In February 2013, GlaxoSmithKline (GSK) announced a commitment to further clinical transparency through the public disclosure of GSK Clinical Study Reports (CSRs) on the GSK Clinical Study Register.

The following guiding principles have been applied to the disclosure:

- *Information will be excluded in order to protect the privacy of patients and all named persons associated with the study*
- *Patient data listings will be completely removed* to protect patient privacy. Anonymized data from each patient may be made available subject to an approved research proposal. For further information please see the Patient Level Data section of the* ***GSK Clinical Study Register****.*
- *Aggregate data will be included; with any direct reference to individual patients excluded*

**Complete removal of patient data listings may mean that page numbers are no longer consecutively numbered*

PASS information

Title	An observational retrospective database analysis to estimate the risk of Multiple Sclerosis (MS) following vaccination with Arepanrix™ in Manitoba, Canada
Version identifier of the final study report	Final Version
Date of last version of the final study report	07 December 2015
EU PAS Register Number	ENCEPP/SDPP/6817
Active substance	J07BB02-AS03-Adjuvanted H1N1 Pandemic Influenza Vaccine
Medicinal product	*Arepanrix*, Pandemic Influenza vaccine (H1N1) Adjuvanted Split influenza virus, inactivated, containing antigen equivalent to A/California/7/2009 (H1N1)v like strain (X-179A)
Product reference	EU/1/10/624/001
Procedure number	EMEA/H/C/001201
Marketing Authorisation Holder	GlaxoSmithKline Biologicals Rue de l'Institut 89 1330 Rixensart, Belgium
Joint PASS	No
Research question and objectives	To assess whether administration of *Arepanrix* during the 2009/2010 H1N1 influenza pandemic was associated with an increased risk of incident MS and other demyelinating conditions not ultimately leading to a MS diagnosis in Manitoba, Canada
Country of study	Canada
Authors	• [redacted] Principal investigator • [redacted] Co-investigator

Marketing authorisation holder for the vaccine under study

Marketing authorisation holder	GlaxoSmithKline Biologicals Rue de l'Institut 89, 1330 Rixensart, Belgium
MAH contact person	[redacted]

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1. ABSTRACT

Title

An observational retrospective database analysis to estimate the risk of multiple sclerosis (MS) following vaccination with *Arepanrix* in Manitoba, Canada.

Date of the abstract: 07 December 2015

Main author: Dr [redacted]

Keywords

Post-authorisation safety study (PASS), *Arepanrix*, H1N1, multiple sclerosis, demyelinating conditions, Manitoba.

Rationale and background

Few observational studies have explored the risk of MS following immunisation with 2009 pandemic H1N1 influenza vaccines. An observational cohort study of adverse events of special interest following vaccination with *Pandemrix* found an increased risk of MS, potentially due to study limitations. This signal lead to further investigating the association between AS03-adjuvanted H1N1 vaccines and occurrence of MS.

Research question and objectives

To assess whether administration of *Arepanrix* was associated with an increased risk of incident MS and "other demyelinating conditions not ultimately leading to a MS diagnosis" in Manitoba, Canada.

Study design

Retrospective, propensity score (PS)-matched cohort study.

Study period

01 October 2009 - 31 December 2012.

Settings

Population-based analysis using de-identified records obtained by linking the electronic database of the Manitoba Immunization Monitoring System (MIMS) with the hospital, physician and prescription claims databases of Manitoba Health (MH).

Subjects and study size

The study population included adults and children above 6 months of age at the time of vaccination, residing in Manitoba and registered with MH during the study period. A vaccinated cohort (N=485,941) comprising all individuals with a MIMS record of H1N1 and/or seasonal influenza vaccination during the influenza season 2009/2010 was

matched on age, gender, place of residence and high-dimensional PS to an unvaccinated cohort comprising individuals registered with MH during the study period but with no MIMS record for H1N1 and seasonal influenza vaccination during the same season. A total of 267,539 subjects (55% of the vaccinated cohort) received *Arepanrix* and another 61,239 (13%) received it concomitantly with a trivalent inactivated seasonal influenza vaccine (TIV).

Variables and data sources

The primary endpoint was the occurrence of MS during the one-year period following administration of *Arepanrix* among the exposed cohort and during an equivalent time period in the unexposed cohort. Data sources consisted of the MIMS, Manitoba Health Population Registry, Drug Program Information Network, Hospital Abstract Database, and the Medical Services database. PS was calculated using logistic regression models that included demographic characteristics, medical history (comorbidities, immune status, vaccine indication, receipt of other vaccines or medications and frequency of healthcare contacts), pregnancy status, pre-existing conditions, and seasonal influenza vaccination.

Results

In the main analysis, the Hazard Ratio (HR) for the association between *Arepanrix* and incident MS was 0.9 (95% Confidence Interval [CI], 0.6-1.4) during the first year of follow-up. Similar estimates were obtained when measured over the entire follow-up period (HR 1.0 [0.8-1.4]) and with further adjustment for receipt of a TIV (HR 0.9 [0.6 - 1.5] and 1.1 [0.8-1.5], one year and anytime following index date, respectively). In age-stratified analyses limited by small numbers, a non-statistically significant increased risk of MS in the 25-49 age group in the first year of follow-up (HR 1.5 [0.8-2.7]) was noted. Hazard ratios for the association between *Arepanrix* and incident demyelinating conditions not ultimately diagnosed as MS were about 0.5 in all analyses.

Discussion

Because of its population-based design and the availability of accurate automated records, this analysis is less susceptible to selection bias and differential misclassification of exposures and outcomes. The availability of a vaccination registry reduced vaccine use measurement errors. The use of validated algorithms limited the risk of misclassification of outcome. One limitation is the lack of information on lifestyle and environmental risk factors for MS, which was addressed by matching on age, gender, place of residence (proxy for ethnicity) and PS, which was calculated using more than 400 covariates. The vaccinated and unvaccinated cohorts were comparable, indicating a reasonable performance of the matching procedure. Finally, the large sample size permitted the calculation of reasonably precise estimates, although in some subgroup analyses, precision was limited by small numbers.

Data of this study are consistent with existing research on the association between influenza vaccination (seasonal and pandemic) and MS. Overall, the range of risk estimates across analyses suggests no evidence of an association between vaccination with A*repanrix* and the incidence of MS or other central nervous system demyelinating conditions not ultimately diagnosed as MS.

Marketing Authorisation Holder for the vaccine under study

GlaxoSmithKline Biologicals
Rue de l'Institut 89, 1330 Rixensart, Belgium

Names and affiliations of principal investigators

Principal Investigator:

- [redacted]

Co-investigator:

- [redacted]

2. LIST OF ABBREVIATIONS

AEFI	Adverse Events Following Immunization
AESI	Adverse Events of Special Interest
AIDS	Acquired Immune Deficiency Syndrome
ARR	Adjusted Rate Ratio
AS03	Adjuvant System 03
ATC	Anatomical Therapeutic Chemical
CI	Confidence Interval
CNS	Central Nervous System
DPIN	Drug Program Information Network
EMA	European Medicines Agency
ERB	Ethical Review Board
EU PAS	European Union Post-Authorisation Studies
GSK	GlaxoSmithKline
GPP	Guidelines for Good Pharmacoepidemiology Practices
H1N1	Hemagglutinin 1 Neurominidase 1
[redacted]	[redacted]
HR	Hazard Ratio
hd-PS	high-dimensional Propensity Score
HIV	Human Immunodeficiency Virus
ICD	International Classification of Diseases
ICD-9-CM	International Classification of Diseases, Ninth Revision, Clinical Modification
ICD-10-CA	International Classification of Diseases, Tenth Revision, Canadian Adaptation
MCHP	Manitoba Centre for Health Policy

MH	Manitoba Health
MHPR	Manitoba Health Population Registry
MIMS	Manitoba Immunization Monitoring System
MS	Multiple Sclerosis
NPV	Negative Predictive Value
PASS	Post Authorization Safety Study
PI	Principal Investigator
PPV	Positive Predictive Value
PS	Propensity Score
Q1	First Quartile
Q3	Third Quartile
SAS	Statistical Analysis System
TIV	Trivalent Inactivated seasonal influenza Vaccine
US	United States (of America)
WHO	World Health Organisation

3. ETHICS

3.1. Independent Ethics Committee or Institutional Review Board

The study protocol and research agreements were reviewed and approved by the [redacted] and the [redacted]

3.2. Ethical conduct of the study

This study was conducted in accordance with Good Pharmacovigilance Practices (GPP) and all applicable regulatory requirements, including the Declaration of Helsinki.

Access to data was subject to approval by [redacted] and by the [redacted]

3.3. Subject information and consent

No patient informed consent was obtained. The patient information in the database utilized is fully anonymized and the research team was not able to make a link between the data and specific individuals. None of the subjects were contacted.

4. INVESTIGATORS

Principle Investigator:

- [redacted]

Co-investigator:

- [redacted]

5. OTHER RESPONSIBLE PARTIES

The present study was initiated following a regulatory commitment from the European Medicines Agency (EMA) to GlaxoSmithKline (GSK) Biologicals. GSK Biologicals has the responsibility for delivering the study report to EMA as per this commitment, and to ensure compliance with the EMA "Guidance for the format and content of the protocol of non-interventional post-authorisation safety studies" (EMA/623947/2012). The protocol and report were developed in a collaborative manner between GSK Biologicals and the Principal Investigator (PI), Dr. [redacted]

As per the Manitoba Centre for Health Policy (MCHP) Guidelines for Public and Private Sponsorship of Research Projects [MCHP, 2011], the PI was responsible for obtaining all necessary study approvals; overall conduct of the study; he is also responsible for

publishing the results in the searchable, peer-reviewed scientific literature. A protocol summary, including the anticipated timing for posting and submission of the results for publication, was posted on the EU PAS register (register # ENCEPP/SDPP/6818), as required by the EMA, and on other publicly available registers (ClinicalTrials.gov # NCT02367222).

6. MILESTONES

Milestones	Planned date	Actual date	Comments
Ethical approvals (from ERB, [redacted] and MCHP)	Not applicable	JAN to MAY-14	Approvals based on Version 1.0 of the protocol dated 19-SEPT-13
Final protocol submitted to EMA	Not applicable	12-MAY-14	Final protocol version dated 05-MAY-14
Registration in the EU PAS Register	Not applicable	01-OCT-14	Register no. ENCEPP/SDPP/6818
Start of data collection	30-SEPT-14	01-JUN-14	Here data collection is gaining access to the MH data repository, as the data is pre-collected
End of data collection	30-NOV-14	30-JUL-14	None
Statistical analysis complete	30-APR-15	30-OCT-15	Includes the process of creation of PS, matching, and generation of variables, in addition to actual analysis
Final report of study results	30-JUN-15	07-DEC-15	None

7. RATIONALE AND BACKGROUND

7.1. Background

Multiple sclerosis (MS) is a chronic, progressively disabling disease of the Central Nervous System (CNS), estimated to affect more than 2.5 million persons worldwide [Dean, 1994]. Canada has among the highest prevalence of MS in the world, with more than 90,000 individuals affected [Beck, 2005; Gilmour and Hofmann, 2010]. It is the most common non-traumatic cause of disability in young adults, and adversely affects employment, social relationships, and quality of life [Nortvedt, 1999; Rao, 1991]. The societal costs of MS exceed those for stroke or Alzheimer's disease. Thus, the burden of MS is substantial for affected individuals and society.

Despite many studies, the aetiology of MS remains unknown [Marrie, 2004]. MS is likely caused by complex interactions between genetic and environmental factors. Putative risk factors that have been commonly studied include infection, vaccinations, stress, occupation, climate, and diet [Marrie, 2004]. Infection has been a putative etiologic agent of particular interest although there has been no reproducible evidence of a transmissible MS agent [Cosby, 1989; Haase, 1981; Hammerschlag, 2000]. The biological plausibility of Epstein-Barr virus as an etiologic factor is increasing, however, suggesting that infectious agents may initiate or perpetuate the disease process.

Similarly, vaccinations have also been considered as etiologic factors for MS. A series of case reports in France raised particular concern about demyelinating events developing after hepatitis B vaccination [DeStefano, 2003]. Ascherio and colleagues conducted a

nested case-control study with data from the Nurses Health Studies in which 192 women with MS were matched to 645 controls. The odds ratio of MS associated with hepatitis B vaccination occurring any time before disease onset was 0.9 (95% Confidence Interval (CI) 0.5–1.6) [Ascherio, 2001]. Case-control and cohort studies have been consistent in showing no association between other childhood vaccinations (measles, mumps, rubella) and MS [Bansil, 1990; Casetta, 1994; Currier, 1996; Zorzon, 2003]. Generally the bulk of scientific evidence does not support an increased risk of developing MS with vaccination perhaps with the exception of the yellow fever vaccine [Farez, 2011].

However, very few published studies have evaluated the association between 2009 pandemic H1N1 vaccination and the risk of developing MS. Vrethem *et al*. reported on a previously healthy young man who developed severe narcolepsy and MS within two months of receiving *Pandemrix* [Vrethem, 2012]. *Pandemrix,* and its Canadian-made equivalent *Arepanrix,* are AS03-adjuvanted split virion pandemic influenza H1N1 vaccines. A large retrospective Swedish record-linkage study reported increased risk of paraesthesia, but not of diagnosed MS, among persons vaccinated with *Pandemrix* [Bardage, 2011; Persson, 2014]. However, the study was limited by the use of non-validated algorithms for the identification of MS from administrative databases and by the inability to distinguish between prevalent and incident cases. Thus, the effect of H1N1 vaccination on MS remains uncertain.

The first confirmed case of pandemic H1N1 infection in the Canadian province of Manitoba was detected on May 3, 2009 [Thompson, 2011]. Like elsewhere in the Northern hemisphere, there were two epidemic waves; one between mid-May and the end of June 2009, and the other during the 2009/10 influenza season, which occurred predominantly between October and December of 2009 [Thompson, 2011; Zarychanski, 2010]. Mass immunization against pandemic H1N1 commenced October 26th 2010 using primarily large-scale vaccination clinics led by public health teams and lasted approximately 8 weeks. Initially, GSK's Canadian-manufactured AS03-adjuvanted 2009 pandemic H1N1 influenza vaccine *Arepanrix* was used to vaccinate adults and children over 6 months of age. Later on, two unadjuvanted pandemic H1N1 vaccines, from CSL Limited and GSK, were offered to pregnant women and children over 10 years of age; however, *Arepanrix* was the only adjuvanted vaccine used in Canada. Trivalent inactivated seasonal influenza vaccine (TIV) were administered as part of the annual influenza immunization program. The live attenuated influenza vaccine was not available in Manitoba during the 2009–2010 season [Mahmud, 2012].

All vaccines were offered free of charge, but limited vaccine supply at the start of the campaign necessitated the development of priority groups for early vaccination. The initial priority group for the H1N1 vaccine in Manitoba included health care workers, Aboriginal persons, pregnant women, children 6-60 months-old, individuals under 65 years of age with chronic medical conditions (including MS), immunocompromised individuals and residents of remote communities [Mahmud, 2011]. On November 18, 2009 the Pandemic H1N1 vaccines were made available to the whole population [Mahmud, 2011].

7.2. Rationale for the study

As outlined above, a limited number of observational studies have explored the risk of MS following pandemic H1N1 influenza vaccination. In most studies, no increased risk was identified. A GSK-supported, observational cohort study of individuals vaccinated with *Pandemrix* as part of the national 2009 H1N1 pandemic immunisation campaign in Sweden, measured incidence rates of Adverse Events of Special Interest (AESIs): anaphylaxis, Bell's palsy, convulsion, demyelination, encephalitis, Guillain-Barré Syndrome, neuritis, any influenza, vasculitis, convulsions in epileptics, autoimmune hepatitis, and MS. For MS, the standardised incidence ratio was significantly increased, which might have been due to the limitations of the study, including potential selection bias and lack of control for residual confounding [unpublished report]. This signal triggered the need to further investigate the potential association between AS03-adjuvanted H1N1 vaccines and the occurrence of MS.

Investigating the signal in the Manitoba settings had the following advantages:

- The burden of MS in Canada is substantial [Beck, 2005; Evans et al, 2013], and the province of Manitoba has one of the highest prevalence of MS with approximately 100 new cases each year [redacted] personal communication], making this region suitable to address the research question;
- This study allowed obtaining complementary data on the safety of *Arepanrix*, an AS03-adjuvanted split virion pandemic influenza H1N1 vaccine similar to *Pandemrix*;
- In the EMA assessment of the draft report of the GSK-supported safety study on the risk of AESIs following vaccination with *Arepanrix* in Manitoba, it was stated that "*No strong signal was observed for demyelination with Arepanrix; indeed higher risk estimates were observed for seasonal trivalent influenza vaccines. However, risk estimates in the subgroup analysis (individuals with autoimmune diseases and those aged 18-64 years) were elevated, with lower 95% confidence levels >1*". This study further explored this matter using a more robust design (propensity score [PS] matching of the cohorts) and a validated case definition, to allow the identification of incident MS cases.

In summary, the aim of the study was to assess whether administration of *Arepanrix* was associated with an increased risk of incident MS in Manitoba, Canada. The availability of a province-wide population-based immunization registry and other linked health care administrative databases provided a unique opportunity to perform this evaluation.

8. RESEARCH QUESTION AND OBJECTIVES

8.1. Primary objective

- To assess whether administration of *Arepanrix* was associated with an increased risk of incident MS.

8.2. Secondary objective

- To assess whether administration of *Arepanrix* was associated with an increased risk of demyelinating events which do not ultimately lead to a diagnosis of MS (i.e., never have a diagnostic claim for MS), including optic neuritis.

8.3. Exploratory objective

- To assess whether administration of unadjuvanted pandemic H1N1 influenza vaccines was associated with an increased risk of incident MS.

9. AMENDMENTS AND UPDATES

None.

10. RESEARCH METHODS

10.1. Study design

This was a retrospective analysis of population-based cohorts of subjects, whose vaccination status and health events before and after vaccination, were recorded in various MH administrative databases. A PS matched cohort analysis was conducted using de-identified records obtained by linking the electronic database of the Manitoba Immunization Monitoring System (MIMS) with the hospital, physician and prescription claims databases of MH.

10.1.1. Rationale for study design

The use of automated administrative databases allows access to a large population of vaccinated individuals. A cohort design using PS matching was adopted to increase comparability between the exposed and unexposed cohorts on known potential confounders.

10.2. Settings

10.2.1. Study period

The study period spanned from 01 October 2009 (beginning of the H1N1 influenza mass vaccination campaign in Canada) to 31 December 2012 (to allow sufficient follow-up time for cases to have a confirmatory diagnosis given the natural history of MS).

10.2.2. Data collected

10.2.2.1. Subjects characteristics

Demographic characteristics such as age, sex, area of residence, socio-economic status were collected. Medical history such as comorbidity, immune status, vaccine indication (e.g., pregnancy, cardiovascular, pulmonary or renal diseases, etc.), receipt of other vaccines or medications and frequency of healthcare contacts was obtained.

Information on pregnancy status and pre-existing conditions was obtained from the Hospital Separation and Physician Claims databases. Previously validated algorithms, based on the frequency of certain International Classification of Diseases (ICD) codes, were used to identify various chronic diseases (Table 1) [Elixhauser, 1998; Lix, 2006]. Immunosuppression was defined as having a diagnosis of Human Immunodeficiency Virus / Acquired Immune Deficiency Syndrome (HIV / AIDS), other immune deficiency disorders or cancer (other than non-melanoma skin cancer), or receiving prescriptions for immunosuppressive drugs (Table 1) [Dublin, 2009]. Auto-immune diseases were defined as ≥1 admission (ICD-10 codes) or ≥2 physician claims (ICD-9 codes) (Table 1). Information on the use of immunosuppressants was obtained from the Drug Program Information Network (DPIN). Pregnancy status was determined from the same databases using disease and tariff codes for different conditions and procedures indicative of ongoing pregnancy or the completion of pregnancy (Table 1) [Hardy, 2004].

10.2.2.2. History of pandemic H1N1 and seasonal influenza vaccines

Information on the receipt of all vaccines, including the pandemic H1N1 and seasonal influenza vaccines, was obtained from MIMS (refer to Table 1 and Section 10.5.2).

10.2.2.3. Case definitions

Multiple Sclerosis

We identified incident cases of diagnosed MS among all included individuals by record linkage with the hospital and physician claims databases and DPIN using a validated algorithm developed by Dr. [redacted] a co-investigator and co-author of this report [Marrie, 2010] (Table 1).

In 2008, Dr. [redacted] and colleagues used Manitoba administrative claims data to identify persons with demyelinating disease using ICD-9 and ICD-10 codes and prescription claims [Marrie, 2010]. To validate the algorithm, questionnaires were mailed to 2000 randomly selected persons with an encounter for demyelinating disease, requesting permission for medical records review. Diagnoses abstracted from medical records were used as the gold standard to evaluate candidate case definitions using administrative data. From 1984-1997, cases of MS using claims data were defined as persons with ≥ 7 hospital or physician claims for MS. From 1998 onward, cases were defined as persons with ≥ 3 hospital, physician or prescription claims for MS. As compared to medical records, this definition had a Positive Predictive Value (PPV) of 80.5% and Negative Predictive Value (NPV) of 75.5% in persons with ≥ 1 claim for demyelinating disease; the NPV is much higher at the population level where more than 98% of the population has no claims for demyelinating disease. The performance of this case definition was recently assessed in Nova Scotia [Marrie et al, 2014]. Applying the case definition of ≥ 3 hospital or physician claims for MS (prescription claims were not available) and comparing it to MS diagnoses from the Dalhousie MS Research Unit database, the PPV was 93% (95% CI: 92-94%).

In the present analysis, we used the validated Manitoba case definition to define a case of MS as a person with ≥ 3 hospital, physician or prescription claims for MS (see Table 1 for details). We considered a case *incident* if there were no physician or hospitalization records indicating a diagnosis of any demyelinating condition between 1971 (the earliest year for which information was available from the electronic databases) and the index date. The date of diagnosis of MS (outcome date) is the date of the first medical contact for any of the MS diagnostic codes.

Other Demyelinating Diseases

Demyelinating events not ultimately leading to MS diagnosis, including optic neuritis, were defined by ≥ 1 hospitalizations or ≥ 2 physician claims at least 30 days apart with no subsequent MS diagnosis (see Table 1 for ICD9/10 codes).

10.3. Subjects

10.3.1. Study population

The study population was comprised of adults and children above 6 months of age (at the time of vaccination) who normally resided in Manitoba and had been registered with MH for at least 1 year before the enrolment period (see Section 10.2.1). To ensure sufficient historical data, all participants were required to have at least one year of insurance coverage before the study period.

10.3.2. Inclusion criteria

The entire population of Manitoba was considered for inclusion.

10.3.3. Exclusion criteria

- Individuals ≤ 6 months of age;
- Having less than one year of insurance coverage before the enrolment period;
- Not registered with MH during the enrolment period;
- Physician or hospitalization records indicating a diagnosis of any demyelinating condition between 1971 (earliest year for which information was available) and the index date.

10.3.4. Cohort identification and creation

The **vaccinated cohort** was assembled by identifying all individuals who had a MIMS record indicating receipt of pandemic H1N1 influenza or TIV/seasonal influenza vaccines (see Table 1 for tariff codes that were used to identify these records) during the **enrolment period**, i.e., between September 15th, 2009, and March 15th, 2010, spanning the period when almost all H1N1 vaccines and TIVs were administered. Individuals who were registered with MH during the study period but did not have MIMS records indicating receipt of the H1N1 or seasonal influenza vaccines constituted the **unvaccinated cohort**.

For additional clarity:

- **Vaccinated cohort:** all individuals with MIMS record of H1N1 and/or seasonal influenza vaccination during the influenza season 2009/2010 (September 15th, 2009 to March 15th, 2010).
- **Unvaccinated cohort:** registered with MH during the study period but with no MIMS record for H1N1 and seasonal influenza vaccination during the influenza season 2009/2010 (September 15th, 2009 to March 15th, 2010).

Based on PSs (see details of the PS model in Section 10.9.2.7.1), each vaccinated individual was matched to an individual who did not receive any influenza vaccines during the study period.

The **index date** was defined as the date of vaccination for vaccinated individuals, and the date of vaccination of the matched vaccinated individual for unvaccinated individuals. For the unvaccinated cohort, the index date was between September 15th, 2009, and March 15th, 2010.

10.4. Variables

10.4.1. Primary endpoint

- Occurrence of MS during the period of one year following administration of *Arepanrix* among an exposed cohort (see Section 10.3.4) and during an equivalent time period in the unexposed cohort.

10.4.2. Secondary endpoints

- Occurrence of MS from administration of *Arepanrix* until 31 December 2012, among an exposed cohort (see Section 10.3.4) and during an equivalent time period in the unexposed cohort.
- Occurrence of demyelinating events which do not ultimately lead to a diagnosis of MS (i.e., never have a diagnostic claim for MS) during the period of one year following administration of *Arepanrix* among an exposed cohort (see Section 10.3.4) and during an equivalent time period in the unexposed cohort such as optic neuritis, acute transverse myelitis, demyelinating disease of CNS unspecified, other acute disseminated demyelination, and neuromyelitis optica.
- Occurrence of demyelinating events which do not ultimately lead to a diagnosis of MS (i.e., never have a diagnostic claim for MS) from administration of *Arepanrix* until 31 December 2012, among an exposed cohort (see Section10.3.4) and during an equivalent time period in the unexposed cohort, such as optic neuritis, acute transverse myelitis, demyelinating disease of CNS unspecified, other acute disseminated demyelination, and neuromyelitis optica.

10.4.3. Exploratory endpoint

- Occurrence of MS during the period of one year following administration of unadjuvanted pandemic H1N1 influenza vaccines among an exposed cohort and during an equivalent time period in the unexposed cohort.

10.5. Data sources

10.5.1. Manitoba Health administrative databases

MH is the publicly funded health insurance agency providing comprehensive health insurance, including coverage for hospital and outpatient physician services, to the province's 1.2 million residents. Coverage is universal (there is no eligibility distinction based on age or income) and participation rates are very high (> 99%) [Singh, 2009]. Only the Royal Canadian Mounted Police and military personnel, whose health benefits are fully covered by the federal government, are not included [Roos, 1993].

For administrative purposes, MH maintains several centralized electronic databases that are linkable using a unique personal health identification number. The completeness and accuracy of the Manitoba administrative database are well established, [Humphries, 2000; Roos, 1993; Young, 1997] and these databases have been used extensively in studies of post-marketing surveillance of various vaccines and drugs [Fedson, 1993; Mahmud, 2011; Mahmud, 2012; Roberts, 1994; Singh, 2009].

10.5.2. Manitoba Immunization Monitoring System

Information on the receipt of all vaccines, including pandemic H1N1 and seasonal influenza vaccines were obtained from MIMS, the population-based province-wide registry recording all immunizations administered to Manitoba residents since 1988

[Roberts, 1996]. Information, including vaccine type and date of immunization, was captured for each immunization event either through direct data entry for vaccines administered by public health staff (who administered the majority of H1N1 vaccines during the pandemic) or using physician claims data for vaccines administered by physicians [Roberts, 1994]. Estimates of the completeness and accuracy of the recorded vaccination information were high [Roberts, 1994]. Vaccination status in the MIMS database did not include information on brand/manufacturer; however, data on the adjuvanted nature of pandemic influenza vaccines that were used in Manitoba were available.

10.5.3. Manitoba Health Population Registry (MHPR)

Eligibility for inclusion in the analysis was determined using the MHPR, a continuously updated registry that stores basic demographic information (e.g., date of birth and sex) on all insured Manitobans, and gathered information on dates and reasons for the initiation and termination of health care coverage (e.g., birth, migration in or out of province and death), and on changes in address and marital status of the insured individuals.

10.5.4. Drug Program Information Network

Information on MS and other relevant diseases and health conditions (see Section 10.2.2.3) was obtained from the hospital and physician claims databases and from the database of the DPIN. The DPIN, in operation since 1995, records all prescription drugs dispensed to Manitoba residents [Kozyrskyj, 1998]. The DPIN database captures data from pharmacy claims for formulary drugs dispensed to all Manitobans even those without prescription drug coverage. Because information is submitted electronically at the "point-of-sale", the accuracy of the recorded prescription information is excellent [Kozyrskyj, 1998].

10.5.5. Hospital Abstract Database

Since 1971, the Hospital Abstracts database record virtually all services provided by hospitals in the province, including admissions and day surgeries [Roos, 1993]. Data collected comprises demographic as well as diagnosis and treatment information including primary diagnosis and service or procedure codes, coded using the ICD, Ninth Revision, Clinical Modification (ICD-9-CM) before April, 2004, and the ICD-10-CA, (Canadian adaptation of the ICD-10 [WHO, 1993]) and the Canadian Classification of Health Interventions [Canadian Institute for Health Information, 2006] afterwards.

10.5.6. The Medical Services database

The Medical Services database, also in operation since 1971, collects similar information, based on physician fee-for-service or shadow billing, on services provided by physicians in offices, hospitals and outpatient departments across the province [Roos, 1993]. Each billing record includes a tariff code and a 3-digit ICD-9 code which identifies the principal diagnosis or main reason for the visit. This database is limited by the lack of more specific ICD codes (4^{th} and 5^{th} digits).

10.6. Bias

Refer to sections 10.9.2.7.1 and 12.2 for a description of potential sources of bias and limitations of the research methods.

10.7. Study size

Based on 400,000 vaccinated individuals (and 400,000 non-vaccinated individuals) and assuming a MS incidence rate of 20/100,000 among non-vaccinated individuals, a conservative assumption given that MS rates among younger adults in Manitoba ranged from 29/100,000 in the 35-39 age-group to 19/100,000 in the 50-54 age-group from 1998 to 2006 [Marrie et al, 2010], the matched cohort analysis was estimated to have >99% power to detect a doubling of the risk (rate ratio [RR]=2) and 81% power to detect 50% increase in risk (RR=1.5) [OpenEpi, 2013; Fleiss, 2003; Kelsey, 1996]. A two-sided test at alpha=0.05 was assumed in all calculations.

10.8. Data transformation

10.8.1. Data management

The final database consisted of data extracted from the databases described in Section 10.5. Record linkage was performed by the employees of the MCHP where these databases are housed. The analytic database was accessed and analysed within the confines of the MCHP's secure computing environment. Data analysis was conducted at the Vaccine and Drug Evaluation Centre using secure terminals directly connected to the MCHP's secure computing environment.

10.9. Statistical methods

10.9.1. Main summary measures

Please refer to section 10.9.2 for a detailed description of each method and corresponding measures (where applicable).

10.9.2. Main statistical methods

10.9.2.1. Hypotheses

Null hypothesis (H0): the incidence of MS in the exposed cohort is equal to the incidence in the non-exposed cohort.

Alternative hypothesis (H1): the incidence of MS in the exposed cohort is not equal to the incidence in the non-exposed cohort.

The same hypotheses were tested for the secondary endpoint (demyelinating events).

10.9.2.2. Analysis Population

The study population for the cohort design comprised all enrolled exposed and unexposed subjects that satisfied the inclusion criteria.

10.9.2.3. Subject disposition

Subject disposition was summarised by computing the number of subjects by type of vaccine received (Table 2; see also Section 10.9.2.4).

10.9.2.4. Demographic and baseline characteristics

Demographic and baseline characteristics of all enrolled subjects (age at enrolment, other vaccination during the previous year, medical history, healthcare resource utilization during the previous year, etc.) were summarized per cohort and overall, using descriptive statistics (Table 3). Frequency tables were generated for categorical variables. Mean, standard error, median, Q1, Q3, and range were provided for continuous variables.

10.9.2.5. Analysis of primary endpoint

The primary analysis compared the incidence rates of MS between the exposed cohort and the unexposed cohort in the year following the index date. Person-time was defined as the period between the index date (see Section 10.3.4) and the earliest of the following events:

- Diagnosis of the outcome of interest;
- Death or loss to follow-up;
- Termination of insurance coverage;
- Receipt of H1N1 vaccine or TIV for the unexposed cohort;
- Receipt of any other vaccine following the index date;
- End of the first year following the index date.

Incidence rates for MS were calculated by dividing the number of cases by person-time. Both crude and age-adjusted incidence rates were calculated. In addition, both crude and age-adjusted incidence rate ratio were calculated.

The corresponding multivariate analysis consisted of Cox regression models (See section 10.9.2.7.2 for details).

10.9.2.6. Analysis of secondary endpoints

The analysis of the incidence of MS until 31 December 2012 used the same statistical approach as the primary analysis.

Exposed person-time was defined as the period between the index date (see Section 10.3.4) and the earliest of the following events:

- Diagnosis of the outcome of interest;
- Death or loss to follow-up;
- Termination of insurance coverage;
- Receipt of H1N1 vaccine or TIV for the unexposed cohort;
- End of study period (31 December 2012).

The secondary analysis also compared the incidence rates of demyelinating events which did not ultimately lead to a diagnosis of MS (including optic neuritis, acute transverse myelitis, demyelinating disease of CNS unspecified, other acute disseminated demyelination, and neuromyelitis optica), between the exposed cohort and the unexposed cohort.

Exploratory analyses were conducted to assess the association between unadjuvanted pandemic influenza vaccine(s) and incidence of MS. These analyses were not considered as confirmatory.

10.9.2.7. Statistical models

10.9.2.7.1. Propensity score model

Due to lack of random assignment of treatments, estimates of treatment effects in observational studies could be biased because the treatment group and the control group might not be comparable with respect to the distribution of important disease (or outcome) predictors (confounders). PS methods are one approach to constructing more comparable groups by limiting comparisons to individuals who had the same propensity to receive the treatment [Rubin, 1997]. PS are defined as the conditional probability of receiving treatment given the value of a set of confounders, and can be estimated using logistic or probit regression models of the association between confounding covariates and the receipt of treatment [Rubin, 1997]. PS methods are especially suitable for post-marketing studies of drug and vaccine safety where the outcomes are typically rare, limiting the utility of conventional multivariate adjustment methods, but the treatment and confounders data are very rich.

We used the high-dimensional Propensity Score (hd-PS) algorithm [Schneeweiss et al, 2009], implemented as a Statistical Analysis System (SAS) macro downloadable from http://www.drugepi.org/dope-downloads/, to calculate a PS for each eligible participant indicating his or her probability of receiving the pandemic vaccine as derived from a logistic regression model that included the receipt of the pandemic vaccine as an dependent variable and more than 400 independent variables including demographic variables (e.g., age, sex, area of residence, socio-economic status), comorbidity and healthcare utilizations variables(e.g., records of admission or physician visits for most common conditions) and prescription drug and vaccine utilization variables.

We used a greedy matching algorithm to pair-match each vaccinated individual with a randomly selected unvaccinated individual with the closest PS. Because of the large sample size, sets of matched individuals were still heterogeneous with regard to age group, gender and area of residence. Consequently, we decided to also match on these variables. So, at the end our cohort was matched with respect to PS, age group, gender and area of residence.

10.9.2.7.2. Time-to-event model

Standard time-to-event (survival) analysis methods were used for most analyses. Time-to-event (onset of MS) was measured from the *index date* to the date of MS onset as defined by the first demyelinating disease code in hospital or physician claims. Individuals were censored on the date of loss to follow-up (e.g., due to death or immigration) or on the study end date (2 years following the index date). In addition, individual observations were censored on the date of any subsequent administration of a different vaccine because any MS cases identified afterwards might have been due to the more recently given vaccine. On the other hand, two vaccines given on the same day (typically, an H1N1 vaccine given concurrently with a TIV) were considered as a single episode. However, in analyses stratified by vaccine type, these episodes were grouped separately (labelled as the "concurrent H1N1/TIV" cohort), and the incidence of MS in this group was compared to that among individuals who received an H1N1 vaccine only (the "H1N1 alone" cohort) or a TIV only (the "TIV alone" cohort).

Cumulative incidence curves of MS were computed separately for each cohort (vaccinated and non-vaccinated) and sub-cohort ("concurrent H1N1/TIV", "H1N1 alone" and "TIV alone"). Numbers permitting, the "H1N1 alone" and ("concurrent H1N1/TIV", sub-cohort was further divided into those who received the adjuvanted H1N1 vaccine and those who received the unadjuvanted H1N1 vaccine.

Cox proportional hazard models, with stratification on the matched pairs, were used to estimate relative risks (hazards ratios) associated with the receipt of the H1N1 vaccine [Cummings, 2003]. Cox models assume that the effect of covariates is constant over time (proportional hazards assumption). We tested this assumption using graphical and formal methods as proposed by Therneau & Grambsch [Therneau, 2000]. If the hazards function was non-proportional over time, interaction terms between time and the appropriate covariates were included in the model. The possibility of effect modification with the receipt of the 2009/10 TIV was assessed, testing for interactions between H1N1 and TIV terms using a likelihood ratio test with a relatively liberal cut-off point for statistical significance ($P < 0.15$).

10.9.2.8. Conduct of analysis

All the analyses were done on the final database.

10.9.3. Missing values

The analyses were based on data from the MH database system. Missing data was not substituted. As for any study using large healthcare databases, it cannot be excluded that some information is not recorded in the database.

10.9.4. Sensitivity analyses

The following subgroup analyses were performed:

- Separate analyses were performed for the following three age groups: ≤24, 25-49 and ≥ 50.

10.9.5. Amendments to the statistical analysis plan

- Analysis for subjects with a history of auto-immune disease other than MS was not conducted due to small numbers.

10.10. Quality control

Data management was performed in accordance with applicable standards and data cleaning procedures. The final study dataset was archived and stored on a secured, limited access, computer platform. The validation of the quality control of the statistical analysis was documented. The final study protocol and the final study report(s) were and will be archived by GSK on a Document management system based on the Documentum platform: Computer Aided Regulatory Submission.

11. RESULTS

11.1. Participants

A total of 485,941 subjects having received one or more doses of the pandemic or seasonal vaccines during the enrolment period constituted the vaccinated cohort (Table 2). Of these, 278,131 (57%) received a pandemic vaccine only, 63,216 (13%) received both a pandemic and a 2009/2010 seasonal vaccine, and 144,594 (30%) received a seasonal vaccine only. In total, 341,347 persons (29% of the total study population) received one or more doses of the pandemic vaccine, which is comparable to overall Canadian data [Gilmour and Hofmann, 2010].

The large majority (96%) of those who received a pandemic vaccine received the adjuvanted vaccine (Arepanrix); 78.4% received it alone, whereas 18% received it in addition to a TIV (Table 2). About 4% of the pandemic vaccine recipients received an unadjuvanted pandemic vaccine (3% alone and about 1% concurrent with an TIV).

11.2. Descriptive data

Table 3 shows participant characteristics by vaccination status and type of vaccine received. As expected, children and younger adults (<35 years of age) dominated (54%)

the pandemic vaccine group, whereas older adults (55+) dominated (78%) the TIV group. Still 62,808 (18%) older adults received the pandemic vaccine. Similar impressions can be gleaned from analyses of birth cohorts with those born after 1994 representing a greater proportion of the pandemic vaccine group.

As a consequence of matching, the vaccinated and unvaccinated groups were quite similar with respect to their socio-demographic (birth season, gender, region of residence, and income) and most clinical characteristics. However, people with chronic illnesses (including cancer and diabetes) constituted a larger percentage of those who received the TIV. Overall, 30% of those who received the TIV had one or more chronic illness (Table 3) compared to 7% of those who received the pandemic vaccine alone and about 13% of those who did not receive any vaccine. As expected, there were more pregnant women in the vaccinated group. As a result of the above patterns, twice as many people in the TIV vaccinated group belonged to a group for which the TIV was recommended (according to recommendations of the Canadian National Advisory Committee on Immunization) compared to the unvaccinated group (76% compared to 33%). The gap was smaller for the pandemic vaccine where 54% of the pandemic vaccine group belonged to a high-priority group compared to 48% of the unvaccinated. The vaccinated were also more likely to have received a 2008/09 TIV and at least one pneumococcal vaccine.

11.3. Outcome data

11.3.1. Multiple sclerosis

Table 4 shows the crude and age-standardized rates (per 100,000 PY) of incident MS during the period of one year following index date by vaccination status. By the end of the first year of follow-up, 106 cases were diagnosed among the unvaccinated, corresponding to an age-standardized rate (ASR) of 24.2 (20.1 – 28.3)/100,000 compared to 69 cases and ASR of 20.2 (15.4 – 24.9)/100,000 among the vaccinated cohort (age-Adjusted Rate Ratio [ARR] = 0.8 (0.3-2.2). Participants who received the pandemic vaccine had a slightly lower ASR at 17.7 (14.1–21.2)/100,000 with an ARR of 0.7 (0.3 – 1.7). Similar rates were observed for adjuvanted and unadjuvanted pandemic vaccine cohorts. The ASR was a bit higher among those who received the TIV alone, 36.8 (25.0 – 48.6)/100,000, with an ARR of 1.5 (0.3 – 6.8) compared to unvaccinated persons. The wide 95% CI indicates the lack of precision of these estimates due to small number of cases in the TIV alone cohort (N=14). No increase of risk was observed among those who received a TIV and pandemic vaccine concurrently.

The average rate of MS over the entire follow-up period (median of about 3 years) were about 20-30% lower than those observed during the first year of follow-up (Table 5). Regardless of vaccine type, ARRs calculated over this period were consistent with lack of an association between vaccine administration and MS. For instance, the ARR for receipt of pandemic vaccine alone was 0.9 (0.3-2.8).

11.3.2. Demyelinating conditions not ultimately diagnosed as MS

Table 6 shows the crude and age-standardized rates (per 100,000 PY) of incident demyelinating conditions not ultimately diagnosed as MS during the period of one year following index date by vaccination status. After 1 year of follow-up, 27 patients met the case definition among the unvaccinated, corresponding to an ASR of 6.9 (2.6 - 11.1)/100,000 compared to 17 cases and ASR of 4.7 (0.0 - 10.6)/100,000 among the vaccinated cohort. Participants who received the pandemic vaccine had an ASR of 5.6 (0.0 - 13.3)/100,000 with an ARR of 0.8 (0.1 - 10.6). No cases were observed among those who received the TIV.

The average rate of these conditions over the entire follow-up period (median of about 3 years) were about 20-30% lower than those observed during the first year of follow-up (Table 7). Generally, ARRs calculated over this period were consistent with lack of an association with vaccine administration except for those who received an unadjuvanted vaccine where the ASR was higher 7.4 (0.0 - 18.0)/100,000, but due to small number of cases (<6), the corresponding ARR (2.1) was very imprecise (0.1 - 39.9).

11.4. Main results

11.4.1. Multiple sclerosis

Table 8 shows estimates of Hazard Ratios (HRs) and 95% CIs of the association between incident MS and vaccine administration during the period of one year following index date by vaccination type. In a model adjusted for PS, age, gender, and area of residence (Model A), there was no evidence of an association with the receipt of any vaccine. The HR for the receipt of *Arepanrix* alone was 0.9 (0.6-1.4), with no change with further adjustment for receipt of a TIV (Model B). The estimates for adjuvanted and unadjuvanted vaccines were comparable. Similarly the receipt of TIV alone or concurrently with pandemic vaccine was not associated with MS diagnosis. Similar patterns were observed when disease occurrence was measured over the entire follow-up period (Table 9).

11.4.2. Demyelinating conditions not ultimately diagnosed as MS

Table 10 and Table 11 show the corresponding results for demyelinating conditions not ultimately diagnosed as MS. There was no evidence of an increased risk of these conditions with the receipt of any pandemic vaccine. The receipt of either TIV alone or concurrently with the adjuvanted pandemic vaccine (*Arepanrix*) was associated with a small increased point estimate (HR about 2) of these conditions and the association persisted after adjusting for receipt of the 08/09 TIV and when repeated for the entire study period. Although consistent, none of these associations were statistically significant or precise, given the small number of cases diagnosed among these groups.

11.5. Other analyses

Table 12 shows estimates of HRs and 95% CIs of the association between incident MS and vaccine administration during the period of one year following index date for 3 different age groups: <=24, 25-49 and 50+. Table 13 shows the corresponding results for the entire follow-up period. There was some evidence of a small increased risk of MS with the receipt of *Arepanrix* among 25-49 year-olds. In the first year of follow-up, the HR (95%CI) was estimated at 1.5 (0.8-2.7) and was slightly lower when measured over the entire study period (1.3 [0.8-2.0]). The results were not statistically significant due to small numbers. Similar findings were seen for the younger age group (Table 13) but not for the 50+ group.

Table 14 and Table 15 show risk estimates of the association between incident MS and vaccine administration during the period of one year following index date, and during any time following index date, respectively, stratified by immunosuppressed status. For the immunosuppressed category, models did not converge due to small numbers of MS events in this group; hence no risk estimates could be computed. There was no evidence of an association in non-immunosuppressed subjects.

11.6. Adverse events/adverse reactions

Individual medical records were not directly examined, and subject reports linked between databases were-de-identified prior to analysis. Therefore, individual case adverse event/adverse reaction reports were not generated.

12. DISCUSSION

12.1. Key results

We found no evidence of an association between vaccination with *Arepanrix* and the incidence of MS or that of other CNS demyelinating conditions that were not ultimately diagnosed as MS.

12.2. Limitations

Because of its population-based design and the availability of accurate automated records of hospitalization, physician utilization, vaccination and prescriptions, [Roberts, 1994] this study is less susceptible to selection bias (the whole population of Manitoba was eligible and available for inclusion in the study) and differential misclassification of exposures and outcomes often seen in observational epidemiologic studies where information on important variables is self-reported. The availability of detailed histories of vaccination, through the unique Manitoba Immunization Registry decreased recall bias and reduced vaccine use measurement errors (e.g., due to patient confusion about what vaccines were received).

While use of administrative databases to measure study variables minimizes the risk of differential misclassification (accuracy of documentation is unlikely to be related to

receipt of the vaccine), it is still possible that these variables are measured with error due, for instance, to coding errors (e.g., using the wrong ICD code). In particular, the ascertainment of MS cases is likely incomplete. We used a validated algorithm with a high NPV (>98%; See Section 10.2.2.3), so it is unlikely that non-MS cases were misclassified as cases. On the other hand, under-ascertainment of MS is a distinct possibility given the relatively low sensitivity of the algorithm and the complexity of diagnosing MS. It is often assumed that this kind of misclassification is non-differential with respect to pH1N1 vaccination because knowledge of pH1N1 vaccination status is unlikely to have directly influenced the way MS was diagnosed or coded. If this assumption is correct, our relative risk estimates of the association with pH1N1 vaccination are accurate even though our absolute MS incidence rates are lower than they should have been. In a cohort study, non-differential misclassification of the outcome does not typically bias the relative risk estimates because it is akin to sampling the same percentage of the cases in each group. The incidence rate of MS among unvaccinated persons measured in this study was comparable to MS rates measured in similarly young populations in studies from Manitoba and elsewhere [Marrie et al, 2010; Kingwell et al, , 2013]. So the magnitude of under-ascertainment is likely not significant.

If, on the other hand, the under-ascertainment of MS was differential, the direction of the error will depend on the nature of the relationship between H1N1 vaccination and the likelihood of MS diagnosis. It is possible that vaccinated individuals are more likely to be diagnosed with MS because receiving the vaccine may indicate better access to healthcare (unlikely in this case) or increased awareness or propensity to seek healthcare services. If that is the case, the benefits of the vaccine in preventing MS may have been masked by the higher rate of disease detection among the vaccinated. But, this kind of bias would not account for the lower risk of MS observed among vaccinated persons in this study.

Both environmental and genetic risk factors, and interactions thereof, could have confounded our analyses [Ascherio, 2012; Kakalacheva, 2011]. One limitation of the present study is the lack of information on lifestyle and environmental factors in our data sources. We attempted to adjust for these (largely unknown) factors by matching on age, gender, place of residence and PS. Matching on place of residence reduces the likelihood of confounding by ethnicity as ethnic minorities (First Nations or migrants) tend to cluster in communities even in large urban centres such as Winnipeg. Smoking information is not available in the Manitoba databases; however, the PS reduces confounding by measured (e.g., access to healthcare services) and unmeasured confounders (e.g., smoking) due to the inclusion of proxy conditions (e.g., smoking-related diseases) in the calculation of the PS. As was described in the results section (Section 11.2), the vaccinated and unvaccinated cohorts were comparable (e.g., in terms of existing morbidity) indicating a reasonable performance of the matching procedure. Because individuals within each matching pair had a similar probability of receiving the vaccine, relative risk estimates derived from the matched cohort analysis are estimated to be less biased with respect to the measured confounders. Residual confounding remains a possibility.

Finally, the relatively large sample size (Section 10.7) permitted the calculation of reasonably precise estimates. However, in some subgroup analyses (e.g., unadjuvanted vaccine), the precision of estimates were limited by small numbers.

12.3. Interpretation

Our findings are consistent with the bulk of scientific evidence in finding no indication that influenza vaccination is associated with an increased risk of MS.

Firstly, seasonal influenza vaccines have not been linked to MS risk. In a systematic review of both RCTs and observational studies that reported on the risk of MS following immunization, there were 4 studies with a total of 14,997 cases and 10,128 controls that reported on the association with influenza vaccination [Farez, 2011]. The pooled OR of developing MS following influenza immunization was 0.97 (95%CI 0.77-1.23) with little evidence of heterogeneity (p= 0.368).

Data on the association with pandemic vaccination is quite limited as very few studies specifically examined the association between the pandemic vaccine and the risk of occurrence of MS. In published (mostly manufacturer-sponsored RCTs) conducted during the pandemic, there were no reports of clinically significant adverse events of the different pandemic vaccine formulations [Manzoli et al, 2011]. Generally, higher frequency of mild to moderate adverse effects was noted with use of adjuvanted vaccines, but there was no evidence of increased risk of serious adverse events such as MS or Guillain-Barré syndrome (a peripheral demyelinating disorder) [Manzoli et al, 2011]. These findings are reassuring, but these trials may have not been large enough to detect a small increase in risk.

Similarly, Adverse Events Following Immunization (AEFI) surveillance systems in Europe and the United States (US) did not detect increased risk of MS with pandemic vaccine use. No increased risk was found in an analysis of the EudraVigilance database which tracked reports of suspected autoimmune disorders following use of either adjuvanted (*Pandemrix* and another 3 products) or unadjuvanted pandemic vaccines. There were reports of MS relapse but they were equally distributed among the adjuvanted (7.9% of all reported AEFIs) and unadjuvanted vaccine groups (7.3%) [Isai et al, 2012]. Similar analysis of the US Vaccine Adverse Event Reporting System found that 9 out of 212 (4%) individuals with serious AEFIs following H1N1 vaccination had a neurologic diagnosis of a demyelinating disorder of unclear etiology, 7 (about 3%) had a diagnosis of demyelinating disorder of unknown etiology and 8 (4%) had a diagnosis of acute disseminated encephalomyelitis. The limitations of AEFI surveillance systems in establishing causal associations are well known.

A large retrospective record-linkage study from Sweden reported an increased risk of paraesthesia, but not of diagnosed MS, among persons vaccinated with *Pandemrix* [Bardage, 2011; Persson, 2014]. Among persons with high-risk of influenza complications who were mostly vaccinated in the first 45 days of the campaign (healthcare workers, children, pregnant women and persons with chronic diseases), the risk of MS was 1.17 (95%CI: 0.82 to 1.66) and the risk estimates were highest within 6 weeks after vaccination (1.35 [0.68 to 2.67]). There was no similar increase of risk

among other groups. The authors attributed the excess risk among high risk groups targeted for early vaccination to possible confounding by underlying comorbidity and vaccine indication.

Finally, other adjuvanted influenza vaccines such as H5N1influenza vaccines, based on similar oil-in-water adjuvants to those used in *Arepanrix*, were found in some RCTs to be more reactogenic than unadjuvanted seasonal vaccines. However, there were no reports of serious AEFIs including MS [Manzoli et al, 2012].

The evidence is less consistent for the association between the pandemic vaccines and MS relapse. In several small RCTs, there were no differences in the incidence of relapses following H1N1 vaccination [Myers et al, 1977; Bamford et al, 1978]. One small study conducted in an MS "relapse clinic" in the United Kingdom evaluated relapses among 30 patients with MS between November 2009 and January 2010, of whom 18 (60%) received the pandemic H1N1 influenza vaccine and/or the seasonal influenza vaccine (40% were unvaccinated) [McNicholas et al, 2011]. Using unconventional design, akin to self-controlled case series design, the relative risk of relapse was 6.0 (95% CI: 1.4-26.2). However, the relative risk was calculated with a historical reference/baseline period without adjustment for time-varying covariates such as influenza strain activity and there was likelihood of selection bias and referral bias.

A subsequent study of 137 relapsing-remitting MS patients from Argentina found that 60 were vaccinated (49 with seasonal trivalent inactivated influenza vaccines (TIVs) and 11 with monovalent H1N1 pandemic vaccine), among which 28 relapse events were observed. Focusing on the 30-day period after the relapse, the risk was not increased (relative risk 0.86; 95% CI: 0.20-0.36). Findings were similar when the risk period was extended to 60 days and 90 days [Farez et al, 2012].

This is consistent with evidence from earlier studies that found no evidence that influenza vaccination is associated with increased risk of MS relapse. Confavreux *et al*. evaluated the risk of MS relapse after vaccination in 643 patients. They did not find any evidence of an increased risk of relapse following vaccination (relative risk 0.71; 95% CI: 0.40-1.26), irrespective of the vaccine including seasonal influenza vaccine [Confavreux, 2001]. In one review that included 5 small studies, the pooled relative risk of relapse following influenza immunization was 1.24 with a 95% CI of 0.89-1.72. There was no evidence of heterogeneity (p = 0.531) [Farez, 2011]. Similarly in an older review that included 4 RCTs and 7 cohort studies [Rutschmann et al, 2002], there was no difference in the RCTs in rates of early (3 to 4 weeks after vaccine/placebo) MS exacerbation (overall rate difference of 0% (95% CI: −6.9% to 6.9%) or late exacerbation (4 to 6 months after vaccine/placebo), 6.1% (95% CI: −4.1 to 16.3%). However, the pooled rate difference for influenza during the 6 months after the intervention was 8.4% (95% CI: −2.5% to 19.3%).

On the other hand, there is a possibility that MS occurrence and relapse might be precipitated by infections including influenza. An ecological analysis of surveillance data from 1986 –1995 found that months of high influenza A activity in the population were often followed by a higher number of MS relapses [Oikonen et al, 2011]. Prevention of influenza and other infections might protect against MS development or relapse. A

Dutch study of MS case series found that MS relapse were more likely to occur following influenza-like illness than following influenza vaccination [Stübgen, 2013]. So it is possible that prevention of influenza using vaccination might actually be reducing the risk of relapse.

The scientific evidence on the association between pandemic vaccination and CNS demyelinating disorders other than MS is even less scarce. In a comprehensive review of published case reports and series, post-marketing surveillance data and observational studies, a diagnosis of optic neuritis was not associated with influenza vaccination. Although there were reports of 13 cases following influenza vaccines, there was no association in two case-control studies [Stübgen, 2013].

12.4. Generalisability

We do not believe our study design or participant selection criteria have reduced the generalizability of our findings to the rest of the Manitoba population. Whether these findings are generalizable to other populations depends on their geographic location, ethnic composition and access to pandemic vaccination. The Manitoba population tends to be typical of many Western populations, especially those in Northern high latitude countries, in terms of MS incidence, ethnic composition (largely European but with significant indigenous and migrant minorities), healthcare systems and even with the timing and epidemiology of the 2009 pandemic and the nature of the public health response to the pandemic.

13. OTHER INFORMATION

None.

14. CONCLUSION

We found no evidence of an association between vaccination with the adjuvanted pandemic vaccine and the incidence of MS or that of other demyelinating conditions that were not ultimately diagnosed as MS.

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16. REPORT TABLES

Table 1 Codes and definitions used in the analyses

ICD codes for multiple sclerosis and other demyelinating events			
Condition	**ICD-9**	**ICD 10-CA**	**Algorithm**
Multiple sclerosis (MS)	340 - Multiple sclerosis	G35 - Multiple sclerosis	≥3 contacts including hospital admissions, physician visits, or MS drugs.
Acute disseminated encephalomyelitis (ADEM)	323.8 - Other causes of encephalitis, myelitis, and encephalomyelitis; 323.9 - Unspecified causes of encephalitis, myelitis, and encephalomyelitis; 323.62 - Other postinfectious encephalitis and encephalomyelitis; 323.63 - Postinfectious myelitis	G04.0 (Acute disseminated encephalitis and encephalomyelitis [ADEM]), G36 - Other acute disseminated demyelination	≥1 hospital admissions
Acute transverse myelitis (ATM)	323.82 - Other causes of myelitis; 341.2 – Acute (transverse) myelitis	G04.8 - Other encephalitis, myelitis and encephalomyelitis; G37.3 - Acute transverse myelitis	≥1 hospital admissions
Demyelinating disease of CNS unspecified	341.9 - Demyelinating disease of central nervous system, unspecified	G37.9 - Demyelinating disease of central nervous system, unspecified	≥1 hospital admissions
Neuromyelitis optica	341.0 - Neuromyelitis optica	G36.0- Neuromyelitis optica	≥1 hospital admissions
Optic neuritis	377.3 – optic neuritis	H46- Optic neuritis	≥1 hospital admissions
Encephalitis, myelitis and encephalomyelitis	323- Encephalitis, myelitis and encephalomyelitis	G04 - Encephalitis, myelitis and encephalomyelitis	≥1 hospital admissions, ≥2 physician visits-30 days apart
Other demyelinating diseases of central nervous system	341- Other demyelinating diseases of central nervous system	G37 - Other demyelinating diseases of central nervous system	≥1 hospital admissions, ≥2 physician visits-30 days apart

Tariff codes for different vaccines	
Tariff code	**Vaccine**
8893	Influenza pandemic H1N1 adjuvanted
8894	Influenza pandemic H1N1 unadjuvanted
8791	Seasonal influenza (TIV)
8961	polyvalent pneumococcal 23
8681	Pneumococcal conjugate PVC7 1st dose
8682	Pneumococcal conjugate PVC7 2nd dose
8683	Pneumococcal conjugate PVC7 3rd dose
8684	Pneumococcal conjugate PVC7 4th dose

Definition of covariates used in the analyses	
Variable	**Definition**
Drugs[†]	
Multiple sclerosis therapy	Interferon beta-1b (L03AB08), Interferon beta-1a (L03AB07), Glatiramer acetate (L03AX13), Natalizumab (L04AA23)
Anti-HIV	Protease inhibitors (J05AE), Nucleoside and nucleotide reverse transcriptase inhibitors (J05AF), Non-nucleoside reverse transcriptase inhibitors (J05AG), Antivirals for treatment of HIV infections, combinations(J05AR)
Diabetes therapy	Drugs used in diabetes (A10)
Immunosuppressants	Antineoplastic agents (L01), Immunosuppressants (L04A)
Systemic steroids	Corticosteroids for systemic use, plain (H02A), Corticosteroids for systemic use, combinations (H02B)
Pregnancy	
Ongoing pregnancy	≥ 1 admission (O10-O16, O20-O29, O30-O48, O94-99, Z32-Z36) OR ≥ 2 physician claims (640-649, V22) OR ≥ 1 tariff code for prenatal services. Must be within ± 30 days of the index date
Completion of Pregnancy	≥1 admission (O8, O65-O75, O80-O84, O85-O92, Z37-Z39) OR ≥ 2 physician claims (650-659, 670-676, 670-676, V27) OR ≥ 1 tariff code for delivery, abortion or postnatal services. Must be within 270 days following the index date
Medical conditions[‡]	
Alcoholism	≥ 1 admission (E52, F10, K70, X45, X65, Y15, Y90, Y91, G31.2, G62.1, G72.1, I42.6, K29.2, K86.0, O35.4, P04.3, R78.0, T50.6, T51.0, T51.1, T51.9, Y57.3, Z50.2, Z71.4, Z72.1, Z81.1, E24.4, E51.2 , Q86.0) OR ≥ 2 physicien claims (303, 291)
Anemia	≥ 1 admission (D50-64) OR ≥ 2 physician claims (280-285)
Asthma	≥ 1 admission (J45, J46) OR ≥ 2 physician claims (493)
Cancer-ex non-melanoma skin	≥ 1 admission (C00-C43, C45-C97) OR ≥ 1 physician claim (140-172, 174-209, 235-239).
Cardiovascular disease	≥1 admission (I00-I99, O11) OR ≥ 2 physician claims (390-459)
Chronic renal failure	≥1 admission (N18, N19, Z49, 12.0 , I13.1, N25.0, Z99.2) OR ≥ 2 physician claims (403-404 586-587)
Chronic respiratory condition	≥ 1 admission (J40-J99) OR ≥ 2 physician claims (490-496, 500-508)
COPD	≥ 1 admission (J40-J44) OR ≥ 2 physician claims (490-492, 496)
Diabetes	≥ 1 admission (E10-E14, O24, G590, G632, H280, H360, M142, M146, N083) OR ≥ 2 physician claims (250) OR ≥ 2 prescriptions for drugs used in treatment of diabetes.
HIV/AIDS	≥ 1 admission (B20-B24, R75, Z21) OR ≥ 2 physician claims (042 V08) OR ≥ 1 prescriptions for drugs used in treatment of HIV.
Hypertension	≥ 1 admission (I10-I15, I67.4 , O11) ≥ 2 physician claims (401-405)
Immune deficiency	≥ 1 admission (D80-D84, D89) OR ≥ 2 physician claims (288, 279)
Immunosuppressed	Having an organ transplant or a diagnosis of HIV/AIDS, other immune deficiency disorders or cancer (other than non-melanoma skin cancer), or receiving prescriptions for immunosuppressants or systemic steroids.
Ischemic Heart diseases	≥ 1 admission (I20-I25) OR ≥ 1 physician claims (410-414)
Obesity	≥ 1 admission (E66) OR ≥ 2 physician claims (278)
Organ transplant	≥ 1 admission (T86, Z94, Y83.0) OR ≥ 2 physician claims (V42)
Stroke	≥ 1 admission (I61, I63, I64, I69, I67.9) OR ≥ 2 physician claims (431,434, 436-438)

Definition of covariates used in the analysis	
Variable	**Definition**
Other chronic heart disease	> 1 admission (I05-I09, I27, I34-I37, I42, I48, I50) OR ≥ 2 physician claims (393-398, 416, 424, 425, 427, 428)
Chronic liver disease	≥ 1 admission (K70, K71.3-K71.5, K71.7, K72.1, K73, K74, K76.9, K75.3, K75.4, K75.81, K75.89) OR ≥ 2 physician claims (571, 572)
Substance abuse	≥ 1 admission (F11-F16, F18-F19) OR ≥ 2 physician claims (292, 304,305)
Chronic disease	Having a diagnosis of diabetes, chronic cardiovascular disease (excluding hypertension), chronic respiratory disease (excluding asthma), chronic renal failure, or chronic liver disease.

Definition of Autoimmune diseases (All based on ≥ 1 admission [ICD-10 codes as below] OR ≥ 2 physician claims (ICD-9 codes as below)		
Disease	**ICD9**	**ICD10**
Pernicious anemia	281	D51.0
Autoimmune hemolytic anemia	283	D59.1
Idiopathic thrombocytopenic purpura	287	D69.3
Thyrotoxicosis	242	E05
Autoimmune thyroiditis	245	E06.3
Type 1 diabetes	250 (AND ≥ 1 prescription [ATC: A10A])	E10
Primary adrenocortical insufficiency	255	E27.1
Guillain–Barre syndrome	357	G61.0
Iridocyclitis	364	H20
Crohn's disease	555	K50
Ulcerative colitis	556	K51
Autoimmune hepatitis		K75.4
Primary biliary cirrhosis		K74.3
Celiac disease	579	K90.0
Pemphigus	694	L10
Pemphigoid	694	L12
Psoriasis vulgaris	696	L40.4
Alopecia areata		L63
Vitiligo		L80
Seropositive rheumatoid arthritis	714	M05–M06
Juvenile arthritis	714	M08
Wegener's granulomatosis	446	M31.3
Polymyositis	710	M33.2
Dermatomyositis	710	M33.0, M33.1
Polymyalgia rheumatica	725	M31.5–6, M35.3
Myasthenia gravis	358	G70.0
Systemic sclerosis	710	M34
Systemic lupus erythematosis	710	M32.1, M32.8,M32.9
Sjogren's syndrome	710	M35.0

Definition of Autoimmune diseases (All based on ≥ 1 admission [ICD-10 codes as below] OR ≥ 2 physician claims (ICD-9 codes as below)		
Ankylosing spondylitis	720	M45

† Drugs were classified based on their Drug Identification Number and the Anatomical Therapeutic Chemical (ATC) Classification System [WHO, 2002].

‡ Based on previously validated chronic disease identification algorithms with modifications [Elixhauser, 1998]. The codes in parentheses are ICD-10-CA codes for hospital admission data and ICD-9-CM codes for physician claims data.

Table 2 Number of participants by vaccination status

Vaccination status	Number	% of Manitoba population	% of vaccinated (A[H1N1]pdm09/TIV)	% of A(H1N1) pdm09 vaccine
Vaccination – overall				
Total Manitoba population	1,178,259	100	-	-
Any influenza (A[H1N1] pdm09 / TIV)	485,941	41.2	100	-
Any A(H1N1)pdm09	341,347	29.0	70.2	100
Adjuvanted A(H1N1)pdm09 (*Arepanrix*)	328,778	27.9	67.7	96.3
Unadjuvanted A(H1N1)pdm09	12,559	1.1	2.6	3.7
Any TIV	207,810	17.6	42.8	-
Vaccine types				
A(H1N1)pdm09 alone	278,131	23.6	57.0	81.5
Concurrent A(H1N1)pdm09 / TIV	63,216	5.4	13.0	18.5
TIV alone	144,594	12.3	30.0	-
Vaccine types -detail				
Adjuvanted A(H1N1)pdm09 (*Arepanrix*) alone	267,539	22.7	55.1	78.4
Concurrent adjuvanted A(H1N1)pdm09 / TIV	61,239	5.2	12.6	17.9
Unadjuvanted A(H1N1)pdm09 alone	10,592	0.9	2.2	3.1
Concurrent unadjuvanted A(H1N1 pdm09 / TIV	1,977	0.2	0.4	0.6
TIV alone	144,594	12.3	29.8	-

A(H1N1)pdm09, Pandemic influenza A (H1N1) strain; TIV, Trivalent Influenza Vaccine

Table 3 Cohort characteristics by vaccination status

Variables	Concurrent adjuvanted A(H1N1)pdm09 / TIV (n=61,239)		Adjuvanted A(H1N1)pdm09 (*Arepanrix*) alone (n=267,539)		Unadjuvanted A(H1N1)pdm09 alone (n=10,592)		Concurrent unadjuvanted A(H1N1 pdm09 / TIV (n=1,977)		TIV alone (n=144,594)		Unvaccinated (n=485,941)		
	N	%	N	%	N	%	N	%	N	%	N	%	P-value
Age group (years)													<.0001
<= 14	10,206	16.7	83,097	31.1	1,245	11.8	109	5.5	4,915	3.4	99,463	20.5	
15 - 34	12,637	20.6	62,261	23.3	4,717	44.5	679	34.3	7,094	4.9	92,008	18.9	
35 - 44	9,272	15.1	38,401	14.4	1,835	17.3	363	18.4	6,463	4.5	51,795	10.7	
45 - 54	12,018	19.6	39,958	14.9	1,452	13.7	389	19.7	12,696	8.8	74,454	15.3	
55+	17,106	27.9	43,822	16.4	1,343	12.7	437	22.1	113,426	78.4	168,221	34.6	
Median age (IQR)	43	24 - 56	31	11 - 49	32	22 - 46	40	28 - 53	69	57 - 78	44	20 - 62	<.0001
Sex													<.0001
Female	31,081	50.8	144,461	54.0	7,352	69.4	1,194	60.4	82,868	57.3	266,956	54.9	
Resides in an urban area													<.0001
Urban	41,916	68.4	146,256	54.7	6,202	58.6	1,604	81.1	96,605	66.8	292,583	60.2	
Region of residence													<.0001
Winnipeg	36,445	59.5	138,466	51.8	5,413	51.1	1,168	59.1	90,466	62.6	271,112	55.8	
North	7,192	11.7	33,040	12.3	456	4.3	125	6.3	3,317	2.3	44,130	9.1	
South	17,602	28.7	96,033	35.9	4,723	44.6	684	34.6	50,811	35.1	170,699	35.1	
Income quintile													<.0001
Q1 (lowest)	9,766	15.9	53,269	19.9	1,803	17.0	279	14.1	27,899	19.3	92,147	19.0	
Q2	11,066	18.1	47,036	17.6	1,833	17.3	355	18.0	27,593	19.1	91,214	18.8	
Q3	10,976	17.9	48,257	18.0	1,766	16.7	358	18.1	28,037	19.4	91,542	18.8	
Q4	12,454	20.3	50,592	18.9	2,373	22.4	447	22.6	27,167	18.8	95,379	19.6	
Q5 (highest)	15,602	25.5	62,320	23.3	2,613	24.7	503	25.4	25,103	17.4	101,411	20.9	
Cannot be calculated	1,375	2.2	6,065	2.3	204	1.9	35	1.8	8,795	6.1	14,248	2.9	
Season of birth													0.0513
Winter	14,538	23.7	63,784	23.8	2,556	24.1	469	23.7	34,416	23.8	114,737	23.6	34,416
Spring	15,931	26.0	69,266	25.9	2,811	26.5	507	25.6	37,296	25.8	124,946	25.7	37,296

Variables	Concurrent adjuvanted A(H1N1)pdm09 / TIV (n=61,239)		Adjuvanted A(H1N1)pdm09 (*Arepanrix*) alone (n=267,539)		Unadjuvanted A(H1N1)pdm09 alone (n=10,592)		Concurrent unadjuvanted A(H1N1 pdm09 / TIV (n=1,977)		TIV alone (n=144,594)		Unvaccinated (n=485,941)		
	N	%	N	%	N	%	N	%	N	%	N	%	P-value
Summer	15,804	25.8	69,202	25.9	2,667	25.2	498	25.2	37,336	25.8	125,972	25.9	37,336
Fall	14,966	24.4	65,287	24.4	2,558	24.2	503	25.4	35,546	24.6	120,286	24.8	35,546
Birth year cohort													<.001
<=1909	s	s	8	0.0	0	0.0	0	0.0	170	0.1	257	0.1	
1910-1919	91	0.1	322	0.1	s	s	0	0.0	5,983	4.1	6,665	1.4	
1920-1929	867	1.4	2,251	0.8	16	0.2	0	0.0	26,692	18.5	29,200	6.0	
1930-1939	2,153	3.5	5,316	2.0	37	0.3	s	s	38,008	26.3	46,538	9.6	
1940-1949	7,797	12.7	19,285	7.2	670	6.3	205	10.4	33,321	23.0	54,280	11.2	
1950-1959	12,633	20.6	36,536	13.7	1,331	12.6	435	22.0	16,654	11.5	69,543	14.3	
1960-1969	10,424	17.0	39,853	14.9	1,514	14.3	352	17.8	9,167	6.3	64,209	13.2	
1970-1979	8,779	14.3	36,412	13.6	2,672	25.2	412	20.8	5,003	3.5	48,478	10.0	
1980-1984	3,221	5.3	13,781	5.2	1,358	12.8	194	9.8	1,888	1.3	26,675	5.5	
1985-1989	2,526	4.1	12,649	4.7	810	7.6	145	7.3	1,586	1.1	20,555	4.2	
1990-1994	2,642	4.3	18,809	7.0	966	9.1	125	6.3	1,258	0.9	21,410	4.4	
1995-1999	3,511	5.7	27,407	10.2	1,135	10.7	101	5.1	1,358	0.9	33,064	6.8	
2000-2004	3,559	5.8	29,937	11.2	54	0.5	s	s	1,526	1.1	35,204	7.2	
2005-2009	3,035	5.0	24,973	9.3	28	0.3	s	s	1,980	1.4	29,863	6.1	
Cancer (excl: non-melanoma skin)													<.0001
Yes	2,390	3.9	6,560	2.5	189	1.8	47	2.4	15,083	10.4	22,834	4.7	
Chronic respiratory diseases													<.0001
Yes	2,940	4.8	8,648	3.2	194	1.8	56	2.8	11,813	8.2	21,236	4.4	
Chronic renal failure													<.0001
Yes	203	0.3	818	0.3	7	0.1	s	s	1,782	1.2	2,966	0.6	
Diabetes													<.0001
Yes	5,056	8.3	12,367	4.6	236	2.2	61	3.1	25,830	17.9	41,340	8.5	

Variables	Concurrent adjuvanted A(H1N1)pdm09 / TIV (n=61,239)		Adjuvanted A(H1N1)pdm09 (*Arepanrix*) alone (n=267,539)		Unadjuvanted A(H1N1)pdm09 alone (n=10,592)		Concurrent unadjuvanted A(H1N1 pdm09 / TIV (n=1,977)		TIV alone (n=144,594)		Unvaccinated (n=485,941)		
	N	%	N	%	N	%	N	%	N	%	N	%	P-value
Immunosuppressed													<.0001
Yes	4,028	6.6	11,541	4.3	322	3.0	78	3.9	20,990	14.5	33,561	6.9	
Ischemic heart disease													<.0001
Yes	1,651	2.7	3,906	1.5	83	0.8	16	0.8	15,955	11.0	21,404	4.4	
Autoimmune diseases													<.0001
Yes	2,669	4.4	6,680	2.5	197	1.9	45	2.3	10,179	7.0	17,661	3.6	
Any chronic diseases													<.0001
Yes	7,485	12.2	18486	6.9	364	3.4	96	4.9	42,909	29.7	65,158	13.4	
Charlson index group													<.0001
0	59,249	96.8	262,072	98.0	10,510	99.2	1,962	99.2	129,809	89.8	460,976	94.9	
1+	1,990	3.2	5,467	2.0	82	0.8	15	0.8	14,785	10.2	24,965	5.1	
Median Charlson index (IQR)	2	1-2	2	1-3	2	1-2	1	1-2	2	1 - 3	2	1 - 3	<.0001
Pregnancy (% of all 15-49 old females)													<.0001
Yes	355	2.4	2,184	3.1	2,857	50.6	330	40.4	816	7.4	5,160	4.9	
Number of hospital admission during past year													<.0001
0	56,720	92.6	250,175	93.5	9,878	93.3	1,850	93.6	126,657	87.6	443,977	91.4	
1	3,578	5.8	13,481	5.0	590	5.6	106	5.4	12,820	8.9	30,544	6.3	
2+	941	1.5	3,883	1.5	124	1.2	21	1.1	5,117	3.5	11,420	2.4	
Median hospital admission during past year (IQR)	1	1-1	1	1-1	1	1-1	1	1-1	1	1 - 2	1	1 - 2	<.0001

Variables	Concurrent adjuvanted A(H1N1)pdm09 / TIV (n=61,239)		Adjuvanted A(H1N1)pdm09 (*Arepanrix*) alone (n=267,539)		Unadjuvanted A(H1N1)pdm09 alone (n=10,592)		Concurrent unadjuvanted A(H1N1 pdm09 / TIV (n=1,977)		TIV alone (n=144,594)		Unvaccinated (n=485,941)		
	N	%	N	%	N	%	N	%	N	%	N	%	P-value
Had >=11 physician visits during past year													<.0001
Yes	14,709	24.0	43,866	16.4	2,306	21.8	489	24.7	69,419	48.0	128,707	26.5	
Median physician visits during past year (IQR)	11	7-16	10	7-15	10	7-15	10	7-15	13	9 - 19	11	8- 17	<.0001
Period of influenza vaccination													<.0001
Early (<=Nov 17, 2009)	45,951	75.0	252,011	94.2	9,732	91.9	1,305	66.0	139,925	96.8	N/A	139,925	
Later (>=Nov 18, 2009)	15,288	25.0	15,528	5.8	860	8.1	672	34.0	4,669	3.2	N/A	4,669	
High priority for A(H1N1)pdm09 vaccine													<.0001
Yes	25,652	41.9	142,909	53.4	6,200	58.5	683	34.5	59,165	40.9	230,948	47.5	
High priority for TIV													<.0001
Yes	12,958	21.2	42,207	15.8	3,231	30.5	419	21.2	105,557	73.0	158,565	32.6	
Receipt of TIV 08/09 vaccine													<.0001
Yes	22,231	36.3	41,896	15.7	1,237	11.7	542	27.4	109,107	75.5	42,071	8.7	
Receipt of pneumococcal vaccine													<.0001
Yes	11,554	18.9	49,203	18.4	286	2.7	64	3.2	87,552	60.6	86,261	17.8	
Median time of entire follow up (days)- MS	1,140	811 – 1,155	749	360 – 1,159	1139	386 – 1,160	1,140	1,133 – 1,153	42	22 – 1,140	1,151	612 – 1,162	<.0001

Variables	Concurrent adjuvanted A(H1N1)pdm09 / TIV (n=61,239)		Adjuvanted A(H1N1)pdm09 (*Arepanrix*) alone (n=267,539)		Unadjuvanted A(H1N1)pdm09 alone (n=10,592)		Concurrent unadjuvanted A(H1N1 pdm09 / TIV (n=1,977)		TIV alone (n=144,594)		Unvaccinated (n=485,941)		
	N	%	N	%	N	%	N	%	N	%	N	%	P-value
Median time of follow up in one year - MS	365	365 - 365	365	360 - 365	365	365 - 365	365	365 - 365	42	22 - 365	365	365 - 365	<.0001

A(H1N1)pdm09, Pandemic influenza A (H1N1) strain; TIV, Trivalent Influenza Vaccine

Table 4 Crude and age-standardized rates (per 100,000 population) of incident multiple sclerosis during the period of one year following index date by vaccination status

Vaccination status	Total Population	Number of events	Rate (95% CI)		Rate Ratio (95% CI)	
			Crude	Age-standardized	Crude	Age-adjusted
Unvaccinated	457,247	106	23.2(19.2 – 28.0)	24.2(20.1 – 28.3)	1	1
Vaccinated (A[H1N1] pdm09 / TIV)	360,417	69	19.1(15.1 – 24.2)	20.2(15.4 – 24.9)	0.8(0.6 – 1.1)	0.8(0.3 – 2.2)
A(H1N1)pdm09 alone	243,697	43	17.6(13.1 – 23.8)	17.7(14.1 – 21.2)	0.8(0.5 – 1.1)	0.7(0.3 – 1.7)
Concurrent A(H1N1)pdm09/TIV	59,174	12	20.3(11.5 – 35.7)	19.4(8.6 – 30.2)	0.9(0.5 – 1.6)	0.8(0.1 – 5.0)
TIV alone	57,546	14	24.3(14.4 – 41.1)	36.8(25.0 – 48.6)	1.0(0.6 – 1.8)	1.5(0.3 – 6.8)
Adjuvanted A(H1N1)pdm09 (*Arepanrix*) alone	233,978	40	17.1(12.5 – 23.3)	17.4(13.8 – 21.1)	0.7(0.5 – 1.1)	0.7(0.3 – 1.7)
Concurrent adjuvanted A(H1N1 pdm09 / TIV	57,293	11	19.2(10.6 – 34.7)	18.3(7.3 – 29.3)	0.8(0.4 – 1.5)	0.8(0.1 – 5.1)
Unadjuvanted A(H1N1)pdm09 alone	9,718	s	s	18.6(8.8 – 28.3)	1.3(0.4 – 4.2)	0.8(0.1 – 4.2)
Concurrent unadjuvanted A(H1N1 pdm09 / TIV	1,881	s	s	37.9(13.8 – 62.0)	2.3(0.3 – 16.4)	1.6(0.1 – 26.6)

s, suppressed due to small sample size (n= 1-5). A(H1N1)pdm09, Pandemic influenza A (H1N1) strain; TIV, Trivalent Influenza Vaccine

Table 5 Crude and age-standardized rates (per 100,000 PY) of incident multiple sclerosis during anytime following index date by vaccination status

Vaccination status	Total person-years	Number of events	Rate (95% CI)		Rate Ratio (95% CI)	
			Crude	Age-standardized	Crude	Age-adjusted
Unvaccinated	1,204,491	188	15.6(13.5 - 18.0)	16.0(13.5 - 18.5)	1	1
Vaccinated (A(H1N1)pdm09/TIV)	876,566	132	15.1(12.7 - 17.9)	15.4(12.4 - 18.4)	1.0(0.8 - 1.2)	1.0(0.5 - 1.9)
A(H1N1)pdm09 alone	563,474	82	14.6(11.7 - 18.1)	14.9(9.3 - 20.5)	0.9(0.7 - 1.2)	0.9(0.3 - 2.8)
Concurrent A(H1N1)pdm09/TIV	164,549	33	20.1(14.3 - 28.2)	18.2(11.8 - 24.6)	1.3(0.9 - 1.9)	1.1(0.4 - 3.6)
TIV alone	148,542	17	11.4(7.1 - 18.4)	16.6(9.5 - 23.8)	0.7(0.4 - 1.2)	1.0(0.3 - 3.9)
Adjuvanted A(H1N1)pdm09 (*Arepanrix*) alone	539,682	78	14.5(11.6 - 18.0)	15.0(9.3 - 20.7)	0.9(0.7 - 1.2)	0.9(0.3 - 2.9)
Concurrent adjuvanted A(H1N1)pdm09/TIV	159,189	32	20.1(14.2 - 28.4)	18.4(11.9 - 24.9)	1.3(0.9 - 1.9)	1.1(0.4 - 3.7)
Unadjuvanted A(H1N1)pdm09 alone	23,793	s	s	9.7(3.6 - 15.8)	1.1(0.4 - 2.9)	0.6(0.1 - 2.6)
Unadjuvanted A(H1N1)pdm09/TIV	5,360	s	s	13.1(0.0 - 27.2)	1.2(0.2 - 8.5)	0.8(0.0 - 13.5)

s, suppressed due to small sample size (n= 1-5). A(H1N1)pdm09, Pandemic influenza A (H1N1) strain; TIV, Trivalent Influenza Vaccine

Table 6 Crude and age-standardized rates (per 100,000 population) of incident demyelinating conditions not ultimately diagnosed as multiple sclerosis during the period of one year following index date by vaccination status

Vaccination status	Total Population	Number of events	Rate (95% CI)		Rate Ratio (95% CI)	
			Crude	Age-standardized	Crude	Age-adjusted
Unvaccinated	456,883	27	5.9(4.1 - 8.6)	6.9(2.6 - 11.1)	1	1
Vaccinated (A(H1N1)pdm09/TIV)	360,123	17	4.7(2.9 - 7.6)	4.7(0.0 - 10.6)	0.8(0.4 - 1.5)	0.7(0.1 - 6.4)
A(H1N1) pdm09 alone	243,493	s	s	5.6(0.0 - 13.3)	0.8(0.4 - 1.6)	0.8(0.1 - 10.6)
Concurrent A(H1N1) pdm09/TIV	59,115	s	s	7.8(0.0 - 17.0)	1.4(0.6 - 3.7)	1.1(0.1 - 15.2)
TIV alone	57,515	0	0	0	N/A	N/A
Adjuvanted A(H1N1)pdm09 (*Arepanrix*) alone	233,784	11	4.7(2.6 - 8.5)	5.4(0.0 - 13.1)	0.8(0.4 - 1.6)	0.8(0.1 - 10.8)
Concurrent adjuvanted A(H1N1)pdm09/TIV	57,236	s	s	8.0(0.0 - 17.3)	1.5(0.6 - 3.8)	1.2(0.1 - 15.5)
Unadjuvanted A(H1N1)pdm09 alone	9,709	s	s	6.2(0.0 - 16.0)	1.7(0.2 - 12.8)	0.9(0.0 - 18.1)
Unadjuvanted A(H1N1)pdm09/TIV	1,879	0	0	0	N/A	N/A

s, suppressed due to small sample size (n= 1-5). A(H1N1)pdm09, Pandemic influenza A (H1N1) strain; TIV, Trivalent Influenza Vaccine

Table 7 Crude and age-standardized rates (per 100,000 PY) of incident demyelinating conditions not ultimately diagnosed as multiple sclerosis anytime following index date by vaccination status

			Rate (95% CI)		Rate Ratio (95% CI)	
Vaccination status	**Total of person-years**	**Number of events**	**Crude**	**Age-standardized**	**Crude**	**Age-adjusted**
Unvaccinated	1,203,667	40	3.3(2.4 - 4.5)	3.6(0.8 - 6.3)	1	1
Vaccinated (A(H1N1)pdm09/TIV)	875,950	25	2.9(1.9 - 4.2)	2.9(0.0 - 7.1)	0.9(0.5 - 1.4)	0.8(0.1 - 6.2)
A(H1N1)pdm09 alone	563,062	17	3.0(1.9 - 4.9)	3.5(0.0 - 9.1)	0.9(0.5 - 1.6)	1.0(0.1 - 10.3)
Concurrent A(H1N1)pdm09/TIV	164,411	s	s	3.8(0.0 - 10.7)	1.1(0.5 - 2.6)	1.1(0.1 - 15.6)
TIV alone	148,477	s	s	0.4(0.0 - 2.1)	0.4(0.1 - 1.7)	0.1(0.0 - 1.0)
Adjuvanted A(H1N1)pdm09 (*Arepanrix*) alone	539,292	15	2.8(1.7 - 4.6)	3.2(0.0 - 8.9)	0.8(0.5 - 1.5)	0.9(0.1 - 10.5)
Concurrent adjuvanted A(H1N1)pdm09/TIV	159,056	6	3.8(1.7 - 8.4)	4.0(0.0 - 10.9)	1.1(0.5 - 2.7)	1.1(0.1 - 16.1)
Unadjuvanted A(H1N1) alone	23,770	s	s	7.4(0.0 - 18.0)	2.5(0.6 - 10.5)	2.1(0.1 - 39.9)
Unadjuvanted A(H1N1)pdm09/TIV	5,356	0	N/A	N/A	N/A	N/A

s, suppressed due to small sample size (n= 1-5). A(H1N1)pdm09, Pandemic influenza A (H1N1) strain; TIV, Trivalent Influenza Vaccine

Table 8 Effect of H1N1/TIV vaccination on occurrence of incident multiple sclerosis during the period of one year following index date

	Model A*			Model B&		
Vaccination status	**Hazard Ratio**	**95% CIs**	**P-value**	**Hazard Ratio**	**95% CIs**	**P-value**
Unvaccinated	**Reference group**			**Reference group**		
Vaccinated (H1N1/TIV)	0.85	0.61 - 1.17	0.321	0.87	0.60 - 1.26	0.460
H1N1 alone	0.88	0.58 - 1.32	0.527	0.91	0.59 - 1.40	0.661
Concurrent H1N1/TIV	0.61	0.29 - 1.29	0.198	0.63	0.30 - 1.36	0.241
TIV alone	1.08	0.51 - 2.29	0.847	1.21	0.51 - 2.89	0.671
H1N1 adj alone	0.89	0.58 - 1.36	0.583	0.92	0.59 - 1.45	0.726
Concurrent H1N1 adj/TIV	0.59	0.27 - 1.28	0.183	0.61	0.28 - 1.34	0.219
H1N1 unadj	0.75	0.17 - 3.35	0.706	0.77	0.17 - 3.46	0.734
H1N1 unadj/TIV	1.00	0.06 - 15.99	1.000	1.10	0.07 - 18.06	0.946

*Model adjusted for propensity scores, age, sex, and area of residence; & Model adjusted for Model A variables plus receipt of TIV 08/09

Table 9 Effect of H1N1/TIV vaccination on occurrence of incident multiple sclerosis during anytime following index date

	Model A*			Model B&		
Vaccination status	**Hazard Ratio**	**95% CIs**	**P-value**	**Hazard Ratio**	**95% CIs**	**P-value**
Unvaccinated	**Reference group**			**Reference group**		
Vaccinated (H1N1/TIV)	1.02	0.80 - 1.31	0.849	1.11	0.84 - 1.46	0.464
H1N1 alone	1.03	0.75 - 1.40	0.873	1.08	0.78 - 1.50	0.637
Concurrent H1N1/TIV	1.07	0.64 - 1.79	0.793	1.17	0.69 - 2.00	0.559
TIV alone	0.94	0.48 - 1.86	0.862	1.17	0.55 - 2.49	0.691
H1N1 adj alone	1.04	0.75 - 1.44	0.805	1.10	0.79 - 1.54	0.580
Concurrent H1N1 adj/TIV	1.07	0.64 - 1.81	0.789	1.17	0.68 - 2.02	0.566
H1N1 unadj	0.80	0.21 - 2.98	0.739	0.83	0.22 - 3.11	0.786
H1N1 unadj/TIV	1.00	0.06 - 15.99	1.000	1.21	0.07 - 19.80	0.895

*Model adjusted for propensity scores, age, sex, and area of residence; & Model adjusted for Model A variables plus receipt of TIV 08/09

Table 10 Effect of H1N1/TIV vaccination on occurrence of incident demyelinating conditions, which do not ultimately lead to multiple sclerosis, during the period of one year following index date

	Model A*			Model B&		
Vaccination status	Hazard Ratio	95% CIs	P-value	Hazard Ratio	95% CIs	P-value
Unvaccinated	Reference group			Reference group		
Vaccinated (H1N1/TIV)	0.63	0.33 - 1.19	0.153	0.62	0.31 - 1.24	0.174
H1N1 alone	0.52	0.25 - 1.09	0.082	0.54	0.26 - 1.15	0.111
Concurrent H1N1/TIV	2.00	0.37 - 10.92	0.423	2.31	0.36 - 15.03	0.381
TIV alone	.	. - .	.	.	. - .	.
H1N1 adj alone	0.50	0.23 - 1.07	0.074	0.52	0.24 - 1.13	0.100
Concurrent H1N1 adj/TIV	2.00	0.37 - 10.92	0.423	2.29	0.35 - 14.84	0.387
H1N1 unadj	1.00	0.06 - 15.99	1.000	1.00	0.06 - 15.99	1.000
H1N1 unadj/TIV	.	. - .	.	.	. - .	.

*Model adjusted for propensity scores, age, sex, and area of residence; & Model adjusted for model A variables plus receipt of TIV 08/09

Table 11 Effect of H1N1/TIV vaccination on occurrence of incident demyelinating conditions, which do not ultimately lead to multiple sclerosis, during anytime following index date

	Model A*			Model B&		
Vaccination status	Hazard Ratio	95% CIs	P-value	Hazard Ratio	95% CIs	P-value
Unvaccinated	Reference group			Reference group		
Vaccinated (H1N1/TIV)	0.67	0.39 - 1.14	0.141	0.67	0.37 - 1.21	0.183
H1N1 alone	0.54	0.29 - 1.00	0.051	0.56	0.30 - 1.07	0.078
Concurrent H1N1/TIV	1.67	0.40 - 6.97	0.484	2.07	0.42 - 10.27	0.373
TIV alone	1.00	0.14 - 7.10	1.000	1.52	0.14 - 16.14	0.729
H1N1 adj alone	0.52	0.27 - 0.99	0.046	0.54	0.28 - 1.05	0.071
Concurrent H1N1 adj/TIV	1.67	0.40 - 6.97	0.484	2.06	0.41 - 10.19	0.378
H1N1 unadj	1.00	0.06 - 15.99	1.000	1.00	0.06 - 15.99	1.000
H1N1 unadj/TIV	.	. - .	.	.	. - .	.

*Model adjusted for propensity scores, age, sex, and area of residence; & Model adjusted for Model A variables plus receipt of TIV 08/09

Table 12 Effect of H1N1/TIV vaccination on occurrence of incident multiple sclerosis during the period of one year following index date stratified by age groups

		Model A*			Model B&		
Age group (years)	Vaccination status	Hazard Ratio	95% CIs	P-value	Hazard Ratio	95% CIs	P-value
	Unvaccinated	Reference group			Reference group		
<= 24	Vaccinated (H1N1/TIV)	0.50	0.05 - 5.51	0.571	0.50	0.05 - 5.51	0.571
	H1N1 alone	.	. - .	.	.	. - .	.
	Concurrent H1N1/TIV	.	. - .	.	.	. - .	.
	TIV alone	.	. - .	.	.	. - .	.
	H1N1 adj alone	.	. - .	.	.	. - .	.
	Concurrent H1N1 adj/TIV	.	. - .	.	.	. - .	.
	H1N1 unadj	.	. - .	.	.	. - .	.
	H1N1 unadj/TIV	.	. - .	.	.	. - .	.
25-49	Vaccinated (H1N1/TIV)	1.03	0.65 - 1.63	0.907	1.01	0.62 - 1.65	0.969
	H1N1 alone	1.43	0.82 - 2.50	0.210	1.41	0.79 - 2.50	0.244
	Concurrent H1N1/TIV	0.33	0.11 - 1.03	0.057	0.33	0.10 - 1.03	0.056
	TIV alone	1.00	0.20 - 4.95	1.000	0.93	0.16 - 5.34	0.934
	H1N1 adj alone	1.50	0.83 - 2.72	0.183	1.49	0.81 - 2.75	0.203
	Concurrent H1N1 adj/TIV	0.27	0.08 - 0.98	0.046	0.27	0.08 - 0.98	0.046
	H1N1 unadj	1.00	0.20 - 4.95	1.000	0.99	0.20 - 4.96	0.991
	H1N1 unadj/TIV	1.00	0.06 - 15.99	1.000	0.97	0.06 - 16.37	0.985
50+	Vaccinated (H1N1/TIV)	0.73	0.38 - 1.38	0.332	0.91	0.39 - 2.14	0.827
	H1N1 alone	0.63	0.20 - 1.91	0.410	0.75	0.23 - 2.45	0.630
	Concurrent H1N1/TIV	0.80	0.21 - 2.98	0.739	0.98	0.24 - 3.94	0.972
	TIV alone	0.78	0.29 - 2.09	0.618	1.14	0.31 - 4.22	0.850
	H1N1 adj alone	0.71	0.23 - 2.25	0.566	0.89	0.26 - 3.05	0.848
	Concurrent H1N1 adj/TIV	0.80	0.21 - 2.98	0.739	1.00	0.25 - 4.03	0.994
	H1N1 unadj	.	. - .	.	.	. - .	.
	H1N1 unadj/TIV	.	. - .	.	.	. - .	.

*Model adjusted for propensity scores, age, sex, and area of residence; & Model adjusted for Model A variables plus receipt of TIV 08/09

Table 13 Effect of H1N1/TIV vaccination on occurrence of incident multiple sclerosis during anytime following index date stratified by age groups

		Model A*			Model B&		
Age group (years)	Vaccination status	Hazard Ratio	95% CIs	P-value	Hazard Ratio	95% CIs	P-value
	Unvaccinated	Reference group			Reference group		
<= 24	Yes	1.43	0.54 - 3.75	0.469	1.29	0.48 - 3.45	0.618
	H1N1 alone	1.50	0.53 - 4.21	0.442	1.33	0.46 - 3.84	0.594
	Concurrent H1N1/TIV	1.00	0.06 - 15.99	1.000	1.00	0.06 - 15.99	1.000
	TIV alone	.	. - .	.	.	. - .	.
	H1N1 adj alone	1.50	0.53 - 4.21	0.442	1.33	0.46 - 3.84	0.594
	Concurrent H1N1 adj/TIV	1.00	0.06 - 15.99	1.000	1.00	0.06 - 15.99	1.000
	H1N1 unadj	.	. - .	.	.	. - .	.
	H1N1 unadj/TIV	.	. - .	.	.	. - .	.
25-49	Yes	1.18	0.84 - 1.65	0.344	1.19	0.83 - 1.70	0.348
	H1N1 alone	1.24	0.82 - 1.88	0.298	1.24	0.81 - 1.89	0.317
	Concurrent H1N1/TIV	1.19	0.61 - 2.31	0.613	1.18	0.60 - 2.33	0.628
	TIV alone	0.60	0.14 - 2.51	0.484	0.59	0.13 - 2.62	0.490
	H1N1 adj alone	1.27	0.83 - 1.95	0.276	1.27	0.81 - 1.97	0.295
	Concurrent H1N1 adj/TIV	1.20	0.60 - 2.38	0.602	1.19	0.60 - 2.40	0.616
	H1N1 unadj	1.00	0.25 - 4.00	1.000	1.00	0.25 - 4.00	0.996
	H1N1 unadj/TIV	1.00	0.06 - 15.99	1.000	0.99	0.06 - 16.21	0.992
50+	Yes	0.88	0.50 - 1.56	0.662	1.36	0.63 - 2.97	0.435
	H1N1 alone	1.00	0.38 - 2.66	1.000	1.37	0.47 - 4.00	0.564
	Concurrent H1N1/TIV	0.86	0.29 - 2.55	0.782	1.26	0.38 - 4.19	0.711
	TIV alone	0.80	0.32 - 2.03	0.638	1.49	0.44 - 5.01	0.521
	H1N1 adj alone	1.14	0.41 - 3.15	0.796	1.65	0.54 - 5.10	0.381
	Concurrent H1N1 adj/TIV	0.86	0.29 - 2.55	0.782	1.29	0.38 - 4.32	0.680
	H1N1 unadj	.	. - .	.	.	. - .	.
	H1N1 unadj/TIV	.	. - .	.	.	. - .	.

*Model adjusted for propensity scores, age, sex, and area of residence; & Model adjusted for Model A variables plus receipt of TIV 08/09

Table 14 Effect of H1N1/TIV vaccination on occurrence of incident multiple sclerosis during the period of one year following index date stratified by status of immunosuppressed conditions

		Model A*			Model B&		
Status of immunosuppression	Vaccination status	Hazard Ratio	95% CIs	P-value	Hazard Ratio	95% CIs	P-value
	Unvaccinated	Reference group			Reference group		
Immunosuppressed	Yes	.	. - .	.	.	. - .	.
	H1N1 alone	.	. - .	.	.	. - .	.
	Concurrent H1N1/TIV	.	. - .	.	.	. - .	.
	TIV alone	.	. - .	.	.	. - .	.
	H1N1 adj alone	.	. - .	.	.	. - .	.
	Concurrent H1N1 adj/TIV	.	. - .	.	.	. - .	.
	H1N1 unadj	.	. - .	.	.	. - .	.
	H1N1 unadj/TIV	.	. - .	.	.	. - .	.
Not immunosuppressed	Yes	0.91	0.64 - 1.29	0.587	0.88	0.60 - 1.32	0.545
	H1N1 alone	0.98	0.63 - 1.51	0.912	0.97	0.62 - 1.54	0.914
	Concurrent H1N1/TIV	0.57	0.24 - 1.36	0.207	0.57	0.24 - 1.37	0.208
	TIV alone	1.11	0.45 - 2.73	0.819	1.11	0.39 - 3.15	0.847
	H1N1 adj alone	1.00	0.63 - 1.58	1.000	1.00	0.62 - 1.62	0.988
	Concurrent H1N1 adj/TIV	0.54	0.21 - 1.35	0.187	0.54	0.21 - 1.35	0.188
	H1N1 unadj	0.75	0.17 - 3.35	0.706	0.75	0.17 - 3.37	0.710
	H1N1 unadj/TIV	1.00	0.06 - 15.99	1.000	1.01	0.06 - 16.58	0.995

*Model adjusted for propensity scores, age, sex, and area of residence; & Model adjusted for Model A variables plus receipt of TIV 08/09

Table 15 Effect of H1N1/TIV vaccination on occurrence of incident multiple sclerosis during anytime following index date stratified by status of immunosuppressed conditions

		Model A*			Model B&		
Status of immunosuppression	**Vaccination status**	**Hazard Ratio**	**95% CIs**	**P-value**	**Hazard Ratio**	**95% CIs**	**P-value**
	Unvaccinated	**Reference group**			**Reference group**		
Immunosuppressed	**Yes**	0.00	0.00 - .	1.000	0.00	0.00 - .	1.000
	H1N1 alone	.	. - .	.	.	. - .	.
	Concurrent H1N1/TIV	.	. - .	.	.	. - .	.
	TIV alone	0.00	0.00 - .	1.000	0.00	0.00 - .	1.000
	H1N1 adj alone	.	. - .	.	.	. - .	.
	Concurrent H1N1 adj/TIV	.	. - .	.	.	. - .	.
	H1N1 unadj	.	. - .	.	.	. - .	.
	H1N1 unadj/TIV	.	. - .	.	.	. - .	.
Not immunosuppressed	**Yes**	1.05	0.80 - 1.37	0.732	1.08	0.81 - 1.45	0.587
	H1N1 alone	1.04	0.75 - 1.45	0.801	1.07	0.76 - 1.50	0.703
	Concurrent H1N1/TIV	1.09	0.62 - 1.91	0.773	1.13	0.63 - 2.01	0.689
	TIV alone	1.00	0.45 - 2.23	1.000	1.11	0.46 - 2.68	0.810
	H1N1 adj alone	1.08	0.77 - 1.51	0.665	1.10	0.78 - 1.57	0.579
	Concurrent H1N1 adj/TIV	1.09	0.61 - 1.95	0.768	1.13	0.63 - 2.03	0.692
	H1N1 unadj	0.60	0.14 - 2.51	0.484	0.61	0.15 - 2.57	0.505
	H1N1 unadj/TIV	1.00	0.06 - 15.99	1.000	1.10	0.07 - 17.89	0.948

*Model adjusted for propensity scores, age, sex, and area of residence; & Model adjusted for Model A variables plus receipt of TIV 08/09

Annex 1 List of stand-alone documents

Number	Document reference number	Date	Title
1.	200405	07-DEC-2015	Annex 1: List of stand-alone documents
2.	200405	07-DEC-2015	Annex 2: Trademarks
3.	200405	07-DEC-2015	Annex 3: Report sign-off

Annex 2 Trademarks

The following trademarks are used in the present report.

Note: In the body of the report (including the synopsis), the names of the vaccines/products and/or medications will be written without the superscript symbol ™ or ® and in italics.

Trademarks of the GlaxoSmithKline group of companies	Generic description
Arepanrix™	AS03-Adjuvanted H1N1 Pandemic Influenza Vaccine
Pandemrix®	Pandemic influenza vaccine (H1N1) (split virion, inactivated, adjuvanted)

Annex 3 Report sign-off

Signature of Principal Investigator

GlaxoSmithKline Biologicals
Vaccine Value and Health Science
Investigator Approval Page

Please note that by signing this page, you take responsibility for the content of the Report, including appendices

STUDY TITLE: An observational retrospective database analysis to estimate the risk of Multiple Sclerosis (MS) following vaccination with Arepanrix™ in Manitoba, Canada

Study: 200405 (EPI-FLU H1N1-014 VS) Development Phase: NA

I have read this report and confirm that to the best of my knowledge it accurately describes the conduct and results of the study.

Name of Investigator:	Dr [redacted]
Affiliation /investigational center:	[redacted]
Signature of Investigator:	_____
Date:	_____

For internal use only

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MODULAR APPENDICES

List of modular appendices available for the study report and ICH-specific appendices - Study Information equivalent numbering.

Modular appendices	ICH numbering
Signatures of principal or coordinating investigator(s) or sponsor's responsible medical officer, depending on the regulatory authority's requirement	16.1.5

Study Protocol

1. PASS INFORMATION

Title	An observational retrospective database analysis to estimate the risk of multiple sclerosis following vaccination with Arepanrix™ in Manitoba, Canada
Protocol version identifier	200405 (EPI-FLU H1N1-014 VS)
Date of last version of the protocol	EMA PASS Final Version 2: 05 May 2014
EU PAS Register No.:	NA (Not applicable)
Active substance	J07BB02-AS03-Adjuvanted H1N1 Pandemic Influenza Vaccine
Medicinal product:	Arepanrix™, Pandemic Influenza vaccine (H1N1) Adjuvanted Split influenza virus, inactivated, containing antigen equivalent to A/California/7/2009 (H1N1)v like strain (X-179A)
Product reference:	EU/1/10/624/001
Procedure number:	EMEA/H/C/001201
Marketing Authorisation Holder(s)	GlaxoSmithKline Biologicals Rue de l'Institut 89 1330 Rixensart, Belgium
Joint PASS	No
Research question and objectives	To assess whether administration of Arepanrix™ during the 2009/2010 H1N1 influenza pandemic was associated with an increased risk of incident multiple sclerosis and other demyelinating conditions not ultimately leading to a multiple sclerosis diagnosis in Manitoba, Canada.
Country of study	Canada

Investigators	Principle Investigator: • [redacted] Co-investigator: • [redacted]

2. MARKETING AUTHORISATION HOLDER

Marketing authorisation holder	GlaxoSmithKline Biologicals Rue de l'Institut 89, 1330 Rixensart, Belgium

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LIST OF ABBREVIATIONS

AESI	Adverse Events Of Special Interest
AIDS	Acquired Immune Deficiency Syndrome
CARS	Computer Aided Regulatory Submission
CCI	Canadian Classification of Health Interventions
CI	Confidence Interval
CNS	Central Nervous System
DPIN	Drug Program Information Network
EMA	European Medicines Agency
EU PAS	European Union Post-Authorisation Studies
GBS	Guillain-Barré syndrome
GSK	GlaxoSmithKline
GPP	Guidelines for Good Pharmacoepidemiology Practices
GVP	Guideline on Good Pharmacovigilance Practices
HIV	Human Immunodeficiency Virus
ICD	International Classification of Diseases
ICD-9-CM	International Classification of Diseases, Ninth Revision, Clinical Modification
ICD-10-CA	International Classification of Diseases, Tenth Revision, Canadian Adaptation
MCHP	Manitoba Centre for Health Policy
MH	Manitoba Health
MHPR	Manitoba Health Population Registry
MIMS	Manitoba Immunization Monitoring System
MS	Multiple Sclerosis
NPV	Negative predictive value

PASS	Post Authorization Safety Study
PI	Principal Investigator
PPV	Positive Predictive Value
PRAC	Pharmacovigilance Risk Assessment Committee
PS	Propensity score
RR	Rate Ratio
SAE	Serious Adverse Event
SAS	Statistical Analysis System
SERM	Safety Evaluation and Risk Management
TIV	Trivalent Inactivated seasonal influenza Vaccine
VVHS	Vaccine Value and Health Science

3. RESPONSIBLE PARTIES

The present study is initiated following a regulatory commitment from the European Medicines Agency (EMA) to GlaxoSmithKline (GSK) Biologicals. GSK Biologicals has the responsibility for delivering the study report to EMA as per this commitment, and to ensure compliance with the EMA "Guidance for the format and content of the protocol of non-interventional post-authorisation safety studies" (EMA/623947/2012). The protocol was developed in a collaborative manner between GSK Biologicals and the Principal Investigator (PI), Dr. [redacted] who wrote the first version of the protocol.

As per the Manitoba Centre for Health Policy (MCHP) Guidelines for Public and Private Sponsorship of Research Projects [MCHP, 2011], the PI is responsible for obtaining all necessary study approvals; overall conduct of the study; and publishing the results in the searchable, peer reviewed scientific literature. A protocol summary, including the anticipated timing for posting and submission of the results for publication, will be posted on-line on the EU PAS register, as required by the EMA, and on other publicly available registers.

4. ABSTRACT

Title	An observational retrospective database analysis to estimate the risk of multiple sclerosis following vaccination with Arepanrix™ in Manitoba, Canada.
Rationale and background	Multiple sclerosis (MS) is a chronic, progressively disabling disease of the central nervous system, estimated to affect more than 2.5 million persons worldwide. Canada has among the highest prevalence of MS in the world, with more than 70,000 individuals affected. The aetiology of MS remains unknown: putative risk factors include genetic susceptibility, infection, vaccination, stress, occupation, climate, and diet. In the Northern hemisphere, there were two epidemic waves of pandemic H1N1influenza; one between mid-May and the end of June 2009, and the other during the 2009/10 influenza season, which occurred predominantly between October and December of 2009. In Canada, mass immunization against pandemic H1N1 commenced October 26th, 2010, using primarily large-scale vaccination clinics led by public health teams and lasted approximately 8 weeks. A limited number of observational studies have explored the risk of neurological and immune-mediated conditions, including MS, following pandemic H1N1 influenza vaccination. In the majority of studies, no increased risk of MS was identified; however, there was a signal in an hypothesis-generating database study supported by GSK in Sweden, that triggered the need to further investigate the potential association between AS03-adjuvanted H1N1 vaccines and the occurrence of MS.
Research question and objectives	**Primary** • To assess whether administration of Arepanrix™ was associated with an increased risk of incident MS. **Secondary** • To assess whether administration of Arepanrix™ was associated with an increased risk of demyelinating events which do not ultimately lead to a diagnosis of MS (i.e., never have a diagnostic claim for MS), including optic neuritis. **Exploratory** • To assess whether administration of unadjuvanted pandemic H1N1 influenza vaccines was associated with an increased risk of incident multiple sclerosis.

Study design	This is an observational, retrospective, matched cohort study using the Manitoba Immunization Monitoring System (MIMS) and the hospital, physician, and prescription claims databases of the MH Database System. The vaccinated cohort will comprise individuals with a MIMS record for pandemic H1N1 influenza vaccination, as well as for Trivalent Inactivated seasonal influenza Vaccine (TIV) to account for potential confounding or effect modification by seasonal influenza vaccination. Individuals with no records of H1N1 or seasonal influenza vaccination will constitute the unvaccinated cohort. Matching will be performed using propensity scores.
Population	Adults and children greater than 6 months of age (at the time of vaccination) who normally reside in Manitoba and who had been registered with MH between September 15, 2009 and March, 15, 2010, spanning the period of time when almost all monovalent H1N1 vaccines were administered, will be eligible for inclusion in the study. To ensure sufficient historical data, all participants will be required to have at least one year of insurance coverage before this period.
Endpoints	Primary endpoint: • Occurrence of MS during the period of one year following administration of Arepanrix™ among an exposed cohort and during an equivalent time period in the unexposed cohort. Secondary endpoints: • Occurrence of MS from administration of Arepanrix™ until 31 December 2012, among an exposed cohort (see Section 9.2.2) and during an equivalent time period in the unexposed cohort. • Occurrence of demyelinating events which do not ultimately lead to a diagnosis of MS during the period of one year following administration of Arepanrix™ among an exposed cohort and during an equivalent time period in the unexposed cohort. This includes optic neuritis. • Occurrence of demyelinating events which do not ultimately lead to a diagnosis of MS (i.e., never have a diagnostic claim for MS) from administration of Arepanrix™ until 31 December 2012, among an exposed cohort(see Section 9.2.2) and during an equivalent time period in the unexposed cohort, such as optic neuritis, acute transverse myelitis, demyelinating disease of central nervous system (CNS) unspecified, other acute disseminated demyelination, and neuromyelitis optica.

Exploratory endpoint:

- Occurrence of MS during the period of one year following administration of unadjuvanted pandemic H1N1 influenza vaccines among an exposed cohort and during an equivalent time period in the unexposed cohort.

Data sources

Eligibility for inclusion in the analysis will be determined using the Manitoba Health Population Registry (MHPR), a continuously updated registry that stores basic demographic information (e.g., date of birth and sex) on all insured Manitobans. A matched cohort analysis will be conducted using de-identified records obtained by linking the electronic database of the MIMS with the hospital, physician and prescription claims databases of MH. Information on MS and other relevant diseases and health conditions will be obtained from the hospital and physician claims databases, the database of the Drug Program Information Network (DPIN), the Hospital Abstracts database and the Medical Services database.

Data Analysis

The incidence rate of MS between the exposed and the unexposed cohort using a sampling approach based on propensity scores will be compared. Each vaccinated individual will be matched to an individual who did not receive any influenza vaccines during the study period. Due to lack of random assignment of treatments, estimates of treatment effects in observational studies can be biased because the treatment group and the control group may not be comparable with respect the distribution of important disease (or outcome) predictors (confounders). Propensity score (PS) methods are one approach to constructing more comparable groups by limiting comparisons to individuals who have the same propensity to receive the treatment. Cox proportional hazard models, with stratification on the PS matched pairs, will be used to estimate relative risks (hazards ratios) associated with vaccination.

Milestones

Provisional milestones for the study depending on Pharmacovigilance Risk Assessment Committee (PRAC) approval and timely approval and release of the dataset by MH will be communicated to EMA as soon as available.

5. AMENDMENTS AND UPDATES

None

6. MILESTONES

Table 1 indicates timelines for the main study milestones. The 18-month period will start upon PRAC confirmation that the present protocol is final. Of note, GSK Biologicals and

the investigator have no control on the exact time required for extraction and release of the dataset by MH; however, these timelines take into account possible delays.

Table 1 Timelines for the main study milestones

Summary of activities	Month (Time 0 is the date the contract is signed)																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Finalize data extraction and analysis plans																		
Obtain study approvals (ERB, [redacted] and MCHP)																		
Construct propensity score and perform matching																		
Data extraction & delivery by MCHP																		
Perform statistical analyses																		
Perform data cleaning and validation tasks																		
Generate & code variables required for analysis																		
Implement analysis and generate results																		
Dissemination and knowledge translation																		
Prepare brief preliminary report and submit for review																		
Prepare manuscripts for publications																		

7. RATIONALE AND BACKGROUND

7.1. Background

Multiple sclerosis (MS) is a chronic, progressively disabling disease of the central nervous system, estimated to affect more than 2.5 million persons worldwide [Dean, 1994]. Canada has among the highest prevalence of MS in the world, with more than 70,000 individuals affected [Beck, 2005]. It is the most common non-traumatic cause of disability in young adults, and adversely affects employment, social relationships, and quality of life [Nortvedt, 1999; Rao, 1991]. The societal costs of MS exceed those for stroke or Alzheimer's disease. Thus, the burden of MS is substantial for affected individuals and society.

Despite multiple studies, the aetiology of MS remains unknown [Marrie, 2004]. MS is conceptualized to be a complex disease; that is, one in which one or multiple environmental factors act on a genetically susceptible individual to cause disease. Putative risk factors that have been commonly studied include infection, vaccinations, stress, occupation, climate, and diet [Marrie, 2004]. Infection has been a putative etiologic agent of particular interest although there has been no reproducible evidence of a transmissible MS agent [Cosby, 1989; Haase, 1981; Hammerschlag, 2000]. The he biologic plausibility of Epstein-Barr virus as an etiologic factor is increasing, however, suggesting that infectious agents may initiate or perpetuate the disease process.

Similarly, vaccinations have also been considered as etiologic factors for MS. A series of case reports in France raised particular concern about demyelinating events developing after hepatitis B vaccination [DeStefano, 2003]. Ascherio and colleagues conducted a nested case-control study with data from the Nurses Health Studies in which 192 women with MS were matched to 645 controls. The odds ratio of MS associated with hepatitis B vaccination occurring any time before disease onset was 0.9 (95% confidence interval

(CI) 0.5–1.6) [Ascherio, 2001]. Case-control and cohort studies have been consistent in showing no association between other childhood vaccinations (measles, mumps, rubella) and MS [Bansil, 1990; Casetta, 1994; Currier, 1996; Zorzon, 2003].

Confavreux et al. evaluated the risk of MS relapse after vaccination in 643 patients. They did not find any evidence of an increased risk of relapse following vaccination (relative risk 0.71; 95% CI: 0.40-1.26), irrespective of the vaccine including seasonal influenza vaccine [Confavreux, 2001]. However, the risk of relapse after pandemic H1N1 vaccine was not evaluated. One small study in the United Kingdom evaluated relapses among 30 patients with MS between November 2009 and January 2010, of whom 18 (60%) received the pandemic H1N1 influenza vaccine and/or the seasonal influenza vaccine (40% were unvaccinated). Using a case-crossover design the relative risk of relapse was 6.0 (95% CI: 1.4-26.2) [McNicholas, 2011]. A subsequent study of 137 relapsing-remitting MS patients from Argentina found that 60 were vaccinated (49 with seasonal TIV and 11 with monovalent H1N1 pandemic vaccine), among which 28 relapse events were observed. Focusing on the 30 day period after the relapse, the risk was not increased (relative risk 0.86; 95% CI: 0.20-0.36). Findings were similar when the risk period was extended to 60 days and 90 days [Farez, 2012].

However, very few published studies have evaluated the association of pandemic H1N1 vaccination and the risk of developing MS. Vrethem et al. reported on a previously healthy young man who developed severe narcolepsy and MS within two months of receiving Pandemrix® [Vrethem, 2012]. Both Pandemrix® and Arepanrix™ are AS03--adjuvanted split virion pandemic influenza H1N1 vaccines, with Pandemrix® being manufactured in Germany and Arepanrix™ manufactured in Canada. A large retrospective Swedish record-linkage study reported increased risk of paraesthesia, but not of diagnosed MS, among persons vaccinated with Pandemrix® [Bardage, 2011; Persson, 2014]. In an analysis of Manitoba's administrative databases, vaccination with Arepanrix™, the AS03-adjuvanted split virion pandemic H1N1 vaccine also manufactured by GSK, was not associated with increased risk of MS diagnosis; like the Swedish study, the [redacted] et al. study was limited by the use of non-validated algorithm for the identification of MS from administrative databases and by the inability to distinguish between prevalent and incident cases. Thus, the impact of H1N1 vaccine on MS remains uncertain.

The first confirmed case of pandemic H1N1 infection in the Canadian province of Manitoba was detected on May 3, 2009 [Thompson, 2011]. Like elsewhere in the Northern hemisphere, there were two epidemic waves; one between mid-May and the end of June 2009, and the other during the 2009/10 influenza season, which occurred predominantly between October and December of 2009 [Thompson, 2011; Zarychanski, 2010]. Mass immunization against pandemic H1N1 commenced October 26th 2010 using primarily large-scale vaccination clinics led by public health teams and lasted approximately 8 weeks. Initially, GSK's Canadian-manufactured AS03-adjuvanted 2009 pandemic H1N1 influenza vaccine Arepanrix™ was used to vaccinate adults and children over 6 months of age. Later on, two unadjuvanted pandemic H1N1 vaccines, from CSL Limited and GSK, were offered to pregnant women and children over 10 years of age; however, Arepanrix™ was the only adjuvanted vaccine used in Canada. The seasonal trivalent inactivated influenza vaccines (TIV) - Fluviral® (GSK) and Vaxigrip®

(Sanofi Pasteur) - were administered as part of the annual influenza immunization program. The live attenuated influenza vaccine was not available in Manitoba during the 2009–2010 season [Mahmud, 2012].

All vaccines were offered free of charge, but limited vaccine supply at the start of the campaign necessitated the development of priority groups for early vaccination. The initial priority group for the H1N1 vaccine in Manitoba included health care workers, Aboriginal persons, pregnant women, children 6-60 months-old, individuals under 65 years of age with chronic medical conditions (including multiple sclerosis), immuno-compromised individuals and residents of remote communities [Mahmud, 2011]. On November 18, 2009 the Pandemic H1N1 vaccines were made available to the whole population [Mahmud, 2011].

7.2. Rationale for the study

As outlined above, a limited number of observational studies have explored the risk of MS following pandemic H1N1 influenza vaccination. In the majority of studies, no increased risk was identified. A GSK-supported, observational cohort study of individuals vaccinated with Pandemrix® as part of the national 2009 H1N1 pandemic immunisation campaign in Sweden, measured incidence rates of Adverse Events of Special Interest (AESIs): anaphylaxis, Bell's palsy, convulsion, demyelination, encephalitis, Guillain-Barré Syndrome (GBS), neuritis, any influenza, vasculitis, convulsions in epileptics, autoimmune hepatitis, and multiple sclerosis. For MS, the standardised incidence ratio was significantly increased, which might have been due to the limitations of the study, including potential selection bias and lack of control for residual confounding [unpublished report].This signal triggered the need to further investigate the potential association between AS03-adjuvanted H1N1 vaccines and the occurrence of MS.

Investigating the signal in the Manitoba settings has the following advantages:

- The burden of MS in Canada is substantial [Beck, 2005], and the province of Manitoba has one of the highest prevalence of MS with approximately 100 new cases each year [[redacted] personal communication], making this region suitable to address the research question;
- This study will allow obtaining important complementary data on the safety of Arepanrix™, an AS03-adjuvanted split virion pandemic influenza H1N1 vaccine very similar to Pandemrix®, on the basis that the two vaccines contain the same antigen and the same adjuvant, hence being expected to produce a similar immune response in vaccinated persons;
- In the EMA assessment of the draft report of the GSK-supported safety study on the risk of AESIs following vaccination with Arepanrix™ in Manitoba, it was stated that "*No strong signal was observed for demyelination with Arepanrix™; indeed higher risk estimates were observed for seasonal trivalent influenza vaccines. However, risk estimates in the subgroup analysis (individuals with autoimmune diseases and those aged 18-64 years) were elevated, with lower 95% confidence levels >1*". This study will further explore this matter using a more robust design (propensity-score

matching of the cohorts) and a validated case definition, allowing the identification of incident MS cases.

In summary, the study purpose will be to assess whether administration of Arepanrix™ was associated with an increased risk of incident MS in Manitoba, Canada. The availability of a province-wide population-based immunization registry and other linked health care administrative databases provides a unique opportunity to perform this evaluation.

8. RESEARCH QUESTION AND OBJECTIVES

8.1. Primary objective

- To assess whether administration of Arepanrix™ was associated with an increased risk of incident MS.

8.2. Secondary objective

- To assess whether administration of Arepanrix™ was associated with an increased risk of demyelinating events which do not ultimately lead to a diagnosis of MS (i.e., never have a diagnostic claim for MS), including optic neuritis.

8.3. Exploratory objective

- To assess whether administration of unadjuvanted pandemic H1N1 influenza vaccines was associated with an increased risk of incident multiple sclerosis.

9. RESEARCH METHODS

9.1. Study design

The proposed study is a retrospective analysis of population-based cohorts of subjects, whose vaccination status and health events before and after vaccination, are recorded in various MH administrative databases. A propensity-score matched cohort analysis will be conducted using de-identified records obtained by linking the electronic database of the Manitoba Immunization Monitoring System (MIMS) with the hospital, physician and prescription claims databases of MH.

9.1.1. Rationale for study design

The use of automated administrative databases allows access to a large population of vaccinated individuals. A cohort design using propensity score (PS) matching was adopted so that the exposed and unexposed cohorts are comparable on known potential confounders; hence, the probability of being vaccinated should be similar in both study groups.

Due to lack of random assignment of treatments, estimates of treatment effects in observational studies can be biased because the treatment group and the control group may not be comparable with respect the distribution of important disease (or outcome)

predictors (confounders). Propensity score methods are one approach to constructing more comparable groups by limiting comparisons to individuals who have the same propensity to receive the treatment [Rubin, 1997]. PS is defined as the conditional probability of receiving treatment given the value of a set of confounders, and can be estimated using logistic or probit regression models of the association between confounding covariates and the receipt of treatment [Rubin, 1997]. PS methods are especially suitable for post-marketing studies of drug and vaccine safety where the outcomes are typically rare, limiting the utility of conventional multivariate adjustment methods, but the treatment and confounders data is very rich.

9.2. Setting and study population

9.2.1. Study population

The study population will be comprised of adults and children above 6 months of age (at the time of vaccination) who normally reside in Manitoba, Canada and who had been registered with MH during the study period (see Section 9.2.5). To ensure sufficient historical data, all participants will be required to have at least one year of insurance coverage before the study period.

9.2.2. Cohort identification and creation

- **Vaccinated cohort:** all individuals with MIMS record of H1N1 and/or seasonal influenza vaccination during the influenza season 2009/2010 (September 15th, 2009 to March 15th, 2010).
- **Unvaccinated cohort:** registered with MH during the study period but with no MIMS record for H1N1 and seasonal influenza vaccination during the influenza season 2009/2010 (September 15th, 2009 to March 15th, 2010).

The **vaccinated cohort** will be assembled by identifying all individuals who had a MIMS record indicating receipt of pandemic H1N1 influenza or TIV/seasonal influenza vaccines (see Table 2 for tariff codes that will be used to identify these records) during the **enrolment period**, i.e., between September 15th, 2009, and March 15th, 2010, spanning the period when almost all H1N1 vaccines and TIVs were administered.

In Manitoba, TIVs were frequently given concurrently or around the same time as the H1N1 vaccines. Gathering information on TIV administration will allow developing models that account for potential confounding or effect modification between the pandemic and seasonal vaccines. In the analysis, cohorts will be stratified by receipt of TIV and H1N1 vaccines. Individuals who were registered with MH during the study period but did not have MIMS records indicating receipt of the H1N1 or seasonal influenza vaccines will constitute the **unvaccinated cohort**.

Table 2 Tariff codes for H1N1 and seasonal influenza vaccines

Tariff code	Vaccine
8893	Influenza pandemic H1N1 adjuvanted
8894	Influenza pandemic H1N1 unadjuvanted
8791	Seasonal influenza (TIV)

Based on propensity scores (see details of the propensity score model in Section 9.7.7), each vaccinated individual will be matched to an individual who did not receive any influenza vaccines during the study period.

The **index date** will be defined as the date of vaccination for vaccinated individuals, and the date of vaccination of the matched vaccinated individual for unvaccinated individuals. For the unvaccinated cohort, the index date will be between September 15th, 2009, and March 15th, 2010.

9.2.3. Inclusion criteria

The entire population of Manitoba is considered for inclusion.

9.2.4. Exclusion criteria

- Individuals ≤6 months of age;
- Having less than one year of insurance coverage before the enrolment period;
- Not registered with MH during the enrolment period;
- Physician or hospitalization records indicating a diagnosis of any demyelinating condition between 1971 (earliest year for which information is available) and the index date.

9.2.5. Study period

- The study period will span from 01 October 2009 (beginning of the H1N1 influenza mass vaccination campaign in Canada) to 31 December 2012 (to allow sufficient follow-up time for cases to have a confirmatory diagnosis given the natural history of MS).

9.3. Endpoints

9.3.1. Primary endpoint

- Occurrence of MS during the period of one year following administration of Arepanrix™ among an exposed cohort (see Section 9.2.2) and during an equivalent time period in the unexposed cohort.

9.3.2. Secondary endpoint

- Occurrence of MS from administration of Arepanrix™ until 31 December 2012, among an exposed cohort (see Section 9.2.2) and during an equivalent time period in the unexposed cohort.
- Occurrence of demyelinating events which do not ultimately lead to a diagnosis of MS (i.e., never have a diagnostic claim for MS) during the period of one year following administration of Arepanrix™ among an exposed cohort(see Section 9.2.2) and during an equivalent time period in the unexposed cohort such as optic neuritis, acute transverse myelitis, demyelinating disease of central nervous system

(CNS) unspecified, other acute disseminated demyelination, and neuromyelitis optica.

- Occurrence of demyelinating events which do not ultimately lead to a diagnosis of MS (i.e., never have a diagnostic claim for MS) from administration of Arepanrix™ until 31 December 2012, among an exposed cohort(see Section 9.2.2) and during an equivalent time period in the unexposed cohort, such as optic neuritis, acute transverse myelitis, demyelinating disease of central nervous system (CNS) unspecified, other acute disseminated demyelination, and neuromyelitis optica.

9.3.3. Exploratory endpoint

- Occurrence of MS during the period of one year following administration of unadjuvanted pandemic H1N1 influenza vaccines among an exposed cohort and during an equivalent time period in the unexposed cohort.

9.3.4. Data to be collected

9.3.4.1. Subjects characteristics

Demographic characteristics such as age, sex, area of residence, socio-economic status will be collected. Medical history such as comorbidity, immune status, vaccine indication (e.g., pregnancy, cardiovascular, pulmonary or renal diseases, etc.), receipt of other vaccines or medications and frequency of healthcare contacts is to be obtained.

Information on pregnancy status and pre-existing conditions will be obtained from the Hospital Separation and Physician Claims databases. Previously validated algorithms, based on the frequency of certain International Classification of Diseases (ICD) codes, will be used to identify various chronic diseases (Table 3) [Elixhauser, 1998; Lix, 2006]. Immunosuppression will be defined as having a diagnosis of Human Immunodeficiency Virus / Acquired Immune Deficiency Syndrome (HIV / AIDS), other immune deficiency disorders or cancer (other than non-melanoma skin cancer), or receiving prescriptions for immunosuppressive drugs (Table 3) [Dublin, 2009]. Information on the use of f immunosuppressants will be obtained from the DPIN. Pregnancy status will be determined from the same databases using disease and tariff codes for different conditions and procedures indicative of ongoing pregnancy or the completion of pregnancy (Table 2) [Hardy, 2004].

9.3.4.2. History of pandemic H1N1 and seasonal influenza vaccines

Information on the receipt of all vaccines, including the pandemic H1N1 and seasonal influenza vaccines will be obtained from MIMS (refer to Table 2 and Section 9.4.2).

9.3.4.3. Case definition

Multiple sclerosis

Incident cases of diagnosed MS among all included individuals will be identified by record linkage with the hospital and physician claims databases and DPIN using a validated algorithm developed by Manitoba's MS registry/clinic [Marrie, 2010].

In 2008, Dr. [redacted] and colleagues used Manitoba administrative claims data to identify persons with demyelinating disease using ICD-9 and ICD-10 codes and prescription claims [Marrie, 2010]. To validate the algorithm, questionnaires were mailed to 2000 randomly selected persons with an encounter for demyelinating disease, requesting permission for medical records review. Diagnoses abstracted from medical records were used as the gold standard to evaluate candidate case definitions using administrative data. From 1984-1997, cases of MS using claims data were defined as persons with ≥7 hospital or physician claims for MS. From 1998 onward, cases were defined as persons with ≥3 hospital, physician or prescription claims for MS. As compared to medical records, this definition had a positive predictive value (PPV) of 80.5% and negative predictive value (NPV) of 75.5% in persons with ≥1 claim for demyelinating disease; the NPV is much higher at the population level where more than 98% of the population has no claims for demyelinating disease. The performance of this case definition was recently assessed in Nova Scotia (confidential unpublished data, [redacted] Applying the case definition of ≥3 hospital or physician claims for MS (prescription claims were not available) and comparing it to MS diagnoses from the Dalhousie Multiple Sclerosis Research Unit database, the PPV was 93% (95% CI: 92-94%).

In the present analysis, a MS case will be defined by ≥3 hospital, physician or prescription claims for MS (see Table 3 for ICD9/10 codes) to define a case of MS. A case will be considered *incident* if there were no physician or hospitalization records indicating a diagnosis of any demyelinating condition between 1971 (the earliest year for which information is available from the electronic databases) and the index date. The date of diagnosis of MS will be considered the date of the first medical contact for any of the MS diagnostic codes.

Other Demyelinating Diseases

Demyelinating events not ultimately leading to MS diagnosis, including optic neuritis, will be defined by ≥1 hospitalizations or ≥2 physician claims at least 30 days apart with no subsequent MS diagnosis (see Table 3 for ICD9/10 codes).

Table 3 Definition of variables used in the analysis

Variable	Definition
Drugs†	
Anti-HIV	Protease inhibitors (J05AE*), Nucleoside and nucleotide reverse transcriptase inhibitors (J05AF*), Non-nucleoside reverse transcriptase inhibitors (J05AG*), Antivirals for treatment of HIV infections, combinations(J05AR*),
Anti-influenza	Neuraminidase inhibitors (J05AH*) or cyclic amines (J05AC*)
For treatment of diabetes	Drugs used in diabetes (A10*)
For treatment of multiple sclerosis	Interferon-beta-1b (Betaseron), Interferon-beta-1a (Avonex), Interferon-beta-1a (Rebif), Glatiramer acetate (Copaxone), and Natalizumab (Tysabri)
Immunosuppressants	Antineoplastic agents (L01*), Immunosuppressants (L04A*)
Systemic antimicrobials	Antibacterials for systemic use(J01*), Antimycotics for systemic use (J02*), Antimycobacterials (J04*)
Systemic steroids	Corticosteroids for systemic use, plain (H02A*), Corticosteroids for systemic use, combinations (H02B*)
Pregnancy	
Ongoing pregnancy	≥ 1 admission (O10-O16, O20-O29, O30-O48, O94-99, Z32-Z36) OR ≥ 2 physician claims (640-649, V22) OR ≥ 1 tariff code for prenatal services. Must be within ± 30 days of the index date [Hardy, 2004].
Completion of Pregnancy	≥1 admission (O8, O65-O75, O80-O84, O85-O92, Z37-Z39) OR ≥ 2 physician claims (650-659, 670-676, 670-676, V27) OR ≥ 1 tariff code for delivery, abortion or postnatal services. Must be within 270 days following the index date [Hardy, 2004].
Medical conditions‡	
Acute disseminated encephalomyelitis	≥ 1 admission (G36.9) OR ≥ 2 physician claims (323) 30 days apart
Acute transverse myelitis	≥ 1 admission (G37) OR ≥ 2 physician claims (323.82) 30 days apart
Alcoholism	≥ 1 admission (E52, F10, K70, X45, X65, Y15, Y90, Y91, G31.2, G62.1, G72.1, I42.6, K29.2, K86.0, O35.4, P04.3, R78.0, T50.6, T51.0, T51.1, T51.9, Y57.3, Z50.2, Z71.4, Z72.1, Z81.1, E24.4, E51.2 , Q86.0) OR ≥ 2 physician claims (303, 291)
Anaemia	≥ 1 admission (D50-64) OR ≥ 2 physician claims (280-285)
Asthma	≥ 1 admission (J45, J46) OR ≥ 2 physician claims (493)
Cancer-ex non-melanoma skin	≥ 1 admission (J40-J99, O24) OR ≥ 2 physician claims (490-496, 500-508)
Cardiovascular disease	≥1 admission (I00-I99, O11) OR ≥ 2 physician claims (390-459)
Chronic renal failure	≥1 admission (N18, N19, Z49, 12.0 , I13.1, N25.0, Z99.2) OR ≥ 2 physician claims (403-404 586-587)
Chronic respiratory condition	≥ 1 admission (J40-J99, O24) OR ≥ 2 physician claims (490-496, 500-508)
COPD	≥ 1 admission (J40-J44, O24) OR ≥ 2 physician claims (490-492, 496)
Demyelinating disease of CNS unspecified	≥ 1 admission (G37.8) OR ≥ 2 physician claims (341.9) 30 days apart
Diabetes	≥ 1 admission (E10-E14, O24, G590, G632, H280, H360, M142, M146, N083) OR ≥ 2 physician claims (250) OR ≥ 2 prescriptions for drugs used in treatment of diabetes.
HIV/AIDS	≥ 1 admission (B20-B24, R75, Z21) OR ≥ 2 physician claims (042 V08) OR ≥ 1 prescriptions for drugs used in treatment of HIV.
Hypertension	≥ 1 admission (I10-I15, I67.4 , O11) ≥ 2 physician claims (401-405)
Immune deficiency	≥ 1 admission (D80-D84, D89) OR ≥ 2 physician claims (288, 279)
Immunosuppressed	Having an organ transplant or a diagnosis of HIV/AIDS, other immune deficiency disorders or cancer (other than non-melanoma skin cancer), or receiving prescriptions for immunosuppressants or systemic steroids.
Ischemic Heart diseases	≥ 1 admission (I20-I25) OR ≥ 1 physician claims (410-414)

Variable	Definition
Multiple sclerosis	≥ 3 contacts including hospital admissions (G35), physician visits (340), or prescriptions for MS (see list of drugs above)
Neuromyelitis optica	≥ 1 admission (G36.0) OR ≥ 2 physician claims (341.0) 30 days apart
Obesity	≥ 1 admission (E66) OR ≥ 2 physician claims (278)
Optic neuritis	≥ 1 admission (H46) OR ≥ 2 physician claims (377.3) 30 days apart
Organ transplant	≥ 1 admission (T86, Z94, Y83.0) OR ≥ 2 physician claims (V42)
Other acute disseminated demyelination	≥ 1 admission (G36)
Stroke	≥ 1 admission (I61, I63, I64, I69, I67.9) OR ≥ 2 physician claims (431,434, 436-438)
Substance abuse	≥ 1 admission (F11-F16, F18-F19) OR ≥ 2 physician claims (292, 304,305)

† Drugs were classified based on their Drug Identification Number and the Anatomical Therapeutic Chemical (ATC) Classification System [WHO, 2002].

‡ Based on previously validated chronic disease identification algorithms with modifications [Elixhauser, 1998]. The codes in parentheses are ICD-10-CA codes for hospital admission data and ICD-9-CM codes for physician claims data.

9.4. Data sources

9.4.1. Manitoba Health (MH) administrative databases

MH is the publicly funded health insurance agency providing comprehensive health insurance, including coverage for hospital and outpatient physician services, to the province's 1.2 million residents. Coverage is universal (there is no eligibility distinction based on age or income) and participation rates are very high (>99%) [Singh, 2009b]. Only the Royal Canadian Mounted Police and military personnel, whose health benefits are fully covered by the federal government, are not included [Roos, 1993].

For administrative purposes, MH maintains several centralized electronic databases that are linkable using a unique Public Health Information Number (PHIN). The completeness and accuracy of the Manitoba administrative database are well established, [Humphries, 2000; Roos, 1993; Young, 1997] and these databases have been used extensively in studies of post-marketing surveillance of various vaccines and drugs [Fedson, 1993; Mahmud, 2011; Mahmud, 2012; Roberts, 1994; Singh, 2009b].

9.4.2. Manitoba Immunization Monitoring System (MIMS)

Information on the receipt of all vaccines, including the pandemic H1N1 and seasonal influenza vaccines will be obtained from MIMS, the population-based province-wide registry recording all immunizations administered to Manitoba residents since 1988 [Roberts, 1996]. Information, including vaccine type and date of immunization, is captured for each immunization event either through direct data entry for vaccines administered by public health staff (who administered the majority of H1N1 vaccines during the pandemic) or using physician claims data for vaccines administered by physicians [Roberts, 1994]. Estimates of the completeness and accuracy of the recorded vaccination information are high [Roberts, 1994]. Vaccination status in the MIMS database does not include information on brand/manufacturer; however, data on the adjuvanted nature of pandemic influenza vaccines that were used in Manitoba is available.

9.4.3. Manitoba Health Population Registry (MHPR)

We will determine eligibility for inclusion in the analysis using the Manitoba Health Population Registry (MHPR), a continuously updated registry that stores basic demographic information (e.g., date of birth and sex) on all insured Manitobans, and gathers information on dates and reasons for the initiation and termination of health care coverage (e.g., birth, migration in or out of province and death), and on changes in address and marital status of the insured individuals.

9.4.4. Drug Program Information Network (DPIN)

Information on MS and other relevant diseases and health conditions (see Section 9.3.4.3) will be obtained from the hospital and physician claims databases (see Section 9.4) and from the database of the Drug Program Information Network (DPIN). The DPIN, in operation since 1995, records all prescription drugs dispensed to Manitoba residents [Kozyrskyj, 1998]. The DPIN database captures data from pharmacy claims for formulary drugs dispensed to all Manitobans even those without prescription drug coverage. Because information is submitted electronically at the "point-of-sale", the accuracy of the recorded prescription information is excellent [Kozyrskyj, 1998].

9.4.5. Hospital Abstract Database

Since 1971, the Hospital Abstracts database recorded virtually all services provided by hospitals in the province, including admissions and day surgeries [Roos, 1993]. The data collected comprise demographic as well as diagnosis and treatment information including primary diagnosis and service or procedure codes, coded using the International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) before April, 2004, and the ICD-10-CA, (Canadian adaptation of the ICD-10 [WHO, 1993]) and the Canadian Classification of Health Interventions (CCI) [Canadian Institute for Health Information, 2006] afterwards.

9.4.6. The Medical Services database

The Medical Services database, also in operation since 1971, collects similar information, based on physician fee-for-service or shadow billing, on services provided by physicians in offices, hospitals and outpatient departments across the province [Roos, 1993]. Each billing record includes a tariff code and a 3-digit ICD-9 code which identifies the principal diagnosis or main reason for the visit. This database is limited by the lack of more specific ICD codes (4^{th} and 5^{th} digits).

9.5. Study size

Based on 400,000 vaccinated individuals (and 400,000 non-vaccinated individuals) and assuming a MS incidence rate of 20/100,000 among non-vaccinated individuals, a conservative assumption given that MS rates among younger adults in Manitoba ranged from 29/100,000 in the 35-39 age-group to 19/100,000 in the 50-54 age-group from 1998 to 2006 (see Table 4), the matched cohort analysis will have >99% power to detect doubling of the risk (rate ratio [RR]=2) and 81% power to detect 50% increase in risk

(RR=1.5) [OpenEpi, 2013; Fleiss, 2003; Kelsey, 1996]. A two-sided test at alpha=0.05 5 was assumed in all calculations.

Table 4 Average Annual Incidence (Inc./100,000 Person-Years) of MS in Manitoba per 100,000 Population by Age and Sex, 1998-2006*

Age (years)	Females			Males			Women: Men		Both		
	No. Cases 1998-2006	Inc.	95% CI	No. Cases 1998-2006	Inc.	95% CI	Rate Ratio	95% CI	No. Cases 1998-2006	Inc.	95% CI
≤24**	96	5.48	4.49, 6.69	17	0.93	0.58, 1.50	5.90	3.52, 9.88	113	3.16	2.62, 3.79
25-29	114	33.6	28.0, 40.4	33	9.73	6.92, 13.7	3.45	2.34, 5.09	147	21.7	18.4, 25.5
30-34	134	38.2	32.2, 45.2	31	8.86	6.23, 12.6	4.31	2.91, 6.37	165	23.6	20.2, 27.4
35-39	186	47.6	41.2, 55.0	43	11.0	8.17, 14.8	4.32	3.10, 6.02	229	29.3	25.8, 33.4
40-44	171	41.5	35.7, 48.2	53	12.8	9.75, 16.7	3.25	2.39, 4.43	224	27.1	23.8, 30.9
45-49	134	34.8	29.4, 41.3	56	14.5	11.2, 18.9	2.40	1.76, 3.28	190	24.7	21.4, 28.4
50-54	89	26.3	21.4, 32.4	41	12.2	8.98, 16.6	2.16	1.49, 3.12	130	19.3	16.2, 22.9
55-59	62	22.6	17.6, 28.9	35	12.8	9.22, 17.9	1.76	1.16, 2.66	97	17.7	14.5, 21.6
60-64	24	11.1	7.41, 16.5	18	8.6	5.39, 13.6	1.29	0.70, 2.38	42	9.82	7.26, 13.3
≥65	37	4.5	3.28, 6.24	17	2.8	1.76, 4.55	1.60	0.90, 2.84	54	3.81	2.91, 4.97
Total	1047	19.8	18.6, 21.1	344	6.7	6.03, 7.45	2.96	2.62, 3.34	1391	13.4	12.7, 14.1

*[Marrie, 2010]
**Age groups collapsed because cell sizes <5 suppressed

Table 5 shows the uptake of influenza vaccination during the 2009/2010 pandemic season in Manitoba.

Table 5 Distribution of vaccinated & unvaccinated individuals in the MIMS by age and gender

		Unvaccinated		H1N1 alone		Conc. H1N1/TIV		TIV alone		All vaccinated	
		N	%	N	%	N	%	N	%	N	%
Age (years)	6 mths-2	9737	3.35	17458	6.04	2379	3.61	2589	1.74	22426	4.46
	3-9	26674	9.19	44127	15.28	5319	8.08	2300	1.55	51746	10.28
	10-17	30735	10.58	42610	14.75	5621	8.54	2157	1.45	50388	10.01
	18-49	117798	40.57	118162	40.91	28007	42.53	19085	12.85	165254	32.84
	50-64	64135	22.09	52534	18.19	19082	28.98	30877	20.78	102493	20.37
	65-74	22356	7.70	9086	3.15	3628	5.51	40494	27.25	53208	10.57
	75+	18940	6.52	4832	1.67	1819	2.76	51075	34.38	57726	11.47
Gender	Female	154758	53.30	157602	54.57	33764	51.27	85312	57.42	276678	54.98
	Male	135617	46.70	131207	45.43	32091	48.73	63265	42.58	226563	45.02

9.6. Data management

The final database will consist of data extracted from the databases described in Section 9.4. Record linkage will be performed by the employees of the MCHP where these databases are housed. The analytic database will be accessed and analysed within the confines of the MCHP's secure computing environment.

9.7. Data analysis

9.7.1. Hypotheses

Null hypothesis (H0): the incidence of MS in the exposed cohort is equal to the incidence in the non-exposed cohort.

Alternative hypothesis (H1): the incidence of MS in the exposed cohort is not equal to the incidence in the non-exposed cohort.

The same hypotheses will be tested for the secondary endpoint: the demyelinating events.

9.7.2. Analysis Population

9.7.2.1. Population for the cohort design

The study population for the cohort design will comprise all enrolled exposed and unexposed subjects that satisfy the inclusion criteria.

9.7.3. Subject disposition

Subject disposition will be summarized by cohort and overall by computing:

- Number of screened subjects.
- Number (%) of non-eligible subjects based on inclusion/exclusion criteria.
- Number of eligible subjects in each cohort (before propensity score matching).
- Number of subjects enrolled in each cohort after propensity score matching.

9.7.4. Demographic and baseline characteristics

Demographic and baseline characteristics of all enrolled subjects (age at enrolment, other vaccination during the previous year, medical history, healthcare resource utilization during the previous year, etc.) will be summarized per cohort and overall, using descriptive statistics.

Duration of follow-up time will be analysed by descriptive statistics.

Frequency tables will be generated for categorical variables.

Mean, standard error, median, Q1, Q3, and range will be provided for continuous variables.

The two cohorts will be compared for their demographic and baseline characteristics using Fisher's exact test or Student t-test.

9.7.5. Analysis of primary endpoint

The primary analysis will compare the incidence rates of MS between the exposed cohort and the unexposed cohort. Results will be presented as the incidence rate ratio and the incidence difference. Exposed person-time will be defined as the period between the index date (see Section 9.2.2) and the earliest of the following events:

- End of study period (defined as 24 months after the index date);
- Death or loss to follow-up;
- Termination of insurance coverage;
- Receipt of H1N1 vaccine or TIV for the unexposed cohort;
- Diagnosis of the outcome of interest.

Incidence rates for MS will be calculated by dividing the number of cases by person-time. Stratified Cox regression models will be used to calculate hazard ratios as a measure of the association between a diagnosis of MS and the receipt of Arepanrix™.

The following subgroup analyses will be performed:

- Analysis for subjects younger/older than 18 years. Numbers permitting, separate analyses will be performed for the following three age groups: <25, 25-49 and ≥50.

- Analysis for subjects with a history of auto-immune disease other than MS. Definitions of auto-immune diseases will be described in the statistical analysis plan.

9.7.6. Analysis of secondary endpoints

The analysis of the incidence of MS until 31 December 2012 will use the same statistical model as the primary analysis.

Exposed person-time will be defined as the period between the index date (see Section 9.2.2) and the earliest of the following events:

- End of study period (31 December 2012);
- Death or loss to follow-up;
- Termination of insurance coverage;
- Diagnosis of the outcome of interest.

Incidence rates of MS will be calculated by dividing the number of cases by person-time. Stratified Cox regression models will be used to calculate hazard ratios as a measure of the association between a diagnosis of MS and the receipt of Arepanrix™. Exposure to 2010-2011 and 2011-2012 seasonal influenza vaccination will be included as a time-dependent covariate.

The secondary analysis will also compare the incidence rates of demyelinating events which do not ultimately lead to a diagnosis of MS (including optic neuritis, acute transverse myelitis, demyelinating disease of CNS unspecified, other acute disseminated demyelination, and neuromyelitis optica), between the exposed cohort and the unexposed cohort.

New cases of demyelinating events which do not ultimately lead to a diagnosis of MS during the study period will be analysed by descriptive statistics per cohort. Incidence rate during the study period will be computed per cohort for each individual disease as the total number of new cases divided by the total person-years, as for the primary endpoint.

Exploratory analyses will be conducted to assess the association between unadjuvanted pandemic influenza vaccine(s) and incidence of MS. These analyses will not be considered as confirmatory.

9.7.7. Statistical models

9.7.7.1. Propensity score model

Due to lack of random assignment of treatments, estimates of treatment effects in observational studies can be biased because the treatment group and the control group may not be comparable with respect the distribution of important disease (or outcome) predictors (confounders). Propensity score (PS) methods are one approach to constructing more comparable groups by limiting comparisons to individuals who have the same propensity to receive the treatment [Rubin, 1997]. PS is defined as the conditional

probability of receiving treatment given the value of a set of confounders, and can be estimated using logistic or probit regression models of the association between confounding covariates and the receipt of treatment [Rubin, 1997]. PS methods are especially suitable for post-marketing studies of drug and vaccine safety where the outcomes are typically rare, limiting the utility of conventional multivariate adjustment methods, but the treatment and confounders data is very rich.

Potential confounders—e.g., age, sex, area of residence, socio-economic status, comorbidity, immune status, vaccine indications (e.g., pregnancy, chronic cardiovascular, pulmonary or renal diseases, etc.), receipt of other vaccines, and frequency of contact with the health care system (as a proxy for health seeking behaviour)—will be included in a logistic regression model fitted to the entire study population, with H1N1 vaccination as the dependent variable. After assessing the model's goodness-of-fit (using the Hosmer-Lemeshow goodness-of-fit test) and discriminative ability (c-statistic, by measuring the area under the receiver–operating curve), estimates of the predicted probability of vaccination (PS) will be derived for each individual. A greedy matching algorithm, as implemented in a widely used Statistical Analysis System (SAS) macro [Parsons, 2001], will be then used to pair-match each vaccinated individual with a randomly selected unvaccinated individual with the closest propensity score. Because individuals within each matching pair have a similar probability of receiving the vaccine, relative risk estimates derived from the matched cohort analysis are less biased with respect to the measured confounders. We will exclude exposed subjects who cannot be accurately matched to unexposed subjects (no available unexposed matches with a PS within ± 0.05). We will explore matching with age group as well if PS does not result in fairly equal distribution by age group.

Although the aim of high-dimension PS matching is to control for potential confounders, there can still be an imbalance between the matched cohorts with regard to some variables after matching. Often, such imbalances are not relevant, for instance, if the variable is causally linked to the occurrence of MS, and therefore cannot confound the relationship between vaccination and MS. Occasionally, matched cohorts are imbalanced with respect to important variables, such as age and gender. Theoretically, such imbalance should not matter because each matched pair has the same propensity for receiving the vaccine (but still one member of the pair received the vaccine whereas the other did not). As such, matching on PS should eliminate the need for adjusting for any confounders in regression models, in the same way that one typically does not need to adjust for confounders in a randomized trial.

However, some epidemiologists are concerned that mismatch in very important predictors such as age might signal significant residual confounding. To address this concern, the quality of the matching will be assessed by:

(1) Examining the frequency distributions of the generated PS for each cohort and excluding individuals who are at the extremes of their corresponding PS distribution and are therefore unlikely have an appropriate match in the other distribution.

(2) Describing the demographic, clinical, etc., characteristics of individuals by vaccination status and category of propensity scores to assess whether any

important potential confounders (e.g., age) are still misbalanced between the exposed and unexposed cohorts. Any such variables will be included in as covariates into the Cox model.

9.7.7.2. Time-to-event model

Standard time-to-event (survival) analysis methods will be used for most analyses. Time-to-event (onset of MS) will be measured from the *index date* to the date of MS onset as defined by the first demyelinating disease code in hospital or physician claims. Individuals will be censored on the date of loss to follow-up (e.g., due to death or immigration) or on the study end date (2 years following the index date). In addition, individual observations will be censored on the date of any subsequent administration of a different because any MS cases identified afterwards might have been due to the more recently given vaccine. On the other hand, two vaccines given on the same day (typically, an H1N1 vaccine given concurrently with a TIV) will be considered as a single episode. However, in analyses stratified by vaccine type, these episodes will be grouped separately (labelled as the "concurrent H1N1/TIV" cohort), and the incidence of MS in this group will be compared to that among individuals who received an H1N1 vaccine only (the "H1N1 alone" cohort) or a TIV only (the "TIV alone" cohort).

Cumulative incidence curves of MS will be computed separately for each cohort (vaccinated and non-vaccinated) and sub-cohort ("concurrent H1N1/TIV", "H1N1 alone" and "TIV alone"). Numbers permitting, the "H1N1 alone" sub-cohort will be further divided into those who received the adjuvanted H1N1 vaccine and those who received the unadjuvanted H1N1 vaccine. Cox proportional hazard models, with stratification on the matched pairs, will be used to estimate relative risks (hazards ratios) associated with the receipt of the H1N1 vaccine [Cummings, 2003]. Cox models assume that the effect of covariates is constant over time (proportional hazards assumption). We will test this assumption using graphical and formal methods as proposed by Therneau & Grambsch [Therneau, 2000]. If the hazards function is non-proportional over time, interaction terms between time and the appropriate covariates will be included in the model. The possibility of effect modification with the receipt of the 2009/10 TIV will be assessed, testing for interactions between H1N1 and TIV terms using a likelihood ratio test with a relatively liberal cut-off point for statistical significance ($P < 0.15$).

9.7.8. Conduct of analysis

9.7.8.1. Sequence of analyses

All the analyses will be done on the final database.

9.7.8.2. Statistical considerations for interim analyses

There is no interim analysis.

9.7.8.3. Changes from planned analyses

Not applicable.

9.8. Quality control

Data management will be performed in accordance with applicable standards and data cleaning procedures. The final study dataset will be archived and stored on a secured, limited access, computer platform. The validation of the quality control of the statistical analysis will be documented. The final study protocol and possible amendments, the final statistical report and the final study report(s) will be archived by GSK on a Document management system based on the Documentum platform: Computer Aided Regulatory Submission (CARS).

9.9. Limitations of the research methods

Major strengths of this study are its population-based design and its relatively large sample size. Because of the availability of accurate automated records of hospitalization, physician utilization, vaccination and prescriptions, [Roberts, 1994] this study is less susceptible to recall bias and differential misclassification of exposures common to observational studies where information on important exposures is self-reported. The whole population of Manitoba is eligible and available for inclusion in the study, so selection bias is not a concern. Ascertainment of cases is virtually complete because all admitted influenza or pneumonia cases in the province are captured by the Hospital Abstract database [Skarsgard, 2000]. The availability of detailed histories of vaccination, through the unique Manitoba Immunization Registry eliminates recall bias, reduces vaccine use measurement errors (e.g., due to patient confusion about what vaccines were received) and permits detailed assessments of the effect of the timing of vaccination on the risk of hospitalization. These detailed analyses will be facilitated by the relatively large sample size (40% of Manitoba's 1.2 million population were vaccinated during the pandemic).

While use of administrative databases to measure exposures minimize the risk of recall bias (differential misclassification), it is still possible that other covariates could be measured with error due to under-reporting and classification and coding errors (e.g., using the wrong ICD code). The completeness and accuracy of the MH database are well established, [Roos, 1993; Young, 1997] and these databases have been used extensively in population-based studies of infectious diseases and vaccines [Mahmud, 2011; Mahmud, 2012; Roberts, 1996; Singh, 2009a; Singh, 2009b]. Generally, the above limitations result in non-differential misclassification because these errors are unlikely to be related to a diagnosis of MS. Typically; non-differential misclassification weakens or masks associations.

Both environmental (including smoking) and genetic (including ethnicity) risk factors, and interactions thereof, have been identified as contributing to the aetiology of MS [Ascherio, 2012; Kakalacheva, 2011]; a limitation of the present study is the inability to control for all risk factors and exposures due to the nature of the data source. Smoking information is not available in the Manitoba databases; however, the propensity score model will include smoking-related diseases such as asthma or COPD. With regards to ethnicity, very limited information is available in the Manitoba databases, as First nations / aboriginal costs of healthcare are covered by the federal government (vs. the provincial system) and access to this information is limited and highly regulated. In Manitoba, there

are geographical clusters of First nations; therefore, cohorts will be matched by area of residence (postal code) as a proxy for ethnicity. Smoking-related diseases and postal code will be added to the risk factors included in the Cox model.

Finally, another potential limitation is related to the validity and timing (potential long lag time between first symptoms and actual diagnosis) of the MS diagnosis. However, prior work in Manitoba and Nova Scotia with the proposed case definition (described above) suggests high predictive values.

10. PROTECTION OF HUMAN SUBJECTS

10.1. Regulatory and ethical considerations, including the informed consent process

The study will be conducted in accordance with all applicable regulatory requirements.

Access to data is subject to approval by [redacted] and by the [redacted]

No patient informed consent will be obtained. The patient information in the database utilized is fully coded and neither the research team nor GSK Biologicals personnel will be able to make a link between the data and specific individuals. None of the subjects will be contacted.

10.2. Data privacy

Data extraction from the various databases will be performed by employees of the Manitoba Centre for Health Policy after obtaining the approvals of the [redacted] and [redacted] who will prepare a de-identified analytic dataset. Thus, the data will not be identifiable by neither the research team nor GSK Biologicals personnel as the key-codes are not available online and never shared with external parties. Final analytic datasets stripped of all identifiers will be accessed and analysed within the confines of the MCHP's secure computing environment.

11. MANAGEMENT AND REPORTING OF ADVERSE EVENTS/ADVERSE REACTIONS

According to the 22 June 2012 EMA/873138/2011 Guideline on Good Pharmacovigilance Practices (GVP), the sponsors of non-interventional studies based on secondary data sources are not required to report adverse events or adverse reactions (VI.C.1.2.1). EPI FLU H1N1-014 is an observational, retrospective, post-authorization safety study, based on data extracted from the MIMS, and the hospital, physician and prescription claims databases of the MH Database system. Individual medical records will not be directly examined, and subject reports linked between databases will be de-identified prior to analysis. Therefore, individual case adverse event/adverse reaction reports will not be generated from this study.

Healthcare providers who treat patients represented in the claims databases are encouraged by Health Canada to submit case reports of adverse events following vaccination. Spontaneous reports of serious adverse events (SAEs) received by GSK are processed according to standard pharmacovigilance procedures, which include reporting of adverse events, adverse reactions, and SAEs, to regulatory authorities.

12. PLANS FOR DISSEMINATING AND COMMUNICATING STUDY RESULTS

To comply with Guidelines for Good Pharmacoepidemiology Practices (GPP) or other applicable guidelines administrative obligations relating to data collection, archiving data, audits, confidentiality and publications must be fulfilled.

12.1. Posting of information on public registers

Study information from this protocol will be posted on public registers (e.g., GSK Clinical Study Register, clinicaltrials.gov) and the European Union Post-Authorisation Studies (EU PAS) register before the start of analysis, as applicable.

12.2. Ownership and publication

12.2.1. Ownership

The source data are the property of MH. The PI will apply for authorisation to use this data for the purpose of the study. All data generated as a result of the analysis are property of the PI.

12.2.2. Posting to the clinical trials registers and publication

The results summary will be posted to the GSK Clinical Study Register and other public registers as applicable, in accordance with regulatory and policy mandated timelines. In addition, a manuscript will be submitted to a peer reviewed journal for publication within the policy defined timelines. In addition, study information will be posted to the GSK Clinical Study Register.

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ANNEX 1. List of stand-alone documents

No.	Document Reference No	Date	Title
1.	200405 (EPI-FLU H1N1-014 VS)	05-MAY-2014	List of stand-alone documents
2.	200405 (EPI-FLU H1N1-014 VS)	05-MAY-2014	ENCePP Checklist for study

ANNEX 2. ENCePP Checklist for study protocols

Section 1: Milestones	**Yes**	**No**	**N/A**	**Page Number(s)**
1.1 Does the protocol specify timelines for				
1.1.1 Start of data collection[1]	☒	☐	☐	17
1.1.2 End of data collection[2]	☒	☐	☐	17
1.1.3 Study progress report(s)	☐	☐	☒	
1.1.4 Interim progress report(s)	☐	☐	☒	29
1.1.5 Registration in the EU PAS register	☒	☐	☐	8, 32
1.1.6 Final report of study results.	☒	☐	☐	32

Comments:

For 1.1.1 (and 1.1.2) – Pending approval of the protocol by the EMA and MH.

For 1.1.3 and 1.1.4 – no progress reports are planned for this study.

For 1.1.5 – the EU PAS register number will be generated at the time of the final version of the protocol.

[1] Date from which information on the first study is first recorded in the study dataset or, in the case of secondary use of data, the date from which data extraction starts.

[2] Date from which the analytical dataset is completely available.

Section 2: Research question	**Yes**	**No**	**N/A**	**Page Number(s)**
2.1 Does the formulation of the research question and objectives clearly explain:				
2.1.1 Why the study is conducted? (e.g. to address an important public health concern, a risk identified in the risk management plan, an emerging safety issue)	☒	☐	☐	12-15
2.1.2 The objective(s) of the study?	☒	☐	☐	15
2.1.3 The target population? (i.e. population or subgroup to whom the study results are intended to be generalised)	☒	☐	☐	16-17
2.1.4 Which formal hypothesis(-es) is (are) to be tested?	☒	☐	☐	25
2.1.5 If applicable, that there is no a priori hypothesis?	☐	☐	☒	

Comments:

Section 3: Study design	**Yes**	**No**	**N/A**	**Page Number(s)**
3.1 Is the study design described? (e.g. cohort, case-control, randomised controlled trial, new or alternative design)	☒	☐	☐	15-16
3.2 Does the protocol specify the primary and secondary (if applicable) endpoint(s) to be investigated?	☒	☐	☐	17-18
3.3 Does the protocol describe the measure(s) of effect? (e.g. relative risk, odds ratio, deaths per 1000 person-years, absolute risk, excess risk, incidence rate ratio, hazard ratio, number needed to harm (NNH) per year)	☒	☐	☐	27-29

Comments:

Section 4: Source and study populations	**Yes**	**No**	**N/A**	**Page Number(s)**
4.1 Is the source population described?	☒	☐	☐	16-17
4.2 Is the planned study population defined in terms of:				
4.2.1 Study time period?	☒	☐	☐	16-17
4.2.2 Age and sex?	☒	☐	☐	16-17
4.2.3 Country of origin?	☒	☐	☐	17
4.2.4 Disease/indication?	☒	☐	☐	17-21
4.2.5 Co-morbidity?	☒	☐	☐	18-19
4.2.6 Seasonality?	☐	☐	☒	
4.3 Does the protocol define how the study population will be sampled from the source population? (e.g. event or inclusion/exclusion criteria)	☒	☐	☐	15-23

Comments:

Section 5: Exposure definition and measurement	**Yes**	**No**	**N/A**	**Page Number(s)**
5.1 Does the protocol describe how exposure is defined and measured? (e.g. operational details for defining and categorising exposure)	☒	☐	☐	15-17
5.2 Does the protocol discuss the validity of exposure measurement? (e.g. precision, accuracy, prospective ascertainment, exposure information recorded before the outcome occurred, use of validation sub-study)	☒	☐	☐	22-24
5.3 Is exposure classified according to time windows? (e.g. current user, former user, non-use)	☒	☐	☐	15-17

Section 5: Exposure definition and measurement	**Yes**	**No**	**N/A**	**Page Number(s)**
5.4 Is exposure classified based on biological mechanism of action and taking into account the pharmacokinetics and pharmacodynamics of the drug?	☐	☐	☒	
5.5 Does the protocol specify whether a dose-dependent or duration-dependent response is measured?	☐	☐	☒	

Comments:

Section 6: Endpoint definition and measurement	**Yes**	**No**	**N/A**	**Page Number(s)**
6.1 Does the protocol describe how the endpoints are defined and measured?	☒	☐	☐	26-27
6.2 Does the protocol discuss the validity of endpoint measurement? (e.g. precision, accuracy, sensitivity, specificity, positive predictive value, prospective or retrospective ascertainment, use of validation sub-study)	☒	☐	☐	27-29

Comments:

Section 7: Confounders and effect modifiers	**Yes**	**No**	**N/A**	**Page Number(s)**
7.1 Does the protocol address known confounders? (e.g. collection of data on known confounders, methods of controlling for known confounders)	☒	☐	☐	15-16, 27-28
7.2 Does the protocol address known effect modifiers? (e.g. collection of data on known effect modifiers, anticipated direction of effect)	☒	☐	☐	29

Comments:

Section 8: Data sources	**Yes**	**No**	**N/A**	**Page Number(s)**
8.1 Does the protocol describe the data source(s) used in the study for the ascertainment of:				
8.1.1 Exposure? (e.g. pharmacy dispensing, general practice prescribing, claims data, self-report, face-to-face interview, etc.)	☒	☐	☐	21-22
8.1.2 Endpoints? (e.g. clinical records, laboratory markers or values, claims data, self-report, patient interview including scales and questionnaires, vital statistics, etc.)	☒	☐	☐	19-21
8.1.3 Covariates?	☒	☐	☐	27-29
8.2 Does the protocol describe the information available from the data source(s) on:				
8.2.1 Exposure? (e.g. date of dispensing, drug quantity, dose, number of days of supply prescription, daily dosage, prescriber)	☒	☐	☐	16-17
8.2.2 Endpoints? (e.g. date of occurrence, multiple event, severity measures related to event)	☒	☐	☐	16-18
8.2.3 Covariates? (e.g. age, sex, clinical and drug use history, co-morbidity, co-medications, life style, etc.)	☒	☐	☐	19-22
8.3 Is a coding system described for:				
8.3.1 Diseases? (e.g. International Classification of Diseases (ICD)-10)	☒	☐	☐	16-21
8.3.2 Endpoints? (e.g. Medical Dictionary for Regulatory Activities (MedDRA) for adverse events)	☒	☐	☐	16-21
8.3.3 Exposure? (e.g., WHO Drug Dictionary, Anatomical Therapeutic Chemical (ATC)Classification System)	☒	☐	☐	16-17

Section 8: Data sources	**Yes**	**No**	**N/A**	**Page Number(s)**
8.4 Is the linkage method between data sources described? (e.g. based on a unique identifier or other)	☒	☐	☐	18-19, 21-22

Comments:

Section 9: Study size and power	**Yes**	**No**	**N/A**	**Page Number(s)**
9.1 Is sample size and/or statistical power calculated?	☒	☐	☐	22-24

Comments:

Section 10: Analysis plan	**Yes**	**No**	**N/A**	**Page Number(s)**
10.1 Does the plan include measurement of excess risks?	☐	☐	☒	
10.2 Is the choice of statistical techniques described?	☒	☐	☐	27-29
10.3 Are descriptive analyses included?	☒	☐	☐	27
10.4 Are stratified analyses included?	☒	☐	☐	16-17 & 26-29
10.5 Does the plan describe methods for adjusting for confounding?	☒	☐	☐	15-16, 27-28
10.6 Does the plan describe methods addressing effect modification?	☒	☐	☐	29

Comments:

Section 11: Data management and quality control	**Yes**	**No**	**N/A**	**Page Number(s)**
11.1 Is information provided on the management of missing data?	☐	☒	☐	
11.2 Does the protocol provide information on data storage? (e.g. software and IT environment, database maintenance and anti-fraud protection, archiving)	☒	☐	☐	29-30
11.3 Are methods of quality assurance described?	☒	☐	☐	29-30
11.4 Does the protocol describe possible quality issues related to the data source(s)?	☒	☐	☐	22, 30
11.5 Is there a system in place for independent review of study results?	☐	☒	☐	

Comments:

Section 12: Limitations	**Yes**	**No**	**N/A**	**Page Number(s)**
12.1 Does the protocol discuss:				
12.1.1 Selection biases?	☒	☐	☐	27-30
12.1.2 Information biases?	☒	☐	☐	27-30
(e.g. anticipated direction and magnitude of such biases, validation sub-study, use of validation and external data, analytical methods)				
12.2 Does the protocol discuss study feasibility? (e.g. sample size, anticipated exposure, duration of follow-up in a cohort study, patient recruitment)	☒	☐	☐	18-25
12.3 Does the protocol address other limitations?	☒	☐	☐	30-31

Comments:

Section 13: Ethical issues	**Yes**	**No**	**N/A**	**Page Number(s)**
13.1 Have requirements of Ethics Committee/Institutional Review Board approval been described?	☒	☐	☐	31
13.2 Has any outcome of an ethical review procedure been addressed?	☐	☒	☐	
13.3 Have data protection requirements been described?	☒	☐	☐	31

Comments:

Section 14: Amendments and deviations	**Yes**	**No**	**N/A**	**Page Number(s)**
14.1 Does the protocol include a section to document future amendments and deviations?	☒	☐	☐	11

Comments:

For 14.1 – none are planned

Section 15: Plans for communication of study results	**Yes**	**No**	**N/A**	**Page Number(s)**
15.1 Are plans described for communicating study results (e.g. to regulatory authorities)?	☒	☐	☐	32
15.2 Are plans described for disseminating study results externally, including publication?	☒	☐	☐	32

Comments:

Name of the main author of the protocol: Dr. [redacted]

Date: 05/MAY/2014

Signature: _____

ANNEX 3. Protocol Investigator Signatory Approval

Protocol Investigator Signatory Approval

eTrack study number and Abbreviated Title	200405 (EPI-FLU H1N1-014 VS)
Date of last version of the protocol	EMA PASS Final Version 2: 05 May 2014
EU PAS Register No.:	NA (Not applicable)
Product reference:	EU/1/10/624/001
Procedure number:	EMEA/H/C/001201
Detailed Title	An observational retrospective database analysis to estimate the risk of multiple sclerosis following vaccination with Arepanrix™ in Manitoba, Canada
Investigator name	Dr. [redacted]
Signature	_____
Date	_____

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