Influenza virus infection and vaccination in Guillain-Barré syndrome and Multiple Sclerosis

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Scientific environment

The work presented in this thesis was carried out at the Department of Neurology, Haukeland University Hospital in Bergen. The academic affiliation was the Department of Clinical Medicine, at the University of Bergen. In addition, the initial work was done in cooperation with a wide range of national and international collaborators; The Norwegian Institute of Public Health, The Brighton Collaboration, European Centre for Disease Prevention and Control, Erasmus MC and VAESCO. Later work was undertaken in cooperation with The Neuro-Rheuma laboratory and The National Competence Centre for MS, at the Haukeland University Hospital in Bergen and The Influenza Centre at the University of Bergen.

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Rien n'est plus puissant qu'une idée dont le temps est venu, Hugo V.

L´air est plein de poignards, Bonaparte N

Introduction

The Influenza virus is a major respiratory tract pathogen causing excess morbidity and mortality, especially in at high-risk individuals. Influenza infection is a vaccine preventable disease (VPD). The public opinion displays cyclical shifts in acceptance of side-effects from immunization as the occurrence of the target disease is reduced due to vaccination itself. However, infection and vaccination may both contribute to induction of autoimmunity. Excess cases of Guillain-Barré syndrome (GBS) were reported during the United States´ 1976 swine flu vaccination campaign and led to campaign halt, and decades later enhanced worldwide surveillance during the pandemic of 2009. Viral infection is proposed as a major contributing factor to the most common autoimmune neurological disease, multiple sclerosis (MS). MS is shown to exacerbate due to influenza infection and these patients are thus high-risk individuals. Many patients with MS receive specific immunomodulatory drugs and some drugs reduce the effect of vaccination. Monitoring seroprotection and administration of booster-doses in cases of insufficient protection therefore seems rational. For these reasons, pharmacovigilance is critical in both vaccinology and neuroimmunology.

Literature search ended ultimo 2017

Abstract

Introduction

The Influenza virus is a major respiratory tract pathogen causing excess morbidity and mortality, especially in at high-risk individuals and it is a vaccine preventable disease. Excess cases of Guillain-Barré syndrome (GBS) were reported during the United States´ 1976 swine flu vaccination campaign. This led to enhanced worldwide surveillance during the pandemic of 2009. Multiple sclerosis (MS) is shown to exacerbate due to influenza infection and these patients are thus high-risk individuals. Patients with MS are treated with specific immunomodulatory drugs that may reduce the effect of vaccination.

Methods

Risk evaluation of GBS during the 2009 pandemic was a collaborative European effort. Cases were classified with the Brighton Collaboration criteria and centralized analyses applied the self-controlled case series method.

The influence of disease-modifying treatments (DMTs) on the efficacy of influenza vaccination in MS patients was investigated in the 2009/2010, 2010/2011 and 2012/2013 influenza seasons by the haemagglutination inhibition assay and was compared to healthy controls.

Results

303 GBS and Miller-Fisher syndrome cases were included. 99 were exposed to influenza A(H1N1)pdm09 vaccination, which was most frequently adjuvanted. In the fully adjusted analyses, the relative incidence of GBS was 1.4 (95% CI: 0.7-2.8) and not significantly elevated after vaccination.

113 MS patients and 216 controls were included in the pandemic of 2009/2010. MS patients had reduced rates of protection compared to controls. Rates of protection were not influenced by interferon beta-1a/1b treatment but among patients receiving

glatiramer acetate, natalizumab and mitoxantrone. A similar pattern emerged after seasonal influenza vaccination in 2010/2011.

90 MS patients and 62 controls were included in the influenza season 2012/2013. No significant differences in rates of protection against H1N1 for interferon beta-1a/1b and glatiramer acetate were observed as compared to controls. Fingolimod and natalizumab displayed reduced rates of protection.

Conclusions

European collaborative vaccine safety studies have a potential in studying rare diseases, and we could rule out with 95% certainty that the number of excess GBS cases after influenza A(H1N1)pdm09 vaccination was more than three per million vaccinees.

MS patients receiving DMTs other than interferon beta, and particularly fingolimod or natalizumab should be considered for a second dose of the vaccine in cases of insufficient protection. Our results further indicate that new immunomodulatory treatment regimens should be systematically evaluated for their influence on influenza-specific vaccine responses.

List of publications

Romio S*, Weibel D*, Dieleman JP, Olberg HK, de Vries CS, Sammon C, Andrews N, Svanström H, Mølgaard-Nielsen D, Hviid A, Lapeyre-Mestre M, Sommet A, Saussier C, Castot A, Heijbel H, Arnheim-Dahlström L, Sparen P, Mosseveld M, Schuemie M, van der Maas N, Jacobs BC, Leino T, Kilpi T, Storsaeter J, Johansen K, Kramarz P, Bonhoeffer J, Sturkenboom MCJM (2014): "Guillain-Barré Syndrome and Adjuvanted Pandemic Influenza A (H1N1) 2009 Vaccines: A Multinational Self-Controlled Case Series in Europe". PLoS ONE Volume: 9. Issue: 1. * equal contribution

Olberg HK, Cox RJ, Nostbakken JK, Aarseth JH, Vedeler CA, Myhr K-M (2014): "Immunotherapies influence the influenza vaccination response in multiple sclerosis patients: an explorative study". Multiple Sclerosis Journal. Volume: 20. Issue: 8. Pages: 1074-1080.

Olberg HK, Eide GE, Cox RJ, Jul-Larsen Å, Larteley Lartey S, Vedeler CA, Myhr K-M (2018): "Antibody response to seasonal influenza vaccination in multiple sclerosis patients receiving immunomodulatory therapy". European Journal of Neurology. Volume 25. Issue 3. Pages 527-534.

Contents

List of abbreviations

ABC tool - Automated Brighton Classification tool

ADVANCE - Accelerated development of vaccine benefit-risk collaboration in Europe

AEFI - Adverse event following immunization

A(H1N1)pdm09 - Influenza A H1N1 Pandemrix 2009 vaccination

APC - Antibody presenting cell

AS - Adjuvant Systems

ASC - Antibody secreting cell

ASIA - Autoimmune/ inflammatory syndrome induced by adjuvants

BBB - Blood-brain barrier

CBC - Complete blood count

CDC - Centers for Disease Control and Prevention

CHMP - Committee for Human Medicinal Products

CPMP – Committee for proprietary medicinal products

CI - Confidence interval

CTL - Cytotoxic T cell

DC - Dendritic cell

DMT - Disease-modifying therapies/ treatments

ECDC - European Centre for Disease Prevention and Control

EMA - European Medicines Agency

EU-ADR - Exploring and Understanding Adverse Drug Reactions Project

GBS - Guillain-Barré syndrome

HA - Haemagglutinin

HAI - Haemagglutination inhibition assay

HLA - Human Leukocyte Antigen

HR - Hazard ratio

Ig - Immunoglobulin

JCV - John Cunningham virus

ILI - Influenza-like-illness

IRA – Immune regulatory abnormalities

IRR - Incidence rate ratio

LAIV - Live attenuated influenza vaccine

LRT - Lower respiratory tract

MIV - Monovalent influenza vaccine

MFS - Miller Fisher syndrome

MF59 - Squalene

MHC - Major histocompatibility complex

MMF - Macrophagic myofasciitis

MMR - Measles, mumps, and rubella vaccine

MN - Microneutralization

MS - Multiple sclerosis

NA - Neuraminidase

NIPH - National Institute of Public Health

NP - Nucleoprotein

PAMP - Pathogen-associated molecular pattern

PIV - Pandemic influenza vaccine

PID - Primary immunodeficiency

PIV SANE - Pandemic Influenza Vaccination Safety Assessment Network Europe

PML - Progressive multifocal leukoencephalopathy

PPMS - Primary progressive multiple sclerosis

PRR - Pattern recognition receptor

RDE - Receptor destroying enzyme

RI - Relative incidence

RR - Relative risk

RRMS - Relapsing remitting multiple sclerosis

RT - Respiratory tract

SAEFI - Serious adverse event following immunization

SID - Secondary immunodeficiency

SCCS - Self-controlled case studies

SPMS - Secondary progressive multiple sclerosis

SYSVAK - Norwegian vaccine registry (System for vaksinasjonskontroll)

T_H - T helper cell

TLRs - Toll-like receptors

TIV - Trivalent influenza vaccine

URTI - Upper respiratory tract infection

UTI - Urinary tract infection

VAESCO - Vaccine Adverse Event Surveillance & Communication

VE - Vaccine effectiveness

VENICE - Vaccine European New Integrated Collaboration Effort

VPD - Vaccine preventable disease

WHO - World Health Organization

1. Influenza virus infection and vaccination in Guillain-Barré syndrome and Multiple Sclerosis

1.1 Influenza virology

Typical symptoms of influenza include elevated temperature, cough, body aches and extreme fatigue, coryza and sore throat (Poehling et al).

1.1.1 A brief history of influenza outbreaks

The influenza virus as causative for acute respiratory disease was described in 412 BCE by Hippocrates (Hirsch). The name is derived from Italian astrologers in the Middle Ages where the periodicity of the disease was related to the "influence of heavenly bodies" (Townsend). Early outbreaks were documented in the 1850´s (Thompson) and 1890´s (Creighton).

In 1918-1919, the H1N1 "Spanish flu" caused at least 40 (Barry) to 50 million deaths (Johnson et al). Mortality rates were highest in healthy young adults due to a possible virus induced "cytokine storm" (Cheung et al), (Kobasa et al). The influenza virus virus was first isolated from humans in 1933 (Smith et al). In 1957 the H2N2 "Asian flu" caused 1-2 million deaths worldwide. The H3N2 "Hong Kong flu" in 1968 was not as severe (Holmes et al). In 1977 the H1N1 "Russian flu" emerged and was similar to the previous circulating virus before the "Asian flu". There were few reports of this H1N1 strain in people older than 26 years and the death rate in affected individuals was low (Gregg et al). The H5N1 "Bird flu" observed in Hong Kong in 1997 conferred > 60% fatality but no sustained human to human transmission has been observed (Chan). In 2003 the "Bird flu" re-emerged in Asia with a mortality rate of 80% (Peiris et al). The H1N1 "Swine flu" virus from 2009 contained a novel constellation of gene segments, which most likely stemmed from triple re-assortment of two or more viruses of swine, human and avian origin (Dawood et al 2009). The influenza A H1N1 virus circulated between 1918 and 1957, and has co-circulated with H3N2 since its reappearance in 1977 until 2009.

1.1.2 Nomenclature

The internationally accepted naming convention for influenza viruses was accepted by WHO in 1979 (WHO).

Four types of influenza viruses exist: A, B, C and D. Human influenza A and B viruses cause seasonal epidemics close to every winter. Type C infections cause a mild respiratory illness and not epidemics. Type D primarily affect cattle and do not cause infection in people. Influenza A viruses are subtyped based on two proteins on the virus surface: hemagglutinin (HA) and neuraminidase (NA). Eighteen different HA subtypes and 11 different NA subtypes exist (H1-H18 and N1-N11). Type B viruses are not subtyped are distinguished into lineages and strains, nowadays one of two lineages: B/Yamagata and B/Victoria (CDC).

1.1.3 Epidemic versus pandemic

Wild waterfowls are thought to be hosts (avian reservoir) to influenza A viruses. Mammals may be sporadically infected, and in rare instances this lead to sustained transmission in the new mammalian host. The greatest risk for zoonotic spread to humans and generation of pandemic or panzootic viruses are thought to come from influenza viruses of domesticated animals and in particular swine (Tong et al).

The influenza viruses´ capacity to mutate causes challenges in disease prevention. Minor changes (point mutations) cause antigenic drift and may overcome the immunity acquired in the previous season resulting in an epidemic. If a major recombination event occurs (antigenic shift), a completely novel virus in a large enough susceptible population - may lead to a pandemic (Lopalco et al). Thus influenza vaccines must be continuously revised (Doherty et al).

The molecular basis of antigenic drift lies in single amino acid substitutions near the receptor binding site of HA or NA in A/H1N1 and A/H3N2 viruses, and possibilities for important antigenic change of seasonal influenza viruses may be more restricted than previously thought (Koel et al).

1.1.4 The burden of influenza

It is estimated that the yearly worldwide deaths from influenza epidemics are 500.000 persons (Stöhr). Seasonal influenza illness ranges from mild to severe and even death. Hospitalization and death occur mainly among high-risk groups. Worldwide, these annual epidemics are estimated to result in about 3 to 5 million cases of severe illness, and about 250 000 to 500 000 deaths (WHO). The latest estimate is up to 650 000 (Iuliano et al).

The annual rate of influenza-associated death in the United States overall during the time period of 1976-2007, ranged from 1.4 to 16.7 deaths per 100.000 persons (CDC). Each year in Europe, seasonal influenza causes 4-50 million symptomatic cases and 15.000-70.000 die of causes associated with influenza (ECDC).

In the pandemic of 2009, global estimates of deaths were in the six-digit figures (Dawood et al 2012). About 61 million cases of influenza A (H1N1) disease were reported in the USA during the 2009 pandemic, including about 274.000 H1N1-related admissions and about 12.470 deaths (Shrestha et al). During the 2009 pandemic, 2.900 deaths directly related to influenza were reported by the EU member states during the first 12 months, but is probably underestimated (Amato-Gauci et al).

1.1.5 Pre-existing immunity to the 2009 pandemic virus

One out of three children was infected with the 2009 pandemic H1N1 virus in the first wave, ten times more than estimated from clinical surveillance. Pre-existing antibodies in older age groups did protect against infection. Children are postulated as a key target group for vaccination, both for their own protection and for the protection of others through herd immunity (Miller E et al).

The pandemic H1N1 2009 virus was antigenically distinct from circulating seasonal H1N1 strains, as only 17% of the known B cell epitopes were conserved between the HA and NA of pandemic H1N1 2009 and circulating influenza strains. The high HI titres detected in vaccinees suggested an anamnestic response, perhaps due to reactivation of cross-reactive memory immune cells (Greenbaum et al).

Virus-specific circulating B-lymphocytes have been found in subjects exposed to the H1N1 1918 strain (Yu et al).

1.1.6 Monitoring of influenza

The Geneva-based World Health Organization influenza surveillance network Global Influenza Surveillance and Response System (GISRS) was established in 1952 and it links four centres, London, Tokyo, Melbourne and Atlanta (WHO). GISRS recommends seasonal influenza vaccine compositions once yearly for the Northern and once yearly for the Southern hemispheres influenza seasons, hence the term biannual recommendation.

The NIPH is a sentinel of the WHO, and monitors influenza and crafts weekly reports from week 40 through 20 in any given season (NIPH).

Laboratory-confirmed influenza implies finding the virus in patients with clinical influenza infection and may be done with a real-time polymerase chain reaction (RT-PCR) (Ellis et al), (Gunson et al).

1.2 Immunological response to antigen and immunization

To understand vaccinology, basic immunological concepts need to be clarified. The immune response to an infectious agent or immunization is broadly divided into two phases; the innate and downstream adaptive responses (Moser et al).

1.2.1 The innate immune system

On exposure to foreign antigens presenting pathogen-associated molecular patterns (PAMPs), the innate immune response by macrophages, monocytes, neutrophils and dendritic cells (DC) with their pattern recognition receptors (PRR) classifies the antigen as a threat or benign (Akira et al). Toll-like receptors (TLRs) are among the recently discovered PRRs. This phase provides no immunologic memory. If recognized as a threat, pro-inflammatory chemokines, cytokines, complement activation and cellular recruitment occurs with the clinical correlate of local

inflammation. Antigen is taken up by innate cells such as DCs, which differentiate into antigen presenting cells (APCs), which are key intermediaries. APCs migrate to the T-cell region of draining lymph nodes which is the site where the phase transitions into the adaptive immune response, which has capacity for inducing memory (Moser et al).

1.2.2 The adaptive immune system

The adaptive immune response consists mainly of lymphocytes; T- and B-cells with their respective T- and B-cell receptors being able to recognize an infinite amount of different antigens stochastically generated through processes of clonal selection (proliferative response following antigen recognition) and somatic hypermutation (multiple mutations in rearranged V, D and J gene segments). Antibodies are secreted by differentiated B cells called plasma cells. Antibodies that bind to surface antigens can activate complement and effector cells, such as phagocytes. Immune memory only occurs if B-cells are helped by T-cells in a so called T-cell dependent response (Moser et al). Purified vaccines are missing the inherent danger-signature associated with a pathogen and this may be responsible for reduced immunogenicity due to reduced or absent antigen-presentation and maturation of DCs (Matzinger).

1.3 Vaccinology

Vaccination is one of the most successful public health interventions ever implemented and continues to have vast impacts in preventing disease and death due to infectious disease worldwide (WHO). Immunization is one of the 10 great public health achievements in the 20th century (CDC). Still, infectious diseases remain the most common cause of death in children less than 5 years of age (Black et al).

1.3.1 History of influenza vaccination

The first influenza vaccine was administered in the US in 1935 (Stokes et al). In 1945 it was licensed for civilians (Francis). These early experimental and non-experimental war-time vaccines contained the whole inactivated virus.

Due to reactogenicity and side-effects, split (subvirion) vaccines were developed in the 1960s. A split vaccine refers to the whole virus treated with a detergent to deconstruct the virus particle into viral subunits and purification and quantification of the viral surface glycoproteins HA and NA. The inherent problem is that the most effective vaccines against the virus are against the most variable viral protein, namely HA. Antibodies that can bind the HA mediate neutralization of the virus (Ellebedy et al).

Most influenza vaccines since the 1970s have been subvirion (split) preparations. They retain immunogenic properties but confer reduced reactogenicity compared to whole virus vaccines (Fiore et al, Current Topics in Microbiology and Immunology 2009).

Split and subunit vaccines are less reactogenic compared with whole pathogens and also often confer reduced immunogenicity. New technologies in the 1990s, including reassortment and cold adaptation made development of successful live attenuated influenza vaccines possible (Bonanni et al).

1.3.2 Principles of influenza vaccination

Key principle of vaccination

The key principle underlying immunization is induction of an immune response capable of providing specific protection from infection or disease and where the risk of acquiring the disease from vaccination has either been reduced or removed. Early vaccines were live-attenuated or whole-pathogen preparations (Zepp), (Bonanni et al). Attenuation is done by desiccation or repeated passage in culture so that the virulence is reduced or removed but the organism remains viable. Whole-pathogen preparations contain inactivated pathogens exposed to high temperatures and these vaccine formulations are still used today. Historically however they have posed difficulties in terms of reactogenicity, potency and efficacy (Cherry).

Live-attenuated vaccines are capable of initiating innate immunity and adaptive responses since they induce mild infection. Live-attenuated vaccines possess the

potential for reversion to virulence, and incomplete inactivation has occasionally caused the disease it aimed to prevent (Zepp).

Inactivated whole virus vaccines are more immunogenic and induce protective antibody responses at a lower antigen dose than other formulations e.g. split virus or subunit vaccines. Whole virus formulations stimulate TLRs of the innate immune system, and explain the relative loss of immunogenicity in split and subunit formulations (Geeraedts et al).

Peak antibody levels are regularly reached within 4 to 6 weeks after influenza vaccination (Osterholm et al). Crucially, most studies on side effects from vaccination have used a cut off period for AEFIs at 6 weeks post vaccination, this will be discussed in detail later.

Rationale of vaccination

The current rationale is based more on individual protection of high risk groups and not herd immunity. Herd immunity requires vaccination of particularly children to reduce spread in the community. Adults in general and especially immunocompromised individuals are highly under-vaccinated (Bernstein et al), (Babcock et al). Further, preemptive vaccine updates may improve influenza vaccine efficacy in previously exposed individuals (Fonville et al).

Efficacy of influenza vaccination

Influenza vaccine effectiveness depends on the matching between the vaccine strain and the circulating strain. In 2009, the only virus in circulation was the pandemic one, thus vaccine efficacy was 78.4% (95% CI 54.4 - 89.8) in patients < 65 years (Valenciano et al). The inactivated influenza vaccine is approximately 70% effective in preventing symptomatic influenza in young adults (Monto et al). During some seasons however, it may be lower than 50% (Lopalco).

Influenza vaccines (LAIV in children and TIV in adults) even when not well matched with the circulating strains, can provide cross protection against non-matching circulating strains (Tricco et al).

In 56 studies of VE from 2004 to 2015, substantial protection was found against H1N1pdm09, H1N1 (pre-2009) and type B, but reduced protection against H3N2. (Belongia et al). The overall effectiveness of parenteral inactivated vaccine against influenza-like illness (ILI) is limited, corresponding to a number needed to vaccinate (NNV) of 40 (95% CI 26 to 128). The overall efficacy of inactivated vaccines in preventing confirmed influenza has a NNV of 71 (95% CI 64 to 80). The difference between these two values depends on the different incidence of ILI and confirmed influenza among the study populations (Demicheli et al 2014).

Comparison of antigenic and genetic evolution of a virus allows for monitoring of antigenic differences among vaccine and circulating strains and thus estimation of the effects of vaccination (Smith et al).

Annual influenza vaccination

One recent study provides the immunological evidence base for continuing annual influenza vaccination in adults (Trieu et al). Antibody titres to H1N1pdm09 persisted above the protective level in both the repeated- and single-vaccination groups. The interferon γ+ (IFN-γ+) and multifunctional CD4+ T-cell responses were maintained in the repeated group but declined significantly in the single-vaccination group. The IFN-γ+CD8+ T cells remained stable in both groups.

Trivalent influenza vaccination

Present day influenza vaccines are usually TIV, meaning three different viral strains; one A/H1N1, one A/H3N2 and one B. Since 2013/2014, quadrivalent vaccines exist with both B lineages Victoria and Yamagata. The WHO´s Global Influenza Surveillance and Response System (GISRS) recommends which strains to be incorporated in the vaccine.

Bivalent influenza vaccine

In 1940, an influenza virus that was antigenically different from influenza A was discovered. This virus was named influenza B. In order to protect against both types of influenza viruses, a bivalent vaccine was developed in 1942 (WHO).

Monovalent influenza vaccines

A vaccine containing only one viral strain is called monovalent, e.g. Pandemrix.

1.3.3 The production cycle of influenza vaccines and authorization

In the northern hemisphere, global influenza surveillance systems lead to selection of viral strains during the first trimester in any given year, with vaccine production by June/ July. This allows vaccination programs to start in autumn. Effective mechanisms of production, distribution and administration ensure high vaccine coverage and are essential in ameliorating a pandemic. 18-24 months are usually required to authorize a medicine product in the EU. EMA advises the European Commission (EC) which in turn grants marketing authorization. A mock-up pandemic influenza vaccine is a vaccine that mimics the future pandemic influenza vaccine in terms of its composition and manufacturing method. Since 2015, data on mock-up vaccines are now defined pandemic preparedness vaccines and is provided continuously to EMA in a “rolling review”, thus vaccines may be authorized within 100 days, which is useful in a pandemic situation. Due to this accelerated authorization procedure, proper post marketing surveillance is critical (Lopalco). Earlier availability of a pandemic vaccine would have prevented more deaths and improved cost savings in the 2009 pandemic (Khazeni et al). Currently, four mock-up vaccines are authorized in the EU (EMA).

1.3.4 The pandemic of 2009

In April 2009 the US CDC reported two cases of novel swine-origin influenza A(H1N1) in children in southern California (CDC). In June 2009, the World Health

Organization (WHO) declared the new influenza of swine origin, A(H1N1) a pandemic (WHO).

In July 2009, the WHO considered spread of the virus unstoppable. Vaccine recommendations were issued for health care workers, in pregnancy, age > 6 months with a chronic medical condition, healthy children and healthy persons > 15 years (WHO). The CDC recommended two doses of 2009 pandemic vaccine for children aged 6 months to 9 years (CDC). The European Union Health Security Committee/ Early Warning and Response System followed suit (HSC/ EWRS).

Initial serological studies showed that recent (2005-2009) seasonal influenza vaccines conferred no protection against the new virus (CDC). Interestingly, seroprotection in the PRC was higher in subjects receiving non-adjuvanted vaccines after both the 1st and the 2nd dose compared to adjuvanted vaccines. Furthermore, whole-virion formulations were less immunogenic than their split-virion counterpart. Children less than 12 years may need a second dose. (Liang et al).

In December 2009, a recommendation was issued on seasonal influenza vaccination by the Council of the EU to reach 75% vaccine coverage rates for the elderly and the risk groups (Council of the European Union). The European Council has repeated these legislations in 2011 (Council of the European Union) and 2014 (Council of the European Union). Although not legally binding, member states are encouraged to monitor vaccine coverage and investigate reasons for low adherence to vaccination. The ECDC started a regular survey in 2006 by funding a network of experts, Vaccine European New Integrated Collaboration Effort (VENICE consortium). Further, the level of cooperation and the limits of EU coordination on serious cross-border threats to health were defined in 2013 (EU Commission).

1.3.5 The 2009 pandemic influenza vaccine

Both MIV 2009/2010 and TIV 2010/2011 contained the same novel H1N1 antigen. The 2009 H1N1 virus was initially homogenous. As the virus drifts, adjuvant will

probably provide broader cross-reactivity and longer lasting antibody responses to protect against future pandemic waves caused by drifted strains. (Galli et al).

The 2009 pandemic vaccine was a monovalent vaccine. The pandemic of 2009 displayed several different vaccine formulations, as well as methods for virus propagation, purification, inactivation, antigen preparation, amount of antigen, adjuvant and other substances. Available formulations were thus monovalent adjuvanted H1N1, monovalent non-adjuvanted H1N1, live attenuated (intranasal) pandemic H1N1 (LAMV) and whole virion H1N1 vaccines. Some were adjuvanted with MF59 only, AS03 only or aluminium only (Wijnans et al, Vaccine 2011).

Cross-reactivity indicated that antibodies capable of neutralizing most influenza subtypes (H1, H3 and H5) might be elicited by vaccination (Li et al). Vaccination with the 2009 pandemic H1N1 vaccine elicits 1918 virus cross-protective antibodies in mice and humans (Medina et al). Recent vaccination, whether with seasonal non-adjuvanted or adjuvanted influenza vaccines, induced little or no cross-reactive antibody response to 2009 H1N1 in any age group. A proportion of older adults had, but persons < 30 years had little evidence of pre-existing cross-reactive antibodies (Hancock et al). TIV may reduce the immunogenicity of pandemic monovalent influenza vaccines (Andrews et al, Vaccine 2011; 29: 7913-7919).

A candidate International Standard for antibody to pandemic H1N1 virus in the HI assays and conversion of HI results allows comparison with other vaccine trials using the same candidate standard and the international WHO collaborative study standard. Seasonal 2009 TIV contained 15 µg HA and the pandemic vaccine only 3.75 µg, which allowed for a fourfold dose-sparing in a restrained manufacturing process. At least 3 months protection was obtained for Pandemrix 2009 vaccination in 84-92% of subjects (Madhun et al).

European authorities and the vaccine manufacturer have recognized that the benefit-risk profile of H1N1/2009/AS03 remains favourable and therefore recommended maintenance of the marketing authorization (EMA).

The prototype for Pandemrix was the H5N1 vaccine and was recommended by EMA in 2008. Efficacy and safety was tested in 5000 adults and 300 children, thus the safety experience was deemed to be limited (Bardage et al). Non-adjuvanted H5N1 pandemic influenza vaccines showed markedly lower immunogenicity than seasonal influenza strains. Adjuvanted H5N1 pandemic influenza vaccines induced improved immunogenicity in all age groups and cross-reactive immunity, thus antigen sparing may occur (Baz et al).

Most of the vaccines were found to elicit a sufficient immune response after one dose in healthy persons from 10 years and above. For children between 6 months and three years, a second dose was recommended. For some vaccines, a second dose was also recommended for children between 3 and 9 years (Girard et al).

Importantly, for vaccine safety studies - vaccine coverage ranged from 0.4% in Slovakia to 59% in Sweden (Mereckiene J et al).

The AS03 seasonal vaccine trials were found to be non-superior to non-adjuvanted vaccines in the elderly (McElhaney et al, 2013). Based on this study, market authorization for adjuvanted TIV could not be achieved.

1.3.6 Guidelines for influenza vaccination in the 2017/2018 season

The current recommendation (2017/ 2018) in Norway is for 1.5 million persons to undergo yearly vaccination due to increased risk of complications due to influenza. These include: Women after week 12 of pregnancy (2nd and 3rd trimester). Pregnant women in 1st trimester which belongs to an additional risk group may be considered. Residents in nursing homes and sheltered accommodation, everyone aged over 65, children and adults with: diabetes mellitus type 1 and 2, chronic respiratory disease, chronic cardiovascular disease, chronic liver failure, chronic renal failure, chronic neurological disease or injury, immunodeficiency disorders, severe obesity (BMI > 40), other severe or chronic illness evaluated on an individual basis by a doctor. Health professionals with patient contact, household contacts of immunosuppressed patients and pig farmers (NIPH).

In 2017/2018, the TIV for the northern hemisphere, in accordance with recommendations from WHO, contains the following viruses: A/Michigan/45/2015 (H1N1)pdm09-like; A/Hong Kong/4801/2014 (H3N2)-like; and B/Brisbane/60/2008-like virus.

In autumn 2013, a new influenza vaccine which is administered as a nasal spray became available in Norway. This live, attenuated influenza vaccine (LAIV) contains viruses that are cold-adapted and temperature sensitive so they cannot cause influenza illness. The vaccine is quadrivalent (two influenza A strains and two influenza B strains). LAIV should not be given to children younger than two years or older than 17 years of age. Since the vaccine contains live viruses, it should not be given to pregnant girls or children or adolescents who are clinically immunodeficient. The quadrivalent vaccine (LAIV) also contains a B/Phuket/3073/2013-like virus (NIPH).

Since 2000, in Ontario, Canada influenza vaccinations were provided for free to all residents aged 6 months or older (Quach et al). In the US, influenza vaccination is and has been recommended to all persons ≥ 6 months since 2010 if not otherwise contraindicated (CDC).

1.3.7 Age specific considerations in vaccination

Children

The immune system in children is immature and newborns have impairment of neonatal monocyte TNF-release in response to an array of TLR ligands (Levy et al).

Further, children are principal disseminators of influenza. Increasing evidence suggests that vaccinating schoolchildren can create herd immunity that indirectly benefits the unvaccinated (Cohen). 50-85% of schoolchildren were vaccinated in Japan during the mid- 70s and 80s and rarely the elderly. The results were that deaths from influenza and pneumonia dropped by at least 10.000 per year (Reichert et al). TIV given to children 3-15 years of age in 50 remote Hutterite communities in Canada resulted in 61% reduction of influenza disease in the respective unvaccinated adult population (Loeb et al).

There is serological evidence that 2 doses of $AS03_B$-adjuvanted pandemic influenza vaccine may be sufficient to maintain protection across 2 influenza seasons. Administration of TIV to children who previously received 2 doses of either pandemic influenza vaccine (split-virion or non-adjuvanted whole-virion monovalent pandemic vaccine) is safe and immunogenic for the H1N1 strain. The study uses microneutralization (MN) titres. (Walker et al). The difference between $AS03_A$ and $AS03_B$ is that B denotes pediatric adjuvant dose. Surprisingly, disparity was found between MN and HI titres in children vaccinated with whole-virion, where MN titres fell after 1 year but was stable after 1 year as measured with the HI method. This was not seen in other groups or other studies.

Annual vaccination of all children aged 5-18 years has been recommended since 2008/2009. Furthermore, two doses of seasonal influenza vaccines are recommended, given 3 weeks apart, for children aged 6 months to 8 years if not previously vaccinated, due to immaturity of the immune system being naive to influenza infection (Fiore et al, Current Topics in Microbiology and Immunology 2009).

PIV administered in 2009 provided some residual protection after 1 year, particularly in the < 5 years age group. TIV administered in 2010 that included the A(H1N1)pdm 2009 strain provided moderate protection (Pebody et al).

One third of the EU countries are targeting children in regards to vaccination (ECDC).

The elderly

The influenza vaccine efficacy (VE) was only 50% in a relatively healthy cohort (Govaert et al). The elderly react poorly to vaccination (Lambert et al). A Cochrane review found VE to be 30% (Beyer et al).

For more than a decade (1990-2008) there was caution regarding rapidly declining antibody titres after vaccination in the elderly. A review study found no compelling evidence for this when compared with young adults, if initial seroprotection was achieved (Skowronski et al).

In elderly people, irrespective of vaccine match, seasonal influenza vaccination is effective against laboratory confirmed influenza during epidemic seasons. Efforts should be renewed worldwide to increase uptake of the vaccine in the elderly (Darvishian et al).

Immune senescence is a phenomenon observed in the elderly where changes in both the innate and adaptive immune system results in reduced response to influenza infection and standard assays of measuring protection. Still important changes in cellular immune mechanisms occur, and the cellular immune response may be a better correlate of protection (McElhaney et al).

The elderly have reduced humoral immunity manifested by increased susceptibility to infections and impaired vaccine responses. Further, the B-cell receptor repertoires become increasingly specialized over decades, but less plastic (de Bourcy et al).

Both cellular and humoral immunity to influenza are affected directly by reduced TLR responses in immune cells from older adults (Bahadoran et al). In aging, impaired TLR function and defects in cytokine production in DCs was strongly associated with poor antibody response to influenza immunization (Panda et al).

1.3.8 The haemagglutination inhibition (HI) method

Gold standard

The gold standard for measuring immunogenicity of the influenza vaccine as recommended by EMA is the haemagglutination inhibition method (HAI) or HI method (EMA).

The first efficacy trial from 1943 found that antibodies to the influenza virus HA surface glycoprotein was a predictor of vaccine efficacy. Higher titres were associated with protection. In the unvaccinated group, 50% of the estimated infections occurred if titres were < 64 (Salk et al). Seroprotection means post vaccination antibody HI titres > 40 that fulfill a 50% probability of clinical protection if exposed to infection (Wood et al).

The HA glycoprotein binds to target cell receptors and is critical to virus infectivity; antibodies to HA inhibit binding and neutralize infectivity (De Jong et al). Antibodies from natural infection only - determines the HAI titre associated with protection (Hobson et al).

Protective antibodies must be functional as in being capable of neutralization or opsonophagocytosis. Clinical correlates of protection may be given in absolute quantities but are mostly provided as relative. In this way most infections are prevented at a certain level, but some will occur above that level due to e.g a large dose of antigenic challenge or deficient host factors. In an instance where the true correlate of protection is unknown or difficult to measure, surrogate tests like antibody measurements must be sufficient as predictors of protection. Cellular responses are more important in the control of established infection (Plotkin). While HAI antibody is the major correlate of protection, postvaccination titres alone should not be used as a surrogate for vaccine efficacy. Laboratory confirmed vaccine failures need to be examined to determine why seemingly protective HAI titres may not be present, like the antineuraminidase antibody or cell-mediated immunity (Ohmit et al). Misclassification of influenza outcomes can occur if only testing for rise in titres and not the virus per se (Petrie et al).

Semi-functional and true functional antibodies

The HI method, however, does not evaluate true functional antibodies even if some authors claim so (Kappos et al). HI antibodies measure antibodies which bind to the HA and inhibit agglutination and are thus semi-functional. A virus neutralization test (VN/ NT) studies titres of antibodies that have the ability to neutralize the virus and stop replication (detection of virus in cell culture supernatant) and may be referred to as true functional antibodies. It is a 72 hour assay. The microneutralization (MN) assay generally yielded higher titres and detected more seroconversions to A/California/04/2009 than the HI assay (Katz et al). MN is usually an overnight assay and measures a broader range of antibodies that neutralize the virus (e.g. antibodies to NP), whereas HI measures a limited set of epitopes in haemagglutination.

Inter-laboratory variation and standardization

The HI test has been accused of insensitivity for antibodies to influenza B and H5N1 viruses and conferring poor reproducibility between laboratories and it is proposed that an International Standard for influenza H5N1 antibody is developed (Stephenson et al, 2007). Use of serological standards has been shown to reduce interlaboratory variation (Stephenson et al, 2009).

Rise and fall in titres

The postulated time point of maximum protection after vaccination is at 21-28 days post vaccination and the steepest drop in antibody and associated protections is at 3 months post vaccination. In addition, vaccine specific immunogenicity might differ from year to year according to the vaccine strains and its antigenic properties including the effect of an adjuvant as described in the original manuscript.

As the HI method is the principal method for evaluation of seroprotection from influenza as recommended by EMA, it should be used to evaluate the immune response to vaccination. However, the assay requires pre and post vaccination serum samples and a qualified laboratory to conduct the assay.

In spite of the above issues, the HI method continues to be the primary test recognized by EU regulators in pandemic influenza vaccine clinical trials (EMA).

1.3.9 Guidelines for vaccine efficacy

EMA issued criteria for influenza vaccines in healthy adults in 1997 that are still used today (The European Agency for the Evaluation of Medicinal Products/ Committee for Proprietary Medicinal Products (CPMP).

All sera shall be assayed for anti-hemagglutinin antibody against the prototype strains by HI tests. Positive and negative sera and reference preparations may be obtained from a reference laboratory. HI < 10 is considered undetectable and is expressed as 5 for calculation purposes. Seroconversion means titres going from seronegative; i.e. from < 40 to ≥ 40, or a fourfold increase. Further considerations are prevaccination

GMT, postvaccination increase in GMT, number of seroconversions, proportion of subjects with a prevaccination titre and proportion of subjects with a postvaccination titre. Blood sampling is to be performed approximately 3 weeks after vaccination. At least one of the following three criteria should be met for each virus strain and each age class:

1. The number of seroconversions or a fourfold increase should be achieved in > 40% of vaccinees if 18-60 years, or ≥ 30% if > 60 years.

2. Mean (group level) GMTs > 2.5 if 18-60 years, or > 2.0 if > 60 years

3. The proportion of subjects with HI ≥ 40 should be achieved in ≥ 70% of vaccinees if 18-60 years, or > 60% if > 60 years.

The FDA and EMA have the same benchmark which is ≥ 70% for two of the three strains.

For an indication that includes use in children from approximately 9 years to < 18 years, a demonstration of vaccine efficacy is not required. Authorisation may be based on a direct comparison of immune responses to the candidate vaccine between subjects aged 9 - < 18 years and young adults or directly against an authorised inactivated non-adjuvanted seasonal influenza vaccine administered to the same age group (EMA).

1.4 Adjuvants

In Latin, *adjuvare* means to help or to aid, and was coined by Gaston Ramon (Bonanni et al). Inactivated and attenuated virus vaccines against influenza often exhibit a decrease or even loss of productivity and efficacy (Jang et al). Vaccines in general, are weakly immunogenic because they are either attenuated forms of the microbe or chemically modified. Adjuvants are integrated with vaccines to enhance the immune response. Advantages are stability before administration, safe usage, biodegradability, antigen-specific immune response, antigen-sparing, cost-effectiveness and easy use. Only a few adjuvants are licensed for prophylactic

vaccines, this is mainly associated with the toxic properties of the adjuvant material (Chauhan et al).

1.4.1 Mode of action

Adjuvants induce the secretion of cytokines, which acts on lymphocytes to augment the immune responses of helper T cells, T_{H1} or T_{H2} cells (Xu-Amano et al). Adjuvants increase immunogenicity by sustained release of antigen at the injection site and thereby stimulate innate immunity (Tritto et al). Adjuvants modulate the innate immune system and contribute to quality, magnitude and duration of the immune response. They are important due to the poor immunostimulatory capabilities of highly purified vaccines, thereby improving immunity in neonates, elderly and immunocompromised (Leroux-Roels).

Aluminium forms an antigen depot at the site of injection, conferring slow release and sustained immune stimulation, enhancing antigen uptake and presentation by APCs, thus increasing antibody production (Burrell et al). Since its inception, aluminium has been the most commonly administered adjuvant.

Highly purified vaccine components frequently lack PAMPS. Adjuvants can act like PAMPs triggering the innate phase with activation and maturation of APCs and initiation of downstream adaptive immune activities (Coffmann et al).

Freund´s incomplete adjuvant, a mineral oil-in-water emulsion, was considered too reactogenic for continued use in humans. The second most common adjuvant is oil-in-water emulsions using oils with improved reactogenicity compared to Freund´s original adjuvant. Several of these use squalene (MF59), a naturally occurring and readily metabolized oil, inducing robust humoral and cellular immune responses (Fox et al) by acting on APCs such as macrophages and DCs directly or indirectly causing elevated antigen presentation (Zhou et al)

Some adjuvant systems contain combinations of adjuvants and have been specifically designed to increase T-cell immune responses. Adjuvant combinations are Adjuvant Systems (AS); AS01, AS02, AS03, and AS04. AS03 is an oil-in-water emulsion

adjuvant system consisting of Vitamin E (α-Tocopherol), surfactant polysorbate 80 and squalene (MF59) (Di Pasquale et al). Published data for the AS03 adjuvant preferentially activates specific T-cell responses and their direct effects are on innate immune cells and effectors and not on adaptive mechanisms (Morel et al). The AS03 in Pandemrix was a novel oil-in-water adjuvant.

An effective adjuvant produces an immune response similar to the natural course of infection. This leads to dose reduction and antigen sparing as well as efficient protection in populations where natural responses are reduced as in infants, the elderly and immunocompromised (Di Pasquale et al).

Combinations of adjuvants may introduce an additive or synergistic effect (Mount et al), (Guy).

1.4.2 Adjuvants used in licensed vaccines

Commonly used adjuvants and their respective application are the following: aluminium (DTP, poliomyelitis, hepatitis A, hepatitis B, meningococcal, pneumococcal), virosomes (hepatitis and influenza), AS04 (hepatitis B, HPV), MF59 (influenza; seasonal for the elderly in Europe, and pandemic), AS03 (influenza pandemic), thermo-reversible oil-in-water (influenza pandemic), ISA51 (therapeutic vaccine for non-small cell lung cancer) (Di Pasquale et al).

Additional components of vaccines are stabilizers, preservatives, residual trace substances (egg proteins, antibiotics or formaldehyde) left over from the manufacturing process (Da Silva et al 2015).

Thimerosal is an ethyl mercury-based preservative used in low doses of multi-dose vials to prevent microbial contamination and it has well established safety records. Study data show no evidence of harm caused by thimerosal in vaccines (CDC).

1.4.3 Safety concerns of adjuvanted vaccines

Components of the vaccine are tested individually early in development, but the bulk of safety assessment considers the final product. Each vaccine combination of

antigen, adjuvant and excipients is unique. Therefore each vaccine requires appropriate individual evaluation and characterization in the target population. Further, the benefit-risk ratio may be different with the same vaccine if used in high-risk populations (e.g. elderly with chronic disease) compared with the young and healthy (Da Silva et al 2015). Side effects associated with adjuvants have to be balanced with the ability to increase immunogenicity.

Pre-clinical studies have limited statistical power to detect potential rare events. Safety risk is evaluated throughout the vaccine life cycle. Benefit-risk ratio under constant review and may change as the target disease comes under control (Di Pasquale et al).

Addition of adjuvants may cause increase in local reactions (Garçon et al). Systemic side effects include fever, headache, nausea, diarrhea, arthralgia, myalgia and lethargy due to activation of innate immune system and downstream inflammatory responses (Petrovsky et al).

Antibodies to squalene were detected in most patients affected of the Gulf war syndrome (Asa et al). Later studies found that squalene is a component of the human body and low titres of anti-squalene antibodies are found in healthy individuals and are not correlated with the anthrax vaccine (Matyas et al) reviewed by (Lippi et al). WHO concluded in 2006 that fears of pathological anti-squalene antibodies were unfounded, but that in other age-groups than older age groups, careful post-marketing follow-up was recommended to detect any vaccine-related adverse events (WHO).

Shoenfeld´s syndrome, also called autoimmune/ inflammatory syndrome induced by adjuvants (ASIA) is part of a clinical and immunological spectrum of non-specific and specific manifestations of autoimmune disease, manly associated with the adjuvants squalene and aluminium hydroxide (Vera-Lastra et al). It includes systemic vasculitis, SLE, RA, inflammatory myopathy and GBS. Accepting ASIA as clinical condition would invalidate influenza vaccination programs. ASIA is accused of being a non-scientific speculation of a theoretical risk undermining vaccination programs

where causality is difficult to prove, as the number of affected patients is small (Kobbe).

Macrophagic myofasciitis (MMF) is microscopic histological lesions that were found in France in biopsies from patients with the symptom constellation of myalgia and fatigue (Cherin et al). These lesions contained aluminium salts, and were shown to persist for up to 10 years (Israeli et al), and since it was found in the usual injection site of vaccines, (the deltoid), it was linked with the administration of aluminium-containing vaccines (Siegrist). In 2008 the WHO Global Advisory Committee of Vaccine Safety (GACVS) issued a statement concluding that "there is no reason to conclude that a health risk exists as a result of administration of aluminium containing vaccines, nor is there any good reason for changing current vaccine practice" (WHO).

An association with narcolepsy was found with AS03 adjuvanted Pandemrix, but not with AS03 adjuvanted Arepanrix, or squalene (MF59) only adjuvanted vaccine (focetria) or non-adjuvanted vaccine. Production of Pandemrix followed the Dresden protocol, and production of Arepanrix followed the Quebec protocol. In Europe, mainly focetria and Pandemrix was administered. The wild type virus and Pandemrix may both have enhanced A(H1N1) pandemic influenza antigen presentation (Ahmed et al 2014).

The increased risk of narcolepsy after vaccination with the AS03 A/H1N1 2009 vaccine indicates a causal association, but the risk might be overestimated by more rapid referral of vaccinated children (Miller E et al, BMJ 2013).

1.4.4 Guidelines for adjuvants and new vaccines

An adjuvant shall not be introduced into a product unless there is satisfactory evidence that it does not affect adversely the safety or potency of the product (FDA).

There must be evidence to demonstrate that the benefit in terms of improvement of the immune response has been achieved without and undue increase in local and systemic adverse reactions. It is recommended that the pre-registration safety

database is large enough, with a minimum sample size of 3000, to detect events occurring in 1/100 - 1/1000 vaccinees (EMA).

1.5 Vaccination and infection in immunosuppression and immunomodulation

There lies an inherent opportunity from studying lessons learnt by related disciplines for vaccination in immunosuppression.

1.5.1 Vaccination in immunosuppression and chronic disease

Available data suggest that most immunocompromised populations are at higher risk of influenza-associated complications, have a general trend toward impaired humoral vaccine responses (although mixed results) and can be safely vaccinated (although longitudinal data are lacking) (Kunisaki et al). A single dose of Pandemrix seemed to be sufficient in most, both children and adults, except in immunocompromised hosts (Örtqvist et al).

The response to vaccination in primary immunodeficiency (PID)/ secondary immunodeficiency (SID)/ immune regulatory abnormalities (IRA) is significantly lower in quantity and quality as compared with the general population (Watcharananan et al). Influenza A H1N1/09 vaccine elicited a similar antibody response in HIV-infected individuals and in control subjects, whereas SOT recipients had an overall lower response. A second dose of the vaccine only moderately improved vaccine immunogenicity in HIV-infected patients. (Manuel et al).

The immunogenicity of the influenza vaccine is overall reduced in immunocompromised patients, although a significant clinical protection from influenza is expected to be obtained with vaccination. Influenza vaccination is safe in immunocompromised patients (Zbinden et al). There are in general no safety concerns regarding killed vaccines in PID, SID and IRA. To protect patients who cannot be vaccinated and/ or respond to vaccination and should be informed on the risk for patient by viral/bacterial shedding in the course of vaccination/infection of close contact. All live vaccines are contraindicated in severe immunodeficiency and if

< 500 CD4 cells/ mm^3 (adults), < 1000 CD4 cells/ mm^3 (1-6 years) or < 1500 CD4 cells/ mm^3 (<1 year) (Shearer et al).

All killed/ inactivated vaccines used in the general population have been recommended in immunocompromised patients as well. Live vaccines will multiply in the host, may be lethal and cause disseminated disease if immunocompromised. Oral polio vaccine OPV might revert to wild type virus years after infection and together with BCG are contraindicated in PID (Rubin et al).

In low-level immunosuppression (low dose or alternate day steroids) live vaccines are to be considered. In high-level immunosuppression (high dose or daily steroids), live vaccines are contraindicated. If anti-CD20 mAb therapy is used, live vaccines should be moved to 4 weeks before to 3 months after therapy (perform review/ test vaccination status before initiation of therapy). In patients that are non-responders by diagnostic vaccination or antibody responses following infections, natural exposure or previous vaccination, live vaccines are contraindicated. Vaccines especially recommended are PPSV23 and/ or PCV, inactivated influenza. Other inactivated vaccines should be considered according to disease-specific expert committees (Eibl et al).

All inactivated vaccines are strongly recommended including influenza, varicella (VAR) and MMR. OPV is contraindicated. Rotavirus and LAIV has special recommendations due to shedding (CDC).

Health care workers (HCW) are recommended for all live and killed vaccines, especially against infectious diseases which constitute a higher risk in the PID/ SID population (Shefer et al).

Addition of the MF59-adjuvant enhances immunogenicity of subunit influenza vaccine in adults with chronic diseases (Baldo et al).

Some vaccines are contraindicated in PID, namely MMR, varicella (VAR), rotavirus and LAIV are contraindicated in PID and SID. SID can be affected by

immunodeficiency to a different extent at different stages during the course of the underlying disease (Eibl et al).

1.5.2 Recommendations of vaccination in multiple sclerosis (MS)

Evidence was found to support strategies to minimize the risk of acquiring infectious diseases that may trigger exacerbations of MS, and the safety of vaccination with influenza, hepatitis B, varicella, tetanus and BCG in MS (Rutschmann et al).

According to the National Multiple Sclerosis Society (NMSS) and American Academy of Neurology (AAN) in association with the CDC, live attenuated vaccines (LAIV) or live vaccines are not recommended due to potential of reversion. Inactivated vaccines are generally considered safe for people with MS, including those who are taking an interferon medication, teriflunomide, glatiramer acetate, fingolimod, alemtuzumab, mitoxantrone, dimethyl fumarate, or natalizumab. It is recommended that only the standard dose be used. MS experts are not in agreement about the risks for a person with MS whose close family member receives a live-virus vaccine (NMSS).

The clinician should inform on the possible risk of relapse after influenza vaccination in RRMS. Offer flu vaccinations to people with MS in accordance with national guidelines, which recommend an individualised approach according to the person's needs (NICE).

The Norwegian Institute of Public Health recommends influenza vaccination in chronic neurological disease (see 1.4.6).

1.5.3 Mode of action and infections specific to the type of DMT

Even after licensure, new treatment associated risks must be monitored thoroughly. Further, as understanding of the disease and therapeutic strategies widen, more implications develop. Interferons and glatiramer acetate seem to carry a low risk of infections. For dimethyl fumarate, fingolimod and natalizumab, PML and opportunistic infections are the most profound risks. A recent review article

(Winkelmann et al) describes effects and infectious side effects, and will be used as a framework when listed categorically in this chapter.

DMTs can modulate or suppress the generation of adaptive immune response and/or the maintenance of immunological memory and may diminish efficacy of vaccines. Large studies on vaccine efficacy are scarce, especially in MS. Systematic prospective studies are virtually absent, and represent a knowledge gap that demands further clinical studies (Loebermann et al)

All non-platform (platform therapies are interferon beta and glatiramer acetate) therapies appear to be associated with an increased risk of herpetic infections and their reactivation, including varicella zoster virus (VZV) in fingolimod and alemtuzumab (Williamson et al, 2015).

Screening of VZV antibody status is recommended in patients about to start fingolimod and alemtuzumab and VZV vaccination has to be considered in VZV-negative patients (Winkelmann et al).

Alemtuzumab - is a monoclonal antibody targeting CD52, depleting T and B lymphocytes shortly after infusion leading to long-lasting changes (repopulation) in adaptive immunity with a preponderance of regulatory cells. This results in leukopenia and long lasting lymphopenia (T cells > B cells). Herpesvirus infections are common. Additional acyclovir treatment is recommended in the first month of treatment as well as monthly complete blood count (CBC) up to 4 years after the last treatment cycle.

Cladribine - is a purine nucleoside which is toxic to some resting and dividing cells. It will enter the market in 2018. An increased risk of opportunistic infections is expected from the high risk of pronounced lymphopenia. There is lack of long term efficacy and safety data, but increased risk of herpetic infections and their reactivation, including VZV have been reported (Holmøy et al).

Dimethyl Fumarate - attenuates proinflammatory activity of T_H1 and T_H17 and by scavenging toxic oxygen metabolites (neuroprotective effect), and causes a reduction

in CD8+ lymphocyte numbers. Lymphocyte numbers decline 30% (leukopenia, lymphopenia) during the first year and are stable thereafter. Single cases of JCV (PML) are reported and especially in combination therapy. CBCs and differential counts are recommended every 3 months. Further, treatment halt is recommended if confirmed leukopenia (< 3 x 10^9 cells/L) or lymphopenia (< 0.5 x 10^9 cells /L) (> 6 months). Alertness for PML is of importance.

Fingolimod - is a sphingosine 1-phosphate receptor functional antagonist. It blocks egress of CCR7+ CD4+ naïve and central memory T cells from the lymph nodes and reversibly redistributes lymphocytes into lymphoid tissue while preserving lymphocyte function. It prevents naïve and central memory T cells from circulating to non-lymphoid tissues such as the CNS. Single cases of JCV (PML) are reported, as well as a reported association with herpesviruses HSV and VZV. Alertness for herpesvirus and infections and PML is of importance. CBCs and differential count are recommended weeks 2, 4 and 12, then every 3 months (more frequent if below 0.6 x 10^9 cells /L).

Glatiramer Acetate - is a synthetic polypeptide that shifts the cytokine profile from a proinflammatory T_H1 to an anti-inflammatory T_H2 phenotype. It deactivates monocytes and macrophages and confers changes in the CBC. Rarely leukocytosis or mild lymphopenia is seen, as well as morphological changes in lymphocytes. Single cases of herpesviruses/ CMV are reported. No opportunistic infections are described. No specific countermeasures are necessary.

Interferon-beta 1a/b - increases production of anti-inflammatory cytokines and suppresses production of proinflammatory cytokines. Further, a reduction of inflammatory lymphocyte migration across the blood-brain barrier (BBB) is seen. This causes leukopenia and especially lymphopenia. Single cases of JCV (PML) have been reported only. There is no overall increased risk of infection. CBC is recommended every 3 months.

Mitoxantrone - is less used today than just a few years ago. It is a type II topoisomerase inhibitor that intercalates with DNA and causes single-strand and

double-strand breaks. It impairs DNA repair, inhibits RNA replication and has anti proliferative effects on macrophages, B cells and T cells, with a preferential effect on helper subsets. It causes bone marrow suppression with resulting leukopenia and lymphopenia. There are reported associations with herpesviruses, but it is not associated with an overall increased risk of viral infection. Weekly CBCs are recommended until normal levels and before each infusion. Exclusion of active or latent infection should be performed before every treatment cycle.

Natalizumab - is a humanized monoclonal anti-α4-integrin antibody which prevents T and NK cells from crossing blood vessel to reach the CNS. It also induces lymphocyte apoptosis. This diminishes immune surveillance in the CNS. There are reported associations with herpesviruses and JCV (PML risk of 4.03 per 1.000 patients). PML risk increases if JCV antibodies are present or in presence of a history of previous DMT and duration > 2 years. There is an overall elevation in the incidence of influenza infections, UTIs, URTs and nasopharyngitis related to therapy. CBCs are recommended every 6 months, as well as JCV antibody status if negative every 6 months. The CSF JCV antibody index aids risk stratification. Alertness for PML is recommended.

Ocrelizumab - is the first drug ever approved for PPMS and will come on the market in 2018. It is the humanized counterpart of rituximab, an anti-CD20 monoclonal antibody leading to depletion of CD20+ B cells by lysis, but only causes a delayed and modest decline of circulating antibodies, which will need to be monitored in patients in the long term (Hohlfeld et al). Data are missing on viral infections but one may extrapolate from rituximab.

Rituximab - is used off-label in MS. It is a chimeric anti CD-20 antibody and depletes CD20+ B cells by lysis which impairs T cell activation and release of proinflammatory cytokines. Vaccine efficacy may be extrapolated from other diseases (Loebermann et al). Single cases of JCV (PML) have been reported, but not in MS.

Teriflunomide - is an antimetabolite and an anti-rheumatic drug that inhibits dihydro-orotate dehydrogenase essential in the *de novo* pyrimidine synthesis of proliferating T and B cells (cytostatic effect). The mode of action is thought to be inhibition of recently activated T lymphocytes. Leukopenia (neutropenia) is seen. Single cases of JCV (PML), as well as single cases of CMV and HCV have been reported. In one study, the majority of infections were URTI, rhinopharyngitis and influenza (Confavreux et al, 2012). Recommended countermeasures are CBCs at months 2, 4 and 6 and every 3 months thereafter. Suspension of treatment is recommended if lymphocyte count < 0.2 x 10^9 cells/ L.

1.5.4 Timing of influenza vaccination in MS

Timing of vaccination is of special importance in immunosuppression (Rubin et al), but no large data series or evidence based recommendation exists for optimal timing of seasonal influenza vaccination during long-term immunomodulation (paper III). Further, little data exists to guide clinical practice on how long the interval should be between vaccination and starting a DMT (Williamson et al, 2016).

Before starting a DMT, missing vaccinations should be sought. Further, vaccination should be performed in advance of DMT initiation (Riminton et al). Vaccines have an important role in the prevention of treatment-associated infections, and should be encouraged before the initiation of DMTs whenever possible. Timing of vaccination should be made according to the manufacturers` recommendations (Loebermann et al). Vaccination should be started as soon as possible to increase the time between vaccination and maximal immune suppression. A conservative recommendation for live vaccines is 4 months before maximum immune suppression (Tamblyn).

Non-simultaneous MMR and VAR are recommended in SID (Klein et al, Pediatrics 2010). Safety margins might be shortened to 4 weeks before immune suppression, depending on the clinical entity.

Vaccination should be avoided when vaccines pose an increased risk and patients with serious relapses should postpone vaccination for 4-6 weeks until recovery or a

return to baseline. Individuals being treated with alemtuzumab should be given the inactivated flu vaccine six weeks before receiving their infusion. It is stated that all necessary vaccinations should be administered at least 6 weeks before a person starts treatment with ocrelizumab (NMSS).

1.6 Pharmacovigilance; monitoring vaccine safety and efficacy

1.6.1 The Brighton Collaboration

In 1978, criteria for GBS were established according to the National Institute of Neurological and Communicative Disorders and Stroke (NINDS). In 2000, the Brighton Collaboration standardized case definitions for AEFIs (Bonhoffer et al). The Brighton Collaboration is the world´s largest network of vaccine-safety experts. It is a global research network which does not advocate for specific vaccines, does not receive funding from specific manufacturers.

1.6.2 Vaccine adverse event surveillance & communication (VAESCO)

VAESCO I was European research network working for the highest quality of vaccine safety data by establishing a shared vaccine safety research infrastructure, conducting collaborative vaccine safety studies that communicated timely and accurate findings.

VAESCO II was established after WHO initiated the European Centre for Disease Prevention and Control (ECDC) and funded the VAESCO II project to investigate background rates of AESIs and the association between pandemic H1N1 vaccines and GBS in Europe (Wijnans et al, 2011). VAESCO II was originally planned as a three-year project for conducting studies on GBS and other safety signals, but was extended in time because of the narcolepsy signal. VAESCO ended in 2012 while the narcolepsy signal was still incompletely evaluated due to resource constraints at ECDC (Chen et al). Pandemic Influenza Vaccination Safety Assessment Network Europe (PIV SANE) was the assigned name for the collaboration between NIPH, VAESCO and ECDC.

1.6.3 Vaccine safety surveillance; pharmacovigilance, vaccinovigilance

As presented in the above section, post marketing surveillance is of great importance. The passive system in the US is VAERS, and EudraVigilance in Europe. Their respective active systems are VSD and VAESCO. The countries lacking infrastructure or resources rely on the WHO Global Advisory Committee on Vaccine Safety (GACVS). National or international expert committees give advice to local authorities. National or international regulatory authorities decides on withdrawal, or changes in indication and safety warnings (Hardt et al). Criticism of passive surveillance is potential under reporting, differential reporting (over reporting of serious AEFIs) and stimulated reporting (over reporting after media coverage of specific AEFIs) (Mailand et al).

1.6.4 Categories of adverse events in immunization

An adverse event (AE) following immunization (AEFI) is any unwanted medical occurrence after immunization - not necessarily causally related to vaccination. The AE may be any clinical sign, laboratory finding, symptom or disease. AEFIs are branded into several categories: vaccine product-related reaction (e.g. extensive limb swelling after DTP), vaccine quality defect-related reaction (e.g. incompletely inactivated poliovirus in the poliovaccine), immunization error-related reaction (e.g. transmission of infection by contaminated multi dose vial), immunization anxiety-related reaction (e.g. vasovagal syncope), and coincidental event (e.g. fever of other cause). An AE will be classified as serious (SAEFI) if death ensues, in a life-threatening situation, in-patient hospitalization or prolongation of hospitalization is required, persistent or significant disability ensues, it is a congenital anomaly, or it requires intervention to prevent permanent impairment or damage. Severe is used to describe the intensity of a specific event (as in mild, moderate or severe) (WHO).

Neurological AEFIs are often severe, and also difficult to assess as they may present in different shapes and forms. Many clinicians are not familiar with the disease group, and there are few neurologists in some parts of the world making neurological AEFIs challenging in clinical vaccinology. GBS is considered a spectrum of

clinicopathological subtypes. A temporal association must be differentiated from causality. A vaccine may be temporally associated with, but is not necessarily the result of administration of a vaccine (Sejvar et al,Vaccine 2011).

1.7 Clinical entities

1.7.1 Guillain-Barré syndrome

Guillain-Barré syndrome (GBS) is the most common and most severe acute autoimmune paralytic neuropathy, with about 100.000 people worldwide contracting the disorder every year (Willison et al). Age-specific background rates have been provided, and incidence in Western countries increases by 20% for every 10-year increase in age and the risk of GBS is higher in males (RR 1.78) (Sejvar et al, Neuroepidemiology 2011). The background rate was found to be 1.1-1.8 per 100.000 per year in Europe and North-America. Rate increases were found in people aged 50 years or more, with lower rates reported in children (< 16 years). 40-70% of cases recorded an infection before onset, with 22-53% an URTI and 6-26% a GI infection. No seasonal variation was found on a global basis (McGrogan et al).

GBS comprises a spectrum of disorders, most commonly acute inflammatory demyelinating polyneuropathy, and is usually preceded by infection or an immune stimulation that induces an aberrant autoimmune response targeting peripheral nerves and their spinal roots. Molecular mimicry between microbial and nerve antigens plays a major role as for the case of *Campylobacter jejuni* infection. However, there is a complex interplay between microbial and host factors that is not well understood. Genetic and environmental factors that influences susceptibility to the disease are unknown. 1-2 weeks after immune stimulation, the acute progression of limb weakness often with sensory and cranial nerve involvement, proceeds to a peak clinical deficit in 2-4 weeks. Diagnosis is based on clinical patterns and is usually straightforward, though there are atypical cases. Diagnostic biomarkers are not available for most variants of the disease and have been major research challenges. All patients need monitoring and supportive care. Early intravenous immunoglobulins

(IVIg) or plasma exchange is of proven benefit. Since 25% are in need of artificial ventilation and many develop autonomic disturbances, patients are often admitted to intensive care. The recovery period may last for months to years as the immune response decays and the peripheral nerve undergoes repair (Willison et al).

Guillain-Barré syndrome is fatal in 3-10% of cases, in some cases due to sudden autonomic failure (Winer et al). 20% are unable to walk unaided after 6 months (Hughes et al). GBS onset at an age above 40 and 50 years confers poorer prognosis (Rajabally et al) as well as severity of paresis and duration of maximal symptoms and presence of axonal damage (Vedeler et al).

Acute hepatitis E was more recently discovered in 5% of Dutch patients with GBS (van den Berg B et al). No vaccine against the hepatitis E virus is currently available outside China (NIPH). GBS has previously been associated with the hepatitis vaccine (Souayah et al).

1.7.2 Multiple sclerosis (MS)

MS is an immune-mediated demyelinating, (Lucchinetti et al) and neurodegenerative (Lassmann 2013), (Mahad et al), (Lassmann 2015) central nervous system disease that can give rise to heterogeneous clinical presentations. It is the most common autoimmune disease of the central nervous system (Havla et al). In histological MS lesions, it has been found that lymphocytes mediate inflammation, demyelination and axonal damage (Goverman). The condition causes cumulative permanent disability, impairs quality of life and shortens life expectancy. Disease modifying therapies (DMTs) are used to attenuate or silence disease activity through long-term modulation of inflammation and normalization of aberrant immune responses. Many of the drugs used in ameliorating MS may unfortunately increase the risk of infection (Winkelmann et al).

1.8 Neurological autoimmunity after infection and vaccination

1.8.1 GBS after influenza virus infection

In GBS patients, infections with *Campylobacter jejuni*, *cytomegalovirus* and *Epstein-Barr virus* were significantly more frequent than in controls (Jacobs et al). A database study with data from 15 years, a 17-fold increase in the risk of GBS was found within one month after influenza-like illness (ILI), and no evidence of an increased risk after seasonal influenza vaccination (Stowe et al 2009). ILI seems to be a relevant triggering event for GBS. A prospective 3 year study found highly statistically significant difference in the rates of GBS occurring between influenza virus circulation periods and noncirculation periods (Grimaldi-Bensouda et al). Another study links GBS with influenza infection and suggests that vaccination had a protective effect against GBS (Verity et al). The risk of GBS after influenza infection has been reported to be as much as 40-70 times higher than the risk of GBS after influenza vaccination (Deeks et al). Seasonal variations were found in one study, with rates being 15% higher in winter and spring (Shui et al). GBS after vaccination is a rare event. In most cases it follows an infection of the GI or URT (Lehmann et al, 2012), (Vedeler et al).

Neurologic complications are relatively common among children admitted with influenza, and can be life-threatening (Khandaker et al). GBS after influenza infection may be higher than risk from vaccination (Sejvar 2013). Greene found no elevated GBS risk after 2009/2010 MIV or 2010/2011 TIV vaccination, but a strong association with a medically-attended infection, and especially respiratory infection. (Greene et al, 2013). Some studies after 1976 have shown a small increased risk of GBS after MIV and TIV, but the risk after influenza infection is several times higher than that after influenza vaccination (Vellozzi et al, Clinical Infectious Diseases 2014).

1.8.2 GBS after influenza vaccination

GBS is the most common passively reported neurological condition after influenza vaccination (Haber et al). One of the earliest studies to link GBS with infection or

vaccination was in 1966 (Leneman). One study found a protective effect of influenza vaccination (Vellozzi et al, American Journal of Public Health 2014). Several studies have found that GBS primarily occurs within 8 weeks of immunization, with most cases occurring within 6 weeks. Evidence favors a causal relation between the 1976 H1N1 influenza vaccine and GBS (Stratton et al 2004). Concerns about the risk of inducing GBS in mass immunization programs do not seem justified by the available epidemiological data (Lehmann et al, 2010). In 2011 it was concluded that the evidence was inadequate for seasonal vaccines; "While the weight of epidemiological evidence does not support a causal link between influenza vaccinations evaluated over the last 30 years, an association cannot be confidently ruled out, particularly for future vaccine strains" (Stratton et al 2012). Two case reports present AIDP and AMAN after pandemic vaccination respectively (Chaari et al), (Kutlesa et al).

1.8.3 Infection and exacerbation in MS

The background rate of severe infections in patients with MS ranges from 0.2 % - 2.6 % (Winkelmann et al). A temporal association between infection and relapse has been found in several studies and common viral-like infections are temporally associated with exacerbations. MS patients are at a noticeably raised risk of serious infections leading to hospital admission and infection-related mortality, even at younger ages (Montgomery et al). Influenza viral infection of autoimmune-prone mice triggers clinical and histological disease (Blackmore et al).

The infection rate in MS was significantly less than in controls suggesting superior immune defenses against common viruses (Sibley et al, 1985). Influenza infections were not followed by a major relapse (n = 6) (Andersen et al). Viral upper respiratory tract infection (URTI) is an important trigger of MS attacks and treatment with interferon beta reduces the attack rate, but not by preventing URTI (Panitch).

One study found MS exacerbation within 6 weeks in 33% after influenza illness and in only 5% after vaccination thus recommending routine annual influenza vaccination to all patients with RRMS (De Keyser et al). Another study found an association between viral infections and exacerbations in MS, but no MRI correlate was found

with the symptomatic or serological risk periods. There were, however, possible confounding effects of interferon beta-1a (Edwards et al). Viral infections may indeed induce plaque formation in MS (Noseworthy et al). Results support an association between a history of infectious mononucleosis and subsequent MS. Further, respiratory tract infections may precipitate disease onset (Marrie et al). One study found a twofold increased risk of relapse with infection and relapses associated with infection seemed to be more prone to cause neurological dysfunction, but no MRI correlate was found (Buljevac et al) with an editorial (Confavreux). After urinary tract infection, viral respiratory tract infection or gastroenteritis, relapse rates increase and MRI shows increased inflammatory activity (Correale et al).

One study found an association between population-level viral epidemics and a higher relapse rate, but only in patients using other medications than interferon (Oikonen et al, 2008). A 10 year study in the pre-interferon era, found MS relapse occurrence to be 6.5-7.1 times higher after peaks in laboratory-confirmed influenza A cases in the general population, with similar findings for EBV infections (Oikonen et al, 2011). The study confirms environmental interaction but no causality.

1.8.4 Safety of vaccination in MS

Vaccination may be introduced as part of the treatment to avoid complications of therapy because avoidance of infection in MS is desirable. Safety of vaccination in patients with MS has been debated for decades.

In 1961, it was publicized that prophylactic inoculation of patients suffering from multiple sclerosis should be restricted to cases of utmost necessity (Pálfy et al). Anecdotes of neurologic dysfunction were reported with different vaccines (Miller H et al), and expert advice on immunization in MS was scarce.

The consensus among neurologists was that immunization could be hazardous in an altered immune system, like in MS. Public health authorities and the pharmaceutical industry was accused of misleading practicing physicians into believing that immunization was safe and proper in chronic neurologic disease. All vaccines were

postulated as problematic in MS, but especially influenza vaccines. In one case with SAEFI, the manufacturer would not produce the quality control records of the batch in question to the court. The Division of Biologics Standards of the National Institutes of Health concluded that almost all of the influenza vaccines between 1966 and 1971 were substandard, ineffective and toxic (Rabin). This was an early extreme outcry for pharmacovigilance.

In a later study, only one out of 93 MS patients showed neurological deterioration after influenza vaccination (Sibley et al, 1976). An early double-blind, placebo controlled study found that influenza vaccination was safe, effective and recommended in MS. Relapse rates were equal in the vaccinated and placebo groups. Efficacy as measured by the HI method was similar as for the general population. Patients with preexisting antibody were less responsive to vaccination than normal controls studied previously (Myers et al). Another study in 1978 concluded that the influenza vaccine was safe and no increased relapse rates were seen (Bamford et al). No exacerbations were found in 11 patients after influenza vaccination and there were less Gd-enhancing lesions in the post vaccination period than the pre vaccination period (Michielsens et al).

In a small study, increased MRI lesion activity was found in one of six patients following influenza vaccination (Salvetti et al). In another study, a clinical protective effect was not demonstrated against flu in 19 patients, although the patients showed higher antibody responses than normal controls (Mokhtarian et al).

The first study to follow 104 MS patients through an entire influenza season (6 months) where influenza infection was accounted for, found that more influenza cases occurred in the vaccinated group than in the placebo group; however, the finding was not statistically significant. No differences in exacerbation rates were found after influenza vaccination when compared to placebo, and the study found influenza immunization safe and recommendable in MS (Miller AE et al). Influenza vaccination in MS neither increases the relapse rate nor worsens disease course. Reduction of viral infections lowers the numbers of exacerbations. Vaccination is

safe and recommended in MS (Merelli et al). An early systematic study found no increase in short-term relapse risk after vaccination with influenza and other common vaccines (tetanus, hepatitis B, influenza, measles, rubella, polio, BCG and typhoid fever) and recommends vaccination in MS (Confavreux et al, 2001). Vaccination against hepatitis B, influenza, tetanus, measles or rubella is not associated with an increased risk of MS or optic neuritis (DeStefano et al). No increased risk of MS onset was found after influenza vaccination or tetanus, but an increased risk of MS onset was found for hepatitis B vaccination (Hernán et al). A pooled analysis found no evidence that influenza vaccination was associated with an increased risk of MS (Farez et al, 2011). In 60 patients, there were no increased relapse rates at days 30, 60 or 90 post influenza vaccination compared to pre vaccination and recommends vaccination in MS (Farez et al, 2012). In 49 patients vaccinated with MIV 2009, TIV 2009 or both, exacerbation did not occur in anyone. The second most common reason for not being vaccinated was that their treating physician had recommended against vaccination due to MS (Auriel et al).

One study (n=18) found an increased relapse risk within 3 weeks of vaccination in 6/7 receiving MIV 2009, 4/8 receiving both MIV and TIV 2009 and in 2/3 receiving TIV alone (McNicholas et al).

A review by the US Institute of Medicine did not find sufficient evidence to accept or reject a causal relationship between the onsets of MS or MS relapse after vaccination against influenza. No evidence of a causal relationship between onset of MS and vaccination against MMR, influenza, hepatitis, HPV, DTP or meningococcal disease (Stratton et al, 2012).

Curiously, BCG vaccination in CIS patients showed that the vaccine significantly reduced enhancing MRI lesions during 6 months prior to starting DMT (Ristori et al). No associations were found for HBV or any vaccination and the risk of CNS demyelination syndromes up to 3 years later. Vaccination of any type (most commonly influenza) was associated with an increased risk within the first 30 days only if < 50 years of age, but not for CIS, MS or ADEM. All associations disappeared

after 30 days. The authors suggest that vaccination (like infection) facilitates the transition to preexisting subclinical autoimmunity and claims no evidence for causality. Results for HPV vaccination were inconclusive (Langer-Gould et al).

Inactivated influenza vaccines are generally considered safe for MS patients. Live attenuated vaccines are engineered to have reduced virulence but carries a risk of infection or reversion to virulence, particularly in the immunocompromised, and should in general be avoided in immunocompromised (LAIV, varicella zoster virus, Sabin, MMR, BCG, typhoid, Yersinia pestis, yellow fever) (Williamson et al, 2016) .

A systematic review found no increased risk of MS or change in relapse risk after influenza vaccination. As MS is immune-mediated, MS after vaccination and infections give rise to speculation of causality. However, it is necessary to underline the importance of not confusing a temporal and causal association. Further, studies investigating risk of MS onset with an unlimited timeframe could dilute a potential association (Mailand et al).

In conclusion, influenza vaccination is safe and recommended in MS (Merelli et al).

1.8.5 Autoimmunity after vaccination

EMA reported that neurological disorders were the second-most-common category of AEFIs reported for Pandemrix after general disorder and administration site reactions (EMA).

A retrospective cohort study of 1 million vaccinated in the pandemic of 2009 found significantly increased relative risks in the early phase of vaccination (≤ 45 days; i.e. mostly in the high risk individuals) for Bell´s palsy, paresthesia and inflammatory bowel disease, but no change in the risk for GBS, MS, type 1 diabetes or rheumatoid arthritis. After the first 45 days of the campaign, they found no statistically significant association between vaccination and autoimmune or neurological diseases. Detailed data on covariates was lacking, with the possibility for confounding. Further, selection bias and surveillance bias were concerns. (Bardage et al).

In 2013, a paper stated explicitly that there was no reliable scientific evidence to suggest a link between autoimmune disease and vaccination, and by contrast, absolute evidence exists that infectious agents can trigger autoimmune diseases (De Martino et al).

Biological plausibility could be based on the fact that narcolepsy is strongly linked to the HLA DQB1*06:02 allele (Nohynek et al) and that this allele is twice as common in northern than in southern Europe (Gonzales-Galarza et al). Pandemrix vaccination is a precipitating factor for narcolepsy, especially in combination with HLA DQB1*06:02 (Szakács et al). Important roles have been found for HLA DQB1*06:02 and the T cell receptor alpha genes as well as two additional genes, Cathepsin H and *TNFSF4/ OX40L*, in disease pathogenesis (Faraco et al). The production process of Pandemrix differed from Arepanrix, where the first showed a) higher amounts of structurally altered viral influenza nucleoproteins (NP), b) detergent-induced antigenic changes of viral NP, recognizable by antibodies from children with narcolepsy, and c) increased antibody response to NP in association with the HLA DBQ1*06:02 risk allele (Vaarala et al). The association of H1N1 vaccination and possibly infection as well as genetic susceptibility, suggest a T-cell mediated autoimmune process in narcolepsy (Partinen et al). A recent review re-states problems with confounding by infection, ascertainment-, recall- and selection biases. Risk was limited to one vaccine only - Pandemrix. During the first year after vaccination, the RR of narcolepsy was increased 5-14-fold in children and adolescents and 2-7-fold in adults. The vaccine attributable risk in children and adolescents was around 1 per 18.400 vaccine doses. There was a possible increased risk into the second year as well, but bias could not be excluded. The benefits of vaccination, however outweighs the risk of narcolepsy (Sarkanen et al).

1.8.6 The hygiene hypothesis

The hypothesis argues that decreased exposure to infections increases the risk of autoimmunity (Münz et al), (Salemi et al). In some circumstances, infections and vaccines may lead to improved tolerance and decreased risk of autoimmunity. Infections are known to cause or augment autoimmunity through T-cells by

molecular mimicry, stimulation of autoreactive T-cells, or enhancement of antigen presentation by polyclonal bystander activation, epitope spreading and adjuvant effect. However, the timing of exposure, antigen type, genetic background, and adjuvants is a rather complex affair (Langer-Gould et al).

1.8.7 The plausible risk window and criteria for causation

A biologically plausible risk window has traditionally been considered 1-42 days (6 weeks) for GBS after vaccination. (Greene et al, 2013). The risk period of AEFIs is often set to 42 days (6 weeks) to be consistent with previous studies (Rowhani-Rahbar et al). Increased risk of GBS after the 1976 swine flu vaccine was found after 6 weeks by some authors (Langmuir et al) but not by others (Breman et al) (Safranek et al).

"The mother of all post vaccination GBS studies" (Schonberger et al) showed relative risks for GBS of 4.0 and 7.6 for the 6- and 8-week postvaccination periods, respectively, with an attributable risk of about 1 case per 100.000 vaccinees. Further, the pandemic did not take place as expected (Salmon et al). A later study used 8 weeks (Hurwitz et al). It was compared to the equivalent 8 week period from the 1976 study. This study describes an early sentinel-neurologist system. They found a non-significant increased relative risk of GBS after vaccination, coinciding with the range of previously published annual incidence figures. Continued analyses of other influenza vaccines will be useful in determining whether the apparent causal relation between GBS and A/New Jersey vaccine was unique.

Previous researchers used either six- or eight week periods to define vaccine associated cases. However, the studies that reported an elevated risk also showed that all or almost all of the risk was within the first six weeks after vaccination (Lasky et al).

Development of autoantibodies by molecular mimicry in the case of bacterial and viral epitopes takes a few days to a week, thus a case of GBS occurring within one week of vaccination is unlikely to be causally related to the vaccine (Poland et al,

Lancet 2013). If immune-mediated physiological responses are suspected, based on biological and epidemiological evidence, time is required to develop and clinically manifest. This means vaccinations occurring < 3 days prior to onset is considered less plausible (Willison), (Schonberger et al), (Hemachuda et al).

Typically, 12 months safety follow-up should be applied for late AEFIs if adjuvanted vaccines (Mastelic et al). This stands in stark contrast to many studies with a cut-off interval of 6 weeks. Twelve months is a maximum risk period of autoimmunity after vaccination. After vaccination, it is unlikely to occur within more than a few months (Da Silva et al, 2013).

As presented, most studies have traditionally utilized a 6-8 week risk window and maximum serum antibody concentration occurs in weeks 4-6. Of note, VAERS has traditionally stopped reporting AEFIs at 6 weeks.

The risk window is largely dependent on type of disease. Immunologically, a T-cell response will be adequate after 4 weeks. For narcolepsy, which is probably an autoimmune disease, since it is strongly associated to HLA, a cut-off is set at 2 years, but in this case there must be several triggers (Partinen et al).

The Hill´s criteria for causation should always be kept in mind when discussing AEFIs. They are: consistency and unbiasedness of findings, strength of association, temporal sequence, biological gradient, specificity, coherence with biological background and previous knowledge, biological plausibility, reasoning by analogy, experimental evidence (Hill).

2. Aims of the studies

2.1 Vaccine safety monitoring during the swine flu pandemic of 2009/2010

Due to the previous association with GBS in 1976, the FDA, WHO and EMA all recommended active monitoring of a potential association between the influenza A(H1N1)pdm09 vaccine and GBS. Further, academia and national public health institutes requested monitoring. The ECDC commissioned the VAESCO for a case control study for rapid initial assessment (Dieleman et al) with a larger scale study carried out in parallel (Paper I). The dataset of Paper I has also been presented in a third congregate global study (Dodd et al) where the Norwegian data were excluded due to true diligence.

2.2 Influenza immunization monitoring in multiple sclerosis during the 2009/2010 and the 2010/2011 seasons

We set out to explore the influence of immunomodulatory treatment on MS patients receiving pandemic H1N1 (swine flu) vaccination in 2009 and seasonal influenza vaccination in 2010, in the respective 2009/2010 and 2010/2011 influenza seasons.

2.3 Influenza immunization monitoring in multiple sclerosis during the 2012/2013 season

As our previous study on MS patients mainly studied long-term protection, ie. 10 months in 2009/2010 and six months in 2010/2011, we decided to explore this in a stricter sampling environment at three, six and 12 months in 2012/2013.

3. Material and methods

3.1 Paper I: Norwegian patient data in European collaborative vaccine safety studies

For patient recruitment, a letter was sent to all Departments of Neurology in Norway, as well as a few hospitals where neurology was admixed with the internal medicine. A contact person was established at all 21 sites in Norway. All hospital medical records of candidate cases in the study inclusion period were mailed, either in the electronic journal system or by regular mail, for retrospective medical history containing clinical information and supplementary investigations (blood, cerebrospinal fluid, electrophysiology and neuroimaging). Data collection closely followed the outline of case definitions and guidelines (Sejvar et al, Vaccine, 2011). To avoid missing out on cases, more than just G61.0 ICD-10 was questioned. Data on vaccination was obtained from the national vaccine registry SYSVAK, where it was possible to discern adjuvanted from non-adjuvanted vaccines. Registration of vaccination was mandatory (Trøgstad et al 2012). Close contact was kept with the National Institute of Public Health (NIPH) all through the study period. The patients' general practitioners were contacted on a regular basis for information on co-variates and prospective disability scoring, weekly for 4 weeks, then monthly for 5 months, then every 3 months. The study population in Norway was 4.4 million at the time of inclusion. The case recruitment period was November 1st 2009 until November 1st 2010. The index date was the onset date of neurological symptoms. When the review failed to assign a case, a senior neurologist was consulted.

3.2 Paper I: Centralized data management and analysis center

Centers from Denmark (DK), Finland (FI), France (FR), Netherlands (NL), Norway (NO), Sweden (SE) and the United Kingdom (UK) used a common protocol and applied the standardized Brighton Collaboration GBS case definition intended for epidemiologic purposes and not as a criterion for treatment (Sejvar JJ et al, Vaccine, 2011) by using the Automated Brighton Classification (ABC) tool ranging from level

1 through 4 (The Brighton Collaboration). Data harmonization, transformation and pooling used methods and tools derived from the EU-ADR project (Coloma et al), now located at EMA (EMA). The total source population was 50 million. Harmonized input files were transformed using a standardized Java-based program Jerboa® version 2.6.0, September 2010, Erasmus University Medical Center, Rotterdam) and was utilized as a data conveyor providing anonymous and aggregated de-identified data. This conferred a shared information technology infrastructure for international data transfer and a central data management and analysis team.

3.3 Paper I: The self-controlled case series method

The study was a prospective self-controlled case series (SCCS). It is a case-only study comparing the incidence of disease during risk and non-risk periods within the same person, thereby controlling for measured and unmeasured confounding factors (between vaccinated and unvaccinated) that remain stable over time (all permanent characteristics of patients, in addition to seasonal variations in risk). The risk window was 42 days after vaccination.

The advantage of SCCS is that it allows powerful statistical analysis based only on the cases admitted and does not require vaccination dates for the entire population cohort cases. In this way it is free from the individual-level confounding that may affect cohort studies. Bias due to altering the schedule of vaccination in unwell patients would underestimate a vaccine effect, however is unavoidable otherwise an RCT is initiated. If vaccinations are missing in a random fashion, records do not bias the relative incidence (RI) calculations but the absolute and attributable risks will be underestimated (Farrington et al, Lancet 1995).

In the SCCS design, the power and simplicity of the cohort method and the economy of the case-control method are combined, while reducing confounding. Similarities exist with the case-crossover design, but it differs in that it is derived from the statistical model of the cohort design (Farrington, Biometrics 1995).

The SCCS is a modified cohort method that estimates relative incidence of rare AEFIs. It requires only a sample of the cases and avoids the need for following large population cohorts and controls. It focuses on the event rates in different periods within each individual´s observation time. It is conditioning (by multinomial log-linear modelling, RRs and CIs are calculated using conditional Poisson regression) the analysis on the total number of events and vaccination history observed for each case. It includes a level for each individual to keep a fixed number of events experienced for each case at its observed value. The statistical power is equivalent to a full cohort method, and outperforms the case-control method when the risk periods after vaccinations are short (it does not include possible long term AEFIs) and vaccine coverage is high. The method also eliminates confounding by variables associated with both the outcome and avoidance of vaccination, and it eliminates variation in background incidence between individuals. To achieve sufficient power, cohort studies must be very large. Case-control studies require smaller sample sizes but are subjectable to bias in choosing controls. Bias in the latter study method may constitute differential ascertainment of recently vaccinated and unvaccinated cases, as well as differential rates of vaccination in high or low risk individuals. The SCCS method handles several dependent exposures and controls for age. Further it avoids the need to specify the prior probabilities of exposure. It controls for both known and unknown patient-specific confounding factors. One paper comparatively evaluates the SCCS, case-control and cohort method (Farrington et al, American Journal of Epidemiology 1996).

The SCCS is a method for where event occurrence censors, curtails, or affects post-event exposures. It allows for exposures whose occurrence or observation is influenced by the event. The method is used for transient point exposures and rare nonrecurring events. The pseudo-likelihood scheme is proposed which makes computations doable in complex models. It allows for the fact that an individual who has had e.g. GBS may be permanently less likely to be vaccinated. The association between time-varying exposures and outcome events is possible using data on cases only. Only cases need to be sampled that are self-matched so that time-invariant confounders are adjusted. The one limiting assumption is that the distribution of

exposure and the observation period must be independent of event times. This inhibits use of the method when occurrence of an event alters the subsequent exposure process or the observations of that process. This occurs for exposures with distribution depending on the event history as well as for terminal events because follow-up, and thus exposure history, is curtailed by the event. It also cannot be used if observation of the exposure process is censored or otherwise disrupted by an event occurrence (Farrington et al, Biostatistics 2009).

SCCS enables control for time-invariant (fixed) individual level confounding. This means any differential vaccine uptake in individuals at risk of GBS is controlled for, which may be difficult to achieve in case-control and cohort designs (Andrews et al, Vaccine 2011; 29: 7878-7882). A critical review of 40 studies utilizing the method was done by the original authors, and in general the method was applied appropriately (Weldeselassie et al).

Occurrence of GBS within 6 weeks of a prior influenza vaccination is a precaution for receiving future influenza vaccines (Fiore et al, Morbidity and Mortality Weekly Report 2009).

3.4 Papers II and III: Patients, controls, DMTs, vaccine specifications and sampling

In the 2009/ 2010 influenza season, 289 patients (mainly RRMS) were invited and 251 controls were recruited. 113 patient sera and 216 control sera were available for analyses. The control group consisted of healthy health care workers. The immunomodulatory categories (DMTs) were glatiramer acetate, interferon beta, natalizumab and mitoxantrone. Both groups mainly received a low dose monovalent split virus AS03 adjuvanted A/California/7/2009 (H1N1). Some patients also received TIV containing A/Brisbane/10/2007 (H3N2-like), A/Brisbane/59/2007 (H1N1-like) and B/Brisbane/60/2008-like strains. The main period for vaccination was from October 2009 to January 2010. Sampling times were at 10 months for patients and at 12 months for the controls.

In the subsequent season of 2010/2011, the same 289 patients and 251 controls were asked for participation, and 49 patients and 73 controls participated with follow-up sera. The same DMTs were prevalent. Only TIV was administered this season, containing A/California/7/2009 (H1N1-like), A/Perth/16/2009 (H3N2-like) and B/Brisbane/60/2008-like strains. The main period for vaccination was from September 2010 to March 2011. Sampling times were at 6 months for both patients and controls.

In 2012/2013, 90 patients (mainly RRMS) were compared to 62 controls without immunotherapy or neurological disease. The prevalent DMTs were fingolimod, glatiramer acetate, interferon beta-1a/1b or natalizumab. Vaccination was against A/California/07/2009 (H1N1)pdm09, A/Victoria/361/2011 (H3N2) and B/Wisconsin/1/2010. The main period for vaccination was from September 2012 to March 2013. Sampling times were at visit 1 (day 0), visit 2 (three months), visit 3 (six months) and visit 4 (12 months).

3.5 Papers II and III: The haemagglutination inhibition method

All samples were stored at − 80°C until used in the blinded analyses. Sera were treated with receptor destroying enzyme (RDE) (one volume of serum was diluted with four volumes of RDE) and tested by the haemagglutination inhibition assay. A twofold dilution series of sera were prepared in phosphate buffered saline (starting dilution 1/10) and incubated with 8 haemagglutinin (HA) units of whole inactivated H1N1 (A/California/7/2009) or H3N2 (A/Perth/16/2009) and in 2012 the H3N2 (A/Victoria/361/2011) viruses for one hour at room temperature. 0.7% turkey erythrocytes were added for 30 minutes before reading. Positive control sera were included in all assays and consisted of a post-infection ferret serum against X179a and a human post-pandemic H1N1 2009 infection plasma (09/194), prepared at the National Institute for Biological Standards and Control as a candidate International Standard for serological assays. Positive control ferret sera against H3N2 were supplied by the International Reagent Resource, Virginia (IRR, VR) and included in all assays.

All sera were tested in duplicate; on up to three separate occasions in 2009 and 2010. The geometric mean titre (GMT) was calculated to reflect the two fold dilutions of sera used in the assay. The H1N1-specific HI titres were standardized according to a conversion factor (collaborative study GMT divided by the laboratory GMT) using the human post pandemic infection plasma. The serum HI titre was expressed as the reciprocal of the highest dilution at which 50% haemagglutination was inhibited and the titres less than 10 were assigned a value of 5, for calculation purposes. This assay is commonly used to measure influenza-specific antibody responses after vaccination. An HI titre ≥ 40 is established as a surrogate correlate of protection and is used in these studies to define protection. The immunogenicity of the Influenza B strain was not tested, due to lack of sensitivity in the HI test.

3.6 Applied statistics in paper II

Cross-tabulation with the Fisher exact test was used to compare the frequency of protection between patient and controls, and rates of protection between different immunomodulatory treatments. We used a logistic regression model to adjust for age and gender. We used SPSS version 20 to perform the analysis.

3.7 Applied statistics in paper III

Descriptive statistics are reported using the mean, geometric mean, standard deviation (SD), frequency counts and per cent (%). Associations between categorical variables were tested using Pearson's chi squared test. Mixed linear regression was performed to analyse the dependency of H1N1 and H3N2, respectively, related to therapy (fingolimod, glatiramer acetate, interferon beta-1a/1b, natalizumab, no treatment compared to controls) adjusted for age and gender assuming an autoregressive correlation of the first order to account for correlation between repeated measurements in each subject. The residuals were examined to check for consistency with the normality assumption (Supplementary tables 1 and 2), and log-transformation of H1N1 and H3N2 was chosen for the analysis. All models were inspected for interactions between time and medication group. The rate of protection,

defined as H1N1 ≥ 40 and H3N2 ≥ 40, respectively, was analysed with respect to the same variables using logistic regression and the generalized estimating equations methodology. Results are reported using the odds ratio (OR) and 95 % confidence interval (CI). SPSS version 23 was used for all statistical analyses. The significance level was set at 0.05 for all tests.

4. Ethics

The REK Vest Committee found the GBS study so important that it was exempt from confidentiality. Their arguments were the following; a written informed consent leading to drop-outs could jeopardize the validity of the study. Further, the risk or absence of risk in regards to a public vaccination program is necessary to erudite which in turn will influence the public trust in future vaccination programs. Thus it was of paramount societal importance that the scientific value was not compromised. De-identified patient health information was also possible to export to EU-countries.

In the MS Influenza vaccine projects of 2009-2011 written consent was obtained by the patients and approved by REK Vest.

In the MS Influenza vaccine project of 2012, written consent was obtained by the patients and approved by REK Vest.

5. Results

5.1 Paper I: Relative incidence of GBS after A(H1N1)pdm09 vaccination

730 potential GBS cases were identified, 427 were excluded leaving 303 cases in the study population. Percentage of vaccinated cases did not change significantly over time. Mean age was 50 years (SD 4.1). Altogether 36% were level 1, 26% level 2, 13% level 3, 25% level 4a. The most frequent disability score was 4 (30.6%). A total of 99 cases were vaccinated with A(H1N1)pdm09, mostly AS03 adjuvanted, before symptom onset. Thirty-six cases developed GBS within 42 days after a first dose. 15 cases developed GBS within 42 days of TIV and 79 cases within 42 days after ILI or URTI. The overall pooled RI: 3.5 (95% CI: 2.2, 5.5), adjusted for calendar month RI: 2.0 (95% CI: 1.2, 3.1), adjusted for contra-indications RI: 1.9 (95% CI: 1.1, 3.2) with the pseudolikelihood method and RI: 1.8 (95% CI: 0.7, 4.7) when considering vaccinated cases only. In a subset of countries (NL, NO, UK) further adjustment for infections (e.g. ILI), TIV and time-dependent covariates was possible. There, the unadjusted pooled RI was 3.2 (95% CI: 1.8, 5.6). RI was lowered to 1.7 (95% CI: 0.8, 3.4) after adjustment for calendar month and 1.4 (95% CI: 0.7, 2.8) after adjustment for ILI, URTI and GI infection which is the main finding. There were no observed significant interactions between GBS and age, infections, TIV or MIV. The formula for the excess cases calculation is exposed rate/ unexposed rate x exposed person time/ total vaccine doses (estimated).

5.2 Paper II: Rates of protection were not influenced by interferon beta treatment

5.2.1 Pandemic H1N1 vaccination in 2009

All MS patients were vaccinated and received DMTs, and for the whole group, only 27.4% had protective titres against H1N1 10 months after vaccination, compared to 43.5% of the controls after 12 months ($p = 0.006$). Adjusted for gender and age in

four categories with logistic regression, the difference was no longer significant. We found reduced protective titres among patients receiving glatiramer acetate (21.6%), natalizumab (23.5%) and mitoxantrone (0.0%) but similar titres for patients receiving interferon beta (44.4%) compared to controls (43.5) (p = 0.002). Self-reported flu symptoms, flu symptoms among family members, oseltamivir or adjustments for mitoxantrone more than 1 year prior to vaccination did not influence the results.

5.2.2 H1N1 antibody specific responses to seasonal influenza vaccination in 2010

In the 2010 season, there were both vaccinated and unvaccinated patients. For the whole group of MS patients, rates of protections were similar to the controls (69.4% versus 71.2%, respectively; p = 0.84). Adjustment for gender and age in logistic regression did not influence the result (p = 0.88). Unvaccinated MS patients had significantly lower rates of protection as compared to vaccinated (40.3% versus 69.4%, respectively; p = 0.004). We found reduced protective titres among patients receiving glatiramer acetate (58.3%), natalizumab (75.0%) and mitoxantrone (25.0%) compared to patients receiving interferon beta (88.2%) (p = 0.05).

5.2.3 H3N2 antibody specific responses to seasonal influenza vaccination in 2010

Rates of protective titres in vaccinated patients were lower than in controls (59.2% versus 79.5%, respectively; p = 0.024) and remained significant after adjusting for age and sex (p = 0.011). We found reduced protective titres among patients receiving glatiramer acetate (41.7%), natalizumab (50.0%) and mitoxantrone (25.0%) compared to patients receiving interferon beta (88.2%) (p = 0.012).

5.2.4 Relapses after vaccination

In 2009, 10 out of 126 patients (7.9%) reported MS exacerbations within two months of vaccination. In 2010, four out of 51 patients (7.8%) reported exacerbations.

5.3 Paper III: Fingolimod and natalizumab confers reduced influenza vaccine efficacy

5.3.1 H1N1 antibody specific responses to seasonal influenza vaccination in 2012/ 2013

In mixed linear regression, ln(H1N1) HI titers showed a significant interaction between time and medication after adjustment for age and gender ($p < 0.001$). Titres increased significantly at all study visits after vaccination compared to pre-vaccination titers in MS patients treated with interferon beta-1a/1b and glatiramer acetate. Fingolimod and natalizumab provided reduced protection at all time points post-vaccination. The group without medication also had significant increase in antibody titers at three and 12 months post-vaccination. The controls showed significant changes in HI titers with time ($p < 0.001$), with significantly higher titers at three months post-vaccination which waned to be significantly lower than pre-vaccination titres at 12 months post-vaccination. Patients without immunotherapy showed a significant increase in GMT antibody titres at three and 12 months, but not at six months.

In logistic regression, adjustment for age and sex gave only marginal changes. The logistic regression of H1N1 specific titers showed a significant interaction between time and medication ($p = 0.001$). There were significant differences between the therapy groups including the controls ($p = 0.007$) pre-vaccination. The pre-vaccination rates of protection were significantly lower in all medication groups compared to the controls. At three months, interferon, glatiramer acetate and the group without treatment showed no significant differences in protection rates as compared to the controls, while protection rates for fingolimod and natalizumab were significantly lower. Also, at six months, interferon and glatiramer acetate showed protection rates similar to the controls, while protection rates for fingolimod, natalizumab and the group without treatment were significantly lower. At 12 months, interferon, glatiramer acetate, natalizumab and those without treatment showed no significant differences in protection rates as compared to the controls, while protection rates in patients on fingolimod were significantly lower.

An increasing age by 10 years gave 20% decrease in titres in a mixed linear model ($p = 0.003$). Gender did not influence the outcome in mixed linear regression ($p = 0.337$).

5.3.2 H3N2 antibody specific responses to seasonal influenza vaccination in 2012/ 2013

The mixed linear regression of H3N2 showed significant differences according to therapy ($p < 0.001$) and time ($p < 0.001$) but not between therapies and time ($p = 0.234$).

In the logistic regression model of protection, the interaction between time and medication was not significant but all medication groups had significantly increased protection rates post-vaccination at all time points compared to pre-vaccination rates ($p < 0.001$). Nevertheless, MS patients were significantly less likely to be protected when compared to the controls at all time points ($p < 0.001$).

Neither age, nor gender affected the rates of protection ($p = 0.974$ and $p = 0.592$, respectively).

6. Discussion

6.1 Paper I: Methodological considerations

In paper I, the main and fully adjusted analysis showed that the RI of GBS was not significantly elevated after influenza A(H1N1)pdm09 vaccination.

6.1.1 The major studies on GBS after influenza vaccination

Several authors of large studies on GBS after influenza vaccination, point to inconsistent and inconclusive evidence of the association to GBS (Salmon et al, Romio et al) (Stowe et al) (Haber et al).

In paper I, based on a source population of 25 million (NL, NO, UK), the fully adjusted RI showed no significant association between the A(H1N1)pdm vaccine and the risk of GBS. Based on the upper limit of the adjusted RIs we could rule out with 95% certainty that the adjuvanted vaccine would have resulted in more than 2 or 3 excess cases of GBS per 1 million vaccinees. Since a major Norwegian hospital did not contribute cases in time, the population size was lowered from 4.8 to 4.4 million corresponding to the size of the missing catchment population, thus reducing a potential selection bias due to incompleteness. Calendar month adjustment is important because it may serve as a proxy for circulating influenza A(H1N1) virus. The pooled estimates were adjusted for calendar month only and 4/ 7 countries did not report covariates after case collection which might introduce confounding by infection. The pooled results could only be used for stratification and not adjustment.

The parallel case control study by Dieleman et al, using age-specific rates from a source population of 50 million, and a background rate of 1.5 cases per 100.000 person-years, found an adjusted odds ratio of 1.0 (0.3, 2.7) where the absolute effect of vaccination based on the CI ranged from one avoided case up to three excess cases within six weeks after vaccination per 1 million vaccines. The study was adjusted for ILI, URTI and TIV. In the Netherlands, two doses of pandemic vaccine were provided for all patients, which seemed to be associated with a higher risk of GBS in

the matched but unadjusted analyses (Dieleman et al). If the vaccine causes both ILI and GBS, adjustments for ILI would mask a detrimental effect of the vaccine (Salmon et al). Results are difficult to interpret because both adjuvant and non-adjuvanted vaccines, vaccination coverage between countries varied, and case ascertainment was not standardized. Their study found an increased risk of GBS after vaccination (De Wals et al).

The third parallel study (Dodd et al) showed a significant association between the vaccine and the risk of GBS, although not in disagreement with international recommendations for the continued use of the vaccine. In the pooled data analysis for the 42 days following exposure, the RI was 2.42 (95% CI 1.58, 3.72) and in the meta-analysis RI was 2.09 (95% CI 1.28, 3.42). Some adjustments were made for concomitant ILI and gastrointestinal illness, but infections were not included as time varying covariates. As stated previously, the Norwegian data set was excluded because it was obtained solely from a specialist network and conferred potential bias. This third parallel study confirmed the difference between adjuvanted and non-adjuvanted vaccines in their association with the risk of GBS (Sturkenboom 2015)

The corresponding US meta-analysis (Salmon et al) included 23 million vaccinees using a self-controlled risk-interval design with data from six different AEFI monitoring systems. They found an incidence rate ratio (IRR) of 2.35 (95% CI 1.42, 4.01, p=0.0003) and 1.6 excess cases per 1 million people vaccinated, still conferring a positive benefit-risk ratio. No ABC level 4 patients were included and 82% had received TIV before MIV. There was however, possible confounding by seasonality or timing of wild type infection. They used a washout period, where they excluded cases with disease onset during days 43-49 in case the risk of GBS extended beyond 42 days. Conceptually, the data behave as if every patient flipped the same coin whether GBS would occur in exposure or comparison period. If increased GBS after MIV, the coin flip would exceed 50% in the exposure period. This probability gives the incidence rate ratio. Week one was included. The background rate applied was one case per 100.000 person-years as well as age-specific background rates.

In another study, the rate of GBS immediately following pH1N1 vaccination was 57% higher than in person-time unexposed to vaccine (adjusted rate ratio = 1.57, 95% confidence interval: 1.02, 2.21), corresponding to 0.74 excess GBS cases per million pH1N1 vaccine doses (95% confidence interval: 0.04, 1.56) (Wise et al).

A meta-analysis of 39 studies (Arias et al) of influenza vaccines administered between 1978 and 2013 (22 seasonal, 16 pandemic, 1 both) found an overall relative risk (RR) of 1.41 (95% CI 1.20, 1.66) for any vaccine. Pandemic vaccines presented a higher risk, RR 1.84 (95% CI 1.36, 2.50) compared to seasonal vaccines, RR 1.22 (95% CI 1.01, 1.48). Pandemic adjuvanted vaccines were not found to be related to a higher risk compared to non-adjuvanted ones. A very interesting observation regarding pandemic vaccines was that the self-controlled design detected higher estimates compared to other designs. In addition the risks for pandemic vaccines appeared higher in Australia and Taiwan, decreases in North America and is slightly lower in Europe. SCCS are considered as more reliable. It is also possible that the risk among different influenza vaccines varies.

A more recent Norwegian study of the old data (Ghaderi et al) with 46 cases using ICD-10 G61.0 only, found an adjusted hazard ratio (HR) of GBS within 42 days after diagnosis of pandemic influenza of 4.89 (95% CI 1.17, 20.36), and thus a significant association, although with a wide CI. The adjusted HR after vaccination was 1.11 (95% CI 0.51, 2.43) and showed no significant association with vaccination. In paper I, we reported 4 additional cases, 50 in total, excluding the patients in a catchment area of 400.000 persons and adjusting the total population accordingly. Using the background rates of this 4 year study, 2.7 per 100.000 person years, an additional 10.8 cases could be expected in our study, but with an increased total population.

Additionally there is different susceptibility across different populations to different infections (e.g. the GBS variant AMAN in Asia) according to genetic polymorphism (McGarvey et al), (Blum et al).

6.1.2 Possible bias in the major GBS studies

Sturkenboom criticizes the studies by Dodd et al and Romio et al for the following: detection bias and ascertainment bias, shortened follow-up time, decreased diagnostic lag times of formerly underdiagnosed diseases and that victim compensation was initiated. She further criticizes that rapid risk assessments were done in the midst of increased public awareness, thus studies were underpowered and media effects were not excluded, so it could not be distinguished between a vaccine and an awareness effect (reporting bias). As long as there is no evidence-based explanation for the biological mechanisms of Pandemrix causing narcolepsy, we should not immediately discard potentially useful adjuvants (Kobbe).

6.1.3 Timing of vaccination coincided with peak wild type virus circulation

Influenza A(H1N1) disease peaked around the same time the H1N1 vaccine was administered (Salmon et al). The timing of initial MIV availability in VSD coincided with the peak of the second wave of the 2009 influenza A(H1N1) pandemic in late October 2009 (Lee et al). TIV 2009-2010 administration mostly preceded this wave (Greene et al, 2013). The vaccination campaign in Norway coincided with the pandemic influenza peak in 2009. Underreporting of influenza infection leads to detection bias. Only 3.6% had a clinical diagnosis. Based on national surveillance data in Norway, the clinical attack rate was ~ 30% (De Blasio et al).

6.1.4 The original association

The original incident happened in 1976 at Fort Dix and was with Hsw1N1/ influenza A/NJ/76. After further negative results reported for an association between 1978 and 1981, an authoritative paper concluded that the epidemiologic association between GBS and influenza vaccination documented in 1976 was unique and that the causative “trigger agent” in the A/New Jersey (swine) influenza vaccine has not been present in subsequent vaccine preparations (Kaplan et al). In 2004, the US Institute of Medicine reviewed published and unpublished studies performed between 1976 and 2002 and concluded that “the evidence is inadequate to accept or reject a causal

relationship between GBS in adults and influenza vaccines administered after 1976." The reason for the increase was still unclear (Stratton et al, 2004). McGrogan et al provides an excellent review from 1980-2008 but no MFS cases are included.

6.1.5 The importance of background rates

The reported background rates differ in published work (Dieleman et al 1.5/100.000, Salmon et al 1.0/100.000, Ghaderi et al 2.7/ 100.000). A low background rate results in lower absolute or attributable risk and conversely higher background rates in higher risks. Some sources (Sejvar et al, Neuroepidemiology, 2011) may be consulted for age specific background rates.

6.1.6 Spontaneous reporting rates

The reporting rate for serious events was significantly higher for the pandemic H1N1 vaccines than for seasonal vaccines for all age groups except for children less than 5 years (Vellozzi et al, Vaccine 2010). Comparing spontaneous reporting rates with rates based on a follow-up study, it was estimated that the spontaneous rates were 322-fold lower than the study rates! (Carvajal et al).

6.1.7 Differences in methodological reporting

Differences in methodology and data presentation render meta-analytic safety analyses of vaccines difficult in the different and relevant age groups (Poland Vaccine 2011). The 2009 experience re-emphasizes need for harmonization of study protocols, surveillance methods and infrastructures to verify signals and test hypothesis. The pandemic has brought us the beginning of an infrastructure for collaborative vaccine safety studies in the EU, USA and globally (Wijnans et al, 2011).

6.1.8 Criticism of the SCCS method and the Brighton criteria

Case-control or cohort designs are susceptible to selection bias and unmeasured confounders. These systematic errors are reduced by the case-series method.

SCCS is however susceptible to bias if vaccination is timed to minimize the risk of an adverse event (Farrington et al, 1996). SCCS have less statistical power than if compared to historical controls because of the typical large amount of historical data available for the comparison group (Greene et al, 2010). However, power struggles may be somewhat overcome when more than 20 million vaccinees are studied, as in the US and European studies. SCCS is prone to seasonality bias (confounding) if it exists. CCS analytically adjusts for seasonality (Fireman et al).

Vaccinated and unvaccinated subjects may differ in underlying risk for adverse events, as well as a different inherent risk of GBS regardless of vaccine receipt, thus their comparison can result in estimates of the risk of vaccine-associated disease that is biased by residual confounding (Hak et al), (Jackson et al).

6.2 Papers II and III: Methodological considerations

In paper II, MS patients had reduced rates of protection compared to controls. Rates of protection were not influenced by interferon beta-1a/1b treatment but among patients receiving glatiramer acetate, natalizumab and mitoxantrone. A similar pattern emerged after seasonal influenza vaccination in 2010/2011. In paper III, no significant differences in rates of protection against H1N1 for interferon beta-1a/1b and glatiramer acetate were observed as compared to controls. Fingolimod and natalizumab displayed reduced rates of protection.

6.2.1 Comparison of findings with the literature

MS patients without immunosuppressive treatment at the time of vaccination mounted a higher relative increase in influenza specific T-cell frequencies measured with the interferon gamma-enzyme-linked immunospot assay (IFNγ-ELISPOT) than the controls and mean antibody responses against influenza A virus were increased in both populations after 2 weeks measured by the HI method. No increase in T-cells responsive to myelin basic protein (MBP) or recombinant myelin oligodendrocyte protein (MOG) was observed, arguing against a general immune stimulation after vaccination (Moriabadi et al).

All DMTs listed below are not investigated for influenza vaccine responses. In these cases, studies on immunization responses by other antigens are provided.

Alemtuzumab

Seasonal influenza vaccine had suboptimal immunogenicity in islet transplant recipients (Silva Jr et al). Further, retained humoral immunity was found to mumps, rubella, varicella and EBV, as well as normal responses to diphtheria, inactivated polio, tetanus and meningococcal vaccines. Vaccination within 2 months of treatment may not be as effective. Live vaccines are advised against following treatment (McCarthy et al).

Cladribine

No data exists on influenza vaccine efficacy. Live vaccines are not recommended (NMSS).

Dimethyl Fumarate

One recent study found preserved immune response to tetanus-diphteria toxoid (Td), polyvalent pneumococcal vaccine and meningococci (von Hehn et al)

Fingolimod

In paper II, no fingolimod patients participated. In paper III, fingolimod-treated patients provided reduced protection at all time points post-vaccination.

Lymphocyte redistribution might confer a reduced immune response, and thus reduced protection after vaccination. However, MS patients have been shown to have few infections and related complications and were able to mount antigen-spcific immune responses in vaccination studies (Mehling et al, Neurology 2011).

Similar response rates to influenza immunization were found in treated and healthy controls and included 14 patients treated with fingolimod (Mehling et al, Annals of Neurology 2011). Seroprotection was measured in the 2008/2009 and the 2009/2010 seasons. It included 14 patients similar to the 15 fingolimod treated patients in our

study. The AS03 adjuvanted pandemic vaccine used in 2009 was manufactured to offer protection at low antigen doses (antigen sparing) and for the longest period of time as possible due to the emergency character of the situation as instigated by the WHO. They measured anti-influenza A and B by ELISA, and a concentration of >10VE/mL was considered as protection, as recommended by the manufacturer, although this is not internationally recognized as a protective titre. Their serum collection times at 0, 7, 14 and 28 days post vaccination were different from our sampling at 0, 3, 6 and 12 months post vaccination. Further, the baseline (pre vaccination) rates of seroprotection were 71% for fingolimod vs 50% in controls for influenza A. The vaccination model used did not take into account the complexity brought by an influenza infection or any other virus infection and was underpowered to evaluate clinical endpoints by an influenza infection. In paper III we measured seroprotection in the 2012/2013 season by the HI method, which is an internationally recognized protective titer. In paper III, the controls had the highest rates of seroprotection pre vaccination. Thus these two studies are not directly comparable.

T cell dependent and independent responses to neoantigens were identical in healthy volunteers in the placebo and 0.5 mg fingolimod groups for anti-keyhole limpet haemocyanin IgG, and comparable for the 23-valent pneumococcal vaccine as well as anti-tetanus toxoid immunogenicity (Boulton et al).

One study provides Class III evidence that patients with MS taking fingolimod did not significantly increase the avidity of the IgG antibody response targeting influenza following influenza vaccination (Mehling et al, 2014).

Response rates to influenza vaccination were reduced at 3 and 6 weeks post vaccination, and against tetanus toxoid at 3 weeks as compared to patients receiving placebo. A larger patient population (n=95 fingolimod vs 43 placebo, all MS patients) was studied during the 2010/2011 season. Many of these patients had already been exposed to the adjuvanted pandemic vaccine which induced durable antibody responses to the California strain (H1N1pdm09). They only studied their patients for up to 6 weeks post vaccination, as 3 weeks is the suggested sampling point for testing

of vaccine immunogenicity by EMA. The HI method was applied. The study provides provide Class I evidence that fingolimod treatment decreases vaccination-induced immune responses compared to placebo. Of note, exclusion criteria were H1N1 vaccination or confirmed or suspected H1N1 infection within 3 months before randomization. However heterogenous vaccines were administered in different countries which may have different constituents, and 2/3 patients were already seropositive for 2/3 strains, rendering only 30% of patients a possibility to seroconvert (Kappos et al).

In the study by Kappos et al, the HI method was applied, and this study is therefore more comparable to our study than the study by Mehling and colleagues. Class I evidence is provided by Kappos et al that fingolimod treatment decreases vaccination-induced immune responses compared to placebo, and is compatible with our results. A somewhat simplified approach when comparing the studies by Mehling et al and Kappos et al with our paper III, one may say that the two studies using the HI method (Kappos et al and paper III) are in agreement, while the study using ELISA (Mehling et al) does not agree with the other two.

Glatiramer Acetate

Scarce data is available on the influenza vaccination response for glatiramer acetate except paper II (Pellegrino et al) and paper III. In paper II, we found a reduced response. On the other hand, in paper III we found a similar response as for interferon-beta treated patients which again was similar to the controls. This may be related to the novelty of the circulating virus in the setting of paper II. Unfortunately the existing literature gives no clues to resolve this, and would be an area of interest in future studies.

Interferon-beta 1a/b

Interferons provide superior consistencies in vaccine efficacy in all our work and all other published studies.

Interferons have an antiviral effect and do not impair immune response in MS (Koerner et al). The first study to show the impact of DMTs in MS patients with and without interferon came in 2003. Both groups mounted similar responses to TIV 2002/2003 as measured by the HI method. Patients on glatiramer acetate within 1 year, or steroids within 1 month were excluded. Sampling was done at day 0, day 21 and day 28. Routine vaccination in MS was suggested (Schwid et al). Influenza vaccination in 26 MS patients clinically and radiologically responding to IFNβ-therapy, frequencies of influenza-specific T cells and concentrations of anti-influenza A and B IgM and IgG increased comparably in MS patients and healthy controls. ELISA and ELISPOT were used. Blood was drawn at day 0, 7, 14 and 28 days after vaccination. Before vaccination, 54% of patients and 64% controls fulfilled the seroprotective criteria for influenza A, as given by the manufacturer. (Mehling et al, 2013).

Mitoxantrone

Little data is available on the effect in influenza vaccination except paper II. Here we found that 0 % of patients were protected after pandemic vaccination and 25 % after seasonal H1N1 vaccination in the following year. Further, paper II is missing timing aspects of vaccination on mitoxantrone, since it is administred every 3 months which could interfere with the response (Pellegrino et al). One reasonable recommendation is that immunization should be done between drug cycles (Loebermann et al).

Natalizumab

This drug might confer a reduced immune response, and thus reduced protection after vaccination.

In paper II, we reported lower protection rates during natalizumab therapy using the HI method in 17 patients vaccinated during 2009/2010 sampled at 10 months and in 8 patients vaccinated in 2010/2011 sampled at 6 months.

In paper III, we found significantly reduced protection rates at 3 and 6 months compared with the controls, but at 12 months the reduced protection rates were more similar to and not significantly different from that of the controls.

TIV was provided in a cohort of 17 RRMS patients vs 10 controls vaccinated in the first quarter of 2010 and sampled at 4, 8 and 12 weeks postvaccination. The method used was an in-house developed ELISA. They found a lower response in titres of anti-influenza A IgG in natalizumab treated patients compared to controls, which is compatible with our results in paper III. The patient's response to influenza B was similar as the controls. We did not analyse the response to influenza B, due to lack of sensitivity in the HI method (Vågberg et al).

Another study introduced a neoantigen, the keyhole limpet hemocyanin (KLH) which is not an influenza antigen on three occasions separated by two weeks, in 30 patients with RRMS and monitored these 28 and 56 days postvaccination by ELISA and found the proportion of responders to be similar in untreated and natalizumab treated MS, and different from our results. Primary immunization (neoantigen) and secondary immunization (recall antigen) responses were not affected (Kaufman et al).

Rituximab

The humoral response (influenza antibody titres and/ or seroconversion or seroprotection rates) was impaired by high-dose i.v. rituximab-treatment in 10/11 studies in rheumatologic patients. Repeated vaccination was not convincing in terms of obtaining protection. It is recommended that future research additionally studies B and T cellular responses (Dos Passos et al).

Teriflunomide

Teriflunomide and vaccination (TERIVA) found sufficient protection to all strains for influenza vaccination in the 2011/2012 season. There was a slightly diminished response in the 14 mg daily group. The lowest seroconversion rates were observed for the H3N2 strain in all 3 groups. A high proportion of patients already had detectable antibodies for each influenza strain at baseline (Bar-Or et al, 2013).

Another study by the same principal author provides Class II evidence that in healthy subjects treated with teriflunomide, antibody titer responses to rabies vaccination are lower than with placebo but sufficient for seroprotection (Bar-Or et al, 2015).

6.2.2 Cellular assays

The field of virology lacks consensus on the correlates of protection for cellular immunity in reducing severe influenza infection, transmission or disease outcome. Unlike serological methods such as the standardized haemagglutination inhibition assay, there is a large degree of variation in both the types of assays and method of reporting cellular outputs. It is critical to standardize assays across sites to facilitate direct comparison between clinical trials (Coughlan et al).

Unfortunately, we did not systematically collect lymphocyte cell counts in any of the studies, which would enable us to answer more questions and better. On the other hand, from clinical experience, most fingolimod treated patients experience lymphopenia at the level of 0.2-0.5 x 10^9/L, but this is not the case for natalizumab treated patients, which usually exhibits a normal lymphocyte count. Thus, a general mechanism of reduced vaccine response due to lymphopenia is not likely. However we cannot rule out that lymphopenia during fingolimod therapy, possibly due to low level of subtypes of lymphocytes, has an effect on the vaccine response and this should be investigated in future studies.

7. Conclusions

7.1 Paper I: Collaborative vaccine safety studies, excess cases of GBS and risk-benefit from vaccination in the healthy population

The safety of the pandemic vaccine was assessed in an unprecedented fashion in the EU. Background rates of 12 adverse events of interest were assessed in eight countries in four months. A CCS and SCCS on GBS were conducted in seven countries. Expert committees at the EMA were informed on a timely basis. The VAESCO experience identified five critical main issues for the future: sustainability, governance, purpose, structure and ethical and administrative approvals (Chen et al).

Paper I proves that pharmacovigilance and collaboration can be mounted basically from scratch on a national, continental, and global (Dodd et al) basis. This was facilitated by some existing infrastructure e.g. SYSVAK. Based on the upper limits of the pooled estimate we could rule out with 95% certainty that the number of excess GBS cases after influenza A(H1N1)pdm09 vaccination would be more than 3 per million vaccinated.

AEFIs (in this case GBS) should be interpreted in the context of protection against morbidity and mortality (Fiore et al, 2010). The risk of GBS due to influenza vaccination outweighs the risk of GBS due to influenza virus infection. The balance of risks favors influenza vaccine over its avoidance - particularly in the setting of pandemic influenza (Poland et al, Lancet 2013). The pandemic vaccination programme prevented 700.000 - 1.500.000 clinical cases of influenza, 4000-10.000 admissions, and more than 200-500 deaths (Borse et al). Frequent hand-washing and correct respiratory hygiene are proven effective in preventing respiratory illness including influenza (Cowling et al). However, vaccination is the mainstay of prevention.

7.2 Paper II: Therapy specific reduced long-term vaccine efficacy in MS

MS patients receiving DMTs other than interferon beta showed reduced influenza vaccination response and a vaccine response analysis should be considered. In cases of insufficient protection, a second dose of the vaccine should be discussed.

7.3 Paper III: Monitoring vaccine efficacy and implications for re-vaccination

MS patients who received fingolimod or natalizumab, but not interferon beta-1a/1b or glatiramer acetate had reduced protection rates after influenza vaccination. These findings suggest that MS patients receiving fingolimod or natalizumab should be considered for a second dose of the vaccine in cases of insufficient protection.

7.4 Papers II and III: Implications for medicine manufacturers

Our results further indicate that new immunomodulatory treatment regimens should be systematically evaluated for the influence on influenza-specific vaccine responses. This feature seems to have been implemented as healthy subjects are exposed to new and forthcoming DMTs (Ufer et al).

Potential side-effects of chronic treatment with second and third generation drugs are unknown. Given the immunosuppressive properties, careful long term monitoring of potential infectious and other side-effects is critical (Bridel et al).

8. Future prospects and re-vaccination

8.1 Feasibility of international studies of rare events

The Accelerated Development of Vaccine benefit-risk Collaboration in Europe (ADVANCE) was formed in 2013 for 5 years (ADVANCE). Currently, no stable funding exists for conducting collaborative vaccine benefit and risk monitoring on a European level. Health Institutes monitors the routine vaccination programs, and pharmacovigilance centres and regulatory authorities monitor product safety. EMA licenses and monitors safety and effectiveness across the EU member states. The agency can request manufacturers of vaccines to conduct post-authorization studies on effectiveness and safety. ECDC supports and give feedback to the member states about vaccination programs. Proper communication between manufacturers and public health organs is not well established (Sturkenboom 2018). A code of conduct has been developed in the interaction between public and private sectors (Kurz et al).

A global surveillance apparatus will facilitate timely and effective evaluation of vaccine safety signals for newly developed vaccines and is shown feasible in 16 countries across all WHO regions (Guillard-Maure et al).

Robust international pharmacovigilance systems may assess potential epidemiological associations between serious and rare AEFIs in any setting. A proof-of-concept study has shown, for the first time, that using standardized procedures is feasible, can produce reliable results and has the potential to characterize differences in risk between vaccine strains that should permit rapid post-marketing evaluation of safety signals for serious and rare AEFIs for new and existing vaccines in all settings (Perez-Vilar et al).

8.2 Re-vaccination and tailored medicine

One study has shown that in healthy persons, revaccination of low responders with the A(H1N1)pdm09 vaccine was required for long-term protection (Pathirana et al).

Young children up to 3 years old who have not previously received an influenza vaccine are recommended for two half doses of vaccine. Older children who have not previously received an influenza vaccine are recommended for two doses of vaccine.

Re-vaccination has not been done systematically in an MS population, thus the effect is less certain. It should therefore be studied in the future to increase efficacy and hopefully duration of protection in MS. To evaluate vaccination success in MS receiving DMTs, antibody testing should be performed 4 weeks after vaccination. If no titre increase, revaccination should be considered (Loebermann et al). I recommend that future studies do sampling at day 0, day 30 and day 90 if feasible.

Optimal criteria for timing and dosing have been included in recommendations for individual vaccines in related disciplines. Determination of serum antibody titres is optional to assess booster requirements (Aikawa et al), (McMahan et al). Semiquantitative assessment of antibody production, e.g. comparison of antibody levels pre and post vaccination could differentiate the level of immune suppression and immune failure. Diagnostic immunization with killed vaccines has mainly been restricted to booster immunization with pneumococcal polysaccharide vaccine. Further studies are needed on treatment indications for vaccination, passive immunoprophylaxis by immunoglobulin replacement and temporary addition of antibiotics, antifungal and antiviral medication. Comparing SID patients with different underlying diseases with comparable immune impairment could provide larger populations for these studies (Eibl et al)

8.3 Possible outcomes of future research

8.3.1 Unanswered questions and challenges

Several questions remain unanswered, such as: what are the specific targetable modes of action at the cellular level in the interaction between the virus, underlying disease, the vaccine with or without adjuvant, and the specific immunomodulation in question.

With rapid advances in biotechnology and biomedical science, DNA vaccines, recombinant vector vaccines, novel types of vaccines, adjuvants and delivery systems are in development and testing. Knowledge on general safety is not abundant and monitoring for rare AEFIs will become important (Chen et al). Further, are universal vaccines on our doorstep (Arnold), and what can we expect from adding nanoparticles into vaccines? (Treuel et al). A CBC with differentials only cover 2% of the immune system, thus 98% is not surveilled (lymph nodes, other lymphoid organs and tissue) (Havla et al). One may therefore ask if we really see the whole picture of the immuneresponse.

Do we have the money available for absolute scrutiny and control of the association between infection, vaccination and neurological disease? A large cohort with repeated exposure assessments, including silent infections and follow-up of is needed. Needless to say, this will be costly and logistically challenging (Trøgstad et al 2017).

Finally, is it possible that MS patients on selected DMTs never will obtain an adequate influenza vaccine efficacy even after a booster-dose? This should be investigated in future studies.

8.3.2 A multidisciplinary approach

Management of DMTs includes history of infections, risks of exposure to microorganisms, concomitant autoimmune disease/ comorbidities, history of immunosuppression and the immune status of the patient. Individual risk stratification when choosing a DMT and rigorous pharmacovigilance is essential. Real life experience has shown that infectious risks and serious adverse events might not be recognized in clinical trials given short follow-up, selected study populations and low numbers treated. Special awareness and a multidisciplinary approach are necessary. Development of new drugs for treatment should also increase safety in terms of risks of infections, ideally preserving natural defense mechanisms (Winkelmann et al). Recent DMTs enable effective treatment; however they are also prone to exhibit side effects. Therefore, interdisciplinary risk management is necessary (Havla et al).

Vaccination may be recommended on an individual basis, after evaluation of the risk-benefit based on assessment of the immunocompetence, based on patient history and laboratory tests: CBC with differentials, subsets of lymphocyte, complement hemolytic activity, complement components, serum Igs immunoglobulin concentrations and IgG subclasses, antibody titres after natural exposure to infection or previous vaccination, response to primary and booster immunization. Functional studies on a cellular level may be necessary (Eibl et al).

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Guillain-Barré Syndrome and Adjuvanted Pandemic Influenza A (H1N1) 2009 Vaccines: A Multinational Self-Controlled Case Series in Europe

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Abstract

Background: The risk of Guillain-Barré syndrome (GBS) following the United States' 1976 swine flu vaccination campaign in the USA led to enhanced active surveillance during the pandemic influenza (A(H1N1)pdm09) immunization campaign. This study aimed to estimate the risk of GBS following influenza A(H1N1)pdm09 vaccination.

Methods: A self-controlled case series (SCCS) analysis was performed in Denmark, Finland, France, Netherlands, Norway, Sweden, and the United Kingdom. Information was collected according to a common protocol and standardised procedures. Cases classified at levels 1–4a of the Brighton Collaboration case definition were included. The risk window was 42 days starting the day after vaccination. Conditional Poisson regression and pooled random effects models estimated adjusted relative incidences (RI). Pseudo likelihood and vaccinated-only methods addressed the potential contraindication for vaccination following GBS.

Results: Three hundred and three (303) GBS and Miller Fisher syndrome cases were included. Ninety-nine (99) were exposed to A(H1N1)pdm09 vaccination, which was most frequently adjuvanted (Pandemrix and Focetria). The unadjusted pooled RI for A(H1N1)pdm09 vaccination and GBS was 3.5 (95% Confidence Interval (CI): 2.2–5.5), based on all countries. This lowered to 2.0 (95% CI: 1.2–3.1) after adjustment for calendartime and to 1.9 (95% CI: 1.1–3.2) when we accounted for contra-indications. In a subset (Netherlands, Norway, and United Kingdom) we further adjusted for other confounders and there the RI decreased from 1.7 (adjusted for calendar month) to 1.4 (95% CI: 0.7–2.8), which is the main finding.

Conclusion: This study illustrates the potential of conducting European collaborative vaccine safety studies. The main, fully adjusted analysis, showed that the RI of GBS was not significantly elevated after influenza A(H1N1)pdm09 vaccination (RI = 1.4 (95% CI: 0.7–2.8). Based on the upper limits of the pooled estimate we can rule out with 95% certainty that the number of excess GBS cases after influenza A(H1N1)pdm09 vaccination would be more than 3 per million vaccinated.

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Competing Interests: Silvana Romio, Daniel Weibel, Henning K Olberg, Nick Andrews, Henrik Svanström, Ditte Mølgaard-Nielsen, Anders Hviid, Maryse Lapeyre, Agnès Sommet, Christel Saussier, Anne Castot, Harald Heijbel, Lisen Arnheim-Dahlström, Mees Mosseveld, Nicoline van der Maas, Bart C Jacobs, Tuija Leino, Jann Storsaeter, Kari Johansen, and Piotr Kramarz have no conflicts of interest. Jeanne P Dieleman has been involved in studies for pharmaceutical companies (i.e., GSK, Sanofi, Astra-Zeneca, Pfizer). None of these had any conflict with the present study. The affiliation (i.e. University of Bath) of Corinne S de Vries and Cormac Sammon has research and consulting contracts in place with Novartis vaccines and with GSK pharmaceuticals. The authors do not personally benefit from these contracts; all financial compensation is to the University of Bath and not to the authors. Martijn Schuemie was employed at the Erasmus University Medical Center, Rotterdam at the time of the study. Since January 1st 2013 (after completion of the study), he is employed at Janssen R&D. Terhi Kilpi is a principal investigator of a nationwide Finish effectiveness study of the 10-valent pneumococcal conjugate vaccine, a collaborative study, for which her institute has received funding from GSK. Par Sparen received a grant from Glaxo Smith Kline in 2010 to for a retrospective, observational register based cohort study to evaluate the safety of GSK Biological's H1N1 pandemic vaccine administered in Sweden according to local vaccination policy. Miriam CJM Sturkenboom: is head of a research group that occasionally conducts research for pharmaceutical companies including Pfizer, EliLilly, Boehringer, AstraZeneca and Novartis. None was related to this topic.This does not alter our adherence to all the PLOS ONE policies on sharing data and materials.

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Introduction

During the influenza A (H1N1) 2009 pandemic, new monovalent adjuvanted and non-adjuvanted influenza A(H1N1)pdm09 vaccines were introduced in Europe. Immunogenicity and safety was in line with the "Committee for medicinal products for human use (CHMP) Note for Guidance", but safety data were limited [1–3]. Vaccination campaigns started in autumn 2009 at the peak of the pandemic in Europe.

A key safety concern identified in planning the pandemic vaccination campaigns was the potential association between Guillain-Barré syndrome (GBS) and influenza vaccines; this concern stemmed from an association observed in the USA in 1976 between swine flu vaccination and GBS [4]. Subsequent prospective surveillance studies and retrospective epidemiological studies on seasonal influenza vaccines used in 1978, 1992, 1993, and beyond showed no or modest increases in the risk of GBS [5–8]. Despite this, the US Food and Drug Administration (FDA), the World Health Organization (WHO) and the European Medicines Agency (EMA) recommended active monitoring of a potential association between the influenza A(H1N1)pdm09 vaccine and GBS.

In Europe, GBS primarily presents as an acute inflammatory demyelinating polyradiculoneuropathy (AIDP) [9]. Three to ten per cent of GBS patients die and an estimated 20% experience continued disability for more than six months [10]. Prospective studies in developed countries have estimated an incidence rate of 2 per 100,000 population per year with an increased risk with age and in males [11]. GBS is thought to be primarily triggered by a preceding respiratory or gastrointestinal infection [12].

The European Centre for Disease prevention and Control (ECDC) commissioned the VAESCO (Vaccine Adverse Events Surveillance and Communication) consortium to study the potential association between influenza A(H1N1)pdm09 vaccine and GBS. A case control study was conducted for a rapid initial assessment with a large-scale more extensive prospective SCCS study carried out in parallel. The VAESCO case control study was based on 104 cases in five European countries and showed no association between A(H1N1)pdm09 vaccine (mostly adjuvanted with AS03) and GBS [13]. In this paper we present the results from the VAESCO SCCS study which included three times the amount of cases.

Methods

Setting and design

The VAESCO consortium conducted a prospective self-controlled case series (SCCS) study to investigate the association between influenza A(H1N1)pdm09 vaccination and GBS. A SCCS is a case-only study comparing the incidence of disease during risk and non-risk periods within the same person, inherently controlling for measured and unmeasured confounding factors that remain stable over time [14].

The VAESCO consortium was initiated and core funded by ECDC with the aim of improving post licensure vaccine safety in Europe. It is coordinated by the Brighton Collaboration Foundation and includes partners from public health organizations, regulatory authorities and academic research institutions in Europe.

Centers from Denmark (DK), Finland (FI), France (FR), Netherlands (NL), Norway (NO), Sweden (SE), and the United Kingdom (UK) contributed to the study. All centers used a common protocol and applied the standardised Brighton Collaboration GBS case definition for case classification [9]. Implementation of the protocol and data collection differed per country based on ethical requirements and the healthcare structure. Data harmonization, transformation, and pooling used methods and tools derived from the EU-ADR (Exploring and Understanding Adverse Drug Reactions) project [15]. Centers created harmonized input files according to well-defined instructions. These data files were generated directly from automated resources or manually using customized electronic case report forms. The harmonized input files were transformed using a standardized JAVA-based program (Jerboa® version 2.6.0, September 2010, Erasmus University Medical Center, Rotterdam, Netherlands). Only anonymous and aggregated de-identified information without dates of disease or exposure were shared for individual patient level data pooling and centralised analysis. Consent forms, original data and Jerboa input files were retained at the local centers. Quality control and verification of transmitted data was done at the central data management and analysis center (Erasmus University) in close collaboration with the other centers. All centers commented on the data and results prior to release.

Source and study population

The total source population exceeded 50 million (M) subjects, with most countries recruiting cases on a national level (NO (4.4 M), SE (9 M), FI (5.5 M), DK (5 M), NL (16 M)). In the UK, the General Practice Research Database (GPRD) (5 M) was used and in France specialized hospitals with a large but undefined catchment area participated. Case recruitment started on 1st November 2009 and lasted maximally until 1st November 2010.

The study population encompassed all cases with GBS or its variant Miller Fisher syndrome with onset of disease during the study period.

Case recruitment-procedures are described in Table 1. Completeness of recruitment was verified retrospectively at the end of the study period by comparing recruited cases with diagnosed case lists (see Table 1). Additional cases identified in this way were

Table 1. Sources of cases, exposure and covariate information per country.

	Cases recruitment	Exposure Information	Covariates during follow-up	Potential bias
DK	Cases were identified from the National Patient Register using primary discharge diagnoses only (ICD-10: G61.0). Case validation based on retrospective chart review.	Vaccination registry	None (only from case hospital charts)	Cases: not all charts available No ability to control for time varying confounders
FI	From hospital Discharge and hospital outpatient records, primary diagnoses (ICD-10 G61.0). Case validation based on retrospective chart review	Vaccination registry	None (only from case hospital charts)	Cases: not all charts available No ability to control for time varying confounders
FR	Cases were identified prospectively through neurologists in 7 reference hospitals in FR. Patients needed to provide informed consent. Completeness was verified against pharmacy data (immunoglobulin prescriptions) and showed incomplete reporting (<50%), Vaccination status of non-reported cases could not be verified since linkage to vaccination registry required consent.	Ad hoc A(H1N1)pdm09 vaccination registry	Hospital charts and interview, only for period prior to GBS	Incompleteness and potential selection bias cannot be excluded. No ability to control for time varying confounders
NL	Cases were identified prospectively through neurologists. Completeness was verified retrospectively by checking against the claims codes in each of the reporting hospitals. Missing patients were included retrospectively in hospitals that were reporting at least one case prospectively.	GP medical record	GP medical record	Small potential for misclassification of exposure since A(H1N1)pdm09 vaccination could also be provided through public health agency for parents of young children
NO	Nationwide neurologist reporting network, group of neurologists. Case validation based on review of GBS experts	Vaccination registry	Neurologists, Hospitals, and GPs	Potential selection due to incompleteness Information on co-variates collected differently for period prior to GBS.
SE	Cases of GBS were identified through seven neurology assessment labs where GBS cases are laboratory confirmed for a population of 9.4 million. Informed consent needed to be obtained from all cases. Completeness of cases was checked in the National Patient Registry for part of the country. Recruitment was incomplete because of delays in consent and non-consent. It was not possible to assess whether this non-response differed by vaccination status and hence selection bias cannot be excluded.	By interview at end of follow-up, recall bias cannot be excluded.	By interview for cases at the end of follow up. change in region over time. Should not be used for adjustment	Consent required, potential selection bias. Recall bias (differential recall over time)
UK	Each case was identified in the General Practice Research Database by using appropriate READ codes (F370.00, F370000, F370100, F370200, F370z00). Case verification was done using any hospital letters, discharge summaries and GPs' notes recorded as free text. No major selection to be expected	Automated GP records, no recall bias. Non-differential misclassification possible since some persons might have been vaccinated outside of GP office.	GP records	Misclassification of cases due to lack of information on test results

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included retrospectively where possible. For each subject, follow-up started at the beginning of the study period or date of birth if born after the start of the study period. Follow-up ended with the end of the study period or death occurring prior to the end of the study period.

The earliest date of onset of neurological symptoms was the index date. If the date of first symptoms could not be retrieved the date of diagnosis or hospitalization was used. Informed consent was required in SE and FR. Case characteristics were obtained from neurologists or from discharge letters and used to classify cases according to the Brighton Collaboration GBS Case Classification using the Automated Brighton Classification (ABC) tool (www.brightoncollaboration.org).

Vaccine Exposure

The primary exposure of interest was vaccination with adjuvanted or non-adjuvanted A(H1N1)pdm09 vaccine as recorded in vaccination registries (FR, DK, FI, NO), General Practitioners' (GP) records (NL, UK), or patient interview (SE). The risk period began the day after vaccination and ended 42 days later. If two doses were administered, the risk period of the first dose ended when the second dose was administered. Brand specific information was collected for each influenza A(H1N1)pdm09 vaccination.

Covariates

Information on several time varying risk factors for GBS was collected during follow-up including seasonal influenza vaccination, influenza-like illness (ILI), upper respiratory tract infections (URTI), and gastrointestinal infections (GI). Each of these covariates was assigned a 42-day risk period. The risk period began on day one of onset of ILI, URTI, or GI or of seasonal influenza vaccine receipt and ended 42 days after onset or exposure. Covariate data were not collected in DK and FI. In FR, covariate data were collected from neurologists at case occurrence for the period prior to GBS only, whereas in SE data on covariates were collected by interview at the end of follow-up. In the UK, NL, and NO general practitioner records were used to collect information on covariates throughout the follow up period; NO also assessed covariates reported by neurologists at the time of case data collection, leading to a potential for differential data collection over time. To adjust for seasonal effects, changes in circulation of the wild type influenza A(H1N1)pdm09 virus and

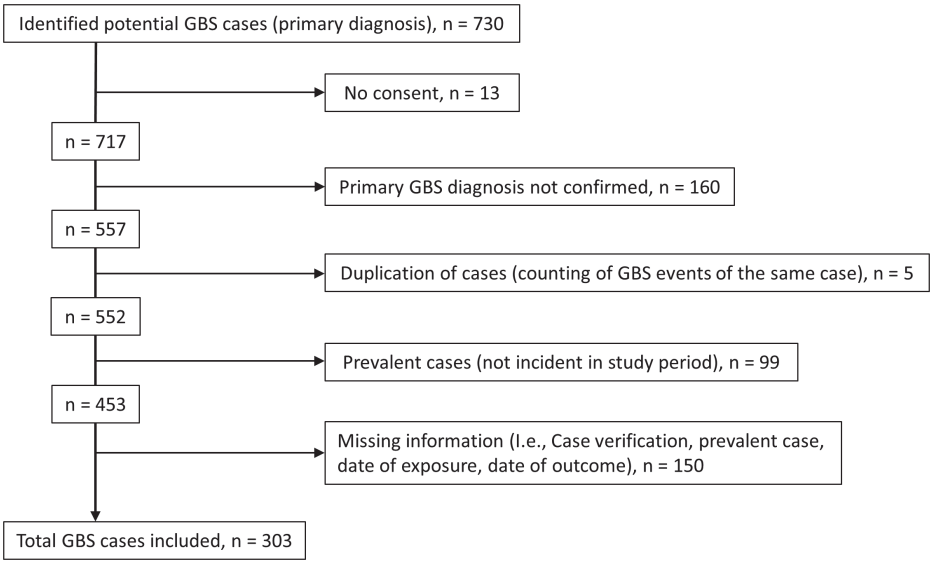

Figure 1. Flowchart of case inclusion.
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differences in case inclusion over the observation period we considered calendar month as a time varying covariate.

Statistical Analysis

The RI for the association between A(H1N1)pdm09 vaccine and GBS was estimated using a conditional Poisson regression analysis. This was done for each country separately. Adjustment for calendar month was possible in all countries, whereas further adjustment for ILI, URTI, GI, and seasonal influenza vaccination was only possible in NL, UK, and NO. Sensitivity analyses were used to assess the effects of misclassification of exposure and confounding. An analysis using vaccinated cases only and an analysis using the pseudo-likelihood approach explored confounding by contra-indication to influenza A(H1N1)pdm09 vaccination [14]. A sub analysis was done to assess the impact of residual confounding by ILI, URTI, seasonal influenza vaccination, and GI infections. Misclassification of the risk period was investigated by applying risk periods smaller than 42 days. In order to study effect modification by infections occurring just prior to GBS onset, stratified analyses were carried out for age, sex, history of GBS,

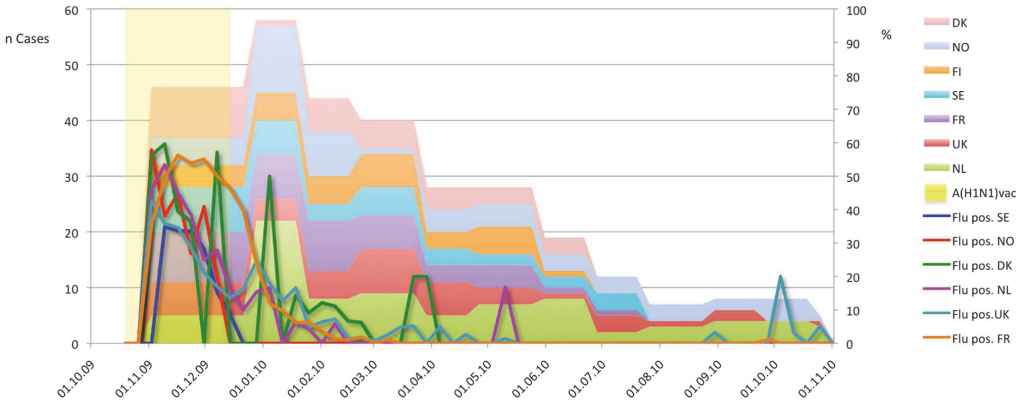

Figure 2. Inclusion of GBS cases (DK, FI, FR, NL, NO, SE, UK), influenza A(H1N1)pdm09 immunization period (influenza A(H1N1)vac), and percentage of flu positive cases among all tested per country (Flu pos. DK, …, Flu pos. UK; Source: ECDC 2011) over total study period.
doi:10.1371/journal.pone.0082222.g002

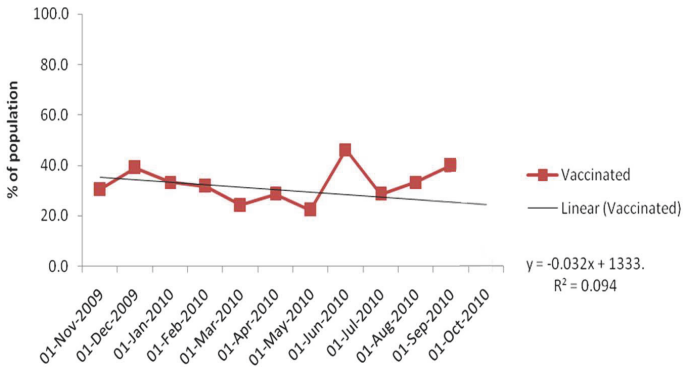

Figure 3. Inclusion of vaccinated cases (% of population) over study period.
doi:10.1371/journal.pone.0082222.g003

and prior infections (ILI, URTI, GI) in UK, NL, and NO. The country specific estimates were pooled applying a random effects model. All analysis used SAS v9.1 (Cary, North Carolina).

Results

In total 730 potential GBS cases were identified during the study period. Of these, 427 cases were excluded (see figure 1), leaving 303 GBS cases in the study population. Case inclusion declined over time from 133 cases in the first three months to 18 in the last three months (Figure 2). The percentage of influenza A (H1N1) pdm09 vaccinated cases did not change significantly over time ($R^2 = 0.094$; Figure 3).

Cases had a mean age of 50 years (SD: 4.1) ranging from 45 (SD: 20.8) years in the NL to 56 (SD: 19.5) years in NO, less than 10% were younger than 20 years. On average the follow-up period was 321 days. Case classification differed by country, primarily depending on the type of data source used for case recruitment. Of all cases, 36% were classified as Brighton Collaboration level 1, 26% as level 2, 13% as level 3, and 25% as level 4a. In 69 cases electrophysiology (mostly AIDP) had either not been performed for diagnosis or was not recorded. On a scale from 0 to 6, with 0 meaning complete physical fitness and 6 meaning death, the disability score was most frequently 4 (30.6%) (Table 2).

Overall, 99 cases (33%) received influenza A(H1N1)pdm09 vaccination, mostly adjuvanted with AS03, before symptom onset (Table 3). Of these, 36 (37%) cases developed GBS within 42 days after a first dose of influenza A(H1N1)pdm09 vaccination whereas 7 cases occurred within the exposure risk window but after a second dose of influenza A(H1N1)pdm09 vaccination.

Few countries could collect data on time-varying covariates over the entire follow-up period. Most countries assessed covariates at the time of case collection, but not afterwards, and therefore these data could not be utilized for adjustments but could be used for stratification. Based on the information collected at case occurrence, 15 cases developed GBS within 42 days after seasonal influenza vaccination and 79 cases developed GBS within 42 days after onset of ILI or URTI (Table 3).

Risk ratio of GBS

The crude country specific RI of GBS during the influenza A(H1N1)pdm09 vaccination risk period compared to the non-risk period varied from a low of 1.6 in FI to a high of 7.7 in DK (based on two exposed cases only), with an overall pooled estimate of 3.5 (95% CI: 2.2 to 5.5). Adjustment for calendar month had a significant impact (RI: 2.0, 95% CI: 1.2 to 3.1). Sensitivity analyses accounting for contra-indication after GBS onset showed a minor change in the calendar month adjusted pooled RI from 2.0 to 1.9 (95% CI: 1.1 to 3.2) when the pseudolikelihood method was used, and 1.8 (95% CI: 0.7 to 4.7) when considering vaccinated cases only (Table 4).

In NL, NO, and the UK where further adjustment for infections, seasonal influenza vaccination, and other time dependent covariates was possible, the RI for the association between influenza A(H1N1)pdm09 vaccination and GBS decreased from the unadjusted pooled RI of 3.2 (95% CI: 1.8 to 5.6) to 1.7 (95% CI: 0.8 to 3.4) after adjustment for calendar month, and to 1.4 (95% CI: 0.7 to 2.8) upon further adjustment for ILI, URTI, and GI.

Sensitivity analyses using different post-exposure risk periods resulted in a calendar month-adjusted pooled RI of 2.3 (95% CI: 1.4 to 3.8) for the first four weeks. The RI was 2.3 (95% CI: 1.2 to 4.4) in the first two weeks and 2.6 (95% CI 1.4 to 4.9) during weeks three to four.

We did not observe statistically significant interactions between age, infections, or seasonal influenza vaccination and the association between the influenza A(H1N1)pdm09 vaccination and GBS (Table 5).

Discussion

Based on a source population of more than 25 million subjects from NL, UK, and NO we found no significant elevated association between the risk of GBS following immunization with an adjuvanted influenza A(H1N1)pdm09 vaccine, when adjusted for all known measurable confounders (RI 1.4, 95% CI: 0.7 to 2.8). This result is very similar to that of the VAESCO consortium case control study, published previously using one third of the cases from fewer countries [13]. In DK, FI, FR and SE we could not adjust for time varying confounders such as infections since data were not collected over the entire follow up period. Pooling data from all seven countries yielded a crude RI of 3.5, which reduced to 2.0 (95% CI: 1.2 to 3.1) after adjustment for calendar month: this pooled estimate still comprises residual confounding by infections. The effect of calendar month may be explained by it being a good proxy for circulation of the wild-type influenza A(H1N1)pdm09 virus (see figure 2).

Table 2. Characteristics of Guillain-Barré syndrome cases.

Characteristic	DK		FI		FR		NL		NO		SE		UK		Total	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	n	%
Cases in study period	31	10.2	29	9.6	41	13.5	80	26.4	50	16.5	32	10.6	40	13.2	303	100
Females	14	45.2	12	41.4	20	48.8	32	40.0	25	50.0	12	37.5	17	42.5	132	43.6
Mean age (SD)[1] [years]	49.2 (20.2)		54.4 (20.8)		50.0 (21.9)		45.0 (20.8)		55.5 (19.5)		51.5 (20.2)		45.4 (20.4)		50.1 (4.1)	
Age ≤4	0	0.0	0	0.0	1	2.4	2	2.5	1	2.0	0	0.0	2	5.0	6	2.0
Age 5–19 years	3	9.7	3	10.3	4	9.8	10	12.5	0	0.0	2	6.3	3	7.5	25	8.3
Age 20–59 years	18	58.1	10	34.5	18	43.9	44	55.0	21	42.0	15	46.9	24	60.0	15	49.5
Age ≥60	10	32.3	16	55.2	18	43.9	24	30.0	28	56.0	15	46.9	11	27.5	122	40.3
Brighton Classification[2]																
1	10	32.3	17	58.6	13	31.7	28	35.0	21	42.0	19	59.4	0	0.0	108	35.6
2	8	25.8	3	10.3	16	39.0	30	37.5	14	28.0	8	25.0	0	0.0	79	26.1
3	4	12.9	7	24.1	7	17.1	11	13.8	5	10.0	5	15.6	0	0.0	39	12.9
4a	9	29.0	2	6.9	5	12.2	10	12.5	10	20.0	0	0.0	40	100.0	76	25.1
Unknown	0	0.0	0	0.0	0	0.0	1	1.3	0	0.0	0	0.0	0	0.0	1	0.3
Electrophysiology																
AIDP[3]	23	74.2	16	55.2	15	36.6	36	45.0	29	58.0	23	71.9	0	0.0	142	46.9
AMAN[4]	0	0.0	0	0.0	1	2.4	6	7.5	3	6.0	0	0.0	0	0.0	10	3.3
AMSAN[5]	1	3.2	2	6.9	0	0.0	4	5.0	1	2.0	6	18.8	2	5.0	16	5.3
Equivocal	0	0.0	0	0.0	6	14.6	9	11.3	7	14.0	1	3.1	0	0.0	23	7.6
Normal	2	6.5	0	0.0	2	4.9	3	3.8	6	12.0	0	0.0	0	0.0	13	4.3
Not performed	5	16.1	11	37.9	12	29.3	20	25.0	3	6.0	2	6.3	0	0.0	53	17.5
Unresponsive nerves	0	0.0	0	0.0	0	0.0	1	1.3	1	2.0	0	0.0	0	0.0	2	0.7
Unknown	0	0.0	0	0.0	5	12.2	1	1.3	0	0.0	0	0.0	38	95.0	44	14.5
GBS disability score[6]																
0	0	0	0	0.0	6	14.6	0	0.0	0	0.0	0	0.0	0	0	6	2.0
1	0	0	0	0.0	0	0.0	5	6.3	7	14.0	1	3.1	0	0	13	4.3
2	0	0	9	31.0	2	4.9	19	23.8	11	22.0	6	18.6	0	0	47	15.5
3	0	0	4	13.8	10	24.4	21	26.3	6	12.0	7	21.9	0	0	48	15.8
4	0	0	13	44.8	11	26.8	20	25.0	17	34.0	10	31.3	0	0	71	23.4
5	0	0	2	6.9	2	4.9	13	16.3	8	16.0	7	21.9	0	0	32	10.6
6	0	0	1	3.4	0	0.0	1	1.3	1	2.0	1	3.1	0	0	4	1.3
Unknown	31	100.0	0	0.0	10	24.4	1	1.3	0	0.0	0	0.0	40	100.0	82	27.1
Index month																
Nov 2009	9	29.0	4	13.8	9	22.0	5	6.3	5	10.0	8	25.0	6	15.0	46	15.2
Dec 2009	1	3.2	5	17.2	8	19.5	22	27.5	12	24.0	6	18.8	4	10.0	58	19.1
Jan 2010	6	19.4	5	17.2	9	22.0	8	10.0	8	16.0	3	9.4	5	12.5	44	14.5
Feb 2010	5	16.1	6	20.7	6	14.6	9	11.3	1	2.0	5	15.6	8	20.0	40	13.2
Mar 2010	4	12.9	3	10.3	3	7.3	5	6.3	4	8.0	3	9.4	6	15.0	28	9.2
Apr 2010	3	9.7	5	17.2	4	9.8	7	8.8	4	8.0	2	6.3	3	7.5	28	9.2
May 2010	3	9.7	1	3.4	1	2.4	8	10.0	3	6.0	2	6.3	1	2.5	19	6.3
Jun 2010	0	0.0	0	0.0	1	2.4	2	2.5	3	6.0	3	9.4	3	7.5	12	4.0
Jul 2010	0	0.0	0	0.0	0	0.0	3	3.8	3	6.0	0	0.0	1	2.5	7	2.3
Aug 2010	0	0.0	0	0.0	0	0.0	4	5.0	2	4.0	0	0.0	2	5.0	8	2.6
Sep 2010	0	0.0	0	0.0	0	0.0	4	5.0	4	8.0	0	0.0	0	0.0	8	2.6
Oct 2010	0	0.0	0	0.0	0	0.0	3	3.8	1	2.0	0	0.0	1	2.5	5	1.7
TOTAL	31		29		41		80		50		32		40		303	

[1]Standard Deviation.
[2]Sejvar J. J. et al. 2011, Guillain-Barre syndrome and Fisher syndrome: case definitions and guidelines for collection, analysis, and presentation of immunization safety data. Vaccine 29(3).
[3]AIDP: acute inflammatory demyelinating polyradiculoneuropathy.

Table 2. Cont.

[4]AMAN: acute motor axonal neuropathy.
[5]AMSAN: acute motor and sensory axonal neuropathy.
[6]Current disability score at the time of case assessment and/or inclusion into the study.
Abbreviations: DK: Denmark; FI: Finland; FR: France; NL: Netherlands; NO: Norway; SE: Sweden; UK: United Kingdom; GBS: Guillain-Barré syndrome.
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This study is unique as it directly pools data on individual patients from seven European countries, using a common protocol, common case definition, common infrastructure, and common data elaboration. The impact of methodological issues that occurred due to differences in implementation of the protocol could be assessed by comparing the association accross countries; the consistency observed is reassuring. Beyond the effect of the influenza A(H1N1)pdm09 vaccination on GBS this study underlines the advantages of collaborative transnational vaccine safety studies. They not only increase the scale of a study, but also allow for consistency- checks across sources in the absence of bias from differences in design and methods. The use of common methods and subsequent pooling reaches far beyond the traditional approach of meta-analyses where rather heterogeneous estimates resulting from different designs, methods, and settings are being pooled.

The data from this VAESCO study are in line with other results from Europe with studies from FR (RI 0.9, 95% CI: 0.1 to 7.6) [16], SE (RI 1.1 95% CI: 0.6 to 1.9) [17], and the UK (RI 1.05, 95% CI: 0.37–2.24) [18], all showing no association. In contrast, a recent report from Germany, where AS03 adjuvanted vaccine was used, showed an increased risk of GBS after vaccination (RI 4.65, 95% CI: 2.17 to 9.98) [19]. German investigators had already started a separate SCCS study and thus elected not to participate in VAESCO. They did not adjust for infections or calendar-time and selection bias could not be excluded since cases originated from a reporting network. Pooling of calendarmonth adjusted RI estimates with the VAESCO study would be possible through meta-analysis to enlarge the scale of the current EU based study. Five studies from the US, where non-adjuvanted influenza A(H1N1)pdm09 vaccines were used, have recently been published. Each of the initial observational studies found an increased RI ranging from 1.6 (95% CI: 1.0 to 2.2) [20], to 2.1 (95% CI: 1.2 to 3.5) [21], to 2.5 (95% CI: 0.42 to 15.0) [22], and to 4.4 (95% CI: 1.3 to 14.2) [23]. Three studies used self-controlled designs but without further adjusting for time-varying confounders [21–23]. The study assessing the lowest RI (1.6 (95% CI: 1.0 to 2.2)) was a cohort study adjusting for age and sex [20]. The highest RI of 4.4 (95% CI: 1.3 to 14.2) was based on data from the US Vaccine Safety Datalink (VSD) project, which was based on 13 vaccinated cases [23]. Salmon et al. recently published a meta-analysis of US studies on the association between influenza A(H1N1)pdm09 vaccines including two unpublished studies and reported a pooled estimate of 2.35 (95%CI: 1.42–4.01) [24]. A SCCS study from Quebec, Canada adjusted for seasonality and contraindication using vaccinated cases only reported a relative risk of 1.9 (95% CI: 1.0 to 3.5) [25]. After the first VSD study, a second VSD study was recently published, investigating specifically the effect of antecedent infections on the relative incidence of GBS following influenza A(H1N1)pdm09 vaccines, using a case centered analysis. This analysis showed the impact of infections as a confounding factor [26]. After adjusting for antecedent infections, there was no evidence for an elevated GBS risk following 2009–10 monovalent/2010–11 trivalent influenza vaccines. However, the association between GBS and antecedent infection was strongly elevated. The effect of infections on the risk of GBS and the potential preventive effect of vaccination on the risk of GBS by preventing influenza was recently discussed by Stowe and Poland [27,28]. This recent evidence underlines the need to adjust for infections as we could do in part of the countries in our analyses.

Owing to its observational nature, our study suffers from limitations that should be considered when interpreting data. In NL and SE, where reporting networks were used, completeness of recruitment was verified by retrospectively comparing included cases with claims made for GBS. In FR and SE informed consent was required which could be another reason for non-inclusion. Finally, since cases were included only if charts/medical records could be reviewed, lack of data could be another source of selection bias. The distribution of vaccinated cases over time showed no significant trend, suggesting changes in the number of cases included over time were not related to exposure and selection bias may be limited (Figure 3).

Information bias may arise from misclassification of the outcome as well as the exposure. Cases recruited directly from neurologists (i.e., FR, NL, NO, and SE) generally had higher levels of diagnostic certainty. In the UK all cases were classified with the lowest Brighton Collaboration case certainty level as information was retrieved retrospectively from GP medical records, which capture information from specialist letters but often lack information on specific test results. In DK cases were classified based on retrospective review of specialist charts resulting in partially missing information. As standardized criteria were used for case classification, misclassification of the outcome will be minimal. In all countries prospectively collected health care records were used to obtain information on exposure, except in SE, which relied on interviews and may have suffered from recall bias. In the NL exposure may have been misclassified in young children (<5 years) who were participating in mass vaccination campaigns, but this will be non-differential and there were very few paediatric cases. Exposure might be misclassified due to misspecification of the risk period. Sensitivity analysis showed no difference in the RI when the risk window was restricted to 15 to 28 days after vaccination (RI 2.6, 95% CI: 1.4 to 4.9); compared to the first two weeks (RI 2.3, 95% CI 1.2 to 4.4) and the risk in a 4-week risk window (RI 2.3, 95% CI 1.4 to 3.8).

We addressed confounding both by design (SCCS controls for time-constant confounders), through adjustments, and sensitivity analyses. GBS could be a contra-indication for influenza A(H1N1)pdm09 vaccine as a similar vaccine had been associated with GBS in the past. To investigate this issue we carried out analyses including only vaccinated subjects and analyses applying the pseudo-likelihood method [14]. The pseudo-likelihood method reduced the calendar-adjusted pooled RI from 2.0 to 1.9 and if only vaccinated cases were included to 1.8, indicating that contra-indications were a minor issue. Calendar month acted as an important confounding factor, not because time itself is a risk factor, but because it may serve as a proxy for influenza A(H1N1)pdm09 circulation, which was highly time-dependent and co-occuring with the mass vaccination campaigns (see figure 2). Adjustment for additional timevarying confounders

Table 3. Guillain-Barré syndrome occurrence during follow-up and during the 6-week (42 days) risk periods following influenza A(H1N1)pdm09 vaccination and infection.

Exposure	DK		FI		FR		NL		NO		SE		UK		TOTAL	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Cases in study period	31	10.2	29	9.6	41	13.5	80	26.4	50	16.5	32	10.6	40	13.2	303	100
Follow-up total (mean) in days	11,286 (364.1)		6,127 (211.3)		11,421 (278.6)		26,322 (329)		17,845 (356.9)		11,471 (358.5)		12,666 (316.7)		97,138 (320.6)	
Cases exposed to influenza A(H1N1)pdm09 vaccine anytime during follow-up period	4	12.9	13	44.8	5	12.2	29	36.3	23	46.0	22	68.8	3	7.5	99	32.7
GBS in influenza A(H1N1)pdm09 vaccination risk period																
1st dose[1)]	2	6.5	4	13.8	2	4.9	10	12.5	8	16.0	9	28.1	1	2.5	36	11.9
2nd dose[1)]	0	0.0	0	0.0	2	4.9	5	6.3	0	0.0	0	0.0	0	0	7	2.3
Cases during risk period following seasonal influenza vaccination	0	0	1	3.4	1	2.4	4	5	4	8	0	0	5	12.5	15	0.05
Cases during risk period following infections (ILI, URTI) risk period	6	19.4	6	20.7	10	24.4	16	20	30	60.0	8	25.0	3	7.5	79	26.11
ILI[1)]	5	16.1	1	3.4	2	4.9	6	7.5	13	26.0	1	3.1	1	2.5	29	9.6
URTI[1)]	1	3.2	5	17.2	8	19.5	10	12.5	17	34.0	7	21.9	2	5	50	16.5

[1)]% per number of cases included per country.

Abbreviations: ILI: influenza like illness; URTI: Upper respiratory tract infection; UK: United Kingdom; NL: Netherlands; FR: France; SE: Sweden; FI: Finland, NO: Norway, DK: Denmark.

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Table 4. Relative incidence estimates for the association between infections, influenza A(H1N1)pdm09 vaccination, seasonal influenza vaccination and Guillain-Barré syndrome.

	DK		FI		FR		NL		NO		SE		UK		Pooled	
	RI	95% CI	RI	95% CI	RI	95% CI	RI	95% CI	RI	95% CI	RI	95% CI	RI	95% CI	RI	95% CI
Covariates																
ILI	NA		NA		NA		10.5	3.0–36.3	30.6	8.6–108	NA		1.8	0.2–16.0	10.4	2.6–41.1
URTI	NA		NA		NA		13.0	4.3–39.2	17.7	6.2–34.7	NA		2.2	0.4–10.6	8.51	3.0–24.0
GI	NA		NA		NA		11.6	2.8–49.4	53.31	6.56–433	NA		2.3	0.2–22.6	11.9	2.5–55.6
Seasonal influenza vaccination	NA		NA		NA		1.2	0.4–4.0	5.5	1.6–18.9	NA		6.0	1.8–19.7	3.9	1.8–8.3
Any influenza A(H1N1)pdm09 vaccination																
Unadjusted	7.7	1.1–54.4	1.6	0.5–5.4	6.4	1.0–40.4	2.7	1.3–5.9	3.9	1.6–9.3	4.8	2.1–11.1	3.3	0.3–36.5	3.5	2.2–5.5
Adjusted for calendar month	3.9	0.5–32.2	1.6	0.5–5.4	2.9	0.4–19.6	1.4	0.6–3.4	1.9	0.7–5.2	2.7	1.0–7.8	2.3	0.2–27.7	2.0	1.2–3.1
Adjustment effect any influenza A(H1N1)pdm09 vaccination in NL, NO, UK																
Adjusted for calendar month only							1.4	0.6–3.4	1.9	0.7–5.2			2.3	0.2–27.7	1.7	0.8–3.4
Fully adjusted (month, ILI/URTI, GI)							1.2	0.5–3.3	1.5	0.5–4.6			1.5	0.1–23.1	1.4	0.7–2.8
Sensitivity analysis on influenza A(H1N1)pdm09 vaccination for contra-indication																
Pseudolikelihood																
1st dose	3.6	0.4–29.5	3.2	0.7–14.6	0.6	0.1–6.7	1.3	0.4–4.0	1.6	0.6–4.3	2.4	0.8–6.9	4.8	0.3–83.9	1.9	1.1–3.2
2nd dose	NA		NA		2.2	0.2–26.3	1.2	0.4–3.4	NA		NA		NA		1.3	0.5–3.4
Vaccinated cases only	NE		2.6	0.2–32.5	NE		1.2	0.2–8.3	1.6	0.3–7.9	2.5	0.4–16.0	NE		1.8	0.7–4.7

Abbreviations: NA: not available or not valid; NE = Not estimable due to small numbers or absence RI: relative incidence; ILI: influenza like illness; URTI: upper respiratory tract infection, GI: gastrointestinal Infection, UK: United Kingdom; NL: Netherlands; FR: France; SE: Sweden; FI: Finland, NO: Norway, DK: Denmark.

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(mainly infections) lowered the pooled calendar-month adjusted RI in NL, NO, and UK from 1.7 to 1.4. This is in line with the effect of control for infections seen by Greene et al [26]. The effect of infections on the risk of GBS differed substantially between countries due to differences in timing and type of data collection methods. In future studies, standardization of covariate exposure

Table 5. Stratified analyses for association between influenza A(H1N1)pdm09 vaccination and Guillain-Barré Syndrome.

	DK		FI		FR		NL		NO		SE		UK		Pooled (random effects)	
	RI	95% CI	RI	95% CI	RI	95% CI	RI	95% CI	RI	95% CI	RI	95% CI	RI	95% CI	RI	95% CI
Changing risk windows																
1–28 days	4.4	(0.5 to 35.6)	1.0	(0.2–4.6)	1.3	(0.1–12.6)	2.5	(1.0–6.4)	2.2	(0.8–6.1)	2.7	(0.9–7.8)	4.2	(0.4–50.2)	2.3[4)]	(1.4–3.8)
1–14 days	7.6	(0.9–61.7)	2.3	(0.5–10.6)	3.4	(0.3–33.3)	2.5	(0.7–9.3)	1.3	(0.3–5.9)	1.0	(0.2–4.7)	10.8	(0.9–133.2)	2.3[5)]	(1.2–4.4)
15–28 days	NE[3)]		0.0		0.0		1.9	(0.7–5.5)	2.5	(0.8–7.8)	3.7	(1.2–11.1)			2.6	(1.4–4.9)
42 day risk window																
19–59 years old	0.0		3.3	(0.5–19.3)			1.0	(0.1–10.7)	0.6	(0.1–5.5)	1.0	(0.2–6.6)			1.3	(0.5–3.6)
older than 59 years	2.3	(0.1–38.0)	0.0		0.0		1.1	(0.3–4.9)	3.5	(1.0–12.6)	7.6	(1.6–35.8)	11.9	(0.4–365.5)	3.2	(1.5–6.9)
Co-morbidities[1)]	0.0		2.5	(0.2–35.5)	0.24		0.0		3.2	(0.6–17.0)	0.0				3.0	(0.7–12.3)
No co-morbidities[1)]	0.0		1.7	(0.4–6.7)	1.7	(0.1–19.6)	1.9	(0.6–6.6)	1.4	(0.4–5.3)	0.0				1.7	(0.8–3.4)
Seasonal influenza vacvination	0.0		3.0	(0.2–50.4)	0.2		0.5	(0.1–3.6)	2.1	(0.2–19.0)	0.0				1.2	(0.3–4.5)
No seasonal influenza vaccination	0.0		1.6	(0.4–6.4)	4.8	(0.3–83.6)	2.2	(0.4–11.2)	1.7	(0.6–5.4)	0.0				1.9	(0.9–4)
ILI, URTI infection	NE		1.1	(0.1–10.6)	2.9	(0.2–51.9)	1.1	(0.1–11.4)	1.4	(0.4–4.8)	3.2	(0.8–14.0)			1.8	(0.8–3.9)
No ILI, URTI infection	2.5	(0.2–34.4)	2.2	(0.5–10.3)	0.0		1.5	(0.4–5.8)	3.6	(0.5–24.3)	2.7	(0.6–13.2)			2.2	(1.1–4.7)

[1)]Malignancy, immune suppression, or autoimmune disorder NE = Not estimable due-small numbers.

Abbreviations: RI, relative incidence; ILI, influenza like illness; URTI, Upper respiratory tract infection; UK, United Kingdom; NL, Netherlands; FR, France; SE, Sweden; FI, Finland, NO, Norway, DK, Denmark.

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reporting will have to be addressed in more detail. Given the variation in the RI of GBS among other countries' A(H1N1)pdm09 vaccinees, these results, as well as the pooled estimate that was adjusted for calendar month only, are likely affected by residual confounding by infections.

Conclusion

This large, multinational SCCS study confirms the results from the initial much smaller VAESCO case control study. In each country, the unadjusted association between influenza A(H1N1)pdm09 vaccine and GBS suggests a possible increase in risk, and adjustment for confounders consistenly lowered this risk. Further adjustment for infections could only be carried out in some countries and demonstrated the effect of confounding by ILI, GI and URTI, which themselves were strong risk factors for GBS. After adjustment we did not observe an association between influenza A(H1N1)pdm09 vaccine and GBS. Based on the upper limit of the confidence interval of both the partially and fully adjusted RI estimates we can rule out with 95% certainty that adjuvanted influenza A(H1N1)pdm09 vaccines (mainly AS03 adjuvanted) would have resulted in more than 2 or 3 excess cases of GBS per 1 million vaccinated persons.

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All information and results concerning the UK in 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 February 2011. However, the interpretation and conclusions contained in this report are those of the authors alone.

Ethical approval

In **France**, the case-control study was approved by the ethics committee (i.e., Comité de Protection des Personnes Sud Ouest et Outre Mer I et II) and participants gave informed consent. In the **Netherlands**, the study was provided with a declaration of no objection from the medical ethics committee of the Erasmus University Medical Center in Rotterdam (MEC-2009-404). Subsequent amendments to collect data entirely anonymously through the GP allowed the inclusion of study subjects without requiring informed consent. In **Denmark** ethics approval was not required, though the National Board of Health approved chart review. In **Sweden** the study was approved by the regional ethics committee, Karolinska Institute, Stockholm, and participants gave written informed consent. In the **UK**, the GPRD Group has obtained ethical approval from a multicenter research ethics committee (MREC) for all purely observational research using GPRD data. Individual studies must be granted approval by an Independent Scientific Advisory Committee (ISAC). This study received ISAC approval (protocol No 10_058). In **Norway**, the Regional Committee for Medical and Health Research Ethics in Western Norway approved national study participation without need for patient consent.

Author Contributions

Wrote the paper: SR DW JPD HKO MCJMS. Study design, data management, data analysis, interpretation of data and results, and reviewing of manuscript: SR DW JPD HKO CSDV CS NA HS DMN AH MLM AS CS AC HH LAD PS MM MS NVDM BCJ TL TK JS KJ PK JB MCJMS. Main pooled statistical data analysis: SR. Main pooled statistical data analysis, advisory role: NA. Scientific coordination of VAESCO consortium: DW JB MCJMS. Development of software for data collection, harmonization, and aggregation across databases: MS MM. European Centre for Disease Prevention and Control, project leader for the GBS and pandemic vaccines: KJ PK.

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III

ORIGINAL ARTICLE

Antibody response to seasonal influenza vaccination in patients with multiple sclerosis receiving immunomodulatory therapy

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Background and purpose: We have previously shown that patients with multiple sclerosis receiving immunomodulatory treatment have reduced seroprotection rates after influenza immunization. The aim of this study was to further investigate the influence of immunomodulatory therapies on the antibody response and seroprotection rates in patients immunized with seasonal influenza vaccine in 2012/2013 compared with healthy controls.

Methods: Ninety patients receiving fingolimod, glatiramer acetate, interferon beta-1a/1b, natalizumab or no therapy were compared with 62 healthy controls. All subjects received the inactivated split virus vaccine in 2012 and serum samples were collected pre-vaccination and 3, 6 and 12 months post-vaccination. The vaccine responses were evaluated by the hemagglutination inhibition assay and adjusted for age and gender.

Results: No significant differences in rates of protection against H1N1 for interferon beta-1a/1b and glatiramer acetate were observed as compared with controls at 3, 6 and 12 months. Fingolimod provided reduced protection at all time points post-vaccination, whereas natalizumab displayed reduced protection at 3 and 6 months. Patients without immunomodulation did not display protection rates that were significantly different from the controls at 3 and 12 months.

Conclusion: These findings suggest that patients with multiple sclerosis receiving fingolimod or natalizumab should be considered for a second dose of the vaccine in cases of insufficient protection. Our results further indicate that new immunomodulatory treatment regimens should be systematically evaluated for their influence on influenza-specific vaccine responses.

Introduction

Influenza is a highly contagious acute respiratory virus causing annual global epidemics resulting in substantial morbidity and mortality particularly in high-risk patients with underlying disease. Vaccination is the main method of prophylaxis, and annual vaccination is recommended for all high-risk patients. In addition to the risk of morbidity and mortality due to the influenza infection itself, it also increases the risk for worsening of multiple sclerosis (MS) [1]. Thus, there is a strong rationale for influenza immunization in patients with MS [2–4]. It has been shown that vaccines do not cause MS, MS relapse or central nervous system demyelination [5], and that inactivated influenza vaccines are considered safe in patients with MS

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[6,7]. Immunomodulatory and immunosuppressive treatments for MS are associated with an increased risk of infection, making treatment of this condition challenging in daily clinical practice [8]. Few data exist on how long one should wait after vaccination before starting a therapy, although it is worth considering measuring the effect of immunization using antibody levels [7].

We have previously shown that the differential immunization effect was dependent on subtype of immunomodulation in patients with MS, where only interferon beta-1a/1b did not influence the protection rate compared with other therapies [9]. In this follow-up study, we analysed the immunogenicity of influenza vaccination in patients with MS during treatment with five different immunomodulatory therapies after seasonal influenza vaccination in 2012/2013, with baseline sampling and follow-up after 3, 6 and 12 months in both the study population and controls.

Materials and methods

Study design

This was a prospective study with follow-up of patients and controls with measurement of the vaccine-specific antibody responses to the influenza A H1N1 and H3N2 2012/2013 influenza vaccine viruses at visit 1 (day 0), visit 2 (3 months), visit 3 (6 months) and visit 4 (12 months).

Patients and controls

Ninety patients with MS (mainly relapsing-remitting) at the Department of Neurology, Haukeland University Hospital, Bergen, Norway were included from December 2012 to February 2013. The patients received one of the following immunomodulatory therapies: fingolimod ($n = 15$), glatiramer acetate ($n = 23$), interferon beta-1a/1b ($n = 25$), natalizumab ($n = 12$) or none. The controls ($n = 62$) were healthcare workers at the Haukeland University Hospital in Bergen without immunotherapy or neurological disease recruited during the same period. Age and gender were recorded for all participants (Table S1). All patients and controls provided written informed consent. The Regional Ethics Committee/REK Vest trial numbers were 2010/745 and 2009/1224. All data were anonymized.

Vaccination

All participants received the trivalent inactivated unadjuvanted split influenza virus vaccine that contained A/California/07/2009 (H1N1)pdm09, A/Victoria/361/2011 (H3N2) and B/Wisconsin/1/2010 (Vaxigrip, Sanofi Pasteur MSD, Lyon, France or Fluarix, GSK, Rixensart, Belgium).

Blood sampling and hemagglutination inhibition assay

Serum samples for analyses of influenza vaccine responses were drawn pre-vaccination (day 0) and 3, 6 and 12 months post-vaccination. Samples were allocated a laboratory number and stored at −80°C until used in the blinded analyses. The numbers of missing blood samples varied throughout the study period, and were highest at 12 months (Tables 1 and 2). Sera were treated with receptor-destroying enzyme (one volume of serum was diluted with four volumes of receptor-destroying enzyme) and tested by the hemagglutination inhibition (HI) assay as described in Madhun *et al.* [10]. In brief, a twofold dilution series of sera was prepared in phosphate-buffered saline (starting dilution 1/10) and incubated with 8 hemagglutinin units of whole inactivated H1N1 (A/California/07/2009) or H3N2 (A/Victoria/361/2011) virus (International Reagent Resource, Manassas, VA, USA) for

Table 1 Post-vaccination comparison of H1N1-specific hemagglutination inhibition (HI) responses after seasonal influenza vaccination in Hordaland, Norway (2012/2013) in 90 patients with multiple sclerosis (MS) and 62 controls[a]

	Day 0			3 months			6 months			12 months		
Group	*n*	GMT	% protected[b]	*n*	GMT	% protected	*n*	GMT	% protected	*n*	GMT	% protected
Controls	53	154.8	90.6	56	206.2	94.6	50	180.4	94.0	54	82.3	70.4
MS	90	36.7	45.6	87	117.0	85.1	76	93.6	76.3	71	63.7	63.4
Fingolimod	15	42.2	40.0	14	87.6	71.4	12	93.6	58.3	9	33.3	22.2
Glatiramer acetate	23	43.2	56.5	23	112.3	91.3	22	112.4	86.4	22	84.8	77.3
Interferon beta-1a/1b	25	28.1	36.0	25	164.2	88.0	19	87.6	84.2	19	89.8	79.0
Natalizumab	12	53.9	50.0	11	115.4	72.7	8	123.9	75.0	9	52.7	55.6
None	15	28.7	46.7	14	92.1	92.9	15	66.9	66.7	12	41.1	50.0
Total	143			143			126			125		

GMT, geometric mean titre. [a]Some patients and controls are missing at different time points. [b]Protection is defined as an HI titre ≥ 40.

Table 2 Post-vaccination comparison of H3N2 hemagglutination inhibition (HI) responses after seasonal influenza vaccination in Hordaland, Norway (2012/2013) in 90 patients with multiple sclerosis (MS) and 62 controls[a]

	Day 0			3 months			6 months			12 months		
Group	*n*	GMT	% protected[b]	*n*	GMT	% protected	*n*	GMT	% protected	*n*	GMT	% protected
Controls	53	44.7	56.6	56	89.8	69.6	50	52.0	58.0	54	50.4	57.4
MS	90	7.7	3.3	86	30.4	33.7	76	23.4	27.6	70	17.0	21.4
Fingolimod	15	7.3	0.0	14	16.8	21.4	12	16.3	8.3	8	12.7	0.0
Glatiramer acetate	23	9.1	8.7	23	26.8	26.1	22	19.9	27.3	22	18.7	27.3
Interferon beta-1a/1b	25	7.3	0.0	25	46.9	44.0	19	38.8	47.4	19	24.7	36.8
Natalizumab	12	7.3	8.3	10	25.8	30.0	9	15.3	11.1	9	10.1	0.0
None	15	7.1	0.0	14	35.0	42.9	14	27.3	28.6	12	14.2	16.7
Total	143	7.4		142			126			124		

GMT, geometric mean titre. [a]Some patients and controls are missing at different time points. [b]Protection is defined as an HI titre ≥ 40.

1 h; 0.7% turkey erythrocytes were added for 30 min before reading. Positive control ferret sera (International Reagent Resource) were included in all assays. All sera were tested in duplicate and the geometric mean titer (GMT) was calculated. The serum HI titer was expressed as the reciprocal of the highest dilution at which 50% hemagglutination was inhibited, and the titers <10 were assigned a value of 5 for calculation purposes. This assay is commonly used to measure influenza-specific antibody responses after vaccination. An HI titer ≥40 is established as a surrogate correlate of protection and is used in this study to define protection. The immunogenicity of the influenza B strain was not tested, due to lack of sensitivity in the HI test.

Statistical methods

Descriptive statistics are reported using the mean, geometric mean, SD, frequency counts and percentage. Associations between categorical variables were tested using Pearson's chi-square test.

Mixed linear regression was performed to analyse the dependency of H1N1 and H3N2, respectively, related to therapy (fingolimod, glatiramer acetate, interferon beta-1a/1b, natalizumab and no treatment compared with controls) adjusted for age and gender assuming an autoregressive correlation of the first order to account for correlation between repeated measurements in each subject. The residuals were examined to check for consistency with the normality assumption (Figs S1 and S2), and log transformation of H1N1 and H3N2 was chosen for the analysis. All models were inspected for interactions between time and medication group.

The rate of protection, defined as H1N1 ≥ 40 and H3N2 ≥ 40, respectively, was analysed with respect to the same variables using logistic regression and the generalized estimating equations methodology. Results are reported using the odds ratio and 95% confidence interval. SPSS version 23.0 (Armonk, NY, USA) was used for all statistical analyses. The significance level was set at 0.05 for all tests.

Results

Demographic variables

Clinical and demographic variables at baseline are shown in Table S1. The MS group was on average about 4 years older and included fewer women than the control group.

H1N1-specific antibody responses to seasonal influenza vaccination

The post-vaccination comparison of the HI response in patients with MS and controls is shown in Table 1. Results from the mixed linear regression are given in Table 3 and results from logistic regression are shown in Table 4.

Age had a negative effect on ln(H1N1) HI titers in the mixed linear model ($P = 0.003$), as shown by an increasing age of 10 years giving an approximate 20% decrease in H1N1-specific antibody titers. In the mixed linear regression, gender did not influence the outcome ($P = 0.337$).

Influence of immunomodulation on the H1N1-specific vaccination response with time

Figure 1 shows the mean H1N1 GMT HI titers for each medication at all visits unadjusted for age and gender on a $\log_2$ scale.

The mixed linear regression of ln(H1N1) HI titers showed a significant interaction between time and medication after adjustment for age and gender ($P < 0.001$). Antibody titers increased significantly at all study visits after vaccination compared with pre-vaccination titers in patients with MS treated with

Table 3 Results from the mixed linear regression analysis of ln(H1N1) and ln(H3N2) hemagglutination inhibition titers after seasonal influenza vaccination in Hordaland, Norway (2012/2013) in 90 patients with multiple sclerosis and 62 controls

	ln(H1N1)			ln(H3N2)		
Variable	*b*	95% CI	*P*	*b*	95% CI	*P*
Intercept	6.29	5.41 to 7.17	<0.001	3.39	2.54 to 4.25	<0.001
Age (years)	−0.02	−0.04 to −0.01	0.003	0.00	−0.02 to 0.02	0.974
Female	−0.20	−0.60 to 0.21	0.337	0.11	−0.28 to 0.49	0.592
Medication			0.014			<0.001
Interferon beta-1a/1b	−1.69	−2.30 to −1.08		−1.01	−1.52 to −0.49	
Glatiramer acetate	−1.03	−1.67 to −0.40		−1.22	−1.75 to −0.68	
Natalizumab	−1.19	−2.00 to −0.38		−1.55	−2.25 to −0.86	
Fingolimod	−1.35	−2.09 to −0.62		−1.49	−2.13 to −0.86	
No medication	−1.39	−2.17 to −0.60		−1.22	−1.90 to −0.54	
Control	0.00	Reference		0.00	Reference	
Time (months)			<0.001			<0.001
0	0.00	Reference		0.00	Reference	
3	0.36	0.14 to 0.58		1.12	0.91 to 1.33	
6	0.27	−0.03 to 0.57		0.76	0.50 to 1.03	
12	−0.54	−0.88 to −0.20		0.59	0.29 to 0.89	
Time × medication			<0.001			0.234[a]
0 × control	0.00	Reference			Not included	
3 × control	0.00	Reference				
6 × control	0.00	Reference				
12 × control	0.00	Reference				
0 × interferon beta-1a/1b	0.00	Reference				
3 × interferon beta-1a/1b	1.41	1.02 to 1.80				
6 × interferon beta-1a/1b	1.09	0.55 to 1.64				
12 × interferon beta-1a/1b	1.81	1.18 to 2.44				
0 × glatiramer acetate	0.00	Reference				
3 × glatiramer acetate	0.60	0.19 to 1.00				
6 × glatiramer acetate	0.68	0.14 to 1.22				
12 × glatiramer acetate	1.21	0.58 to 1.83				
0 × natalizumab	0.00	Reference				
3 × natalizumab	0.41	−0.11 to 0.93				
6 × natalizumab	0.33	−0.41 to 1.06				
12 × natalizumab	0.66	−0.19 to 1.50				
0 × fingolimod	0.00	Reference				
3 × fingolimod	0.38	−0.09 to 0.86				
6 × fingolimod	0.39	−0.26 to 1.04				
12 × fingolimod	0.52	−0.29 to 1.32				
0 × no medication	0.00	Reference				
3 × no medication	0.94	0.47 to 1.42				
6 × no medication	0.58	−0.05 to 1.20				
12 × no medication	1.16	0.41 to 1.91				

b, estimated regression coefficient; CI, confidence interval. [a]From model with interaction term included.

interferon beta-1a/1b and glatiramer acetate. Fingolimod and natalizumab provided reduced protection at all time points post-vaccination. The group without medication also had significant increase in antibody titers at 3 and 12 months post-vaccination. The controls showed significant changes in HI titers with time ($P < 0.001$), with significantly higher titers at 3 months post-vaccination, which decreased to be significantly lower than pre-vaccination titers at 12 months post-vaccination. The absence of immunotherapy provided a significant increase in GMT at 3 and 12 months, but not at 6 months.

An HI titer ≥40 is considered protective. For analyses of protection (H1N1 ≥ 40) using the logistic regression model, adjustment for age and sex gave only marginal changes. The logistic regression of H1N1-specific titers showed a significant interaction between time and medication ($P = 0.001$). There were significant differences between the therapy groups including the controls ($P = 0.007$) pre-vaccination. The pre-vaccination rates of protection were significantly lower in all medication groups compared with the controls. At 3 months, which is the time of the peak antibody response, interferon, glatiramer acetate

Table 4 Results from logistic regression analysis of protection rates against influenza H1N1and H3N2 viruses after seasonal influenza vaccination in Hordaland, Norway (2012/2013) in 90 patients with multiple sclerosis and 62 controls

	H1N1 ≥ 40			H3N2 ≥ 40		
Variable[a]	OR[b]	95% CI	*P*[c]	OR	95% CI	*P*
Intercept	8.71	3.69 to 20.56	<0.001	0.86	0.53 to 1.42	<0.001
Medication			0.007			<0.001
Interferon beta-1a/1b				0.23	0.11 to 0.50	
Glatiramer acetate				0.14	0.05 to 0.39	
Natalizumab				0.07	0.02 to 0.23	
Fingolimod				0.06	0.02 to 0.20	
No medication				0.16	0.06 to 0.41	
Control				1.00	Reference	
Time (months)			<0.001			<0.001
0	1.00	Reference		1.00	Reference	
3	2.36	0.88 to 6.33		3.88	2.54 to 5.91	
6	2.28	0.82 to 6.32		2.45	1.53 to 3.93	
12	0.29	0.12 to 0.67		2.03	1.31 to 3.14	
Time × medication			0.001		Not included	n.s.[d]
0 × control	1.00	Reference				
0 × interferon beta-1a/1b	0.06	0.02 to 0.21				
0 × glatiramer acetate	0.15	0.05 to 0.49				
0 × natalizumab	0.11	0.03 to 0.48				
0 × fingolimod	0.08	0.02 to 0.29				
0 × no medication	0.10	0.03 to 0.38				
3 × control	1.00	Reference				
3 × interferon beta-1a/1b	0.36	0.06 to 2.02				
3 × glatiramer acetate	0.51	0.08 to 3.46				
3 × natalizumab	0.13	0.02 to 0.74				
3 × fingolimod	0.12	0.02 to 0.65				
3 × no medication	0.79	0.05 to 12.77				
6 × control	1.00	Reference				
6 × interferon beta-1a/1b	0.35	0.06 to 2.16				
6 × glatiramer acetate	0.33	0.06 to 1.91				
6 × natalizumab	0.09	0.01 to 0.56				
6 × fingolimod	0.06	0.01 to 0.33				
6 × no medication	0.10	0.02 to 0.53				
12 × control	1.00	Reference				
12 × interferon beta-1a/1b	1.74	0.48 to 6.31				
12 × glatiramer acetate	1.36	0.43 to 4.31				
12 × natalizumab	0.62	0.16 to 2.43				
12 × fingolimod	0.19	0.05 to 0.69				
12 × no medication	0.50	0.14 to 1.73				

CI, confidence interval; n.s., not significant; OR, odds ratio. *P* value from likelihood ratio test. [a]For both H1N1 and H3N2 adjusting for age (n.s.) and sex (n.s.) gave marginal changes. [b]From model without main effect from medication, to obtain time-specific estimates for each medication vs. controls. [c]From model with main effects and interaction. [d]The model with interaction between time and medication could not be estimated due to numerical problems. However, analysis with four groups (interferon, non-interferon, no medication and controls) showed no significant interaction.

and the group without treatment showed no significant differences in protection rates as compared with the controls, whereas protection rates for fingolimod and natalizumab were significantly lower. Also, at 6 months, interferon and glatiramer acetate showed protection rates that were not significantly different from the controls, whereas protection rates for fingolimod, natalizumab and the group without treatment were significantly lower. At 12 months, interferon, glatiramer acetate, natalizumab and those without treatment showed no significant differences in protection rates as compared with the controls, whereas protection rates in patients on fingolimod were significantly lower.

H3N2-specific antibody responses to seasonal influenza vaccination

The post-vaccination comparison of the HI response in patients with MS and controls is shown in Table 2. Results from the mixed linear regression are given in Table 3 and the logistic regression is shown in Table 4.

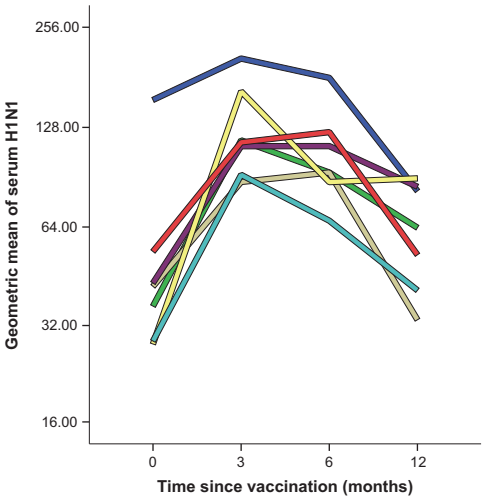

Figure 1 Geometric mean hemagglutination inhibition serum antibody titers to H1N1 plotted on $\log_2$ scale according to time since vaccination and medication including multiple sclerosis (MS) vs. control group. Blue, controls; green, MS; brown, fingolimod; purple, glatiramer acetate; yellow, interferon beta-1a/1b; red, natalizumab; pale blue, none.

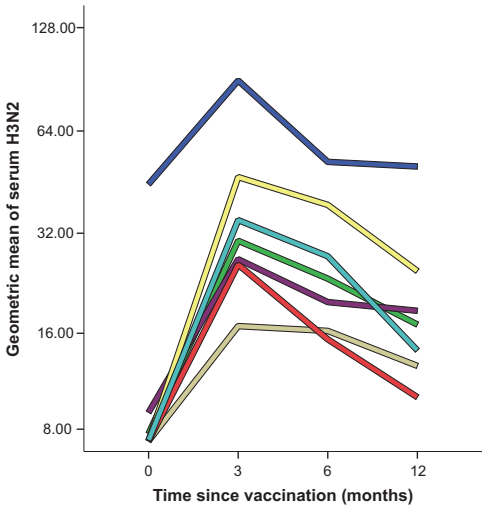

Figure 2 Geometric mean hemagglutination inhibition serum antibody titers to H3N2 plotted on $\log_2$ scale according to time since vaccination and medication including multiple sclerosis (MS) vs. control group. Blue, controls; green, MS; brown, fingolimod; purple, glatiramer acetate; yellow, interferon beta-1a/1b; red, natalizumab; pale blue, none.

Neither age nor gender affected the rates of protection ($P = 0.974$ and $P = 0.592$, respectively).

Influence of immunomodulation on the H3N2-specific vaccination response with time

Figure 2 shows the mean H3N2 GMT HI titers for each therapy at all visits unadjusted for age and gender on a $\log_2$ scale.

The mixed linear regression of H3N2 showed significant differences according to therapy ($P < 0.001$) and time ($P < 0.001$) but not between therapies and time ($P = 0.234$).

In the logistic regression model of protection, the interaction between time and medication was not significant but all medication groups had significantly increased protection rates post-vaccination at all time points compared with pre-vaccination rates ($P < 0.001$). Nevertheless, patients with MS were significantly less likely to be protected when compared with the controls at all time points ($P < 0.001$).

Discussion

We have previously shown that patients with MS receiving immunomodulatory treatment except for interferon beta-1a/1b had reduced antibody responses and protection rates after influenza immunization. Samples were drawn at an average of 10 months after the pandemic swine influenza vaccination in 2009 and 6 months after the seasonal influenza vaccination in 2010. In the present study, we have extended our work to evaluate the influence of different immunomodulatory therapies in patients with MS immunized with seasonal influenza vaccine in 2012/2013 on the time course of the antibody response and protection rates compared with healthy controls. Compared with the controls, our data show significantly reduced protection rates in patients receiving fingolimod and natalizumab, whereas such reduction could not be demonstrated in patients receiving interferon beta-1a/1b, glatiramer acetate or in untreated patients.

One previous study has reported no influence of fingolimod on the protective response after the influenza vaccination periods in the 2008/2009 and 2009/2010 seasons by enzyme-linked immunosorbent assay analyses in 14 patients and sampling times 0, 7, 14 and 28 days post-vaccination [11]. Another study reported reduced response rates after 3 and 6 weeks with the HI method in 95 patients during the 2010/2011 season [12]. Our results based on the HI method are

consistent with reduced protection at all time points post-vaccination, similar to the latter study. Fingolimod is a sphingosine 1-phosphate receptor functional antagonist that blocks egress of CCR7+ CD4+ naive and central memory T cells from the lymph nodes. The effect on the immune system is lymphocyte redistribution [8], which might confer a reduced immune response and thus reduced protection after vaccination.

For glatiramer acetate, few data exist except from our previous explorative study where we found possible reduced long-term protection [9]. In the present study, we found protection rates for glatiramer acetate that were similar to interferon beta-1a/1b, which might be due to more patients receiving this drug in the present study as compared with our pilot study.

Rates of protection after influenza immunization in patients receiving interferon beta have been reported to be unchanged in several studies [3,9,13,14], as confirmed in the present study. This applies for all post-vaccination time points.

We reported lower protection rates during natalizumab therapy in our previous study using the HI method in 17 patients vaccinated during 2009/2010 sampled at 10 months and in 8 patients vaccinated in 2010/2011 sampled at 6 months [9]. Others have, however, found unchanged protection if vaccinated with a strongly immunogenic protein but not an influenza antigen in 30 patients sampled at 28 and 56 days post-vaccination and measured by enzyme-linked immunosorbent assay [15]. Another study reported reduced vaccine response to influenza A but not to influenza B compared with controls, which is compatible with our results. This study included 17 patients in the 2010/2011 season sampled at 4, 8 and 12 weeks post-vaccination measured by an in-house-developed enzyme-linked immunosorbent assay [16]. In the present study we found significantly reduced protection rates at 3 and 6 months compared with the controls, but at 12 months the reduced protection rates were more similar to and not significantly different from that of the controls. We did not analyse the response to influenza B, due to lack of sensitivity in the HI method. Natalizumab is a humanized monoclonal anti-α4-integrin antibody that prevents T and NK cells from crossing blood vessels to reach affected organs and also induces lymphocyte apoptosis. The effect on the immune system is diminished immune surveillance in the central nervous system [8]. The trials that led to approval of the drug for relapsing-remitting MS (AFFIRM and SENTINEL) showed an overall elevation in the incidence of infections, including influenza. This might confer a reduced response and thus reduced protection after vaccination.

The controls had significantly higher pre-vaccination H1N1 protection rates, but this was probably attributable to the receipt of the same vaccine yearly for the last 4 years. Additionally, the antigenicity of the circulating H1N1 virus has been unchanged since the pandemic of 2009. Further, the pandemic influenza vaccine in 2009 contained the AS03 adjuvant that potentiated the immunogenicity of the vaccine to provide increased HI antibody titers and additional protection with a longer lasting response for up to 3 years. Thus, any differences between MS and controls in this study are probably underestimated.

All rates of protection against H3N2 were lower pre- and post-vaccination as previously reported, but this was also the case for the controls. In particular, the H3N2 pre-vaccination titers in all patients indicated that they had either not been previously exposed to the virus or alternatively had high antibody waning rates leading to lower protective titers. Additionally, the H3N2 virus antigenicity has changed frequently since 1968. The patient group with interferon beta-1a/1b showed the highest increase, but no conclusion could be reached for any of the medications according to time.

Vaccines play an important role in the prevention of treatment-associated infections, and are encouraged in MS and in particular before the initiation of disease-modifying therapies whenever possible [17], although no large data series or evidence-based recommendation exists for optimal timing of seasonal influenza vaccination during long-term immunomodulation.

Conclusion

Patients with MS who received fingolimod or natalizumab, but not interferon beta-1a/1b or glatiramer acetate, had reduced protection rates after influenza vaccination. These findings suggest that patients with MS receiving fingolimod or natalizumab should be considered for a second dose of the vaccine in cases of insufficient protection. Our results further indicate that new immunomodulatory treatment regimens should be systematically evaluated for influence on influenza-specific vaccine responses.

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Disclosure of conflicts of interest

GSK donated vaccine antigen for immune response analysis in 2009 outside the submitted work. K.-M.M. reports unrestricted grants and/or scientific advisory board or speaker honoraria from Almirall, Biogen, Genzyme, Merck, Novartis and Roche outside the submitted work. The other authors declare no financial or other conflicts of interest.

Ethical approval

All patients and controls provided written informed consent. The Regional Ethics Committee/REK Vest trial numbers were 2010/745 and 2009/1224. All data were anonymized.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Clinical and demographic variables of patients and controls during seasonal influenza vaccination in the county of Hordaland, Norway (2012/2013).

Figures S1 and S2. Histograms of residuals for ln (H1N1) and ln(H3N2) in Table 3.

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