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Narcolepsy associated with Pandemrix influenza vaccine: A systematic review and meta-analysis

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DISSERTATION in PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE MASTERS OF PUBLIC HEALTH

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DECLARATION

I, Leif Lakoma, hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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ABSTRACT

This systematic review and meta-analysis forms the mini-dissertation part of the primarily coursework degree of Master of Public Health at the University of Cape Town. The review is divided into three parts. The first comprises the research protocol which outlines the proposed methods and scope of the systematic review. The second component is a structured literature review elaborating further on the research topic and state of existing knowledge thereof. The final and third part presents the submission-ready manuscript.

The aim of this review was to systematically explore the scientific literature regarding the association between narcolepsy and the Pandemrix pandemic influenza vaccine. This particular association has been one of the most-studied presumed vaccine adverse effects of the last decade and has greatly affected the discussion on vaccine safety and vaccine hesitancy to date. Despite several studies having been published investigating the association, no systematic review of these studies had been performed prior to embarking on this review. Thus this review also serves to update the evidence with additional studies published after the initial review.

Part A - Protocol

1. Background

Narcolepsy is a chronic neurological disease characterised by unusual daytime sleepiness and sudden abrupt episodes of falling asleep while carrying out activities of daily living. Around 70 per cent of individuals with narcolepsy also exhibit cataplexy, a sudden loss in muscle tone than can occur at any time during the day. [1] Symptoms commonly first appear in childhood and adolescence. The exact cause for narcolepsy remains unknown, but it is thought to be of autoimmune origin. Most individuals with narcolepsy have insufficient levels of hypocretin, a neurotransmitter normally promoting wakefulness. In those exhibiting cataplexy this has also been shown to coincide with the loss of brain cells producing hypocretin. [2] Most cases of narcolepsy are sporadic and the affected individuals have no prior family history of the illness. History and clinical examination are required to exclude other diagnoses. A polysomnogram is often required to reach a definitive diagnosis. Cataplexy, when occurring, if often a key symptom not commonly seen in other conditions. [1]

Studies conducted in Finland and the Sweden found an increased risk of narcolepsy associated with the Pandemrix vaccine [3,4]. Produced by GlaxoSmithKline in response to the pandemic H1N1 influenza in 2009, Pandemrix was one of eight vaccines licenced within the EU/EEA for pandemic influenza [5]. Of these, three were authorised through the central procedure in the EU under the European Medicines Agency (EMA) and the European Commission: Pandemrix, Focetria and Celvapan while the remaining five were authorised for national use in individual countries. [6] No prior reports of such association had been made before the pandemic H1N1 influenza in 2009 [3].

Pandemrix was the most commonly used vaccine in Europe. The European Medicines Agency (EMA) has estimated that in August 2010 at least 38.6 million people in the EU/EEA had been vaccinated with 30.5 million of them having been administered Pandemrix, more than 6.5 million Focectria and more than 560 000 Celvapan. The highest rates of immunizing children and adolescents with Pandemrix were in Finland, Sweden, Norway and Ireland. [7]

In 2010, some months after the vaccinations had started, an increasing amount of

cases of narcolepsy was observed in both Finland and Sweden, countries where Pandemrix was the sole product used for inoculation against the pandemic influenza. An existing association between Pandemrix and narcolepsy was later confirmed in both countries [3,4]. In Finland, a 12.7-fold increased risk in children and adolescents aged between 4 and 19 years was observed within 8 months after vaccination when compared to people in the same age group that were not vaccinated [3]. By July 2014 a total of 1333 separate cases of narcolepsy associated with the pandemic influenza vaccines had been reported to the EMA EudraVigilance database. These cases originated from 18 different countries with 61.9 per cent coming from Finland and Sweden. Pandemrix was associated with 95.3 per cent of the cases, Arepanrix with 1.6 per cent, Focetria with 0.5 per cent and Celvapan with 0.1 per cent. The EudraVigilance database does not, however, collect enough information to confirm diagnosis as per the Brighton Collaboration case definition used commonly with narcolepsy. [8]

Of the three vaccines authorised through the central procedure only Pandemrix and Focetria contained an adjuvant. The main purpose of adjuvants is to increase and prolong the effect of a vaccine, and most importantly to reduce the overall amount of antigen needed. Only one of the adjuvants, the AS03 included in Pandemrix, had never been used before. [9] In the United States, no adjuvant-containing influenza vaccines of any kind were used and accordingly no increased risk of narcolepsy was observed [10]. In China, on the other hand, use of any pandemic influenza vaccine was minimal and there seemed to nonetheless be an increase in the incidence of narcolepsy related to the pandemic itself [11].

In this review we seek to examine narcolepsy as an adverse event following vaccination with Pandemrix. No systematic review of the literature regarding this particular association between Pandemrix and narcolepsy has so far been done. With the rising number of studies being conducted reporting conflicting results, such a systematic review would be useful. The results of the review can be important both for assessing vaccine safety and preventing their use from being hindered by unnecessary fears. The review will provide further use in critically appraising the available evidence which is currently solely based on observational studies

inherently subject to various bias and confounding [12].

2. Objectives

To identify, retrieve and assess all existing studies evaluating the association between the Pandemrix pandemic influenza vaccine and narcolepsy.

3. Methods

3.1 Criteria for considering studies for this review

3.1.1 Types of studies

Randomised controlled trials, cohort studies, case-control studies and cross-sectional studies will be considered.

3.1.2 Types of participants

Any population will be considered. Although the association has been mostly observed in children and adolescents, no age restriction is set.

3.1.3 Types of interventions

Pandemrix pandemic influenza vaccine.

3.1.4 Comparison

For comparison we will use any other pandemic influenza vaccine or the prior prevalence of narcolepsy in the given population.

3.1.5 Types of outcome measures

3.1.5.1 Primary outcomes

The primary outcome of interest is narcolepsy as per the case definition of either the Brighton Collaboration [13] or the International Classification of Sleep Disorders (ICSD-2) [14].

3.1.5.2 Secondary outcomes

There are no secondary outcomes to be evaluated.

3.2 Search methods for identification of studies

3.2.1 Electronic searches

A comprehensive search will be undertaken to identify both published and unpublished articles. There is no language restriction. The search strategy for electronic databases will incorporate both medical subject headings (MeSH) and free-text terms, and will be adapted to suit each particular database using applicable controlled vocabulary (Appendix 1).

Electronic searches will be conducted in PubMed, Scopus and the Cochrane Library. The following trials registers will also be searched: ClinicalTrials.gov, EU Clinical Trials Register, Pan African Clinical Trial Registry and the Cochrane Central Register of Controlled Trials (CENTRAL). Where possible, key authors will be contacted for unpublished studies and corresponding data.

Articles must be published after September 27th 2006, the date of registration of the patent for Pandemrix.

3.2.2 Searching other resources

The reference lists of all the final included studies will be viewed to detect any additional studies.

3.3 Data collection and analysis

3.3.1 Selection of studies

Two reviewers will independently apply the inclusion criteria on all identified articles. To initially identify any relevant articles the same two reviewers will screen the titles and abstracts of any studies found using the search terms. A third independent reviewer will be used to arbitrate in case a disagreement occurs. A table for included studies will be provided in the final review with reasons for exclusion of studies.

3.3.2 Data extraction and management

Data will be extracted and managed using Review Manager 5.3 or similar software. Prior to use the extraction form will be piloted on at least five studies identified randomly from the list of included studies.

Data will be extracted on the following:

- Study design
- Publication status
- Date of study
- Location of study
- Characteristics of participants
- Methodological quality of study

3.3.3 Assessment of risk of bias in included studies

After relevant data have been extracted from the included studies, risk of bias will be assessed by using an appropriate tool specific for the study design. The Newcastle-Ottawa scale will be used for cohort and case-control studies. The tool by Hoy et al. as modified by Werfalli [15] will be used for prevalence studies. Finally, the Cochrane Collaboration's tool [16] will be applied to any randomised controlled trials.

3.3.4 Dealing with missing data

Where necessary, authors of included papers will be contacted for possible missing and/or corresponding data.

3.3.5 Assessment of reporting biases

The review will incorporate published data, and thus, is subject to publication bias. We will use a funnel plot to investigate the risk of publication bias by intervention type, provided that ten or more studies are included in the analysis for each intervention type. We will critically examine the funnel plot for asymmetry both visually and with the use of formal tests. For the dichotomous outcome (narcolepsy), we will use the test by Harbord et al. [17]. In situations where asymmetry is detected by either test or by visual assessment, we will perform further exploratory analyses to investigate reasons for the phenomenon. This will include reviewing the included studies for small sample size studies and their intervention effect.

3.3.6 Data synthesis

The findings in each included study will first be described in order to examine any common patterns. We will also note the frequency of the adverse events and any possible causal relationships between them and the participants' characteristics according to the primary study report. We will express the results of each study as a risk ratio with a corresponding 95 per cent confidence interval for dichotomous data,

or as a mean difference and its standard deviation for continuous data. We will group studies that compare broadly similar types of outcome to get feasible results on an overall estimate of effect. Random-effects meta-analysis will be preferred due to anticipated heterogeneity in study results. The proportion of narcolepsy across different studies will be pooled in a meta-analysis using Stata 13 statistical software. In case we encounter variation in reported outcome measures between the studies we will not pool the results, but rather summarize the findings in a narrative format.

Heterogeneity between the studies will be explored using the I^2 statistic and its confidence intervals. If the level of heterogeneity appears substantial, we will explore reasons for this through sub-group analyses. Reasons for outliers will be explored by rechecking the accuracy of data extraction and studying the particular included study characteristics in greater detail.

3.3.7 Subgroup analysis

Subgroup analyses may be conducted, if possible, taking into account, but not limited to, age, gender, socioeconomic characteristics, definition of narcolepsy, setting of the studies and dosage of the vaccine.

3.3.8 Sensitivity analysis

Sensitivity analyses will be conducted to explore the source of heterogeneity, i.e. to determine the impact of specific studies on a pooled estimate by exclusion of studies with low quality scores and thus higher risk of bias.

4. Declarations of interest

No conflict of interest.

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6. Appendices

Appendix 1: Search Terms

The following search strategy will be used for the all the databases:

#1 Narcolepsy [MeSH]

#2 Vaccines [MeSH]

#3 Vaccination [MeSH]

#4 Narcolepsy [Text Word]

#5 Vaccine [Text Word]

#6 Vaccines [Text Word]

#7 Vaccination [Text Word]

#8 (#1 OR #4) AND (#2 OR #3 OR #5 OR #6 OR #7)

Appendix 2: Data Extraction Form

Data Extraction Form (as per Cochrane Public Health Group template)

Study ID:	Data extractor:	Date form completed:
Citation:		

1. General Information

Publication type	Journal Article ☐ Abstract ☐ Other (specify) _____
Country of study:	
Funding source of study:	Potential conflict of interest from funding? Y / N / unclear

2. Study Eligibility

Study Characteristics			**Page/ Para/ Figure #**
Type of study	☐ Randomised Controlled Trial ☐ Cohort Study ☐ Case-Control Study	☐ Prevalence Study ☐ Case Report ☐ Other	
	Does the study design meet the criteria for inclusion?	Yes ☐ No ☐ →**Exclude** Unclear ☐	
Participants	Describe the participants included:		
	Do the participants meet the criteria for inclusion?	Yes ☐ No ☐ →**Exclude** Unclear ☐	

Types of intervention	Does the study include Pandemrix as an intervention?	Yes ☐ No ☐	
	Does the intervention meet the criteria for inclusion?	Yes ☐ No ☐ →**Exclude** Unclear ☐	
Types of outcome measures	Is narcolepsy an outcome measure?	Yes ☐ No ☐	
	If so, what case definition is used?	☐ Brighton Collaboration ☐ ICSD-2 ☐ Other (specify) _____	
	Do the outcome measures meet the criteria for inclusion?	Yes ☐ No ☐ →**Exclude** Unclear ☐	

Summary of Assessment for Inclusion

Include in review ☐	**Exclude from review** ☐
Independently assessed, and then compared? Yes ☐ No ☐	Differences resolved Yes ☐ No ☐
Request further details? Yes ☐ No ☐	Contact details of authors:
Notes:	

DO NOT PROCEED IF PAPER EXCLUDED FROM REVIEW

3. Study details

Study intention	Descriptions as stated in the report/paper	Page/ Para/ Figure #
Aim of study	*What was the study designed to assess? Are these clearly stated?*	

Participants *Include if relevant*	Include information for each group (i.e. intervention and controls) under study	Page/ Para/ Figure #
• Age group		
• Location		
• Other characteristics		
• Follow-up period		

435 **4. Outcomes**

Question	Outcome 1	Page/ Para/ Figure #	Outcome 2	Page/ Para/ Figure #
Outcome definition				
Unit of measurement				
State the outcome				
Is it a reliable outcome measure?				
Is there adequate power for this outcome?				

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438 **5. Other relevant information**

Key conclusions of the study authors		
References to other relevant studies		
Additional notes by review authors		

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Part B – Literature review

1. Background

Vaccinations are important in protecting us from many otherwise deadly infections, some of which have already perhaps become forgotten. Fear of vaccine adverse events can reduce vaccine uptake below the critical level needed to protect a population. Any reported adverse event needs to thus be carefully examined to maintain trust of the general populace and to safeguard the efficacy of vaccines in the future. In this review we examine narcolepsy as an adverse event related to vaccination. No prior reports of such association had been made before the pandemic H1N1 influenza in 2009 [1].

No systematic review of the literature regarding this particular association between Pandemrix and narcolepsy had so far been done prior to the start of this review, although one was subsequently published during the current review [2]. With the rising number of studies being conducted, at times with conflicting results, systematic reviews and meta-analyses are important in summarizing all the available information. The results of the review can be important both for assessing vaccine safety and preventing their use from being hindered by unnecessary fears.

For this review electronic searches on the following databases were performed to find relevant articles: PubMed, Scopus, Cochrane Library and Google Scholar. The keywords used were narcolepsy and vaccination. The search was not limited to articles published in scientific journals, and scientific papers released by agencies such as the European Medicines Agency (EMA) were also considered. There was no restriction placed on study design, but only literature in English language was included.

2. Narcolepsy as a disease

Narcolepsy is a chronic neurological disease characterised by unusual daytime sleepiness and sudden abrupt episodes of falling asleep while carrying out activities of daily living. Around 70 per cent of individuals with narcolepsy also exhibit cataplexy, a sudden loss in muscle tone than can occur at any time during the day. [3] Symptoms commonly first appear in childhood and adolescence. The exact cause

for narcolepsy remains unknown, but it is thought to have an autoimmune basis. Most individuals with narcolepsy have insufficient levels of hypocretin, a neurotransmitter normally promoting wakefulness. In those exhibiting cataplexy this has also been shown to coincide with the loss of brain cells producing hypocretin. [4] Most cases of narcolepsy are sporadic and the affected individuals have no prior family history of the illness. History and clinical examination is required to exclude other diagnoses. A polysomnogram is often required to reach a definitive diagnosis. Cataplexy, when present, is often a key symptom not commonly seen in other conditions. [3]

3. Epidemiology of narcolepsy

Knowledge of the incidence of narcolepsy has been limited but one study estimated it to be 0.74 per 100 000 person-years for narcolepsy with cataplexy in the US [5]. A similar figure of 0.7 per 100 000 person-years was also estimated based on register data in Finland [1]. This would put it on par with multiple sclerosis, another disease of the central nervous system with an estimated incidence of similar magnitude [6]. Narcolepsy is mostly diagnosed in children and adolescents with a peak incidence around 15 years of age [7]. It is thought to affect both females and males in equal numbers, although a slight predominance in men has been noted in some studies. [8]

4. Diagnosis of narcolepsy

Narcolepsy is a disease typical suspected on a clinical basis. Two tests are then performed to confirm this diagnosis, namely the polysomnogram (PSG) and the multiple sleep latency test (MSLT). The latter is usually performed the following day after the night-time PSG has first been successfully completed. The MSLT involves the patient being encouraged to fall asleep every 2 hours throughout the day with patients affected by narcolepsy commonly falling asleep repeatedly in less than 8 minutes. A positive MSLT also includes the patient experiencing rapid eye movement (REM) sleep on at least two occasions during these episodes. [9]

Especially the MSLT part of the test relies heavily on being performed under correct conditions to provide accurate diagnosis [10].

As there are multiple other conditions producing possibly similar symptoms as with narcolepsy, the use of a proper case definition is highly important. The most used case definition in clinical settings has been the International Classification of Sleep Disorders [11] and especially its more easily accessible second iteration (ICSD-2). After the link between narcolepsy and the pandemic H1N1 influenza vaccine was established another standardised case definition was created by the Brighton Collaboration [12]. The latter has since been the case definition of choice for studies examining the association between narcolepsy and vaccination, and is to some extent an extension of the ICSD case definition. Both of the case definitions also allow diagnosis based on low levels of the neurotransmitter hypocretin in the cerebrospinal fluid (CSF) in addition to typical clinical symptoms when PSG or MSLT is not available. [11][12]

5. Cause of narcolepsy

The human immune system has for long been thought to play a role in the pathogenesis of narcolepsy [13]. The discovery of the neurotransmitter hypocretin (also called orexin) has so far been one of the most important steps in unravelling the origins of the disease [14][15]. Lack of hypocretin has been shown to result in narcolepsy in both animals [16] and humans [17]. The observed loss of hypocretin-producing nerve cells in the hypothalamus resulting in low levels of the neurotransmitter in the cerebrospinal fluid (CSF) eventually gave rise to the hypothesis that narcolepsy is a disease of autoimmune origin [18]. The autoimmune origins are further supported by the fact that narcolepsy has one of the strongest known associations with the human leukocyte antigen (HLA) complex with nearly all patients carrying the HLA DQB1*06:02 gene variant [19].

In addition to the suspected autoimmune origin of the disease, onset of narcolepsy has been for long theorized to be triggered by environmental factors such as prior exposure to respiratory tract infections. Susceptibility to narcolepsy has been shown

to increase during upper airway infections cause by the bacterium *Streptococcus pyogenes*, with high levels of antibodies against the bacterium detected close to onset of disease [20]. Most importantly, the onset of new narcolepsy cases in children has been shown to be strongly linked to seasonal patterns of upper airway infections in China with a surge of new cases during the H1N1 pandemic of 2009. Of the new cases diagnosed following the pandemic almost none had received any kind of pandemic H1N1 vaccination. [21]

The exact mechanism for the loss of hypocretin-producing nerve cells and the pathogenesis of narcolepsy still remains to be uncovered. It has also been theorized that this mechanism might be difficult to unravel due to lack of autoantibodies and epitope spreading in the disease [22].

6. Vaccination during the 2009 influenza pandemic

Influenza poses a significant global health issue and is also major economic burden to countries afflicted by it. The virus itself changes on a constant basis and new vaccines are commonly rolled out twice a year. The vaccines are generally considered to give a moderate to high protection against the disease, and as such can provide considerable benefit to societies distributing them. [23]

Occasionally a new influenza strain can lead to a pandemic and the role of vaccination becomes ever more important. The pandemic H1N1 influenza in 2009 led to mass vaccination campaigns in various countries. By late 2009, eight different vaccines for pandemic influenza had been licenced within the EU/EEA: Cantgrip by Cantacuzino, Celltura by Novartis, Celvapan by Baxter, Fluval P by Omnivest, Focetria by Novartis, Pandemrix by GlaxoSmithKline, Panenza by Sanofi Pasteur and PanvaxH1N1 by CSL [24]. Of the eight licenced vaccines three were authorised through the central procedure in the EU under the European Medicines Agency (EMA) and the European Commission: Pandemrix, Focetria and Celvapan. The other five vaccines were authorised for national use in individual countries. [25]

Pandemrix was the most used vaccine in Europe. The European Medicines Agency (EMA) has estimated that as of August 2010 at least 38.6 million people in the

EU/EEA had been vaccinated with 30.5 million of them having been administered Pandemrix, more than 6.5 million Focectria and more than 560 000 Celvapan. The highest rates of immunizing children and adolescents with Pandemrix were in Finland, Sweden, Norway and Ireland. [26]

Two of the three vaccines authorised through the central procedure, Pandemrix and Focetria, contained an adjuvant. The main purpose of adjuvants is to increase and prolong the effect of a vaccine, and most importantly to reduce the overall amount of antigen needed. One of the adjuvants, the AS03 included in Pandemrix, had never been used before. [27]

Arepanrix, a vaccine similar to Pandemrix and also manufactured by GlaxoSmithKline was authorised for use in Canada. It contained the same AS03 adjuvant and had a similar yet not identical manufacturing process. [26] A total of 12 million people were vaccinated with Arepanrix in Canada. [25]

7. Link between Pandemrix and narcolepsy

In 2010, some months after the vaccinations had started, an increasing amount of cases of narcolepsy was observed both in Finland and Sweden, both countries where Pandemrix was the sole product used for inoculation against the disease. The two were seemingly linked together, and distribution of the vaccine ceased soon after. An existing association between Pandemrix and narcolepsy was later confirmed in both countries [1][28]. In Finland, a 12.7-fold increased risk in children and adolescents aged between 4 and 19 years was observed within 8 months after vaccination when compared to people in the same age group that were not vaccinated [1].

By July 2014 a total of 1333 separate cases of narcolepsy associated with the pandemic influenza vaccines had been reported to the EMA EudraVigilance database. These cases originated from 18 different countries with 61.9 per cent coming from Finland and Sweden. Pandemrix was associated with 95.3 per cent of the cases, Arepanrix with 1.6 per cent, Focetria with 0.5 per cent and Celvapan with 0.1 per cent. The EudraVigilance database does not however collect enough

information to confirm diagnosis as per the Brighton Collaboration case definition used commonly with narcolepsy. [29]

Since then there have been several studies from other countries citing similar results. Studies conducted in France and the United Kingdom found also an increased risk of narcolepsy associated with the Pandemrix vaccine [30][31]. Paired with this, no apparent observed increased risk of narcolepsy seemed to be associated with Focetria which was noted to contain the adjuvant MF59, a similar oil-in-water based emulsion adjuvant than what AS03 is [32].

8. The missing causality

The most likely culprit for the surge of new narcolepsy cases associated with Pandemrix was thought to be the novel adjuvant AS03 included in the vaccine. This was fitting with the fact that no increased risk of narcolepsy was observed with Focetria containing a different adjuvant [32]. In the United States, only non-adjuvanted influenza vaccines were used and accordingly no increased risk of narcolepsy was observed [33].

In China, on the other hand, use of any pandemic influenza vaccine was minimal and there seemed to nonetheless be an increase in the incidence of narcolepsy related to the pandemic itself [21]. Another factor contradicting with the premise of an adjuvant causing the onset of narcolepsy is that no risk of similar magnitude was found to be associated with Arepanrix, the other pandemic influenza vaccine produced by GlaxoSmithKline (GSK) and containing the same adjuvant AS03 [34]. It had a similar yet not identical manufacturing process, and was produced by GSK in Canada whereas Pandemrix was produced by in Germany [26].

It was later proposed that the reason for the increased incidence of narcolepsy associated with the vaccine might not be the adjuvant, but rather a nucleoprotein of the inactivated virus mimicking a part of a neurotransmitter receptor and thus inducing an adverse immune reaction responsible for outset of the disease. This particular nucleoprotein A was especially prevalent in the Pandemrix vaccine, and less so in the other adjuvant-containing vaccines such as Focetria and Arepanrix.

[35]. Serum from Finnish narcolepsy patients vaccinated with Pandemrix was shown to create antibodies that bound to cells engineered to display human hypocretin receptor 2. The serum of Italians vaccinated with Focetria did not create such antibodies. [36][35]

The exact mechanism for vaccine-induced narcolepsy will probably still remain hidden for some time. One such mechanism has been theorized to be molecular mimicry, where cross-reactive H1N1-spesific CD4+ T-cells target hypocretin-producing nerve cells in the hypothalamus causing their destruction [37]. More recently there has also been a case report suggesting that a patient could have developed narcolepsy due to a yellow fever vaccine containing proteins that share a similar structure with both the neurotransmitter and its receptor [38].

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Part C - Manuscript

Abstract

Background: Narcolepsy is a chronic neurological disease characterised by unusual daytime sleepiness and sudden abrupt episodes of falling asleep while carrying out activities of daily living. The aetiology of the disease remains unclear, but it is suspected to have autoimmune origin. Studies conducted in Finland and the Sweden found an increased risk of narcolepsy associated with the Pandemrix pandemic influenza vaccine used during the pandemic H1N1 influenza in 2009. The association between narcolepsy and the Pandemrix vaccine was later confirmed in other countries, and although several studies have since been published no systematic review had been done to pool and critically assess the results.

Objectives: The aim of this review is to systematically explore the scientific literature regarding the association between narcolepsy and the Pandemrix pandemic influenza vaccine.

Method: We conducted a comprehensive search in several electronic databases to identify relevant articles. After applying inclusion criteria the final studies where then chosen for meta-analysis. Data was pooled using statistical software and the risk of bias was assessed though appropriate tools for each study design.

Results: Individuals vaccinated with Pandemrix were 3.75 times more likely to develop narcolepsy when compared to individuals not vaccinated with Pandemrix (Risk Ratio = 3.75 (95% confidence interval (CI), 2.43 to 5.79; 5 studies, 6,786,864 participants). The review was limited by the small amount of studies fulfilling the inclusion criteria and considerable heterogeneity among them produced by varying study designs and outcome measures.

Conclusion: Further well-designed prospective cohort studies in the field of immunology and molecular biology are still needed to prove the causality in the association between narcolepsy and the Pandemrix vaccine.

Keywords: narcolepsy, Pandemrix, influenza, vaccine adverse effects

1. Background

Narcolepsy is a chronic neurological disease characterised by unusual daytime sleepiness and sudden abrupt episodes of falling asleep while doing something else. Around 70 per cent of individuals with narcolepsy also exhibit cataplexy, a sudden loss in muscle tone than can occur at any time during the day. [1] Symptoms commonly first appear in childhood and adolescence. The exact cause for narcolepsy remains unknown, but it is thought to be of autoimmune origin. Most individuals with narcolepsy have insufficient levels of hypocretin, a neurotransmitter normally promoting wakefulness. In those exhibiting cataplexy this has also been shown to coincide with the loss of brain cells producing hypocretin. [2] Most cases of narcolepsy are sporadic and the affected individuals have no prior family history of the illness. History and clinical examination is required to exclude other diagnoses. A polysomnogram is often required to reach a definitive diagnosis. Cataplexy, when occurring, if often a key symptom not commonly seen in other conditions. [1]

Studies conducted in Finland and Sweden found an increased risk of narcolepsy associated with the Pandemrix vaccine [3,4]. Produced by GlaxoSmithKline in response to the pandemic H1N1 influenza in 2009, Pandemrix, was one of eight vaccines licenced within the EU/EEA for pandemic influenza [5]. Of these, three were authorised through the central procedure in the EU under the European Medicines Agency (EMA) and the European Commission: Pandemrix, Focetria and Celvapan while the remaining five were authorised for national use in individual countries. [6] No prior reports of such association had been made before the pandemic H1N1 influenza in 2009 [3].

Pandemrix was the most commonly used vaccine in Europe. The European Medicines Agency (EMA) has estimated that as of August 2010 at least 38.6 million people in the EU/EEA had been vaccinated with 30.5 million of them having been administered Pandemrix, more than 6.5 million Focectria and more than 560 000 Celvapan. The highest rates of immunizing children and adolescents with Pandemrix were in Finland, Sweden, Norway and Ireland. [7]

In 2010 an increasing amount of cases of narcolepsy was observed in both Finland and Sweden, countries where Pandemrix was the sole product used for inoculation against the pandemic influenza. An existing association between Pandemrix and narcolepsy was later confirmed in both countries [3,4]. In Finland, a 12.7-fold increased risk in children and adolescents aged between 4 and 19 years was observed within 8 months after vaccination when compared to people in the same age group that were not vaccinated [3]. By July 2014 a total of 1333 separate cases of narcolepsy associated with the pandemic influenza vaccines had been reported to the EMA EudraVigilance database. These cases originated from 18 different countries with 61.9 per cent coming from Finland and Sweden. Ninety-three per cent of the cases were reported due to exposure to Pandemrix, 1.6 per cent to Arepanrix, 0.5 per cent to Focetria and 0.1 per cent to Celvapan. The EudraVigilance database does not, however, collect enough information to confirm diagnosis as per the Brighton Collaboration case definition used commonly with narcolepsy. [8]

In this review we seek to examine narcolepsy as an adverse event following vaccination with Pandemrix. No systematic review of the literature regarding this particular association between Pandemrix and narcolepsy had so far been done, although one was published during our review [9]. With the rising number of studies being conducted reporting conflicting results, such a systematic review would be useful. The results of the review can be important both for assessing vaccine safety and preventing their use from being hindered by unnecessary fears. The review will provide further use in critically appraising the available evidence which is currently solely based on observational studies inherently subject to various bias and confounding.

2. Methods

The review protocol has been published in the PROSPERO international prospective register of systematic reviews: https://www.crd.york.ac.uk/prospero/ (registration number CRD 42017081359)

2.1 Criteria for considering studies for this review

2.1.1 Types of studies

Randomised controlled trials, cohort studies, case-control studies and cross-sectional studies were considered.

2.1.2 Types of participants

Any population was considered. Although the association has been mostly observed in children and adolescents, no age restriction was set.

2.1.3 Types of interventions

Pandemrix pandemic influenza vaccine.

2.1.4 Comparison

Any other pandemic influenza vaccine or the prior prevalence of narcolepsy in the given population was used.

2.1.5 Types of outcome measures

2.1.5.1 Primary outcomes

The primary outcome of interest is narcolepsy as per the case definition of either the Brighton Collaboration [10] or the International Classification of Sleep Disorders (ICSD-2) [11].

2.1.5.2 Secondary outcomes

There are no secondary outcomes.

2.2 Search methods for identification of studies

2.2.1 Electronic searches

A comprehensive search was undertaken to identify articles. There was no language restriction, but the search was limited to published literature due to time constraints imposed by the nature of this review. The search strategy for electronic databases incorporated both medical subject headings (MeSH) and free-text terms, and was adapted to suit each particular database using applicable controlled vocabulary (Appendix 1).

Electronic searches were conducted on November 1st 2018 in PubMed, Scopus and the Cochrane Library. The following trials registers were also searched: ClinicalTrials.gov, EU Clinical Trials Register, Pan African Clinical Trial Registry and the Cochrane Central Register of Controlled Trials (CENTRAL). The search was restricted to the period on or after September 28th 2006 which was when the patent for Pandemrix was registered.

2.2.2 Searching other resources

The reference lists of all the final included studies were viewed to detect any possible additional studies. No studies matching the inclusion criteria set in the protocol were found.

2.3 Data collection and analysis

2.3.1 Selection of studies

The titles and abstracts of all studies found using the search terms were screened to identify any relevant articles. The inclusion criteria were then applied on all the initially identified articles. Further details on the selection process and a table for included studies can be found in the results section.

2.3.2 Data extraction and management

Data were extracted using the Cochrane Public Health Group Data Extraction and Assessment form modified for this purpose (Appendix 2).

Data were extracted on the following:

- Study design
- Publication status
- Date of study
- Location of study
- Characteristics of participants
- Methodological quality of study

2.3.3 Assessment of risk of bias in included studies

After relevant data were extracted from the included studies, risk of bias was assessed using the appropriate tool specific for the study design. The Newcastle-Ottawa scale was used for both cohort and case-control studies (tables 1 and 2).

Table 1: Newcastle – Ottawa quality assessment scale for cohort studies.

Selection (4 points)
1. Representativeness of the exposed cohort
2. Selection of the non-exposed cohort
3. Ascertainment of exposure
4. Demonstration that outcome of interest was not present at start of study
Comparability (2 points)
5. Comparability of cohorts on the basis of the design or analysis
Outcome (3 points)
6. Assessment of outcome
7. Was follow-up long enough for outcomes to occur
8. Adequacy of follow up of cohorts

2.3.4 Dealing with missing data

The review was conducted using existing data – there was no need to contact authors for missing data.

2.3.5 Assessment of reporting biases

We intended to use funnel plots to investigate the risk of publication bias by intervention type, where 10 or more studies were included in the analysis for a particular intervention type. Unfortunately, the results were not amenable to this assessment.

942 Table 2: Newcastle – Ottawa quality assessment scale for case-control studies.

Selection (4 points)
1. Is the case definition adequate?
2. Representativeness of the cases
3. Selection of controls
4. Definition of controls
Comparability (2 points)
5. Comparability of cases and controls on the basis of the design or analysis
Exposure (3 points)
6. Ascertainment of exposure
7. Same method of ascertainment for cases and controls
8. Non-response rate

943

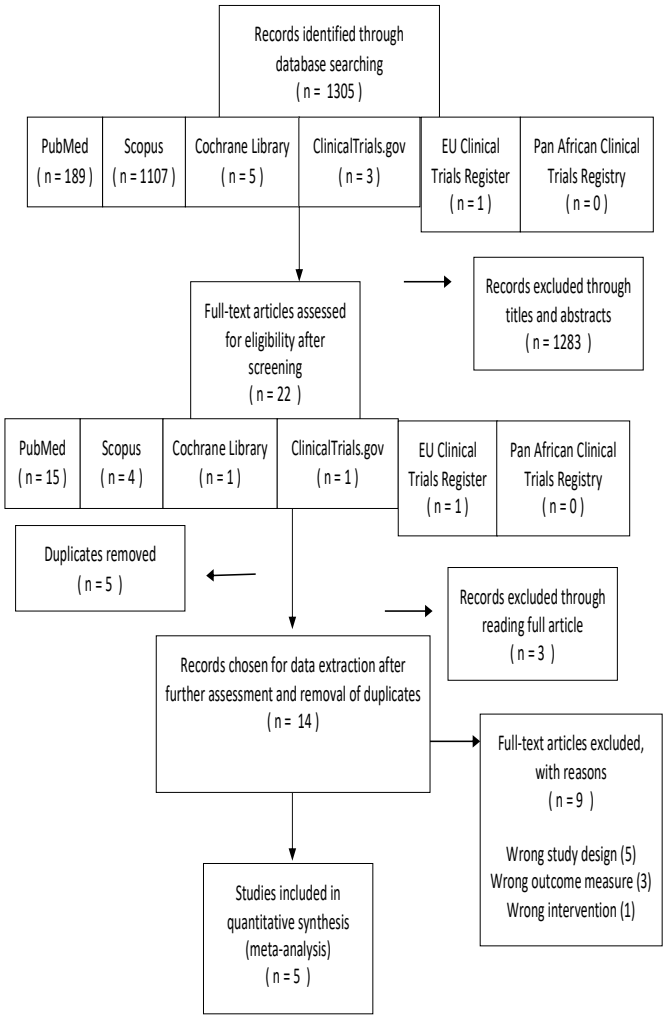
944

3. Results

3.1 Description of studies

Results of the literature search are presented according to the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) scheme (Figure 1). Database searches returned 1305 items of which the majority came through Scopus (1107) and PubMed (189). One-thousand, two hundred and eighty-three records were excluded after reviewing titles and/or abstract. Twenty-two articles were potentially eligible and were subject to full-text assessment. After removing five duplicates and excluding three articles though full-text assessment, a final 14 records were chosen for this review and subjected to data extraction. Of these, five were amenable to meta-analysis, as per the protocol. Five articles were excluded due to improper study design, three for wrong outcome measures and one for wrong intervention. Finally, the reference lists of all included articles were reviewed, but no other studies fitting the inclusion criteria were found. The included articles and their characteristics can be seen in Table 3.

All of the included studies took place in Europe, where Pandemrix was mostly used. Two of them were case-control studies with limited number of participants [12,13], while the other three were registry-based cohort studies [3,14,15]. The outcome measure reported in the studies varied significantly.



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967 Figure 1. Results of the conducted literature search.

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Study ID	Country	Study design	Age	Participants	Follow-up	Measure	Outcome (95% CI)
Dauvilliers 2013	France	Case-Control	Any	194	Oct 2009 to Apr 2011	Odds ratio	4.4 (2.0-9.7)
Nohynek 2012	Finland	Cohort	4-19	915,854	Jan 2009 to Aug 2010	Rate ratio	12.7 (6.1-30.8)
O'Flanagan 2014	Ireland	Cohort	Any	4,231,923	Apr 2009 to Dec 2010	Risk ratio	13.9 (5.2-37.2) for children 20.4 (1.8-225.0) for adults
Oberle I 2017	Germany	Case-Control	5-55	368	Jan 2005 onwards	Odds ratio	4.5 (2.0-9.9)
Trogstad 2017	Norway	Cohort	3-29	1,638,526	Jan 2008 to Dec 2012	Hazard ratio	5.53 (3.01-10.15)

970 Table 3: Included articles and their characteristics.

971

972 ### 3.2 Assessment of risk of bias

973 As only cohort and case-control studies were included in the meta-analysis, the
974 Newcastle-Ottawa scale [16] was used to assess all of them, with appropriate
975 adjustment according to the study design. Results of the risk of bias assessment can
976 be seen in Table 4.
977

978 Table 4: Risk of Bias evaluation of included studies according to the Newcastle-Ottawa scale.

Study ID	Selection	Comparability	Outcome/Exposure
Dauvilliers 2013	★★	★★	★★
Nohynek 2012	★★★★	★★	★★★
O'Flanagan 2014	★★★★		★★★
Oberle I 2017	★★★★	★★	★★
Trogstad 2017	★★★★		★★★

979

980

981 One of the included studies was seen not to have satisfyingly representative cases
982 and proper community controls [12]. Two of the studies did not state proper
983 adjustment for confounders [14,15]. Furthermore, one of the case-control studies
984 was seen as lacking in ascertainment of exposure [12] and another had a varying
985 non-response rate in its case and control groups [13].

986

3.3 Data synthesis

Individuals vaccinated with Pandemrix were 3.75 times more likely to develop narcolepsy when compared to individuals not vaccinated with Pandemrix (Risk Ratio = 3.75 (95% confidence interval (CI), 2.43 to 5.79; 5 studies, 6,786,864 participants). The results of the quantitative data synthesis can be seen in Figure 2.

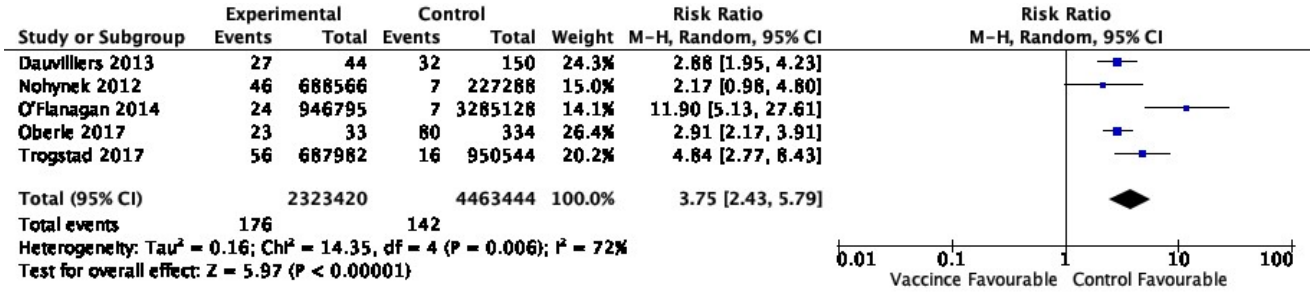

Figure 2. Vaccine-associated narcolepsy (all studies).

We conducted sub-analyses in an attempt to explain the heterogeneity of $I^2 = 72\%$. Excluding the studies with unclear adjust for confounders [14,15] maintained an increased risk of narcolepsy (Risk Ratio = 2.83 (95% confidence interval (CI), 2.26 to 3.55; 3 studies, 916,415 participants; $I^2 = 0\%$) (Figure 3).

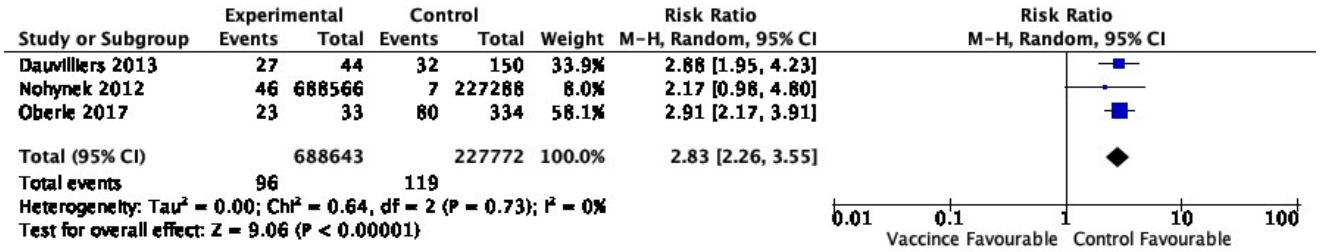

Figure 3. Quantitative data synthesis of studies with adjustment for confounders.

4. Discussion

The results of this systematic review provide statistically significant evidence for individuals vaccinated with Pandemrix having an increased risk to develop narcolepsy when compared with individuals not vaccinated with Pandemrix.

These results mirror those of an earlier systematic review [9] which reported relative risk increases of 5 to 14-fold in children and 2 to 7-fold in adults. This review represents an update on the earlier review with the inclusion of two later studies [13,15]. However, the exact mechanism through which narcolepsy is caused remains to be uncovered. Thus far it has been concluded that the vaccine adjuvant is not to be blamed, as no increased risk was observed with the similar vaccine, Arepanrix produced by the same manufacturer and containing an identical adjuvant [17].

The I^2 statistic for heterogeneity was 72% indicating considerable heterogeneity among the study outcomes. The variation may be explained by the three cohort studies based on data gathered from national registries [3,14,15] having a considerably larger participant count when compared to the case-control studies [12,13]. Even so, sub-analyses removing studies with these possible biases produced results maintaining the outcome of vaccine-associated narcolepsy with statistically significant results, thus giving weight to the pooled result. Notably, the event count among the control groups was however much smaller.

The review was meant to systematically assess the current knowledge regarding this particular association, as no systematic review had so far been published at that time, although one was published during the conduct thereof [9]. The increased risk of narcolepsy associated with Pandemrix has been one of the most widely discussed topics in vaccine adverse events during the past decade, and is still continuously referred to by both vaccine advocates and vaccine hesitants.

The review is limited by the small amount of studies fulfilling the final inclusion criteria and heterogeneity among these studies especially regarding study design and outcome measures. The strength of the final review is the systematic nature in which the available literature was searched and no published studies on the subject should have been overlooked.

5. Conclusions

Further well-designed prospective cohort studies in the field of immunology and molecular biology are still needed to prove the causality in the association between narcolepsy and the Pandemrix vaccine. As additional studies are continuously published and the amount of evidence slowly accumulates, any systemic reviews regarding this topic will surely need to be updated in the forthcoming years.

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7. Appendices

Appendix 1: Search Terms

The following search strategy was used for the all the databases:

#1 Narcolepsy [MeSH]

#2 Vaccines [MeSH]

#3 Vaccination [MeSH]

#4 Narcolepsy [Text Word]

#5 Vaccine [Text Word]

#6 Vaccines [Text Word]

#7 Vaccination [Text Word]

#8 (#1 OR #4) AND (#2 OR #3 OR #5 OR #6 OR #7)

Appendix 2: Data Extraction Form

Data Extraction Form (as per Cochrane Public Health Group template)

Study ID:	Data extractor:	Date form completed:
Citation:		

1. General Information

Publication type	Journal Article ☐ Abstract ☐ Other (specify) _____
Country of study:	
Funding source of study:	Potential conflict of interest from funding? Y / N / unclear

2. Study Eligibility

Study Characteristics			**Page/ Para/ Figure #**
Type of study	☐ Randomised Controlled Trial ☐ Cohort Study ☐ Case-Control Study	☐ Prevalence Study ☐ Case Report ☐ Other	
	Does the study design meet the criteria for inclusion?	Yes ☐ No ☐ →**Exclude** Unclear ☐	
Participants	Describe the participants included:		
	Do the participants meet the criteria for inclusion?	Yes ☐ No ☐ →**Exclude** Unclear ☐	

Types of intervention	Does the study include Pandemrix as an intervention?	Yes ☐ No ☐	
	Does the intervention meet the criteria for inclusion?	Yes ☐ No ☐ →**Exclude** Unclear ☐	
Types of outcome measures	Is narcolepsy an outcome measure?	Yes ☐ No ☐	
	If so, what case definition is used?	☐ Brighton Collaboration ☐ ICSD-2 ☐ Other (specify) _____	
	Do the outcome measures meet the criteria for inclusion?	Yes ☐ No ☐ →**Exclude** Unclear ☐	

Summary of Assessment for Inclusion

Include in review ☐	**Exclude from review** ☐
Independently assessed, and then compared? Yes ☐ No ☐	Differences resolved Yes ☐ No ☐
Request further details? Yes ☐ No ☐	Contact details of authors:
Notes:	

DO NOT PROCEED IF PAPER EXCLUDED FROM REVIEW

3. Study details

Study intention	Descriptions as stated in the report/paper	Page/ Para/ Figure #
Aim of study	*What was the study designed to assess? Are these clearly stated?*	

Participants *Include if relevant*	Include information for each group (i.e. intervention and controls) under study	Page/ Para/ Figure #
• Age group		
• Location		
• Other characteristics		
• Follow-up period		

4. Outcomes

Question	Outcome 1	Page/ Para/ Figure #	Outcome 2	Page/ Para/ Figure #
Outcome definition				
Unit of measurement				
State the outcome				
Is it a reliable outcome measure?				
Is there adequate power for this outcome?				

5. Other relevant information

Key conclusions of the study authors		
References to other relevant studies		
Additional notes by review authors		