before media attention (27, 30) or included sensitivity analyses using such a reduced study period (34) in order to exclude detection bias. Additionally, studies used primary index dates that were thought to be less susceptible to such a bias including onset of symptoms (26, 31), first contact with health care (30, 34) or referral to specialist care (27, 32). The simulations described in chapter 7 suggest that limiting the analysis to subjects with an onset date prior to media attention will not eliminate the effect of detection bias, since all patients need to be diagnosed to be included, which is where the bias arises. When limiting cases to those with an EDS date before media attention, Nohynek et al. found that the RR increased from 11.4 to 12.7 (30) and O'Flanagan et al. found that the RR increased from 13.0 to 14.5 (33). Since only diagnosed subjects can be included as cases, detection bias will be unavoidable if the onset-to-diagnosis interval is shorter in vaccinated individuals.

The only analysis which would exclude the presence of the detection bias is one that excludes all cases diagnosed after start of awareness on the association – however the power in this scenario will be too limited to provide interpretable results. Miller et al. did censor those diagnosed after media attention, suggesting that media attention in the UK did not start until July 2011, thereby retaining sufficient power for their analyses (31). However, professional awareness also in the UK is likely started much earlier and could similarly have resulted into a shortening of the onset to diagnosis interval in vaccinated persons.

Five years after the original signal emerged the exact contribution of Pandemrix to the occurrence of clusters of narcolepsy cases in Europe remains unclear. The simulations illustrated that it is possible that the levels of increased risk observed were at least partially due to bias. So far, these biases have not yet been adequately addressed and therefore it is inappropriate to conclude on causality.

To exclude the presence of differential exposure misclassification would require knowledge of the actual dates of symptom onset – impossible to obtain retrospectively – or an understanding of differential health seeking behaviour and 'diagnosis risks' between vaccinated and unvaccinated individuals.

Potentially, patterns of narcolepsy diagnosis rates in vaccinated and unvaccinated individuals over time can help to detect the presence of these biases and maybe even help

in modelling their impact in specific situations. As also suggested by Miller et al., long term follow-up of vaccinated cohorts will be important to clarify the risk of narcolepsy associated with Pandemrix (31).

It is not unthinkable that a similar scenario could unfold in future, i.e. a signal emerges following a mass vaccination campaign in which a difficult to diagnose condition with a delayed onset is linked to exposure to a new vaccine. The simulations have shed some light on how study design can be improved to deal with biases in these situations.

In future analyses of safety signals for diseases with long latency periods for which observation times are limited the effect of limited case capture together with fixed person-time denominators should be recognized, as should the changes in exposed and unexposed person-time denominators with changing vaccination coverage. Chapter 7 illustrates that the case-control design provides less biased estimates in these circumstances as it does not require the calculation of person-time. Finally, the simulations described in chapter 7 illustrate the importance of not only measuring background rates of adverse events of special interest but also having insight in the background onset-to-diagnosis interval.

Recommendations:

4. Population cohorts should be followed over several years to monitor patterns of narcolepsy diagnosis rates and elucidate the attributable risk of narcolepsy associated with Pandemrix.

A balancing act: final considerations and future perspectives.

The studies described in this thesis are a fraction of the enormous volume of work that was conducted to shed light on the performance of the different pandemic influenza A(H1N1)pdm09 vaccines. This experience gained during the 2009/2010 influenza A(H1N1) pandemic has helped to improve the regulation of influenza vaccines in Europe where a move is made towards more evidence based regulation. As discussed in chapter 9, existing approaches for establishing efficacy of influenza vaccines are abandoned in favour of a more diversified and evidence-based approach prior to licensing and an increased focus on the monitoring of the benefits and risks post licensure. These changes are expected to result in an improved understanding of the performance of new influenza vaccines and a better quality of evidence to inform decision making on the use of these vaccines. However, as also highlighted in chapter 9, for pandemic influenza vaccines the pre-licensure requirements are limited by the feasibility of obtaining data on efficacy and data on the safety and immune response in specific risk groups such as children and pregnant women. Although there might

be a clear wish – and arguably a clear need - to obtain this data, there are ethical concerns around conducting randomised controlled trials in children evaluating a vaccine for which there is no measurable threat, thus no clear benefit. Efficacy data cannot be obtained until a pandemic influenza strain is circulating. New, unexpected, adverse events can occur with every mass vaccination campaign. Therefore in a next pandemic again we will have to rely on existing systems to monitor the safety and effectiveness of the pandemic influenza vaccines. The greatest legacy of the 2009/2010 influenza A(H1N1) pandemic has undeniably been the collaboration in Europe to monitor of the effectiveness and safety of the pandemic influenza vaccines. European collaborative initiatives such as I-MOVE (55-57), of which chapter 2 was a part, and VAESCO (27, 58-60), of which chapter 6 was a part, delivered important contributions to the understanding of the benefits and risks of the pandemic influenza A(H1N1)pdm09 vaccines used in Europe.

Improving benefit risk monitoring infrastructure in Europe

The initiatives mentioned in the paragraph above have brought the beginnings of a lasting infrastructure in Europe to monitor the performance of vaccines. Despite this success, five years on we are still faced with limited availability of an infrastructure in Europe for timely and consistent evaluation of the effectiveness and safety of new pandemic influenza vaccines.

Vaccination registries are not operational in all countries and regions within Europe (61). Where they are in place it is not always possible to link these to outcome data such as electronic health care data. For example, in the Netherlands information on childhood vaccination is collected in a registration system named Praeventis [www.rivm.nl] whilst influenza vaccination is provided by and recorded by GPs and health outcomes are recorded in a variety of databases. The different sources of data are not easily linked on an individual basis. These systems can therefore be helpful in identifying a signal, however evaluating an existing signal will be more challenging. As highlighted in chapter 3, efforts should be made during the inter-pandemic period to improve the prospective collection of information on vaccine exposure that can be linked to health outcomes on an individual level.

Some countries and regions in Europe do have linked databases available for the benefit risk monitoring of influenza vaccines, however, underlying populations are often too small to rapidly evaluate safety signals of rare adverse events. Moreover, it is not good practice to confirm a signal in the same population in which it was identified. Therefore, even these countries will benefit from improved systems across the whole of Europe. Finally, although the VAESCO project did pave the way, at present there is no common infrastructure that will allow the systematic evaluation of vaccines across Europe, applying a single methodology, standardized collection of information on population, outcomes and exposures and a standardized analysis and reporting of findings.

The diversity in Europe in healthcare provision, vaccination policies, vaccines used, influenza epidemiology, and recording of vaccination and health outcomes provides enormous challenges in the development of such an infrastructure and ultimately to the monitoring of the benefits and risks of vaccines and vaccination. This diversity also provides a unique opportunity for the monitoring of vaccines. Comparisons between countries in which different vaccines are used, different target groups are vaccinated, or different vaccine coverage is achieved can help to understand associations as demonstrated in chapter 6. For this to be successful however the underlying reasons for the diversity do need to be well understood.

To add complexity to this situation multiple stakeholders are involved in the benefit risk monitoring. Manufacturers, or marketing authorisation holders, are responsible for their products, regulators guard these products, and public health institutes are responsible for the programmes in which the vaccines are used. Undoubtedly these different groups will have different perspectives on the relevance of different health outcomes, the impact of vaccination and the importance of identified risks. Furthermore, healthcare professionals who administer vaccines and the public whom the vaccines are intended to protect will also have important perspectives on the benefits of vaccination and the importance of risks. To improve the benefit risk monitoring process and communication surrounding the benefit risk of vaccines, an understanding of the different perspectives of these stakeholders is essential.

Initiatives such as the ADVANCE project (62) have been started to further study methods and enhance collaboration to improve benefit risk monitoring of vaccines in Europe. However, ultimately these are research projects with a finite life and although they provide an urgently needed service they do not substitute for permanent institutions and infrastructure. They cannot address the big elephant in the room: who is ultimately responsible for the development, running and maintenance of a vaccine monitoring infrastructure. Who will finance such an infrastructure, who will 'own' the data and who can decide when and how to evaluate the benefit risk of a certain vaccine, i.e. push the analysis button? Whilst research projects are ongoing, and to some degree are starting these discussions, European regulators, public health institutes and health policy makers need to come together to start addressing and owning these issues.

Finally, it is not possible to predict when and how a future pandemic will evolve. And although we might now have considerable experience to inform the safety profile of the existing influenza vaccines, with a new pandemic virus and a new mass-vaccination programme we need to be prepared for the occurrence of new safety signals. The experience of narcolepsy has taught us that it is very helpful to have a good understanding of the epidemiology of potential adverse events in Europe. Although impossible to pinpoint what adverse events will be of interest, considering the experience of influenza and (adjuvanted) influenza vaccines focus should be on neurological events and disorders with a

potential auto-immune aetiology. The inter-pandemic period should be used to collect data on diagnosis rates in different age groups, populations, countries and improve the understanding of differences between European countries in recording and diagnosing these conditions.

Recommendations:

5. The inter-pandemic period should be used to develop and improve systems for monitoring effectiveness and safety in Europe.
6. The recording of influenza vaccination in the Netherlands should be improved and methods developed to link this information to databases with health outcomes and prospectively collected information on potential confounding variables.
7. Diagnosis rates of rare but serious conditions that are either neurological in nature or have a potential autoimmune aetiology should be collected. An understanding in differences between countries in recording and diagnosing of these conditions should be gained, as well as insight in the onset to diagnosis interval for these events of special interest.

Adjuvanted vaccines

The 2009/2010 influenza A(H1N1) pandemic was the first time that adjuvanted influenza vaccines were used in such a large and diverse population. When discussing the benefits and risks of pandemic influenza vaccines in Europe, the usefulness and safety of adjuvants in these vaccines cannot be ignored.

Adjuvanted influenza vaccines are far from new, nor are the discussions surrounding their safety. In a manuscript on the future of influenza vaccines published in 1969, Stuart-Harris (63) provided the following reflection on adjuvanted influenza vaccines:

"*It is quite obvious that so long as there is any question of using inactivated whole influenza vaccine or a split product subcutaneously, methods of enhancing the antibody response are still much to be desired. […]*

The history of the use of drugs in man indicates the great need for caution before risks are dismissed as negligible. Until a fuller understanding of the mechanism of adjuvant action has been obtained, it is clear that unusual and unwanted effects may continue to occur whatever the materials which are used. However, adjuvant vaccines seem certain to have a future in programmes of human immunization and further basic research work is necessary."

These words could have just as easily been written today. The adjuvants used now are different, however the challenges in influenza vaccination and uncertainties surrounding the safety of adjuvanted vaccines remain.

There is a need for improved (pandemic) influenza vaccines. In a pandemic situation, theoretically and simplistically put, the population will be unprimed by the new virus and therefore the pandemic influenza vaccine will have to be successful in priming risk groups. Evidence points out that inactivated split or subunit vaccines have a limited ability to successfully do this when natural priming by influenza virus is absent (64, 65) (also see chapter 8). Although live attenuated influenza vaccines form an alternative, these are not licensed in young children due to an increased risk of wheezing leading to hospitalisation in children under the age of two years (66-69). Secondly, the manufacturing capacity for influenza vaccines worldwide is limited and vaccines that spare the amount of antigen are therefore needed to ensure improved access to vaccines during a pandemic (70). For these reasons adjuvanted influenza vaccines will undoubtedly play a role in a future pandemic. As described in chapter 8 oil-in-water adjuvanted vaccines exhibit improved immunogenicity, both quantitative as qualitative, translating into improved efficacy and resulting in less antigen use. The reactogenicity to these vaccines is however also increased and this will need to be carefully considered for each vaccine separately when determining the optimal dosage and vaccination schedule. What should be underlined above all is that the uncertainties regarding rare but serious adverse events, such as the association between Pandemrix and narcolepsy, need to be addressed and fully investigated if we are to move forward with these vaccines - especially for the target group in which there is a most urgent need for improved influenza vaccines: young children. Until we fully understand how these adjuvants work, in particular in children with immature, developing, immune systems, basic research to increase our understanding is needed. This fundamental research should however acknowledge the uncertainties that exist surrounding the association between Pandemrix and narcolepsy.

Possibilities to evaluate signals are mostly limited to observational studies. Unless a biological mechanism for the observed adverse event is known, these studies involve a great deal of guess work on risk windows, potential confounding factors and alternative explanations for an observed association. Moreover, the subjective nature of inference from observational data is not always evident and it is up to investigators to clearly communicate the limitations of their studies and the potential consequences of these limitations. As a result, wrong conclusions could easily be drawn from observational studies. These wrong conclusions could potentially have disastrous consequences, either by exposing people to undue safety risks through continued vaccination or through a loss in confidence in vaccination which could lead to a drop in vaccination coverage and renewed outbreaks of infectious diseases. Once communicated these messages will be difficult to change.

Considering the potential impact of decisions regarding the safety of a vaccine, or conclusions on causality between a vaccine and an adverse event, the evidence underlying these decisions should be strong, obtained using rigorous methods, and be robust and valid. This will always remain challenging in an alert situation as was the case with Pandemrix and narcolepsy. Whilst regulators should continue to be critical towards the evidence obtained and continue to strive to obtain robust evidence, they will need to act even if faced with these uncertainties and limitations. In these situations, it will be helpful to evaluate the potential impact of uncertainties or speculated biases. Secondly, when communicating on regulatory actions in response to safety signals existing uncertainties and their potential impact should be clearly communicated.

Narcolepsy is a debilitating chronic condition resulting in life long disability, affecting school performance and the ability to function in society. There is no known cure. Understanding the exact role that Pandemrix may have played in the surge of narcolepsy diagnoses seen in several European countries is crucial for those affected.

Recommendations:

8. As adjuvanted vaccines are indispensable for future vaccination programmes further research work is necessary to better understand their mechanism of action and the relationship with the occurrence of auto-immune disease.
9. If regulatory action is considered necessary without the presence of strong and robust evidence on identified risks, the potential impact of uncertainties and speculated biases should be evaluated.
10. When communicating on regulatory actions in response to safety signals existing uncertainties and their potential impact should be clearly communicated

Concluding remarks

Vaccination is one of the most beneficial public health interventions, having led to the eradication of diseases worldwide (71), and provides an efficient way to protect populations during pandemics (72). Vaccination however can adversely affect those who are vaccinated and severe and serious adverse reactions to vaccination do occur. As these reactions are usually rare, they are difficult to predict and study. Yet, accurate assessment of potential associations between adverse events and vaccination is crucial to ensure the safety of vaccination and to maintain public confidence in vaccination programs. Loss of public confidence in vaccination can result in outbreaks of infectious disease (73, 74) whilst allowing continued exposure of individuals to a vaccine that can trigger severe disease is

unacceptable (75). This is the challenge that benefit risk assessment needs to address : providing accurate data to clearly communicate the benefits and the risks of vaccination on which to base regulatory decisions, to inform those responsible for the design and implementation of vaccination programmes, to inform health policy makers on the potential need for additional preventative actions, to inform health professionals and arguably most importantly to inform the public. This communication should clearly and transparently identify not only the known benefits and risks but also the uncertainties and why, despite the uncertainties and known risks, the decision makers – be it regulators or public health officials – deem the benefit risk balance to be positive.

References

1. Summary of Product Characteristics for Focetria, Influenza vaccine H1N1v (surface antigen, inactivated, adjuvanted). Last update on www.ema.europa.eu on 05/10/2010. . In: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000710/WC500023749.pdf, editor. 04-01-2011.

2. Orenstein EW, De Serres G, Haber MJ, Shay DK, Bridges CB, Gargiullo P, et al. Methodologic issues regarding the use of three observational study designs to assess influenza vaccine effectiveness. International journal of epidemiology. 2007 Jun;36(3):623-31. PubMed PMID: 17403908.

3. Valenciano M, Kissling E, Team IMC-CS. Early estimates of seasonal influenza vaccine effectiveness in Europe: results from the I-MOVE multicentre case-control study, 2012/13. Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin. 2013;18(7):3. PubMed PMID: 23449183.

4. Ohmit SE, Thompson MG, Petrie JG, Thaker SN, Jackson ML, Belongia EA, et al. Influenza vaccine effectiveness in the 2011-2012 season: protection against each circulating virus and the effect of prior vaccination on estimates. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2014 Feb;58(3):319-27. PubMed PMID: 24235265. Pubmed Central PMCID: 4007111.

5. Foppa IM, Haber M, Ferdinands JM, Shay DK. The case test-negative design for studies of the effectiveness of influenza vaccine. Vaccine. 2013 Jun 26;31(30):3104-9. PubMed PMID: 23624093.

6. Jackson ML, Nelson JC. The test-negative design for estimating influenza vaccine effectiveness. Vaccine. 2013 Apr 19;31(17):2165-8. PubMed PMID: 23499601.

7. Jackson ML, Rothman KJ. Effects of imperfect test sensitivity and specificity on observational studies of influenza vaccine effectiveness. Vaccine. 2015 Mar 10;33(11):1313-6. PubMed PMID: 25659280.

8. Valenciano M, Kissling E, Reuss A, Jimenez-Jorge S, Horvath JK, Donnell JM, et al. The European I-MOVE Multicentre 2013-2014 Case-Control Study. Homogeneous moderate influenza vaccine effectiveness against A(H1N1)pdm09 and heterogenous results by country against A(H3N2). Vaccine. 2015 Jun 4;33(24):2813-22. PubMed PMID: 25936723.

9. Black S. Safety and effectiveness of MF-59 adjuvanted influenza vaccines in children and adults. Vaccine. 2015 Jun 8;33 Suppl 2:B3-5. PubMed PMID: 26022564.

10. Bone A, Guthmann J-P, Nicolau J, Lévy-Bruhl D. Population and risk group uptake of H1N1 influenza vaccine in mainland France 2009-2010: Results of a national vaccination campaign. Vaccine. 2010;28(51):8157-61.

11. Salmon DA, Akhtar A, Mergler MJ, Vannice KS, Izurieta HS, Ball R, et al. Immunization-safety monitoring systems for the 2009 H1N1 monovalent influenza vaccination program. Paediatrics. 2011;127(Suppl 1:):S78-86.

12. Bardage C, Persson I, Ortqvist A, Bergman U, Ludvigsson JF, Granath F. Neurological and autoimmune disorders after vaccination against pandemic influenza A (H1N1) with a monovalent adjuvanted vaccine: population based cohort study in Stockholm, Sweden. Bmj. 2011;343:d5956. PubMed PMID: 21994316. Pubmed Central PMCID: 3192001.

13. National Institute for Health and Welfare (THL). National Institute for Health and Welfare recommends discontinuation of Pandemrix vaccinations 25 Aug 2010 Available from: http://www.thl.fi/en_US/web/en/pressrelease?id=22930.

14. Medical Products Agency. The MPA investigates reports of narcolepsy in patients vaccinated with Pandemrix 2010 [cited 2013 09/10/2013]. Available from: http://www.lakemedelsverket.se/english/All-news/NYHETER-2010/The-MPA-investigates-reports-of-narcolepsy-in-patients-vaccinated-with-Pandemrix/.

15. European Medicines Agency. Pandemrix : EPAR - Public assessment report.

16. World Health Organisation. Pandemrix® vaccine and increased risk of narcolepsy 2011 [cited 2013 20/11]. Available from: http://www.euro.who.int/en/health-topics/communicable-diseases/influenza/news/news/2011/02/pandemrix-vaccine-and-increased-risk-of-narcolepsy.

17. European Medicines Agency. Committee for Medicinal Products for Human Use (CHMP) Assessment report - Pandemrix Influenza vaccine (H1N1) (split virion, inactivated, adjuvanted) A/California/7/2009 (H1N1)v like strain (x-179a) - Procedure No.: EMEA/H/C/000832/A20/0045. 21 July 2011.

18. Weldeselassie YG, Whitaker HJ, Farrington CP. Use of the self-controlled case-series method in vaccine safety studies: review and recommendations for best practice. Epidemiology and infection. 2011 Dec;139(12):1805-17. PubMed PMID: 21849099.

19. Whitaker HJ, Hocine MN, Farrington CP. The methodology of self-controlled case series studies. Stat Methods Med Res 2009 February 1, 2009;18(1):7-26.

20. Stowe J, Andrews N, Wise L, Miller E. Bell's palsy and parenteral inactivated influenza vaccine. Hum Vaccin. 2006 May-Jun;2(3):110-2. PubMed PMID: 17012908. Epub 2006/10/03. eng.

21. National Institute for Health and Welfare recommends discontinuation of Pandemrix vaccinations 25 Aug 2010 Available from: http://www.thl.fi/en_US/web/en/pressrelease?id=22930.

22. EMA. European Medicines Agency starts review of Pandemrix 27 August 2010. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Press_release/2010/08/WC500096005.pdf.

23. European Medicines Agency. European Medicines Agency starts review of Pandemrix. 27 August 2010 [cited 2012 22/03/2012]. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Press_release/2010/08/WC500096005.pdf.

24. European Medicines Agency. Press release: European Medicines Agency recommends restricting use of Pandemrix 21 July 2011. Available from: http://www.ema.europa.eu/ema/index.jsp?curl=pages/news_and_events/news/2011/07/news_detail_001312.jsp&murl=menus/news_and_events/news_and_events.jsp&mid=WC0b01ac058004d5c1&jsenabled=true.

25. Medical Products Agency. Narcolepsy – Case inventory study in Sweden 2009-2010 2011. Available from: http://www.lakemedelsverket.se/english/All-news/NYHETER-2011/Report-from-an-epidemiological-study-in-Sweden-on-vaccination-with-Pandemrix-and-narcolepsy-/.

26. Medical Products Agency. A registry based comparative cohort study in four Swedish counties of the risk for narcolepsy after vaccination with Pandemrix - A first and preliminary report, by the 2011 [March 28,2011]. Available from: www.mpa.se

27. European Centre for Disease Prevention and Control. Narcolepsy in association with pandemic influenza vaccination (a multi-country European epidemiological investigation). Stockholm: September 2012.

28. Montplaisir J, Petit D, Quinn MJ, Ouakki M, Deceuninck G, Desautels A, et al. Risk of Narcolepsy Associated with Inactivated Adjuvanted (AS03) A/H1N1 (2009) Pandemic Influenza Vaccine in Quebec. PloS one. 2014;9(9):e108489. PubMed PMID: 25264897. Pubmed Central PMCID: 4180737.

29. Persson I, Granath F, Askling J, Ludvigsson JF, Olsson T, Feltelius N. Risks of neurological and immune-related diseases, including narcolepsy, after vaccination with Pandemrix: a population- and registry-based cohort study with over 2 years of follow-up. Journal of internal medicine. 2013 Oct 17. PubMed PMID: 24134219.

30. Nohynek H, Jokinen J, Partinen M, Vaarala O, Kirjavainen T, Sundman J, et al. AS03 adjuvanted AH1N1 vaccine associated with an abrupt increase in the incidence of childhood narcolepsy in Finland. PloS one. 2012;7(3):e33536. PubMed PMID: 22470453. Pubmed Central PMCID: 3314666.

31. Miller E, Andrews N, Stellitano L, Stowe J, Winstone AM, Shneerson J, et al. Risk of narcolepsy in children and young people receiving AS03 adjuvanted pandemic A/H1N1 2009

influenza vaccine: retrospective analysis. BMJ. 2013;346:f794. PubMed PMID: 23444425. Epub 2013/02/28. eng.

32. Dauvilliers Y, Arnulf I, Lecendreux M, Monaca Charley C, Franco P, Drouot X, et al. Increased risk of narcolepsy in children and adults after pandemic H1N1 vaccination in France. Brain : a journal of neurology. 2013 Aug;136(Pt 8):2486-96. PubMed PMID: 23884811.

33. O'Flanagan D, Barret A, Foley M, Cotter S, Bonner C, Crowe C, et al. Investigation of an association between onset of narcolepsy and vaccination with pandemic influenza vaccine, Ireland April 2009-December 2010. Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin. 2014;19(17). PubMed PMID: 24821121.

34. O'Flanagan D, Bonner C, Crowe C, Lynch B, Sweeney B, Gilvarry J, et al. Investigation of an increase in the incidence of narcolepsy in children and adolescents in 2009 and 2010. Final Report of National Narcolepsy Study Steering Committee. Department of Health, Ireland. 2012.

35. Medical Products Agency. Registry study confirms increased risk of narcolepsy after vaccination with Pandemrix in children and adolescents and shows an increased risk in young adults 2013 [cited 2013 09/10/2013]. Available from: http://www.lakemedelsverket.se/english/All-news/NYHETER-2013/Registry-study-confirms-increased-risk-of-narcolepsy-after-vaccination-with-Pandemrix-in-children-and-adolescents-and-shows-an-increased-risk-in-young-adults/.

36. National Institute for Health and Welfare (THL). Increased risk of narcolepsy observed also among adults vaccinated with Pandemrix in Finland 2013 [cited 2013 09/10/2013]. Available from: http://www.thl.fi/en_US/web/en/pressrelease?id=33516.

37. Morris K. Implications of narcolepsy link with swine-influenza vaccine. The Lancet Infectious diseases. 2013 May;13(5):396-7. PubMed PMID: 23755397.

38. Thebault S, Vincent A, Gringras P. Narcolepsy and H1N1 vaccination: a link? Current opinion in pulmonary medicine. 2013 Nov;19(6):587-93. PubMed PMID: 24048081.

39. World Health Organization. Causality assessment of adverse event following immunization (AEFI): user manual for the revised WHO classification. 2013.

40. Guilleminault C, Mignot E, Partinen M. Controversies in the diagnosis of narcolepsy. Sleep. 1994 Dec;17(8 Suppl):S1-6. PubMed PMID: 7701189.

41. Morrish E, King MA, Smith IE, Shneerson JM. Factors associated with a delay in the diagnosis of narcolepsy. Sleep medicine. 2004 Jan;5(1):37-41. PubMed PMID: 14725825. Epub 2004/01/17. eng.

42. Dauvilliers Y, Montplaisir J, Molinari N, Carlander B, Ondze B, Besset A, et al. Age at onset of narcolepsy in two large populations of patients in France and Quebec. Neurology. 2001;57:2029-33.

43. Poli F, Overeem S, Lammers GJ, Plazzi G, Lecendreux M, Bassetti CL, et al. Narcolepsy as an adverse event following immunization: case definition and guidelines for data collection, analysis and presentation. Vaccine. 2013 Jan 30;31(6):994-1007. PubMed PMID: 23246545.

44. Peterson PC, Husain AM. Pediatric narcolepsy. Brain & development. 2008 Nov;30(10):609-23. PubMed PMID: 18375081.

45. Overeem S, Black JL, 3rd, Lammers GJ. Narcolepsy: immunological aspects. Sleep medicine reviews. 2008 Apr;12(2):95-107. PubMed PMID: 18291691. Pubmed Central PMCID: 2366136.

46. Luca G, Haba-Rubio J, Dauvilliers Y, Lammers GJ, Overeem S, Donjacour CE, et al. Clinical, polysomnographic and genome-wide association analyses of narcolepsy with cataplexy: a European Narcolepsy Network study. Journal of sleep research. 2013 Oct;22(5):482-95. PubMed PMID: 23496005.

47. Kauta SR, Marcus CL. Cases of pediatric narcolepsy after misdiagnoses. Pediatric neurology. 2012 Nov;47(5):362-5. PubMed PMID: 23044019.

48. Singh AK, Mahlios J, Mignot E. Genetic association, seasonal infections and autoimmune basis of narcolepsy. Journal of autoimmunity. 2013 Jun;43:26-31. PubMed PMID: 23497937.

49. Ahmed SS, Schur PH, Macdonald NE, Steinman L. Narcolepsy, 2009 A(H1N1) pandemic influenza, and pandemic influenza vaccinations: What is known and unknown about the neurological

disorder, the role for autoimmunity, and vaccine adjuvants. Journal of autoimmunity. 2014 Feb 18. PubMed PMID: 24559657.

50. Barker CI, Snape MD. Pandemic influenza A H1N1 vaccines and narcolepsy: vaccine safety surveillance in action. The Lancet Infectious diseases. 2014 Mar;14(3):227-38. PubMed PMID: 24360892.

51. Kall A. The Pandemrix - narcolepsy tragedy: how it started and what we know today. Acta paediatrica. 2013 Jan;102(1):2-4. PubMed PMID: 23016673.

52. Partinen M, Saarenpaa-Heikkila O, Ilveskoski I, Hublin C, Linna M, Olsen P, et al. Increased incidence and clinical picture of childhood narcolepsy following the 2009 H1N1 pandemic vaccination campaign in Finland. PloS one. 2012;7(3):e33723. PubMed PMID: 22470463. Pubmed Central PMCID: 3314680. Epub 2012/04/04. eng.

53. THL. National Institute for Health and Welfare recommends discontinuation of Pandemrix vaccinations 25 Aug 2010 Available from: http://www.thl.fi/en_US/web/en/pressrelease?id=22930.

54. Andrews N, Miller E, Taylor B, Lingam R, Simmons A, Stowe J, et al. Recall bias, MMR, and autism. Archives of disease in childhood. 2002 Dec;87(6):493-4. PubMed PMID: 12456546. Pubmed Central PMCID: 1755823.

55. Kissling E, Valenciano M, Cohen JM, Orozszi B, Barret AS, Rizzo C, et al. Estimates of pandemic influenza vaccine effectiveness in Europe, 2009-10: results of the I-MOVE multicentre case-control study [abstract]. European Scientific Conference on Applied Infections Disease Epidemiology; Abs. N 432010.

56. Valenciano M, Kissling E, Cohen JM, Oroszi B, Barret AS, Rizzo C, et al. Estimates of Pandemic Influenza Vaccine Effectiveness in Europe, 2009-2010: Results of Influenza Monitoring Vaccine Effectiveness in Europe (I-MOVE) Multicentre Case-Control Study. . PLoS Med. 2011;8:e1000388.

57. Wijnans L, Dieleman J, Voordouw B, Sturkenboom M. Effectiveness of MF59 adjuvanted influenza A(H1N1)pdm09 vaccine in risk groups in the Netherlands. PloS one. 2013;8(4):e63156. PubMed PMID: 23646191. Pubmed Central PMCID: 3640023.

58. Dieleman J, Romio S, Johansen K, Weibel D, Bonhoeffer J, Sturkenboom M. Guillain-Barre syndrome and adjuvanted pandemic influenza A (H1N1) 2009 vaccine: multinational case-control study in Europe. BMJ. 2011;343:d3908. PubMed PMID: 21750072. Pubmed Central PMCID: 3134565. Epub 2011/07/14. eng.

59. Wijnans L, Lecomte C, de Vries C, Weibel D, Sammon C, Hviid A, et al. The incidence of narcolepsy in Europe: before, during, and after the influenza A(H1N1)pdm09 pandemic and vaccination campaigns. Vaccine. 2013 Feb 6;31(8):1246-54. PubMed PMID: 23246544. Epub 2012/12/19. eng.

60. Romio S, Weibel D, Dieleman JP, Olberg HK, de Vries CS, Sammon C, et al. Guillain-Barre syndrome and adjuvanted pandemic influenza A (H1N1) 2009 vaccines: a multinational self-controlled case series in Europe. PloS one. 2014;9(1):e82222. PubMed PMID: 24404128. Pubmed Central PMCID: 3880265.

61. Pebody R. Vaccine registers--experiences from Europe and elsewhere. Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin. 2012;17(17). PubMed PMID: 22551493.

62. the ADVANCE Consortium. ADVANCE – Accelerated development of vaccine benefit-risk collaboration in Europe [cited 2015 27 06]. Available from: http://www.advance-vaccines.eu/.

63. Stuart-Harris CH. Adjuvant influenza vaccines. Bull World Health Organ. 1969;41(3):617-21. PubMed PMID: 5309486. Pubmed Central PMCID: 2427695. Epub 1969/01/01. eng.

64. El-Madhun AS, Cox RJ, Haaheim LR. The effect of age and natural priming on the IgG and IgA subclass responses after parenteral influenza vaccination. The Journal of infectious diseases. 1999 Oct;180(4):1356-60. PubMed PMID: 10479172.

65. el-Madhun AS, Cox RJ, Soreide A, Olofsson J, Haaheim LR. Systemic and mucosal immune responses in young children and adults after parenteral influenza vaccination. The Journal of infectious diseases. 1998 Oct;178(4):933-9. PubMed PMID: 9806018.

66. MedImmune. FluMist®, Influenza Virus Vaccine Live, Intranasal. Highlights of prescribing information Gaithersburg, MD2007. Available from: http://www.medimmune.com/pdf/products/flumist_pi.pdf.

67. Belshe RB, Ambrose CS, Yi T. Safety and efficacy of live attenuated influenza vaccine in children 2–7 years of age. Vaccine. 2008;26, Supplement 4(0):D10-D6.

68. Belshe RB, Edwards KM, Vesikari T, Black SV, Walker RE, Hultquist M, et al. Live attenuated versus inactivated influenza vaccine in infants and young children. The New England journal of medicine. 2007 Feb 15;356(7):685-96. PubMed PMID: 17301299. Epub 2007/02/16. eng.

69. European Medicines Agency. European Public assessment report – Fluenz 2011 [cited 2013 05-01]. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/001101/WC500103711.pdf.

70. Kieny MP, Costa A, Hombach J, Carrasco P, Pervikov Y, Salisbury D, et al. A global pandemic influenza vaccine action plan. Vaccine. 2006 Sep 29;24(40-41):6367-70. PubMed PMID: 17240560.

71. Plotkin S, Plotkin S. A short history of vaccination. In: Plotkin S, Orenstein W, editors. Vaccines. 4th ed. Philadelphia: WB Saunders; 2004. p. 1-15.

72. Ferguson NM, Cummings DA, Fraser C, Cajka JC, Cooley PC, Burke DS. Strategies for mitigating an influenza pandemic Nature 2006;442:448–52.

73. Coughlan S, Connell J, Cohen B, Jin L, Hall WW. Suboptimal measles-mumps-rubella vaccination coverage facilitates an imported measles outbreak in ireland. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2002 Jul 1;35(1):84-6. PubMed PMID: 12060880.

74. Vivancos R, Keenan A, Farmer S, Atkinson J, Coffey E, Dardamissis E, et al. An ongoing large outbreak of measles in Merseyside, England, January to June 2012. Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin. 2012;17(29). PubMed PMID: 22835470.

75. Larson HJ, Cooper LZ, Eskola J, Katz SL, Ratzan S. Addressing the vaccine confidence gap. Lancet. 2011 Aug 6;378(9790):526-35. PubMed PMID: 21664679.

Appendices

Samenvatting in het Nederlands

De baten en risico's van pandemische griep vaccins

In 2009 werd de wereld geconfronteerd met de eerste pandemie van de 21ste eeuw. In een rap tempo werden er vaccins ontwikkeld en geregistreerd door de verschillende regulatoire autoriteiten. Voor deze vaccins gold dat er een zekere mate van onzekerheid was betreffende de baten en de risico's – de werkzaamheid was afgeleid van de immune response en de veiligheid was bepaald in klinische studies met gezonde vrijwilligers, voornamelijk volwassenen. Om deze onzekerheden te adresseren, werd de doeltreffendheid van vaccinatie opgevolgd in verschillende studies, en werd de veiligheid van vaccinatie nauwlettend gevolgd terwijl de vaccinatie programma's werden uitgerold. Dit proefschrift bespreekt verschillende studies die zowel de doeltreffendheid, of te wel de baten, als de risico's evalueren.

Hoofdstuk 1 geeft een algemene inleiding in pandemische griep en griepvaccins, de registratie van pandemische griepvaccins en de uitdagingen hierin. Het hoofdstuk wordt afgesloten met een kort overzicht van de verschillende hoofdstukken in het proefschrift.

De eerste sectie van dit proefschrift omvat studies die de baten van pandemische griepvaccinaties evalueren.

Hoofdstuk 2 betreft een studie van de doeltreffendheid van vaccinatie met een MF59rTM geadjuveerd influenza A(H1N1)pdm09 vaccin welk in Nederland was gebruikt om risico groepen en ouderen te beschermen tegen de gevolgen van de pandemische griep. De bescherming tegen zowel *griep achtige ziekte* als tegen laboratorium bevestigde influenza A(H1N1)pdm infectie werd bepaald door middel van een cohort studie (*griep achtige ziekte*) en een daarin in genest patiënt-controle studie (influenza A(H1N1)pdm infectie). De cohort studie omvatte 121.446 patiënten die in aanmerking kwamen voor het pandemische griep vaccin gegeven via de huisarts. Voor de genest patiënt-controle studie werden 41 huisartsen praktijken uitgerust met keel en neuswatten. Indien een patiënt met griepachtige ziekte verschijnselen zich zou melden bij een van deze praktijken, die meededen in het patiënt controle onderzoek, werd bij deze patiënt een neus en keel wat afgenomen. De afgenomen keel en neuswatten werden opgestuurd naar het virologisch

laboratorium van het Erasmus MC. Hier werd bepaald of de desbetreffende patiënt geïnfecteerd was met een griep virus en, zo ja, of dit het influenza A(H1N1)pdm virus betrof.

De doeltreffendheid van vaccinatie in het voorkomen van griepachtige ziekte binnen de cohort studie was 17.3% (95%CI: -8.5% - 36.9%). Zowel de ernst van onderliggend lijden als zorg zoekend gedrag bleken de relatie tussen vaccinatie en het voorkomen van griepachtige ziekte te vertekenen. Na het corrigeren voor deze twee variabelen bleek de doeltreffendheid van vaccinatie 20.8% (95%CI: -5.4% - 40.5%). De doeltreffendheid was het hoogst in volwassenen tot 50 jaar (59%, 95%CI: 23% - 78%). Doordat vaccinatie relatief laat gedurende de pandemie op weg kwam werden er slechts 45 keel en neuswatten ontvangen. Hiervan waren er negen positief voor influenza virus. De doeltreffendheid van vaccinatie in het voorkomen van RT-PCR bevestigde influenza infectie was 73.3% (95%CI: 4.8% - 92.5%). De doeltreffendheid tegen RT-PCR bevestigde influenza A(H1N1)pdm09 infectie was 88% (95%CI: 25% - 98%). Gezien het laag aantal patiënten waarop deze schattingen zijn gebaseerd worden ze weinig robuust geacht. Daarnaast, door het lage aantal patiënten in het patiënt controle onderzoek kon er geen correctie voor confounders plaatsvinden. Deze studie heeft wel uitgewezen dat een cohort studie met daarin genest een patiënt controle studie een haalbare en mogelijk bruikbaar alternatief ontwerp is voor toekomstige onderzoeken naar de doeltreffendheid van influenza vaccins.

In **hoofdstuk 3** wordt een studie beschreven die de doeltreffendheid van het MF59^{rTM} geadjuveerd influenza A(H1N1)pdm09 vaccin in het voorkomen van hospitalisatie met laboratorium bevestigde influenza A(H1N1)pdm09 infectie bepaald. Patiënten die gedurende de pandemie met laboratorium bevestigde influenza A(H1N1)pdm09 infectie opgenomen waren in een ziekenhuis werden genotificeerd en, de geanonimiseerde gegevens, opgenomen in de OSIRIS database. Van deze gehospitaliseerde patiënten kwamen 149 in aanmerkingen voor een pandemisch griep vaccin. Deze werden vervolgens gematched op leeftijd, geslacht en onderliggend lijden aan 28.238 personen uit de in hoofdstuk 2 beschreven cohort studie. Met conditionele logistische regressie werd de doeltreffendheid van vaccinatie geschat. Daarnaast werden er verschillende sensitiviteitsanalyses uitgevoerd waarbij de invloed van de ernst van het onderliggend lijden werd geëvalueerd en daarnaast bekeken werd wat de invloed van verschillende aannames betreffende missende vaccinatie gegevens was. Van 46% (n=68) van de gehospitaliseerde patiënten was het bekend dat zij tenminste een vaccinatie hadden ontvangen. Hiervan was de exacte datum van vaccinatie

slechts bekend voor 49% (n=33). De patiënten voor wie de datum van vaccinatie bekend waren wat betreft patiënt karakteristieken vergelijkbaar met de patiënten voor wie dit niet bekend was. Er werd aangenomen dat de datums van vaccinatie ook vergelijkbaar waren tussen de patiënten met deze gegevens en de patiënten zonder deze gegevens.

Van de gehospitaliseerde patiënten was 22% tijdig gevaccineerd tegen influenza A(H1N1)pdm09, in vergelijking met 28% van de controle personen. Er was dus een significant aantal doorbraak infecties. Van de gehospitaliseerde patiënten waren er zeven overleden. Hiervan waren vier gevaccineerd, waarvan er voor één bekend was dat deze vaccinatie slechts enkele dagen voor optreden symptomen was ontvangen. Voor twee werd aangenomen dat deze tijdig gevaccineerd waren. De doeltreffendheid van vaccinatie met het MF59 geadjuveerd influenza A(H1N1)pdm09 vaccin in het voorkomen van hospitalisatie werd geschat op 19% (95%CI: -28-49). Indien dit beperkt werd tot patiënten en controle personen met ernstig onderliggend lijden was dit 49% (95%CI: 16-69). Deze bevindingen suggereren dat het MF59 geadjuveerde influenza A(H1N1)pdm09 vaccin slechts beperkte bescherming bood tegen hospitalisatie met influenza A(H1N1)pdm09.

De tweede sectie van dit proefschrift omvat studies naar de risico's van pandemische griep vaccins.

Door de bestaande onzekerheden omtrent de veiligheid van de pandemische influenza A(H1N1)pdm09 vaccins ten tijde van het registreren van deze vaccins werd er een uitgebreide monitoring van de veiligheid van de vaccins opgezet. Dit door middel van het stimuleren van de bestaande nationale en internationale passieve bewakingssystemen (bijv EudraVigilance, World Health Organization-Uppsala Monitoring Centre, de Amerikaanse Vaccine Adverse Events Reporting System, het Canadese bijwerkingen na immunisatie surveillance systeem) en door middel van nieuwe actieve surveillance-activiteiten in de Verenigde Staten en de Europese Unie.

In **hoofdstuk 4** wordt de veiligheid van pandemische influenza A(H1N1)pdm09 vaccins in kinderen en adolescenten besproken aan de hand van klinische studies, observationele studies en rapportages van bijwerkingen. Er werden 25 klinische studies met daarin 10.505 kinderen en adolescenten tussen de 6 maanden en 23 jaar geïdentificeerd waarin de veiligheid van verscheidene pandemische influenza vaccins werd beschreven. De studies bevatten zowel gezonde kinderen en adolescenten als kinderen en adolescenten met onderliggend lijden. Daarnaast waren er circa 13.000 rapportages van bijwerkingen. De verscheidenheid in de

meetmethodes en rapportage van bijwerkingen in de verschillende studies en publicaties maakt dat de analyse van veiligheid over de studies en publicaties heen niet mogelijk was. Hierdoor is er relatief weinig geleerd over de verschillen tussen vaccins wat betreft het veiligheidsprofiel. Er zou meerwaarde gegeven kunnen worden aan individuele studies als men zich, bij het uitvoeren van studies, aan bestaande richtlijnen zou houden voor het verzamelen en rapporteren van veiligheidsgegevens in kinderen en adolescenten. Wel heeft de pandemie geresulteerd in een beginnende infrastructuur voor verbeterde internationale samenwerking in studies naar de veiligheid van vaccins.

In de maanden na de 2009/2010 influenza A (H1N1) pandemie waren er twee belangrijke veiligheids- signalen: een verhoogde incidentie van Bellse parese werd gemeld bij personen die een AS03 geadjuveerd influenza A (H1N1) pdm09 vaccin, Pandemrix, hadden ontvangen in een Zweedse studie. Daarnaast werden er verhoogde gevallen van narcolepsie waargenomen bij kinderen en adolescenten na blootstelling aan hetzelfde vaccin in Zweden en Finland. Pandemrix is een geïnactiveerd gesplitst virion AS03 adjuvans H1N1pdm09 vaccin dat werd gebruikt in 38 landen over de hele wereld tijdens de 2009/2010 influenza A (H1N1) pandemie. In de Europese Unie was Pandemrix het voornaamst gebruikte vaccin met meer dan 30 miljoen doses toegediend en een zeer hoge dekkingsgraad met name in de Scandinavische landen.

Hoofdstuk 5 gaat in op het signaal van Bellse parese na vaccinatie met Pandemrix. In een patiënt-controle onderzoek, waarbij patiënten hun eigen controle vormden werd de associatie tussen influenza vaccins met een A/California/7/2009 (H1N1)-achtige griepstam (waaronder Pandemrix) en het voorkomen van Bellse parese geschat. De studie was gesitueerd in een in het Verenigd Koninkrijk huisartsen database – The Health Improvement Network (THIN) genaamd. Bellse parese, ook wel aangezichtsverlamming van Bell genoemd, is een plotseling optredende perifere verlamming welk in het verleden vaker in verband is gebracht met influenza vaccinatie.

Alle nieuwe gevallen van Bellse parese die tussen 1 juni 2009 en 20 juni 2013 optraden in de THIN database werden geïncludeerd. De relatieve incidentie van Bellse parese gedurende de 6 weken na vaccinatie met pandemische of seizoens-influenza vaccins werd bepaald. Analyses werden gecorrigeerd voor seizoen en geïdentificeerde confounders. We vonden een incidentie van Bellse parese van 38,7 per 100.000 persoonsjaren. Zowel acute respiratoire infecties als zwangerschappen bleken confounders te zijn. Naar correctie voor confounders was de relatieve

incidentie van Bellse parese gedurende de 42 dagen na vaccinatie met een influenza-vaccin 0,77 (95% CI: 0,65-0,91). Deze was niet significant verschillend gedurende de 42 dagen na de seizoens-vaccin (0,70, 95% CI: 0,58-0,84) of pandemisch vaccin (0,67, 95% CI: 0,44-1,03). Er waren aanwijzingen dat het schijnbaar beschermend effect van vaccinatie op het voorkomen van Bellse parese vooral gedreven werd door vrouwen in de leeftijd van 45 tot 65 jaar, en personen boven de 65 jaar. In andere groepen werd geen beschermend effect gezien. Concluderend, in deze studie werd geen bewijs voor een verhoogde incidentie van Bellse parese volgende vaccinatie met een vaccin bevattende een A/California/7/2009 (H1N1)-achtige griepstam gezien, noch voor monovalente influenza A(H1N1)pdm09 griepvaccin. In tegendeel, de relatieve incidentie van Bellse parese gedurende de 6 weken na vaccinatie was verlaagd. Het is onduidelijk wat dit verminderde incidentie zou kunnen verklaren.

In hoofdstuk 6 en 7 wordt dieper ingegaan op het signaal van narcolepsie na vaccinatie met Pandemrix.

Hoofdstuk 6 beschrijft de achtergrond incidentie van narcolepsie in verschillende databases uit zes Europese landen gedurende de periode 2000 tot 2010. Er werden 2.608 incidente gevallen van narcolepsie geïdentificeerd in 280 miljoen persoonsjaren. De gepoolde incidentie was 0,93 (95% CI: 0,90-0,97) per 100.000 persoonsjaren. Er waren pieken tussen de 15-30 jaar oud (vrouwen> mannen) en rond de 60 jaar. In de leeftijdsgroep van 5-19 jarigen werd een significant verhoogde incidentie na het begin van de pandemische vaccinatie programma's in vergelijking met de periode hier vóór gezien in Denemarken (relatieve incidentie:1,9, 95% CI: 1,1-3,1), in Finland (6,4, 95% CI: 4,2-9,7) en in Zweden (7,5, 95% CI: 5,2-10,7). Verificatie van narcolepsie diagnoses in Nederland had een significant effect op het patroon van de incidentie in de tijd. Deze studie naar het verloop van achtergrond incidentie biedt nuttige inzichten rondom een veiligheidssignaal. De verhoogde incidentie van narcolepsie na het begin van de vaccinatie programma's zoals gemeld vanuit Zweden en Finland worden ook waargenomen in deze studie. Een toegenomen incidentie in narcolepsie diagnoses werd niet waargenomen in alle landen of kwam niet overeen met pandemische influenza A(H1N1) pdm09 vaccinatie. De verhoogde incidentie na start van de vaccinatie programma's gezien in Denemarken kan moeilijk verklaard worden door vaccinatie met Pandemrix aangezien de dekkingsgraad hier zeer laag was. Dit wijst erop dat er mogelijk andere factoren dan vaccinatie een rol spelen in de geziene toename in narcolepsie diagnoses in Europa.

Verschillende epidemiologische studies hebben een associatie tussen Pandemrix en narcolepsie gerapporteerd. Aangezien deze studies werden uitgevoerd ten tijde van een toegenomen bewustzijn omtrent narcolepsie en een mogelijk verband met pandemische vaccinatie, en aangezien deze studie observationeel waren en een relatief korte observatie tijd hadden en daarbij narcolepsie een moeilijk te diagnosticeren aandoening is, is het mogelijk dat de gevonden relatieve risico's in deze studies deels verklaard kunnen worden veranderingen in het vaststellen van narcolepsie diagnoses en het vaststellen van blootstelling aan Pandemrix over tijd. In **hoofdstuk *7*** wordt dit voorbeeld gebruikt om, met behulp van simulaties, methodologische aanbevelingen te maken voor toekomstige vaccin veiligheidsstudies die onder eenzelfde omstandigheden moeten worden uitgevoerd.

Het effect van twee mogelijke bronnen van bias op de associatie tussen Pandemrix en narcolepsie wordt in **hoofdstuk *7*** gesimuleerd: 1) detectie bias, wat wordt gedefinieerd als het versneld vaststellen van narcolepsie in kinderen die zijn blootgesteld aan Pandemrix en 2) differentiële misclassificatie van vaccinatie, gedefinieerd als het optreden van de eerste symptomen van narcolepsie ten onrechte toewijzen tot na vaccinatie. Hierdoor wordt iemand ten onrechte toegewezen als gevaccineerd bij het optreden van de eerste symptomen. De rol van deze twee bronnen van bias werd bestudeerd in een gesimuleerde populatie van 100.000 kinderen. De incidentie van narcolepsie en narcolepsie diagnose, en pandemische vaccinatie werd gesimuleerd gebaseerd op beschikbare gegevens. De gesimuleerde datasets werden geanalyseerd met behulp van een cohort en patiënt-controle analyse methode.

Het relatieve risico van narcolepsie bij gevaccineerde versus niet-gevaccineerde kinderen kan oplopen tot 28,4 in aanwezigheid van de twee gesimuleerde bronnen van bias. De bias had minder effect wanneer vaccinatiegraad hoger was, de onderliggende interval van start symptomen tot narcolepsie diagnose korter was, of de observatie periode werd verlengd. De patiënt controle methode was beter bestand tegen deze bronnen van bias dan het cohort onderzoek. De simulatie toonde aan dat bij afwezigheid van een werkelijke associatie tussen vaccin en narcolepsie, aanwezigheid van detectie bias en differentiële misclassificatie van vaccinatie een verklaring zou kunnen zijn voor de verhoogde risico's gezien in de verschillende gepubliceerde studies. De simulaties leveren nuttige inzichten voor het ontwerp en de interpretatie van toekomstige vaccin veiligheidsstudies.

De 2009/2010 influenza A(H1N1) pandemie was de eerste keer dat er op grote schaal gebruik werd gemaakt van geadjuveerde influenza vaccins. Deze vaccins

hebben het voordeel dat ze het immuunsysteem sterker stimuleren dan conventionele influenza vaccins, waardoor er een betere response te verwachten is en/of er minder antigen in het vaccin nodig is. **Hoofdstuk 8** beschouwt recente klinische studies met geadjuveerde influenza vaccins in kinderen jonger dan 3 jaar. Voor deze leeftijdsgroep is er beperkt bewijs omtrent de werkzaamheid en doeltreffendheid van conventionele influenza vaccins. De evidentie die er is wijst uit dat deze conventionele, subunit of split geïnactiveerde, influenza vaccins suboptimale bescherming bieden in jonge kinderen. Mogelijk bieden geadjuveerde vaccins voor deze leeftijdsgroep een werkzaam alternatief. Olie-in-water geadjuveerde vaccins lijken een effectief alternatief welk mogelijk een dringende behoefte in de jongste, immunologisch naïeve kinderen kunnen vullen. De beperkte studies beschikbaar wijzen op een sterk verbeterde immunogeniciteit, zowel kwantitatief als kwalitatief, en een verbeterde werkzaamheid. Echter, hiertegenover staat een verhoogde reactogeniciteit welk zorgvuldig moet worden meegewogen in het bepalen van de optimale dosering voor ieder vaccin. Wat moet vooral moet worden benadrukt is dat de onzekerheden ten aanzien van zeldzame maar ernstige bijwerkingen, zoals de associatie tussen Pandemrix en narcolepsie (hoofdstuk 6 en hoofdstuk 7), moeten worden aangepakt mochten deze geadjuveerde influenza vaccins verder worden ontwikkeld voor jonge kinderen. Totdat er een goed begrip is hoe deze adjuvantia werken bij kinderen met een nog ontwikkelend immuunsysteem moet er geïnvesteerd worden in fundamenteel onderzoek. Tegelijkertijd zullen andere mogelijkheden om de immuunrespons en werkzaamheid bij jonge, veelal immunologisch naïeve, kinderen verder moeten worden ontwikkeld.

Een van de belangrijkste regulatoire gevolgen van de 2009/2010 pandemie in Europa is de ontwikkeling van een nieuwe richtlijn voor het evalueren van influenza vaccins. De belangrijkste veranderingen in het klinische gedeelte van de nieuwe influenza-richtlijn worden in **hoofdstuk 9** besproken. De achtergrond van deze veranderingen wordt bediscussieerd en er wordt bekeken of de richtlijn zal leiden tot een beter inzicht in de karakteristieken van seizoens-, zoönotische en pandemische influenza vaccins gedurende het regulatoire evaluatie proces. De nieuwe richtlijn neemt een nieuwe benadering voor de toetsing van influenza vaccins. Eerdere toetsingscriteria gebaseerd op serologische drempelwaarden worden afgeschaft en er is in plaats een meer gediversifieerde aanpak voor het meten en presenteren van de immuunrespons na vaccinatie gekomen. Daarnaast wordt er nu geëist dat werkzaamheid tegen klinische eindpunten voor jonge kinderen, onder de drie jaar, wordt vastgesteld voor registratie van een nieuw seizoens-vaccin. Daarnaast is er meer aandacht voor het bewaken van de baten en risico's van

influenza vaccins na registratie. Hoewel het daadwerkelijk de verwachting is dat deze nieuwe aanpak zal leiden tot een verbeterd inzicht in het functioneren van nieuwe influenza vaccins zijn er uitdagingen, zoals het gebrek aan gestandaardiseerde assays en serologische correlaten voor bescherming, die niet kunnen worden overkomen door betere richtlijnen alleen. De erkenning van deze uitdagingen heeft geresulteerd in een aantal samenwerkingsinitiatieven in Europa waar de academische wereld, fabrikanten, volksgezondheidsinstituten en regulatoire autoriteiten samenwerken om de evaluatie van influenza vaccins in de toekomst te vereenvoudigen en te verbeteren.

Tenslotte worden in **hoofdstuk 10** de belangrijkste bevindingen van de verschillende studies bediscussieerd.

Abbreviations

ADEM	acute disseminated encephalomyelitis
ADRS	Australian Adverse Drug Reactions System
AEFI	adverse events following immunization
AESIs	Adverse events of special interest
ARI	acute respiratory infection
CAEFISS	Canadian Adverse Events Following Immunization Surveillance System
CDC	Centres for Disease Control
CHMP	Committee for Human Medicinal Products
CI	confidence interval
ECDC	European Centre for Disease Control
EDS	excessive daytime sleepiness
EMA	European Medicines Agency
EPARs	electronic public assessment reports
FDA	Food and Drug Administration
GBS	Guillain-Barré syndrome
GCP	Good Clinical Practice
GMT	geometric mean titre
GPs	general practitioners
HA	haemagglutinin
HI	haemagglutination inhibition
HLA	Human Leukocyte Antigen
HR	hazard ratio
ICPC	International classification of primary care
ICSRs	individual case safety reports
ILI	influenza like illness
IMPACT	Immunization Monitoring Program ACTive
IPCI	Integrated Primary Care Information
LAIVs	live attenuated influenza vaccines
MN	microneutralisation
MSLT	multiple sleep latency test
NA	neuraminidase
NL	Netherlands
OR	odds ratio

ORS	oculo-respiratory syndrome
PPV	positive predictive value
PRAC	Pharmacovigilance Risk Assessment Committee
PRISM	Post-Licensure Rapid Immunization Safety Monitoring
PY	person years
RCDCs	reverse cumulative distribution curves
RI	relative incidence
RMP	Risk Management Plan
RNA	ribonucleic acid
RR	Relative Risk
RT-PCR	reverse transcription polymerase chain reaction
SAGE	Strategic Advisory Group of Experts
SOC	system organ class
SRH	serial radial haemolysis
UK	United Kingdom
UMLS®	Unified Medical Language System®
US	United States
VAERS	Vaccine Adverse Events Reporting System
VAESCO	Vaccine Adverse Event Surveillance & Communication
VE	vaccine effectiveness
VN	virus neutralisation
VSD	Vaccine Safety Datalink
WHO	World Health Organization
WHO-UMC	World Health Organization-Uppsala Monitoring Centre

Codes for Data Extraction

Chapter 5 Bell's palsy after influenza(H1N1)pdm09 vaccines

Read code for Bell's palsy

F31..00	Facial nerve disorders
F310.00	Bell's (facial) palsy

AHD codes for influenza vaccination

1002090105	Influenza A H1N1v
1002090104	Influenza A H1N1v unknown brand (other health provider)
1002090103	Influenza A H1N1v (other health provider)
1002090102	Influenza A H1N1v (other health provider)
1002090101	Influenza A H1N1v
1002090100	Influenza A H1N1v unknown brand
1002090000	Influenza

READ codes for influenza vaccination

65E..00	Influenza vaccination
65E0.00	First pandemic influenza vaccination
65E1.00	Second pandemic influenza vaccination
65E2.00	Influenza vacc othr hlth prov
65E3.00	1st pan flu vac othr hlth prov
65E4.00	2nd pan flu vac othr hlth prov
65E5.00	CELVAPAN - first influenza A (H1N1v) 2009 vaccination given
65E6.00	CELVAPAN - second influenza A (H1N1v) 2009 vaccination given

65E7.00	CELVAPAN - 1st flu A (H1N1v) 2009 vacc by othr hlth provider
65E8.00	CELVAPAN - 2nd flu A (H1N1v) 2009 vacc by othr hlth provider
65E9.00	PANDEMRIX - first influenza A (H1N1v) 2009 vaccination given
65EA.00	PANDEMRIX - second influenza A (H1N1v) 2009 vaccination give
65EB.00	PANDEMRIX - 1st flu A (H1N1v) 2009 vac by othr hlth provider
65EC.00	PANDEMRIX - 2nd flu A (H1N1v) 2009 vac by othr hlth provider

READ codes for ARI

'H00..00'	'H03..00'	'H042000'	'H060700'	'H01yz00'	'H040000'
'H00..11'	'H03..11'	'H042100'	'H060800'	'H01z.00'	'H040100'
'H00..12'	'H03..12'	'H042z00'	'H060900'	'H02..00'	'H040200'
'H00..13'	'H030.00'	'H043.00'	'H060A00'	'H02..11'	'H040300'
'H00..15'	'H031.00'	'H043.11'	'H060B00'	'H02..12'	'H040400'
'H00..16'	'H032.00'	'H043000'	'H060C00'	'H02..13'	'H040500'
'H01..00'	'H033.00'	'H043100'	'H060D00'	'H020.00'	'H040600'
'H01..11'	'H034.00'	'H043200'	'H060E00'	'H021.00'	'H040w00'
'H010.00'	'H035.00'	'H043211'	'H060F00'	'H022.00'	'H040x00'
'H010.11'	'H035000'	'H043z00'	'H060v00'	'H023.00'	'H040z00'
'H011.00'	'H035100'	'H044.00'	'H060w00'	'H023000'	'H041.00'
'H012.00'	'H035z00'	'H04z.00'	'H060x00'	'H023100'	'H041000'
'H013.00'	'H036.00'	'H05..00'	'H060z00'	'H023z00'	'H041100'
'H014.00'	'H03z.00'	'H050.00'	'H061.00'	'H024.00'	'H041z00'
'H01y.00'	'H04..00'	'H051.00'	'H061000'	'H025.00'	'H042.00'
'H01y000'	'H040.00'	'H052.00'	'H061100'	'H02z.00'	'H042.11'
'H053.00'	'H061200'	'H05z.11'	'H061600'	'H060.11'	'H06z000'
'H055.00'	'H061300'	'H05z.12'	'H061z00'	'H060000'	'H06z011'
'H05y.00'	'H061400'	'H06..00'	'H062.00'	'H060100'	'H06z100'
'H05z.00'	'H061500'	'H060.00'	'H06z.00'	'H060200'	'H06z111'
'H060300'	'H06z112'	'H060400'	'H07..00'	'H060600'	'H0z..00'
'H060500'	'H0y..00'				

READ codes for delivery date

'63...00'	'63...00'	'639..00'	'63A..00'	'632..00'	'63D..00'
'6331.00'	'633..00'	'633a.00'	'6341.00'	'6342.00'	'63E2.00'
'635..11'	'7F10.00'	'7F10000'	'7F10100'	'7F10y00'	'7F10z00'
'7F10z11'	'7F10z12'	'7F11.00'	'7F11000'	'7F11100'	'7F11200'
'7F11300'	'7F11y00'	'7F11z00'	'7F12.00'	'7F12000'	'7F12100'
'7F12111'	'7F12y00'	'7F12z00'	'7F13.00'	'7F13000'	7F13100 '
'7F13111'	'7F13200'	'7F13300'	'7F13y00'	'7F13z00'	7F14.00'
'7F14100'	'7F14y00'	'7F14z00'	'7F15.00'	'7F15000'	'7F14000'
'7F15100'	'7F15y00'	'7F15z00'	'7F16.00'	'7F16000'	'7F16200'
'7F16300'	'7F16400'	'7F16500'	'7F16600'	'7F16700'	'7F16800'
'7F16900'	'7F16A00'	'7F16B00'	'7F16y00'	'7F16z00'	'7F17.00'
'7F17000'	'7F17100'	'7F17200'	'7F17300'	'7F17y00'	'7F17z00'
'7F18.00'	'7F18000'	'7F18100'	'7F16100'	'7F18y00'	'7F18z00'
'7F19.00'	'7F19000'	'7F19100'	'7F19y00'	'7F19z00'	'7F1A.00'
'7F17.11'	'7F17.12'	'L34..00'	'L398.00'	'L398300'	'L398400'
'Ly0..00'					

READ codes for H1N1 / influenza infection

H27..00	Influenza
H270100	Influenza with pneumonia, influenza virus identified
H271100	Influenza with pharyngitis
H2A..11	Influenza A (H1N1
H271z00	Influenza with respiratory manifestations NOS
H27yz00	Influenza with other manifestations NOS
H27z.11	Flu like illness
H27z.12	Influenza like illness
H2A..00	Influenza due to Influenza A virus subtype H1N1
16L..00	Influenza-like symptoms
H2...00	Pneumonia and influenza
H270.00	Influenza with pneumonia
H271.00	Influenza with other respiratory manifestation
H27y.00	Influenza with other manifestations
H27z.00	Influenza NOS

H27y100	Influenza with gastrointestinal tract involvement
H270000	Influenza with bronchopneumonia
H270z00	Influenza with pneumonia NOS
H271000	Influenza with laryngitis
H27y000	Influenza with encephalopathy
4J3L.00	Influenza A virus H1N1 subtype detected
4J3M.00	Influenza A virus H1N1 subtype not detected
4JDb.00	Influenza (A&B
4JU0.00	Influenza H1 virus detected
4JU5.00	Influenza B virus detected
4JU4.00	Influenza A virus, other or untyped strain detected
4JU1.00	Influenza H2 virus detected
4JU2.00	Influenza H3 virus detected
43dF.00	Influenza A antibody level
43dG.00	Influenza B antibody level

List of Publications

Publications based on studies described in this thesis

Wijnans, L., B. Voordouw. A review of the changes to the licensing of influenza vaccines in Europe. *Accepted for publication* Influenza and Other Respiratory Viruses

Wijnans, L., J. Dieleman, B. Voordouw, M. Sturkenboom. Effectiveness of MF59TM adjuvanted A(H1N1)pdm09 vaccine in risk groups and the elderly in the Netherlands. Plos ONE. Apr 30;8(4):e63156. doi: 10.1371/journal.pone.0063156.

Wijnans, L., D. Weibel, M. Sturkenboom. Adjuvanted versus non-adjuvanted influenza vaccines in young children: comparing results from recent clinical trials Clinical Investigation, April 2013, Vol. 3, No. 4, Pages 395-408.

Wijnans, L., C. Lecomte, C. de Vries, D. Weibel, C. Sammon, A. Hviid, H. Svanström, D. Mølgaard-Nielsen, H. Heijbel, L. Arnheim Dahlström, J. Hallgren, P. Sparen, P. Jennum, M. Mosseveld, M. Schuemie, N. van der Maas, M. Partinen, S. Romio, F. Trotta, C. Santuccio, A. Menna, G. Plazzi, K. Kaveh Moghadam, S. Ferro, G.J. Lammers, S. Overeem, K. Johansen, P. Kramarz, J. Bonhoeffer, M. Sturkenboom. The incidence of narcolepsy in Europe: before, during, and after the influenza A(H1N1)pdm09 pandemic and vaccination campaigns. Vaccine. 2013 Feb 6;31(8):1246-54.

Wijnans, L., S. De Bie, J. Dieleman, J. Bonhoeffer, M. Sturkenboom. Safety of pandemic H1N1 vaccines in children and adolescents. Vaccine 2011; 29(43), p7559-7571

Steens, A., E. Wijnans, J. Dieleman, M. Sturkenboom, M. van der Sande, W. van der Hoek. Effectiveness of a MF-59TM adjuvanted pandemic influenza vaccine to prevent 2009 influenza A/H1N1-related hospitalization: a matched case-control study. BMC infectious diseases. 2011; 11(1): 196.

Other relevant publications

Jacobs, B.C., L. Wijnans, M. Sturkenboom, N. Van der Maas. Guillain-barré syndroom en het nieuwe influenza A (H1N1) virus. Ned. Tijdsch. Geneesk. 2009; 153: A1490

PhD Portfolio

Name	Eleonora G Wijnans
Erasmus MC Department	Medical Informatics
PhD period	April 2009 – December 2015
Promotores	Prof.dr. M.C.J.M. Sturkenboom
Copromotores	Dr. A.C.G. Voordouw
	Dr. D.M. Weibel

PhD Training

Presentations

2012	Regulatory Challenges for Influenza Vaccines. Summer School on Influenza, 2nd edition Siena, Italy.
2011	Estimating the effectiveness of pandemic influenza vaccination in a Dutch population 27th International Conference on Pharmacoepidemiology and Therapeutic Risk Management, 13-17 August 2011, ICPE, Chicago, US. (Poster)
2010	Effectiveness of pandemic influenza vaccination in a Dutch population. 26th International Conference on Pharmacoepidemiology and Therapeutic Risk Management, 19-22 August 2010, ICPE, Brighton UK.(Poster)

International conferences and symposia

2011	27th International Conference on Pharmacoepidemiology and Therapeutic Risk Management, 13-17 August 2011, ICPE, Chicago, US
2012	International meeting on influenza vaccine effectiveness; 3-4 December 2012, CICG Geneva, Switzerland

Courses, seminars and workshops

2009-2011	Research seminars of Medical Informatics, Erasmus University Medical Centre, Rotterdam, The Netherlands
2012	Workshop on correlates for protection and serological assays for influenza vaccines 30-31 May 2012: European Medicines Agency, London, United Kingdom

Other

Peer-reviewing of papers

2012 – 2015	*Vaccine*
2013	*Sleep disorders*
2012	*BMC Public Health*
2012	*Influenza and other Respiratory Viruses*

Acknowledgements

No one walks alone. This thesis in front of you is not the work of a single person as the cover might suggest. It is the result of connections made on many different levels. If it weren't for the people who inspired me, motivated me, kept me going and showed me the right direction when I was lost the last dot on this page would have never been placed.

Mijn dank gaat in het bijzonder uit naar prof.dr. Miriam Sturkenboom, Dr. Bettie Voordouw, Dr. Daniel Weibel en Dr. Jeanne Dieleman.

Miriam, bedankt voor het vertrouwen dat je hebt gegeven en het creëren van de mogelijkheid om dit werk te beginnen en te volbrengen. Jouw input is van heel veel waarde geweest bij de totstandkoming van de verschillende studies en het begrijpen van de soms bijzondere resultaten. Ik heb enorm veel respect in hoe je je staande houdt in de vaak politieke en emotionele sferen waar de discussies over vaccin veiligheid zich in begeven en je vasthoudendheid om te blijven bij de feiten.

Bettie. Zonder jou enthousiasme was ik nooit bij het CBG gaan werken. Zonder jou doortastendheid was ik nooit begonnen aan de I-MOVE studie. Zonder jou push was ik nooit in de VWP gestapt om de influenza richtlijn te schrijven. Het mag duidelijk zijn – you are to blame. Daarvoor ben ik je zeer dankbaar. Je bent inspirerend. Je weet de mensen om je heen te motiveren en bent een enorme doorzetter, wat blijkt uit dat men eindelijk afscheid heeft durven nemen van de CHMP criteria en de annual update trials.

Daniel. Thank you for your relaxed outlook and positive encouragements.

En natuurlijk Jeanne. Ik heb je de afgelopen jaren enorm gemist, wat onderstreept dat ik tijdens die eerste twee jaar zoveel van je heb mogen leren. Dank voor al je hulp.

To all my co-authors – thank you for the wonderful and fruitful collaboration.

My thanks goes out to all the GPs and patients contributing to the primary health care databases IPCI and THIN.

Collega's binnen IPCI, Medische Informatica en de pharmaco-epi group. Door halverwege mijn promotie uit Nederland weg te gaan heb ik wezenlijk maar twee jaar op de vakgroep Medische Informatica doorgebracht. In die periode daarna heb ik de afdeling erg gemist, niet alleen vanwege de gezelligheid maar toch ook zeker als klankbord of hulp als mijn programma's weer eens vastliepen. De fijne omgang tussen collega's en de bereidheid om zelfs op afstand te helpen waar mogelijk heeft me enorm geholpen. Dank aan – in no particular order - Marjolein, Sandra, Caitlin, Anne, Ingrid, Vera, Roelof, Seppe, Preci, Kartini, Rikje, Maria, Inge, Eline, Gwen, Daan, Fatma, Katia, Nico, Preci, Ana, Osemeke, Toke, Carmen, Maria, Gianluca, Silvana.

Desiree, bedankt voor het helpen bij het regelen van de promotie. Carmen, bedankt voor het organiseren van alle meetings met mijn begeleiders – no simple feat. Mees en Marcel – jullie ondersteuning is onmisbaar geweest, en onbetaalbaar. Mijn dank is groot. En Ann, zonder jouw enorme inzet geen i-move studie. Daarvoor mijn dank. Kris – altijd een feest, altijd gezellig – we gaan nog wel een keer een wijntje drinken. En Ria – hoe fijn was het om met jou die eerste studie op te zetten, en hoe leerzaam is dat geweest. Veel dank hiervoor. En hoe fijn is het om ook dit jaar weer bij jou en Jan welkom te zijn in Hary – dat gaan we hopelijk nog vaak herhalen want er valt nog heel veel te schilderen, heel veel wijn te proeven en van heel veel gezelligheid te genieten. En Tineke, uiteraard – zonder jou was het vaak maar een saaie bedoening geweest - bedankt voor de gezelligheid en de vrolijkheid.

Thanks to my roommates of Ee-2116 – Ana, Carmen, Nico and Preci – it was a joy sharing an office with you those first years. Nico – foxpro held, jij hebt me vaak uit de brand geholpen en de vroege ochtenden op de vakgroep stukken aangenamer gemaakt. Preci, thank you for your humour – now that you have your Nederlandse paspoort there are no valid excuses not to come and visit me anymore – so see you soon in London!

Caitlin – it has been fantastic working with you – and the simulations is truly the most exciting bit of work I've ever been involved in. I know you have your secret stats hero's you worship from a far –you are now officially one my stats hero. I do hope we get to do more work together in future.

Nico, Sandra, Kartini en Inge – ook jullie hadden het plezier om zowel bij het CBG te werken als bij het Erasmus onderzoek te doen en daarom extra veel begrip en steun voor de moeilijkheden dat dit met zich mee bracht. Maar ook dubbel de gezelligheid – zowel in Rotterdam als in Den Haag en later Utrecht.

Het CBG heeft mij de kans gegeven om te promoveren, mij het vertrouwen gegeven en mij de tijd gegeven. Dat is bijzonder, en daarvoor ben ik het CBG zeer dankbaar. Bert Hiemstra, jij bent hierin instrumentaal geweest – daarvoor in het bijzonder voor jou veel dank. Daarnaast wil ik mijn collega´s van FT4 toch zeker even noemen. Tékla, Charlotte, Ron, Jan S, Nancy, Rob, Michel, Barbara, Jan W, Carla, Eric, Ineke, Edith, Angela, Hannelore, Chaya, Roya, Mariette, Fons, Marianne, Joost, Nouri, Saskia, Jens, Nienke, Robin. Het is een enorme fijne club om mee te werken, en zonder de flexibiliteit en het geduld van velen van jullie was het combineren van mijn werk in Den-Haag/Utrecht/Groombridge en het onderzoek doen in Rotterdam onmogelijk geweest. Daarvoor mijn dank.

Sandra en Inge – het is niet verwonderlijk dat ik wilde dat juist jullie vandaag naast me zouden staan. Of het nou kwam omdat we allemaal een vleugje regulatoir, biomedisch onderzoek en een krul in het haar hebben of dat het gewoon zo ontzettend gezellig was in

Chicago dat we nooit meer zonder willen, jullie hebben allebei en heel bijzonder plekje in mijn leven gekregen en we delen allang niet meer alleen de werk sores maar ook bij de mooie en minder mooie momenten staan jullie daar. Dank voor de fijne vriendschap. En Kartini – wat heerlijk dat jij hier ook naast me staat. Ik vind het super om met je samen te werken – je bent een geweldig nuchter mens en ik kan altijd erg met je lachen. Dat we dat nog lekker vaak mogen doen.

Dear Viv, thank you so much for your beautiful artwork.

Lieve Pappa, lieve Mamma. Jullie zijn het begin. Jullie zijn de basis. Jullie hebben mij gegeven waardoor ik kan zijn wie ik ben en daarvoor ben ik ontzettend dankbaar. Zonder jullie steun had ik hier nooit gestaan. Pap, de ritjes naar Rotterdam waren ontzettend gezellig, altijd een fijn begin van de dag en toch heerlijk dat we dat nog hebben mee kunnen pikken. Dikke knoes.

Anne-Marije – lief zusje. Vol bewondering zie ik hoe jij de uitdagingen die het leven jou toe werpt blijft aangaan en door blijft vechten. Je bent een enorme inspiratie voor me. Altijd naar boven blijven kijken, de sterren blijven zien.

Girls. Soraya, Flora and Molly. I couldn't really start to say how happy and grateful I am that you are such an important part of my life, not only as sisters to Fenne and Jacob and as Andy's daughters. Moving to the UK has not been easy, making Groombridge our home has been incredible hard work, but in our minds there was never really any other option than to move close to you and we feel so lucky that we are where we are today. You are such amazing strong independent young women and it has been wonderful to see you find your feet and make your way in this mad world. Thank you for everything.

Lieve Jacob, lieve Fenne – stralend middelpunt van mijn leven. Wat een vreugd en lol hebben jullie gebracht. Wat een blijdschap. Nu dit hoofdstuk afgesloten is gaan we gewoon nog veel vaker hutten in de tuin bouwen, op onze knieën lego kastelen maken, vuurtjes stoken, dammen bouwen, dansen op de tafel en heel hard in alle plassen springen.

And finally, Andy – mijn maatje, mijn vriend, mijn alles. Thank you for being there and being you – for all your support and encouragement. For letting me be me. Now we have found our little piece of paradise I hope we can sit back and relax for a bit whilst we watch it grow. Life with you is so much better. It's exactly where I want to be. (And thank you for diligently checking all my English writing again and again – you are the best editor).

About the author

Leonoor Wijnans was born on 6 March 1979 in Utrecht, the Netherlands. She completed her VWO at the Rijnlands Lyceum in Oegstgeest in 1997. In that same year she started her degree in Biology at Wageningen University.

During her studies she specialized in the ecology of malaria vectors at the Laboratory for Entomology under Professor Willem Takken. In 2000 she spent a six month period studying socio-economic factors influencing malaria transmission in the Brazilian Amazon as an intern with the Ministry of Health of Brazil. Following this she undertook a research project evaluating the population dynamics of malaria mosquitoes in the Netherlands in order to evaluate the risk of malaria returning to the Netherlands. Finally, Leonoor spent six months on an internship with the International Water Management Institute in Colombo, Sri Lanka, studying factors influencing malaria transmission in rural areas of Southern Sri Lanka.

After obtaining her MSc in Biology from Wageningen University in 2002, she joined the International Institute of Tropical Agriculture in Yaoundé, Cameroon where she spent two years working within a project aiming to develop an Integrated Pest Management programme for the African Root and Tuber Scale.

In 2005 she undertook a Master's degree in Public Health in Developing Countries at the London School of Hygiene and Tropical Medicine, writing a dissertation on malaria in school children under the supervision of Dr. Siân Clarke. Leonoor obtained her degree in 2006. Following this, she spent several months in the Indian Himalaya's working with small grass root organisations on programmes to improve reproductive and child health care in remote rural areas.

Since 2008 she has been working as a clinical assessor with the Medicines Evaluation Board, assessing the safety and efficacy of anti-infective drugs and vaccines. In 2014 she was elected a member of the Vaccine Working Party of the Committee for Medicinal Products for Human Use of the European Medicines Agency.

In April 2009 she began the work described in this thesis with the department of Medical Informatics of the Erasmus University and Medical Centre. She has been supervised by dr. Jeanne Dieleman, dr. Bettie Voordouw, dr. Daniel Weibel and prof. dr. Miriam Sturkenboom.

Leonoor is married to Andy Cherry. They live together with their two children, Jacob and Fenne, and their mad springer spaniel, Ziggy, in Groombridge, United Kingdom.

In 2009 and 2010 the world experienced the first influenza pandemic of the 21st century. As the new influenza A(H1N1)pdm09 virus spread across the world, vaccines were being produced and licensed at an unprecedented scale and speed. In Europe, adjuvanted and non-adjuvanted H1N1pdm09 vaccines were licensed through fast track procedures with the first centrally registered vaccines authorized at the end of September 2009.

Due to the nature of pandemic influenza, uncertainties surrounding the benefit risk balance for pandemic influenza vaccines remained at the time of licensing. These uncertainties were addressed in the post-licensure phase through additional studies and alternative monitoring efforts. Whilst the monitoring of effectiveness and safety of vaccines is challenging under most circumstances it is particularly so when vaccination is rolled out within a developing pandemic at the scale seen in 2009 and 2010.

The work described in this thesis was conducted in the wake of the 2009/2010 pandemic. It consists of several studies that evaluate the benefits and risks of pandemic influenza vaccines used across Europe. The experience with pandemic influenza vaccines is collated and lessons drawn to improve the future monitoring of the benefits and risks of influenza vaccines.