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Is there an Association between the H1N1 Influenza Pandemic Vaccination and the Manifestation of Narcolepsy?

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Abstract

After the mass-vaccination campaign during the influenza A (H1N1) 2009 pandemic, a significant increase in narcolepsy incidence was observed initially in Scandinavia, later in other European countries and recently also in Canada. Narcolepsy is a sleep disease caused by the loss of hypocretin-producing cells in the hypothalamus. Almost all narcolepsy patients carry the HLA-DQB1*0602 allele, giving a link to an autoimmune-mediated process.

Most of the observed narcolepsy cases were correlated to the vaccination with Pandemrix, the most frequently used vaccine in the EU, and a slight connection to Arepanrix was also detected, which was distributed in Canada. Both vaccines were adjuvanted with AS03, suggesting a possible link between AS03 and narcolepsy. No narcolepsy cases were detected with MF59-adjuvanted or non-adjuvanted influenza vaccines. Recent studies reported differences between Pandemrix and Arepanrix and suggested the vaccine rather than the adjuvant as a suspect for narcolepsy development following vaccination. In addition, in China an increase of narcolepsy cases was reported to occur in absence of vaccination. Possible factors and potential additive effects that may have triggered narcolepsy after the pandemic vaccination are being reviewed in this paper.

Influenza virus

Influenza viruses belong to the Orthomyxoviridae family and are divided into three classes: A, B and C. They are spherical particles with a diameter of 100-200 nm. The viruses are enveloped with two surface antigens, Hemagglutinin (HA or H) and Neuraminidase (NA or N). Hemagglutinin, which is the major projection on the influenza virus surface, binds to the host cell receptors and promotes the fusion between the virus envelope and the host cell, whereas neuraminidase is responsible for the release of progeny viruses by cleavage of the terminal sialic acid [1,2]. The influenza virus A has 16 different types of hemagglutinin (H1–H16) and 9 different types of neuraminidase (N1–N9) [3]. Recently, a novel type of hemagglutinin (H17) was identified in bats [4]. Based on the combinations of hemagglutinin and neuraminidase types on the cell surface, the viruses are classified in diverse subtypes such as H1N1, H3N2 or H5N1 [3].

The core of influenza viruses contains a negativesense singlestranded RNA genome that is composed of eight separate gene segments [1]. Two types of mutations can occur within the genome. First, an antigenic drift those are point mutations within the genome causing mild changes in the surface antigens and thus creating a new variant of an existing strain. Second, an antigenic shift that is a new recombination of gene segments which can occur when a host is infected with influenza viruses from different species (human, birds, pigs), leading to a formation of a completely new strain. In many cases, pigs are the mixing vehicles [5,6].

Narcolepsy

Narcolepsy is a rare sleep disease characterized by the classical tetrad of excessive daytime sleepiness, hypnagogic hallucinations, sleep paralysis and cataplexy [7]. Excessive daytime sleepiness is the main and often first complaint of narcolepsy patients, meaning that the affected persons have sudden and unexpected sleep attacks during the daytime. Another complaint is that hypnagogic hallucinations occur – these are dream-like perceptions at sleep onset. In addition, narcolepsy patients suffer from sleep paralysis; this is a temporal inability to make voluntary movements and can occur while falling asleep or immediately after awaking. The only specific symptom of narcolepsy is cataplexy which is brief episodes of muscle weakness triggered by strong emotions such as laughing or joking [8-10].

Narcolepsy is estimated to have a prevalence of 0.03-0.05% in the general population [11,12]. The age of onset lies between 15 and 40 years and shows a bimodal distribution with the biggest peak at around 15 years and a second peak at around 36 years [13]. Narcolepsy has not a clear pattern of inheritance with just very rare exceptions. Only 1-2% of the first-degree relatives of people with narcolepsy develops narcolepsy with cataplexy [14]. Also monozygotic twins are most frequently discordant for narcolepsy [14]. Narcolepsy is often misdiagnosed and it can take many years (8-10 years) until the exact diagnose is made [15,16]. The symptoms can be falsely attributed to epilepsy or depression [17].

Influenza A (H1N1) 2009 pandemic

Pandemic influenza occurs when a new emerging influenza strain turns out to be infectious to humans and transmissible to man [6,18].

In the last century, three influenza pandemics occurred: A (H1N1) in 1918, A (H2N2) in 1957 and A (H3N2) in 1968 [19]. The most severe influenza pandemic was detected in 1918 with estimated 50 million deaths or more [20], the highest mortality rate of any disease outbreak in the recorded history [21].

In April 2009, a novel variant of the influenza A (H1N1) virus was identified in Mexico [22,23] and caused a high reported case fatality ratio [24]. In parallel, the new influenza strain was isolated also from individuals in the USA [25]. The virus contained a unique combination of gene segments that had previously not been found among influenza viruses. Two gene segments of the new variant were derived from the Eurasian swine virus that originated from the avian influenza virus. Three segments came from the classical swine influenza virus. The remaining three gene segments were derived from the triple reassortant swine virus that originated from different lineages of avian viruses [26].

The infection spread fast around the world and on June 11, 2009, the World Health Organization (WHO) declared the global influenza A (H1N1) pandemic [27], the first one of the 21st century. The pandemic H1N1 became the dominant influenza strain in 2009 [28]. However, most of the cases were mild and self-limiting [29] and mortality was not as high as estimated (seasonal influenza causes 250,000-500,000 annual deaths worldwide [30]). By the end of the pandemic (August 10, 2010), at least 18,448 laboratory-confirmed deaths were reported by the WHO as associated with the new virus strain [31]. In addition, another statistical approach was applied to quantify the global burden of the 2009 pandemic influenza A (H1N1). Here, an age-stratified probabilistic model was used that estimates mortality rates as the product of symptomatic attack rates and the risk of death given infection (case fatality rate). With this probabilistic multiplier approach, it was estimated that worldwide 201,200 respiratory deaths occurred and additional 83,300 cardiovascular deaths were assumed to be associated with the 2009 pandemic influenza A (H1N1) [32,33]. The data from this approach were not based on PCR-confirmed influenza cases. Unlike the seasonal influenza, the new influenza affected a larger number of people younger than 65 years old [33].

As a response to the pandemic, the WHO planned a high-scale vaccine production which had to be accomplished within 5–6 months after the first identification and isolation of the new pandemic virus [34,35]. It was proposed that 4.9 billion vaccine doses could be produced within one year [35].

Influenza A (H1N1) 2009 pandemic vaccines

By mid-September 2009, the first pandemic influenza vaccines were available. Most of them contained whole-virion or split-virion-based antigens. To ensure a global supply, many vaccines were adjuvanted as an antigen-sparing strategy [35].

Pandemrix (GlaxoSmithKline) is a split-virion vaccine made in eggs, containing 3.75 μg of hemagglutinin type 1 (H1), and was adjuvanted with AS03 [36-38].

That was the first time that AS03 was used in a licensed vaccine [36], as it had shown a high tolerability and immunogenicity in clinical trials [39-41]. Pandemrix was given to at least 30.8 million individuals in the EU [38] and was thus the most used vaccine in Europe.

Pandemrix was licensed by the so called Mock-up procedure at the EMA; initially a H5N1 mock-up vaccine was licensed [42] that was later – upon emergence of the H1N1 pandemic strain -adapted to the H1N1 strain by means of a variation procedure [43]. For further information [44]. Clinical data available for both strain vaccines are described in these reports [42-44]. Most studies with the prepandemic H5N1 influenza vaccine adjuvanted with AS03 (Prepandrix) were performed with people aged between 18-60 years, in two studies elderly aged more than 60 years were included and one study was performed with children aged 3-9 years [45]. Children and adolescence aged between 9 and 18 years were not included in any of the studies, an age group mostly affected after the pandemic influenza vaccination.

Focetria (Novartis) contained 7.5 μg of H1 and was adjuvanted with MF59 [36-38]. It was given to more than 6 million people [46]. In contrast to AS03, MF59 had been used in seasonal vaccines for many years and after 45 million doses applied, no safety concerns have been observed so far [47,48].

In the USA, live-attenuated or inactivated influenza A (H1N1) monovalent vaccines were approved and none of them was adjuvanted [49].

In Canada, the AS03-adjuvanted vaccine Arepanrix (GlaxoSmithKline) was licensed [50,51] and 4.8 million doses were distributed in Canada [52]. Arepanrix contained the same adjuvant as Pandemrix, but the antigens in these vaccines were isolated using different antigen purification procedures (Dresden protocol for Pandemrix and Quebec protocol for Arepanrix) [53]. In addition, Pandemrix antigen preparations contained excipients that were not included in Arepanrix such as polysorbate 80, octoxynol 10 and magnesium chloride [36]. In a recent study, the differences between Pandemrix and Arepanrix were investigated using two dimensions difference gel electrophoresis (2D-DIGE) and mass spectrometry (MS). Interestingly, the H1 at aa 146N was found to be 10-fold higher deamidated in Arepanrix than in Pandemrix [54]. Another study revealed that Pandemrix and Arepanrix have antigenic differences. Pandemrix contained higher amounts of a structurally altered form of the influenza virus nucleoprotein (NP) than Arepanrix. In addition, it was observed that children with narcolepsy have antibodies against the NP antigen derived from Pandemrix compared to healthy children and the antibody response was associated with the HLA-DQB1*0602 allele [55].

Adjuvants

Adjuvants are components that are included in vaccines to enhance antibody responses to vaccine antigens [56,57]. Furthermore, they allow reducing the antigen amount, which is especially valued when more than one vaccine dose per vacciniation is needed to induce sufficient antibody titers. Therefore, the addition of adjuvants increases the global supply of a vaccine in the case of a pandemic [58-61].

Adjuvants can be classified into the two groups of delivery and immunomodulatory systems. Delivery systems promote an effective uptake of antigens and their presentation to antigen-presenting cells (APCs), leading to enhanced humoral immunity [62]. Immunomodulatory systems, in contrast, are able to stimulate the immune system directly. They interact with specific receptors, such as toll-like receptors (TLR) on APCs leading to the induction of T helper cells and thus to the activation of the cellular immune response [63,64].

There are only very few adjuvants that have been licensed for use in humans. The most commonly used ones are the traditional aluminium

salts (alum) and squalene-based oil-in-water emulsions (MF59 and AS03) [65,66].

Aluminum salts have been used for more than 70 years, but it still remains unclear how they exactly work. They are thought to act as depot systems, meaning that they catch antigens and keep them at the local injection site for days or weeks. In this way, the antigens can be slowly presented and processed by the immune system [66]. It was observed that alum enhances the humoral immune responses and has a very good safety profile [67]. However, in several studies, aluminum had a lower adjuvanticity (adjuvant effect on vaccine immunogenicity) compared to MF59 [68-74], AS03 [75,76] or other adjuvants [77] based on the measurement of hemagglutination inhibition and microneutralization antibody titers.

MF59 (Novartis) is licensed on the European market since 1997. MF59 is composed of 9.75 mg squalene, 1.175 mg polysorbate 80 (Tween 80) and 1.175 mg sorbitan trioleate 85 [78]. Squalene oil is a natural component and synthetic precursor to cholesterol and steroid hormones. After emulsification, the oil droplets have a size of about 160 nm and are stabilized by polysorbate 80 and sorbitan trioleate 85 [79,80]. In pre-clinical studies it has been observed that MF59 adjuvant is safe, efficacious and well tolerated in humans. MF59 significantly increased the antibody titers against influenza compared to non-adjuvanted flu vaccines. In addition, it induced enhanced immune responses against heterovariant flu strains and also allowed a significant reduction in the antigen concentration. The mode of action of MF59 includes the following steps. After the injection of MF59, local muscle cells and macrophages are activated and respond by creating a cytokine and chemokine environment. This results in a migration of immune cells from the blood stream into the activated environment of the muscle. In addition, also monocytes and granulocytes secrete chemokines upon contact with MF59, leading to a further influx of chemokine gradient. All recruited cells can take up the adjuvant and antigen and transport them to the lymph nodes, where they can interact with antigen-specific T-cells and activate the adaptive immune response. Thus, MF59 enhances the number of antigens presented to T-cells, resulting finally in greater vaccine potency [81-84].

The adjuvant System 03 (AS03) from GlaxoSmithKline is another squalene-based adjuvant and is composed of 10.69 mg squalene, 4.86 mg polysorbate 80 (Tween 80) and, in contrast to other oil-in-water emulsions, it additionally includes 11.86 mg DL α-tocopherol per one dose (0.5 ml) [42,78,83]. αTocopherol, the most bioavailable form of vitamin E, is an anti-oxidant that exhibits immunomodulatory properties [85,86]. The amount of α-tocopherol included in the adjuvant is comparable to the amount of α-tocopherol in the daily diet. However, compared to MF59, AS03 contains higher amounts of the detergent polysorbate 80 which might improve the solubility and resorption of α-tocopherol into neuronal cells as described for the delivery of estradiol in rat brains [87] and thus facilitate the intake of AS03. In clinical trials, AS03-adjuvanted H5N1 influenza vaccines promoted high antibody and T-cell responses [88,89] The analyses of AS03's mode of action revealed that AS03 acts as an immunomodulatory system. The presence of α-tocopherol in AS03 enhanced the antigen-specific adaptive responses, increased the cytokine production and antigen loading in monocytes as well as the recruitment of granulocytes in the lymph nodes [83].

Narcolepsy-molecular pathology

As a cause for narcolepsy, the deficiency of the neurotransmitter hypocretin (orexin) was observed. Hypocretin was identified for the first time in 1998 by two groups almost simultaneously. Louis de Lecea and colleagues determined the hypocretin gene and observed that it is expressed exclusively in the lateral hypothalamus area (LHA) and encodes a precursor molecule for two related peptides having a structural similarity to the gut hormone secretin [90]. Based on this, the derived peptides were called hypocretin 1 and 2 (hypocretin=hypothalamic secretin). In Sakurai et al., the peptides were named orexin A and B to reflect the orexigenic (appetite-stimulating) activity of these hormones (Greek: orexis=appetite) and described also their receptors [91]. Hypocretin and orexin are the same peptides and both terms are used synonymously in literature.

Hypocretin 1 and 2 are produced from a common 131 amino acid long precursor polypeptide, prepro-hypocretin, with proteolytic processing by prohormone convertases. Hypocretin 1 is a 33 amino acid peptide with an amino (N)-terminal pyroglutamyl residue, two intra-chain disulphide bonds and carboxy (C)-terminal amidation. The human hypocretin 1 sequence is identical to the mouse, rat, bovine and porcine hypocretin 1, suggesting high conservation throughout evolution. Hypocretin 2 is a 28 amino acid long, C-terminally amidated linear peptide and is 46% (13/28) identical in sequence to hypocretin 1. The C-terminal part of hypocretin 2 is very similar to that of hypocretin 1 (73%; 11/15), whereas the N-teminal part is variable. Hypocretins bind to two orphan G-protein-coupled receptors named hypocretin receptor 1 and 2 (orexin receptor 1 and 2). Hypocretin receptor 1 has a greater affinity for hypocretin 1 than hypocretin 2 by one order of magnitude, whereas hypocretin receptor 2 is a non-selective receptor for both peptides. The receptors are expressed in the entire central nervous system (CNS).

There are only 70,000-90,000 neurons producing hypocretin [92,93]. Hypocretin neurons originate in the hypothalamus, but they project widely to the olfactory bulb, cerebral cortex, thalamus, hypothalamus and brainstem and more densely to the locus coeruleus, tuberomamillary nucleus, raphe nucleus and bulbar reticular formation [94-96]. Thus, hypocretin neurons influence multiple brain areas and are involved in the coordination of feeding, emotions, reward, arousal, drug addiction and energy homeostasis [97-100].

Initially, hypocretins were recognized as regulators of feeding behaviour, first because they are produced exclusively in the lateral hypothalamus, a region known as the feeding centre, and second because of the observation that the injection of hypocretins during the light period induces feeding behavior in rats and mice [91,101,102]. The following year, in cloning studies of a naturally occurring familial canine narcolepsy model, it was determined that canine narcolepsy is caused by null mutation of the hypocretin receptor 2 gene [103]. Almost at the same time, a prepro-hypocretin knock-out mouse was described to have sleep abnormalities similar to human narcolepsy [104], indicating that hypocretin is also involved in sleep regulation. After the observations in dogs and mice, the hypocretin system was analyzed in human narcolepsy. In systematic screenings of mutations in patients with narcolepsy, only one patient was observed to have a mutation in his hypocretin genes, but this patient was atypical with a very early disease onset at 6 months of age [92]. Hence, most of the human narcolepsy cases are not caused by gene mutations. Further studies determined that almost all people with narcolepsy had undetectable hypocretin 1 levels in their cerebrospinal fluid (CSF), leading to the conclusion that narcolepsy is caused by a deficiency in

the hypocretin production [93,105]. Two studies demonstrated the loss of hypocretin cells in the narcolepsy brain tissue. In one study, peptide radioimmunoassays in the post-mortem brain tissue of narcoleptic patients were performed and the results indicated a loss of prepro-hypocretin RNA and hypocretin peptides, representing significant differences when compared with control patients [92]. In the second study, immunocytochemistry experiments revealed an 85-95% reduction of hypocretin neurons in narcolepsy brains [106]. In both studies it was also observed that melanin-concentrating hormone (MCH) neurons, which neighbor on hypocretin cells, were not affected [92,106], indicating that the loss of hypocretin-producing cells is selective. In addition, markers that colocolize in hypocretin-containing neurons in the hypothalamus, such as neuronal activity-regulated pentraxin (NARP), dynorphin and most recently, insulin-like growth factor binding protein 3 (IGFBP3), were also found to be deficient, indicating a cell loss rather than a lack of hypocretin expression [107-109].

CSF hypocretin 1 levels lower than 110 pg/ml have a high positive predictive value for narcolepsy (94%). Hypocretin 1 levels in patients with sleep disorders other than narcolepsy or control patients are almost always above 200 pg/ml [110]. To assess narcolepsy patients, low hypocretin 1 levels seem to be a specific indicator. Thus, the measurements of hypocretin 1 levels in the CSF are now used to diagnose narcolepsy [111]. All diagnoses of narcolepsy are included in the newest version of the international classification of sleep disorders-third edition (ICSD-3) [112].

In addition to the hypocretin deficiency, narcolepsy is tightly associated with a specific human leukocyte antigen (HLA) allele. 90-100% of narcolepsy patients carry the HLA-DQ0602, a heterodimer encoded by DQA1*0102 (α-chain) and DQB1*0602 (β-chain) [113-115]. This allele is not limited to narcoleptic patients, as it can be found in 12-38% of people among different ethnic groups [116,117]. Because of this high association with the HLA-DQ0602, narcolepsy is thought to be caused by an autoimmune-mediated process [8,118]. So far, no classical auto-antibodies and no increase in oligoclonal CSF bands were found in narcolepsy patients [119]. However, it is assumed that T-cell-mediated autoimmunity targeting hypocretin neurons could lead to the loss of hypocretin and thus to the development of narcolepsy [120,121].

Crystal structure analysis revealed that DQB1*0602 binds to the N-terminal part of the prepro-hypocretin (first 13 amino acids) with a high affinity. It was observed that the narcolepsy-associated DQB1*0602 molecule differs in only 9 residues from the DQB1*0601 molecule which is protective against narcolepsy. Five of these residues contribute to binding pockets. The hypocretin peptide is presented in the DQB1*0602 binding groove, harbouring the peptide side chains in the P1, P4, P6 and P9 pockets, with more effect on the polymorphism on the P4 and P9 pockets. At the P9 pocket, the residue at position 38 is changed from alanine in DQB1*0602 to valine in DQB1*0601, which is a very conservative change. The change from tyrosine (DQB1*0602) to aspartic acid (DQB1*0601) in P9 at residue 37 introduces significantly more negative charge and could have an impact on the hydrogen bonding in this pocket, but this may modulate the anchor specificity rarely. The P4 binding pocket is larger in DQB1*0602 than in DQB1*0601 which results from the polymorphism at residues 13 and 26. Here, glycine is changed to alanine and leucine to tyrosine in DQB1*0601 compared to DQB1*0602, respectively. The side chains at the positions 13 and 26 are larger in DQB1*0601 compared to DQB1*0602 and cause thus steric clashes with the hypocretin peptide so that hypocretin can no longer be accommodated. These findings indicate that the P4 pocket and, to a certain extent, the P9 pocket differ significantly between DQB1*0602 and DQB1*0601. DQB1*0602 can bind and present larger hypocretinspecific fragments with higher affinity compared to DQB1*0601 [122]. These longer self-peptides could be mistakenly recognized as foreign by the T-cells, leading to the destruction of hypocretin-producing cells by an apoptotic process [123]. So far, however, there are no data confirming this hypothesis.

Epidemiology

The first suggestions of a link between vaccination and narcolepsy were obtained from the Swedish Medical Agency reporting about 6 narcolepsy cases in adolescents aged between 12–16 years within just a few weeks following the vaccination with Pandemrix, whereas in previous years just a few new cases per year were determined [124]. Soon after, up to 14 further narcolepsy cases were reported to the Finnish National Institute for Health and Welfare [125,126]. Consequently, the Institute recommended to stop the use of Pandemrix until the possible link is clarified [127,128]. The European Centre for Disease Prevention and Control (ECDC) and the Vaccine Adverse Event Surveillance and Communication Consortium (VAESCO) started to investigate the association between the immunization with Pandemrix and narcolepsy in eight European countries [129]. The investigations were performed on a national level and the countries carried out their own studies.

A cohort study made in Sweden revealed a 6.6-fold increase of narcolepsy cases in individuals vaccinated with Pandemrix, compared to those not vaccinated, with an absolute risk of 3.6 per 100,000 vaccinated subjects [124]. In Finland a retrospective cohort study observed a 12.7-fold increase in narcolepsy in 4-19 year old children and adolescents with an onset approximately two months after the vaccination with Pandemrix compared to the unvaccinated individuals in the same age group. The absolute risk was 6.3 in 100,000 vaccinated persons [130]. In Finland and Sweden, Pandemrix was the only vaccine administered. The vaccination coverage in these countries was very high. In Sweden approximately 67% of the population was vaccinated [124] and in Finland the vaccination coverage in children and adolescents was 75% [125]. The reason for this is that Finland and Sweden recommended the vaccination to their entire population, whereas other EU member states (Denmark, Italy, the Netherlands and the United Kingdom) recommended vaccination only to selected risk groups [131]. In July 2011, the VAESCO report appeared and could not confirm an increase in narcolepsy diagnosis outside of Sweden and Finland [132]. Based on the Swedish and Finnish data, the European Medicines Agency (EMA) concluded on the one hand that the benefit-to-risk ratio of Pandemrix remains positive and Pandemrix continues to be licensed in the EU [126]. On the other hand, the EMA officially recommended the restriction of the use of Pandemrix so that this vaccine shall no longer be administered to persons <20 years of age [133]. Moreover, the incidents of narcolepsy after vaccination with Pandemrix have been included in the "Summary of product characteristics" [134].

However, in the next years, also other European countries gave additional reports about an abrupt increase of narcolepsy cases after the pandemic vaccination, confirming the initial data from Sweden and Finland and indicating that the association is not limited to those populations. In February 2013, England reported a peak of increased evidence of increased incidence of narcolepsy following the vaccination with Pandemrix which was observed in retrospective

analysis. Here, a relative risk of 14.4 was detected in children and adolescents aged between 4-18 years. The vaccination coverage with Pandemrix was 37% in the risk group aged between 2-15 years and around 24% in healthy children aged less than 5 years. The relative risk found in England was consistent with the relative risk reported from Finland. However, the attributable risk in England was 1.8 in 100,000 and was lower than in Finland (6.3 in 100,000) which could be due to differences in the genetic susceptibility and higher vaccine coverage in children and adolescents in Finland [135].

A retrospective population-based cohort study made in Ireland found a 13.9-fold higher risk of narcolepsy in children and adolescents vaccinated with Pandemrix compared to unvaccinated individuals. Vaccination coverage with Pandemrix was 40.3% in children aged 0-4, 37.8% in those aged 5-19 and 14.3% in individuals aged 20 and over. The absolute attributable risk to Pandemrix vaccine was 5.3 in 100,000, consistent with the data from Finland [136]. In the studies from England and Ireland, the general increase in narcolepsy incidence was distinguished from the narcolepsy increase associated with Pandemrix vaccination. The ratios were calculated based only on the values of narcolepsy cases linked to the vaccination with Pandemrix.

In France, the first post-H1N1 cases of narcolepsy (n=6) were reported at the Sleep Center in Montpellier, France, just within a few months after the vaccination onset (Nov 2009 - Jan 2010) which was an unusual observation. In five of these cases, the vaccine Pandemrix was used [137]. Subsequently, a case-control study made in France revealed a 6.5-fold increase of narcolepsy in subjects aged less than 18 years. In contrast to the other reports, in France a 4.7-fold increase was observed also in individuals aged 18 and over. This study confirmed the initial signs. The odds ratios were calculated for the general increase of narcolepsy following the H1N1 pandemic vaccination, including both vaccines used in France (Pandemrix and the non-adjuvanted vaccine Panenza). However, the biggest increase of narcolepsy was observed after vaccination with Pandemrix (87% of positive outcomes). 8.8% of the population was vaccinated, of which about 89% aged 9 years and over received Pandemrix [138].

In Norway, a 10-fold increase in narcolepsy was detected in a study including children and adolescents aged between 4 and 19 years receiving Pandemrix. The vaccination coverage in this age group was 50% and the highest incidence was observed within 6 months of vaccination onset. The absolute risk of Pandemrix was 10 per 100,000 vaccinated persons [139].

Furthermore, detailed follow-up studies from Western Sweden revealed a 25-fold increase of narcolepsy cases following the vaccination with Pandemrix. This higher incidence compared to the initial data from Sweden could be due to better case ascertainment and longer follow-up [140].

In Canada, another AS03-adjuvanted vaccine (Arepanrix) was administered. In 2010, the first post-H1N1 narcolepsy onset cases (n=4) were reported at the Sleep Disorder Centre in Montreal, Canada, within just two months after the pandemic vaccination. All of the affected persons were vaccinated with Arepanrix [137].

Recently, a review about post-marketing safety surveillance data dealing with adverse events following immunization (AEFIs) was published by the Public Health System in Canada. Here, no narcolepsy cases were reported to the passive AEFI reporting system following vaccination with Arepanrix in individuals aged 29 years and younger in ontario, canada [52]. However, a few weeks later another publication appeared, describing the results of a retrospective population-based study that was carried out in the Canadian province quebec. Here, the relative risks were calculated in a cohort analysis by a self-controlled case series (SCCS) and with a case-control method. A relative risk of 1.48 (3.21 among persons less than 20 years of age and 0.73 among adults) was detected using the case-control method and 2.07 in the SCCS following the administration with Arepanrix. The absolute risk was very moderate with 0.1 adverse events in 100,000 individuals. At the end of the vaccination campaign, 57% of persons 6 months of age or older had been immunized of which 96% received Arepanrix [141].

During the 2009 pandemic, also an MF59-adjuvanted influenza A (H1N1) vaccine was administered. Analyses of the clinical trials and pharmacovigilance databases clearly revealed that there was no case of narcolepsy associated with MF59-adjuvanted A (H1N1) pandemic or other MF59-adjuvanted influenza vaccines [142].

In contrast to Europe and Canada, in the USA only non-adjuvanted vaccines were distributed. Within about 3 months after vaccination onset, 4 narcolepsy cases were observed at the Stanford Center for Sleep, USA. Two of these cases had been vaccinated in Europe with Pandemrix; the other two cases had received a non-adjuvanted vaccine in the USA [137]. A population-based cohort study undertaken in the USA with persons younger than 30 years did not reveal any association between the influenza (H1N1) 2009 pandemic virus strain-containing vaccines used in the USA and narcolepsy [143]. In addition, also in South Korea, the vaccination with MF59-adjuvanted and non-adjuvanted H1N1 pandemic vaccines had no link to narcolepsy as observed in ecological studies [144]. However, a retrospective study performed in Beijing, China, describes a 3-fold increase in narcolepsy cases following the influenza (H1N1) infection in 2009 in absence of vaccination [145].

Potential link between 2009 Pandemic Vaccination and Narcolepsy

An increased incidence of narcolepsy was observed after the influenza A (H1N1) 2009 pandemic vaccination. More than 800 people have been affected [146], most of them children and young adults. While the absolute risk was low, the relative risk was significantly raised. The mechanisms underlying such a link were presumed in the literature, including either a specific immune response to H1N1 or a stimulation of the immune system through the adjuvant AS03, leading to an autoimmune process [137,147]. However, there were no conclusive data available describing such a mechanism.

Recently, a study was carried out to identify a possible mechanism that could be a factor leading to the development of narcolepsy after the vaccination with the AS03-adjuvanted pandemic vaccine Pandemrix [148]. So far, there has been no increase in narcolepsy cases after vaccination with the MF59-adjuvanted A (H1N1) pandemic vaccine [142]. In contrast to MF59, AS03 additionally contains high amounts of αtocopherol [83]. In light of this, it was suspected that αtocopherol could have played a role in the observed increased incidence of narcolepsy following the vaccination.

It was observed that αtocopherol activates the transcription factor NFE2-related factor 2 (Nrf2) [149,150]. In its inactive state, Nrf2 is associated with its inhibitor Keap1, leading to a permanent and rapid degradation of Nrf2. After Nrf2 is activated, it dissociates from Keap1 and translocates into the nucleus. Here it binds to the antioxidant response element (ARE) in the promoter regions of different cytoprotective genes, i. e. the NAD(P)H-dependent quinone oxidoreductase 1 (NQO1) or the glytamatecysteine ligase catalytic

subunit (GCLC) [151]. In addition, genes encoding for the catalytic active β-subunits (β1, β2 and β5) of the constitutive proteasome are target genes of Nrf2 [152,153]. The increased expression rate of proteasomal subunits is reflected in an enhanced proteasomal activity [152,154]. This was described for the β5 subunit (PSMB5) [152] in more detail. It was found that hypocretin also has an ARE site in its promoter region and could thus be activated in an Nrf2-dependent-manner. In addition, there is a crosstalk between Nrf2 and the transcription factor NF-kB (nuclear factor kappa-light-chain-enhancer of activated B cells), leading to a decreased NF-kB activity when Nrf2 is activated and vice versa.

It was observed that the AS03 component α-tocopherol activates Nrf2 in neuronal cells leading on the one hand to an enhanced expression of hypocretin and on the other hand to a higher expression rate of PSMB5 resulting in increased proteasomal activity. This leads to a strong turnover of hypocretin and the formation of many hypocretin-specific peptides in the cells. Moreover, elevated activation of Nrf2 is associated with a decreased activity of NF-κB that results in an increased sensitivity to apoptotic stimuli. In case of a genetic predisposition (DQB1*0602) that is found in almost all narcolepsy patients, α-tocopherol could confer to the development of narcolepsy by activation of Nrf2 that leads to increased hypocretin levels in the cells and as well to an enhanced proteasomal activity resulting in a strong turnover of hypocretin. The longer hypocretin-derived fragments can be bound and presented by DQB1*0602 on the cell surface. These cells can be recognized by the immune system and due to their increased sensitivity to apoptotic stimuli they can be destroyed, finally leading to a lack of hypocretin which is the trigger for narcolepsy.

In Canada the vaccine Arepanrix was licensed that was also adjuvanted with AS03. The observed increase of narcolepsy in Canada was less significant than in European countries (0.1 versus 1.8-10 per 100,000). The genetic predisposition in Canada is as common as in Northern Europe, but the vaccination coverage in Canada was not as high as in Scandinavia in the age group from 4-19 years [130]. Furthermore, the isolation of the vaccine antigens for Pandemrix and Arepanrix were prepared using different manufacturing processes [53] and differences between these vaccines were recently reported [54,55], having a possible impact on the adjuvant effect. The data from Canada indicate in addition, albeit only slightly, that α-tocopherol included in AS03 could favour the development of narcolepsy after the vaccination with an AS03-adjuvanted vaccine, as there was no association reported with narcolepsy after the administration of MF59-adjuvanted or non-adjuvanted vaccines [142-144].

Narcolepsy is a complex disease, involving many factors. α-Tocopherol alone was probably not able to induce the onset of narcolepsy. AS03 contains in addition high amounts of Tween 80 which could facilitate the intake of α-tocopherol into neuronal cells as described recently for the delivery of estradiol in rat brains [87]. An important role in the development of narcolepsy plays the genetic predisposition (DQB1*0602), as almost every narcolepsy patient carries this HLA allele [113-115]. The release of a variety of cytokines triggered by the vaccination is an additional factor in the multifactorial process of the occurrence of narcolepsy after a vaccination [77]. It was reported that in China a 3-fold increase of narcolepsy incidence was detected following the influenza A (H1N1) pandemic. The majority of the affected individuals were not vaccinated [145]. In the following years, a reduction in narcolepsy cases was observed due to changes in the viral strain, suggesting a possible role of influenza A (H1N1) 2009 virus antigens in the development of narcolepsy. It cannot be ruled out that the virus antigen could confer to the development of narcolepsy. If this is so, the addition of the adjuvant AS03 could increase this effect.

The resulting question is whether a similar problem can be prevented in the future? Unfortunately this might be one of the risks we have to take for active ingredients-such as adjuvants-for which at the time of licensure no broad data basis is available. However, the experiences gained with recent licensing procedures shows that nowadays licensing is mostly based on data from analysis of large cohorts (eg; 30.000-40.000 for HPV vaccines). However, even these enhanced numbers would not have been sufficient to detect the narcolepsy risk pre-licensure. One has to bear in mind that cases of narcolepsy as observed are extremely rare and not traceable even with very high numbers of subjects in clinical trials pre-licensure (eg even with more than 100.000 subjects in clinical trials this would not have been detected). Although there is evidence based on cell culture experiments that the adjuvant AS03 and especially α-tocopherol as a component of AS03 could have played a crucial role in the observed increased incidence of narcolepsy after the pandemic vaccination campaign, there are still a variety of open questions that have to be analyzed by additional experimental studies with a focus on animal models that allow to analyze the effect of AS03 on the hypocretin expression, processing and presentation of hypocretin-specific peptides in the context of the human HLA-DQA*0102, HLA-DQB*0602. This model would allow whther the mechanisms described in the cell culture model [148] can contribute to the Pandemrix-associated manifestation of narcolepsy.

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