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NARCOLEPSY:
CLINICAL PICTURE, DIAGNOSTICS, AND ASSOCIATION WITH H1N1 VACCINATION

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ACADEMIC DISSERTATION

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To my family

ABSTRACT

Objectives: After the 2009-2010 pandemic H1N1 vaccination campaign, a large number of new narcolepsy cases suddenly appeared in countries where the AS03-adjuvanted Pandemrix vaccination was used. An increased incidence of narcolepsy after the 2009/2010 H1N1 influenza season was observed also in China, where vaccine coverage was very low. However, epidemiological studies are prone to various biases and confounders. Furthermore, there is evidence from animal studies that H1N1 virus infection per se might be able to manifest a narcolepsy-like phenotype. Therefore, some controversy exists in the association between vaccination and narcolepsy. Our first aim was to systematically analyze the magnitude of the risk of narcolepsy after Pandemrix vaccination and to examine whether an increased association emerged with any other vaccine or H1N1 virus infection (Study I).

H1N-vaccine-associated narcolepsy (pNC) cases had very abrupt onset, short diagnostic delay, and common psychiatric comorbidity, which warranted thorough analysis of the phenotype and characteristics of the disease. In Studies II and III, we aimed to determine whether differences were present in clinical, polysomnographic (PSG), or actigraphic (ACT) characteristics between pNC and sporadic narcolepsy (sNC). Moreover, clinical evolution of pNC was analyzed.

Diagnosis of narcolepsy can be challenging since neurophysiological sleep studies are not 100% accurate for narcolepsy. Furthermore, lumbar puncture to measure hypocretin level (HCRT) is an invasive procedure that some patients refuse to undergo. Patient-reported outcomes or questionnaires to measure narcolepsy symptoms or tools to help in diagnostics remain scarce. The Ullanlinna Narcolepsy Scale (UNS) was developed for population screening for narcolepsy, but the structure of the questionnaire could allow its use in the clinical population as well. In Study IV, we wanted to validate the UNS in diagnostics of narcolepsy.

Methods: Study I is a comprehensive systematic review and meta-analysis of the risk of pNC. In Study II, PSG and ACT characteristics of 69 pNC and 57 sNC subjects were analyzed. In Study III, 26 pNC patients completed the modified Basic Nordic Sleep Questionnaire near onset of the disease and at the follow-up at least two years later. We specifically analyzed the results from UNS, Epworth Sleepiness Scale (ESS), Rimon's Brief Depression Scale (RDS), and WHO-5 Well-Being Index. Follow-up results were compared with 25 subjects with sNC. In Study IV, we reviewed sleep questionnaires of 89 patients with narcolepsy type 1 (NT1), 10 with narcolepsy type 2 (NT2), 37 with sleep apnea, 56 with restless legs syndrome or periodic limb movement disorder, 51 with other sleep-related disorders, and 24 with other hypersomnias (Kleine-Levin syndrome, idiopathic hypersomnia, or hypersomnia not otherwise specified).

Results: The relative risk of narcolepsy was increased 5- to 14-fold in children and adolescents and 2- to 7-fold in adults in the countries where Pandemrix vaccine was used widely (Finland, Sweden, Norway, France, England, Ireland) or in certain age groups (< 5 years, in the Netherlands). The vaccine-attributable risk in children and adolescents was 1 per 18,400 vaccines. Studies from Finland and Sweden suggest that the risk was increased two years after the vaccination, but this result needs to be interpreted with caution because of possible biases.

Patients with pNC had shorter diagnostic delays, were diagnosed younger, had lower periodic limb movement index during sleep, and had earlier sleep-wake rhythm than sNC patients, but otherwise there were no significant differences in ACT and PSG parameters between the patient groups.

In pNC patients in Study III, RDS points decreased significantly, indicating less symptoms of depression (mean (M) difference -3.5, 95% confidence interval (CI) [-5.5, -1.3], P = .003). At follow-up, the median of body mass index increased from 20.8 kgm^{-2} to 23.4 kgm^{-2} (P < .001). There were no significant differences in other sleep scores. However, variation in questionnaire scores at follow-up was wide. pNC subjects with very low or undetectable HCRT had higher scores in UNS and ESS than those with HCRT between 20 and 110 pg/mL (UNS M = 24.4, 95% CI 20.4, 28.4 vs. 18.8, 95% CI 15.0, 22.5, P = .048; ESS M = 17.2, 95% CI 14.4, 20.0 vs. 13.1, 95% CI 11.4, 14.9, P = .040). The most disabling symptoms were excessive daytime sleepiness and fragmented nocturnal sleep. At the follow-up, there were no significant differences between the scores in pNC and sNC.

In Study IV, UNS score in NT1 (M = 22.0, 95% CI 20.4, 23.6, range 9-43) was higher than in other disorders, including NT2 (M = 13.7, 95% CI 10.3, 17.1, P = .0013). Sensitivity and specificity of the UNS in separating NT1 from other disorders were 83.5-85.4% and 84.1-87.6%, respectively (cut-off point 14). Positive and negative predictive values were 77.6% and 92.3%, respectively. The UNS had a strong negative correlation with hypocretin-1 levels (r_s = -.564, P < .001) and mean sleep latency in MSLT (r_s = -.608, P < .001).

Conclusions: The risk of narcolepsy was clearly increased after immunization with Pandemrix vaccine especially in children and adolescents, but to a lesser degree also in adults. The risk was associated only with Pandemrix, not with any other vaccine. The clinical, PSG, and ACT characteristics of pNC and sNC are similar, implying that pNC is probably not its own disease entity, instead being the same disease as sNC. The clinical evolution and severity of symptoms in pNC are highly variable. Even though there seems to be correlation between UNS scores and hypocretin levels in cerebrospinal fluid, the degree of hypocretin deficiency hardly explains all of the variation in the clinical phenotype. The UNS is a feasible tool in the diagnostic procedure of narcolepsy. The cut-off point of 14 predicts narcolepsy well. If UNS score is below nine, narcolepsy is very unlikely.

TIIVISTELMÄ

Tavoitteet: Talven 2009-2010 H1N1 influenssapandemian rokotekampanjan jälkeen ilmeni huomattavan paljon uusia narkolepsiatapauksia maissa, joissa käytettiin AS03-adjuvantilla varustettua Pandemrix-rokotetta. Toisaalta lisääntyneet tautitapaukset matalan rokotekattavuuden Kiinassa, havainnoiviin tutkimuksiin liittyvä harhan mahdollisuus sekä eläinkokeissa todettu H1N1-viruksen mahdollinen kyky aiheuttaa narkolepsian kaltainen oirekuva aiheuttivat jonkin verran hämmennystä varsinaisesta rokotteen roolista narkolepsian synnyssä. Ensimmäisen tutkimuksen tavoitteenamme olikin analysoida systemaattisesti rokotenarkolepsian riskin suuruutta ja tutkia liittyikö muihin rokotteisiin tai influenssavirusinfektioon vastaavanlaista riskiä.

H1N1-rokotteen jälkeiseen narkolepsiaan (pNC) sairastuneiden sairauden alku oli hyvin äkillinen ja voimakas, ja viive oireiden alusta diagnoosiin oli selvästi lyhyempi kuin aiemmissa tutkimuksissa on raportoitu (keskimääräinen viive on voinut olla jopa yli 10 vuotta). Sairastuneilla oli myös runsaasti psykiatrista oireilua. Näin ollen sairauden oirekuvan ja luonteen tarkempi tarkastelu oli tarpeen. Toisessa ja kolmannessa osatyössä tavoitteenamme oli tutkia rokotenarkolepsian kliinisneurofysiologisia löydöksiä, oirekuvaa ja sen kehittymistä.

Narkolepsian diagnostiikka voi myös olla haastavaa tutkimusmenetelmien epätarkkuuden, kajoavuuden (lannepisto) ja vaihtelevan oirekuvan vuoksi. Potilaslähtöisiä tulosmittareita (patient-reported outcome measures) tai kyselylomakkeita narkolepsian oirekuvan kartoittamiseksi ei kuitenkaan ei juuri ole. Ullanlinnan narkolepsia-asteikko (UNS) on vuonna 1994 julkaistu kysymyssarja narkolepsian seulomiseksi väestöstä epidemiologisia tutkimuksia varten, mutta kyselylomakkeen rakenne saattaisi sallia sen käytön myös kliinisessä työssä. Neljännessä tutkimuksessa tavoitteenamme oli validoida UNS myös kliinisessä potilasjoukossa.

Menetelmät: Tutkimus I on laaja-alainen, systemaattinen kirjallisuuskatsaus ja meta-analyysi rokotenarkolepsian riskistä. Tutkimuksessa II vertasimme 69 rokotenarkolepsiaa ja 57 tavanomaista narkolepsiaa sairastavien unipolygrafia- (PSG) ja aktigrafia-rekisteröintejä (ACT). Tutkimuksessa III tarkastelimme 26 rokotenarkolepsiaa sairastavan potilaan oireita kyselymittareilla, seurasimme oirekuvan kehittymistä kaksi vuotta myöhemmin ja vertasimme tuloksia tavanomaista narkolepsiaa sairastaviin. Tutkimuksessa IV kävimme läpi 89 tyypin 1 narkolepsiaa (NT1), 10 tyypin 2 narkolepsiaa (NT2), 24 muuta liikaunisuussairautta, 37 uniapneaa, 56 levottomia jalkoja ja 51 muuta unihäiriötä sairastavan henkilön unikyselylomakkeet.

Tulokset: Narkolepsian suhteellinen riski lisääntyi lapsilla 5-14- ja aikuisilla 2-7-kertaiseksi niissä maissa, joissa käytettiin Pandemrix-rokotetta laaja-alaisesti (Suomi, Ruotsi, Norja, Ranska, Englanti, Irlanti) tai tietyissä ikäryhmissä (Alankomaissa alle 5-vuotiailla). Rokotteeseen liittyvä riski lapsilla ja nuorilla oli 1 tautitapaus 18400 rokotettua kohden. Rokotteeseen liittyvä riski oli koholla 2 vuotta rokotuksesta, joskin tähän tulokseen liittyy epävarmuustekijöitä.

Rokotenarkolepsiaan sairastuneilla diagnostinen viive oli lyhyempi ja diagnoosi saatiin nuorempana tavanomaiseen (sporadiseen, sNC) narkolepsiaan verrattuna. Heillä oli myös tavanomaista vähemmän yöllisiä jaksottaisia raajaliikkeitä ja aikaisempi vuorokausirytmi, mutta muutoin erot PSG:ssä ja ACT:ssa olivat vähäisiä.

Tutkimuksessa III Lyhyen kartoittavan depressioasteikon pisteet laskivat merkitsevästi seuranta-aikana (keskiarvojen (ka) ero -3.5, 95% luottamusväli (lv) -5.5, 1.3, P = .003) viitaten vähäisempiin masennusoireisiin. Painoindeksin mediaani nousi 20.8 kgm^{-2}:sta 23.4 kgm^{-2}:ään, P < .001). Muissa tuloksissa ei ollut merkitseviä eroja, mutta tulosten vaihtelu oli suurta. Niillä tutkittavilla, joilla selkäydinnesteen hypokretiini-tasot olivat mittaamattomissa tai hyvin matalat (< 20 pg/ml) UNS:n ja Epworthin uneliaisuusasteikon (ESS) pisteet olivat korkeampia kuin niillä, joilla ne olivat 20-110 pg/ml (UNS ka = 24.4, 95% lv 20.4, 28.4 vs. 18.8, 95% lv 15.0, 22.5, P = .048; ESS ka =17.2, 95% lv 14.4, 20.0 vs. 13.1, 95% lv 11.4, 14.9, P = .040). Haittaavimmat oireet olivat päiväväsymys ja rikkonainen yöuni. Seurantakäynnillä ei havaittu eroja pNC:n ja sNC:n välillä.

Tutkimuksessa IV UNS-pisteet olivat NT1:ssa selvästi korkeammat kuin muissa ryhmissä (ka = 22.0, 95% lv 20.4, 23.6, R 9-43) ml. NT2 (ka = 13.7, 95% lv 10.3, 17.1, P = .0013). UNS:n herkkyys muiden sairauksien erottamisessa oli 83.5-85.4% ja tarkkuus 84.1-87.6%. UNS:lla oli voimakas negatiivinen korrelaatio hypokretiinitasoihin (r_s = -.564, P < .001) ja univiiveeseen nukahtamisviivetutkimuksessa (r_s = -.608, P < .001).

Johtopäätökset: Tyypin 1 narkolepsian riski lisääntyi selvästi Pandemrix-rokotteen jälkeen erityisesti lapsilla ja nuorilla, mutta lievemmissä määrin myös aikuisilla. Riski liittyi vain Pandemrix-rokotteeseen, ei muihin rokotteisiin tai influenssavirukseen. Rokotenarkolepsian kliinisneurofysiologisten tutkimusten tulokset ja kliininen oirekuva ovat tavanomaista narkolepsiaa vastaavat, joten tautien taustamekanismit ovat todennäköisesti samankaltaisia. Vaikka oirekuvan ja hypokretiinitasojen välillä näyttäisi olevan yhteys, hypokretiini-vajeen voimakkuus ei täysin selitä vaihtelevaa oirekuvaa sairastuneiden välillä. Ullanlinnan narkolepsia-asteikko on käyttökelpoinen menetelmä myös kliinisessä työssä. Katkaisupistemäärä 14 ennustaa hyvin narkolepsiaa. Tutkittavilla, joilla UNS-pisteet ovat alle yhdeksän, narkolepsian todennäköisyys on erittäin pieni.

CONTENTS

CORRIGENDUM

Corrigendum to Sarkanen, T (2019). "Narcolepsy: clinical picture, diagnostics, and association with H1N1 vaccination". Doctoral dissertation.

The following four references are missing from the reference list and their reference numbers in the text are erroneous. On page 11, reference number 89 should be 88a. On page 13, second paragraph, reference numbers 99, 100, and 101 should be 96a, 96b, and 96c, correspondingly.

88a. Luo G, Ambati A, Lin L, Bonvalet M, Partinen M, Ji X, Maecker HT, Mignot EJ-M. Autoimmunity to hypocretin and molecular mimicry to flu in type 1 narcolepsy. Proceedings of the National Academy of Sciences. 2018;115:E12323-E12332.

96a. Ahmed SS, Volkmuth W, Duca J, Corti L, Pallaoro M, Pezzicoli A, Karle A, Rigat F, Rappuoli R, Narasimhan V, Julkunen I, Vuorela A, Vaarala O, Nohynek H, Pasini FL, Montomoli E, Trombetta C, Adams CM, Rothbard J, Steinman L. 2015. Antibodies to influenza nucleoprotein cross-react with human hypocretin receptor 2. Sci Transl Med. 2015;7:294ra105

96b. Luo G, Lin L, Jacob L, Bonvalet M, Ambati A, Plazzi G, Pizza F, Leib R, Adams CM, Partinen M, Mignot EJ-M. Absence of anti-hypocretin receptor 2 autoantibodies in post Pandemrix narcolepsy cases. PLoS One. 2017;12:e0187305.

96c. Giannoccaro MP, Waters P, Pizza F, Liguori R, Plazzi G, Vincent A. Antibodies Against Hypocretin Receptor 2 Are Rare in Narcolepsy. Sleep. 2017;40.

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications:

I   Sarkanen T, Alakuijala A, Dauvilliers Y, Partinen M. Incidence of narcolepsy after H1N1 influenza and vaccinations: Systematic review and meta-analysis. Sleep Med Rev 2018;38:177-186.

II   Alakuijala A, Sarkanen T, Partinen M. Polysomnographic and actigraphic characteristics of patients with H1N1-vaccine-related and sporadic narcolepsy. Sleep Med 2015;16:39-44.

III   Sarkanen T, Alakuijala A, Partinen M. Clinical course of H1N1-vaccine-related narcolepsy. Sleep Med 2016;19:17-22.

IV   Sarkanen T, Alakuijala A, Partinen M. Ullanlinna Narcolepsy Scale in diagnosis of narcolepsy. Sleep. 2019;42:zsy238.

The publications are referred to in the text by their Roman numerals and have been presented here with the permission of their copyright holders. In addition, some unpublished material is presented.

ABBREVIATIONS

ACT	Actigraphy
ADHD	Attention-deficit/hyperactivity disorder
AHI	Apnea-hypopnea index
ANSM	Agence Nationale de Sécurité des Médicaments et des Produits de Santé
BCC	Brighton Collaboration criteria
BMI	Body mass index
BNSQ	Basic Nordic Sleep Questionnaire
CC	case control
CI	Confidence interval
CNS	Central nervous system
CPL	Cataplexy
CPL/week	Cataplectic attacks per week
CSF	Cerebrospinal fluid
CTSH	Cathepsin H
DCSAD	Diagnostic Classification of Sleep and Arousal Disorders
DP1	Prostaglandin D2 receptor 1
ECDC	European Centre for Disease Control and Prevention
EDS	Excessive daytime sleepiness
ES	Effect size
ESS	Epworth Sleepiness Scale
FRI	Movement and fragmentation index
Hcrt2	Hypocretin receptor 2 gene
HLA	Human Leukocyte Antigen
HPSC	Health Protection Surveillance Centre, Ireland
HS	Hypersomnia
HSCVRC	Helsinki Sleep Clinic, Vitalmed Research Center, Helsinki, Finland
ICSD-1	International Classification of Sleep Disorders, 1st version
ICSD-2	International Classification of Sleep Disorders, 2nd revision
ICSD-3	International Classification of Sleep Disorders, 3rd revision
IVIG	Intravenous immunoglobulin
L5	Lowest 5 [hours of activity]
LPT	Lateral pontine tectum
M	Mean
M10	Maximal 10 [hours of activity]
mBNSQ	Modified Basic Nordic Sleep Questionnaire
MCH	Melanin-concentrating hormone

Mdn	Median
MeSH	Medical Subject Heading (US National Library of Medicine, NIH)
MPA	Medical Products Agency, Sweden
MSL	Mean sleep latency
MSLT	Multiple sleep latency test
MWT	Maintenance of wakefulness test
N/A	Not applicable
NA	Not available
NEI/ αMSH	Glutamic acid–isoleucine/α-melanocyte-stimulating hormone
NPV	Negative predictive value
NREM	Non-rapid eye movement
NT1	Narcolepsy type 1
NT2	Narcolepsy type 2
NR	Not reported
NS	Non-significant
OR	Odds ratio
OSA	Obstructive sleep apnea
OSRD	Other sleep-related disorders
OX40L	Tumor necrosis factor (ligand) superfamily member 4
P2RY11	Purinergic receptor subtype $P2RY_{11}$
PLMD	Periodic limb movement disorder
PLMS	Periodic limb movements in sleep
PLMSI	Periodic limb movements in sleep index
pNC	H1N1-vaccine-associated (Pandemrix) narcolepsy
pNT1	H1N1-vaccine-associated (Pandemrix) narcolepsy type 1
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PROM	Patient-reported outcome measure
PPV	Positive predictive value
PSG	Polysomnography
RBD	Rapid eye movement sleep behavior disorder
RC	Register cohort
RDS	Rimon's Brief Depression Scale
REM	Rapid eye movement
RLS	Restless legs syndrome
ROC	Receiver operating characteristic
RR	Relative risk
S-CCS	Self-controlled case series
SD	Standard deviation
SLD	Sublaterodorsal nucleus
sNC	Non-H1N1-vaccine-related (sporadic) narcolepsy

SNS	Swiss Narcolepsy Scale
sNT1	Non-H1N1-vaccine-related (sporadic) narcolepsy type 1
SOREMP	Sleep onset rapid eye movement sleep period
TCRA	T-cell receptor alpha locus
THL	National Institute for Health and Welfare, Finland
TRIB2	Anti-Tribbles homolog 2
UNS	Ullanlinna Narcolepsy Scale
VAESCO	Vaccine Adverse Event Surveillance & Communication
vlPAG	Ventrolateral periaqueductal gray matter
WHO5	5-item World Health Organization Well-Being Scale

1 INTRODUCTION

Narcolepsy is a fascinating, yet for the individual patient usually a highly disabling disease.[1] It is a rare central disorder of hypersomnolence that opens a window into understanding sleep, sleep disorders, the underlying neurobiology, and neuroimmunology.[2-4]

In August 2010, reports from Finland and Sweden indicated that there was an unprecedented increase in the number of new narcolepsy cases.[5,6] The first cases encountered were linked to the 2009-2010 pandemic H1N1 vaccination campaign by Professor Markku Partinen at the Helsinki Sleep Clinic in spring 2010. Afterwards, a similar increase was observed in numerous studies from countries where the AS03-adjuvanted Pandemrix vaccine was used.[7-12] The studies were, however, criticized for possible biases such as confounding by natural H1N1 infection.[13-16] Interestingly, an increased incidence of narcolepsy was seen in 2010 also in the Beijing area in China where vaccine coverage was very low.[17] In addition, in Quebec, Canada, where another, almost identical AS03-adjuvanted vaccine Arepanrix was used, an increase in the risk of narcolepsy was minimal if even noticeable.[18] There is some limited evidence from translational studies that H1N1 virus infection per se could target the hypothalamic hypocretin-producing neurons crucial for the development of narcolepsy syndrome.[19,20] Systematic evaluation of all available data from published studies, articles, and reports with an estimation of the magnitude of the risk is needed.

Previously, the diagnostic delay of sporadic narcolepsy had been rather long, over 10 years in some studies.[21,22] By contrast, Many H1N1-vaccine-associated narcolepsy (pNC) cases had a very abrupt onset and a short diagnostic delay even before the heightened awareness of the disease.[23,24] Psychiatric comorbidity seemed to be very common as well. This raised multiple questions such as could these patients have a more severe form of narcolepsy or could their symptoms be caused by e.g. autoimmune encephalitis or other more extensive neuronal injury?

Finally, methods for diagnosing narcolepsy are far from perfect. Taking a proper clinical history is indispensable, but available neurophysiological sleep recordings are not 100% sensitive or specific for narcolepsy.[25,26] Lumbar puncture provides the highest degree of accuracy for narcolepsy diagnosis, but it is an invasive procedure that not all patients are eager to undergo. If properly validated, patient-reported outcome measures, such as screening and diagnostic questionnaires, could help clinicians in interpreting the reliability of these studies and assessing quantitatively a priori probability of a positive finding. This is an important aspect for both an individual patient to get the right diagnosis and researchers in epidemiological studies to correctly recognize true cases. The Ullanlinna Narcolepsy Scale (UNS)

is a tool developed for screening of narcolepsy in the general population that was published already in 1994.[27] However, its psychometric properties could allow its use in complementing clinical interview in the diagnostics of narcolepsy syndrome. These kinds of validation studies have, however, been lacking.

In this thesis, we sought to fill the gaps in the knowledge of these previously mentioned issues. First, we conducted a comprehensive systematic review and meta-analysis of all the available literature, including published articles, healthcare official reports, and other documents, on the incidence of narcolepsy after the pandemic H1N1 vaccination campaign with different vaccines and the relationship with the H1N1 virus itself. Second, we analyzed thoroughly the clinical, polysomnography (PSG), and actigraphy (ACT) data of pNC and non-vaccine-related, sporadic narcolepsy (sNC), and analyzed the differences between the two groups. In addition, the clinical course of pNC was followed in a prospective follow-up study. Third, we validated the UNS also in the diagnostic process of narcolepsy in the clinic and compared its performance with the Epworth Sleepiness Scale (ESS) and the Swiss Narcolepsy Scale (SNS).

The beginning of the thesis is committed to a literature review of the history, phenotype, diagnostics, and epidemiology of narcolepsy. It is followed by a description of methods, results, and discussion for each study. Final conclusions are then presented in the summary section.

2 REVIEW OF THE LITERATURE

2.1 History of narcolepsy

Narcolepsy is a relatively young disease with regard to written descriptions and research in the field. The first clinical reports of patients suffering from irresistible sleep attacks accompanied by other typical symptoms of narcolepsy date back to the end of the 19th century. The importance of the hypothalamus in regulation of sleep was discovered already in the 1920s, but it was not until the change in the millennia and the finding of the hypocretin/orexin neuropeptide family that the biochemical basis for narcolepsy was identified. After the H1N1 pandemic in 2010, the scientific community has made great strides in understanding of the pathophysiological mechanism of narcolepsy. However, we are still far from finding a cure for the disease, i.e. replacement therapy for hypocretin loss. Nor do we have the means to prevent occurrence of the disease or halt disease progression after it has been triggered.

2.1.1 First clinical descriptions of narcolepsy

Karl Friedrich Otto Westphal (1833-1890) was a German professor of psychiatry who contributed widely to Western medicine (Figure 1). He was active especially in research of nervous system disorders, being the first to describe e.g. agoraphobia and tabes dorsalis. In 1877, Westphal presented two cases with irresistible sleep attacks accompanied by sudden spells where the patient lost muscle tone but not consciousness.[28,29] This is considered the first clinical description of narcolepsy syndrome, although there are earlier case reports of irresistible sleep attacks by e.g. Willis from the 17th century, but it is uncertain whether these are true narcolepsy cases or a consequence of some other disease.[29] In 1880, French Jean Baptiste Edouard Gélineau (1828-1906) published a report titled "De la narcolepsie" on a 38-year-old man with frequent attacks where he seemed to fall asleep even at Dr. Gélineau's office (Figure 2).[29,30] These attacks were also provoked by emotional triggers. Dr. Gélineau's patient, however, had restful nighttime sleep, which in light of current knowledge is uncommon in narcolepsy and also contradictory to the cases reported by Professor Westphal. In addition, actual sleep attacks in narcolepsy are usually not triggered by emotions (unlike cataplexy), and during cataplexy, patients usually do not fall asleep, although they may seem drowsy. However, Gélineau was the first to suggest the term "*narcolepsie*", derived from the Latinized forms of originally Greek words *narke-* (meaning stupor or numbness) and *-lepsis* (a seizure or an attack).

Figure 1. Karl F. O. Westphal (1833-1890)[a]

D[r] JEAN-BAPTISTE-ÉDOUARD GÉLINEAU
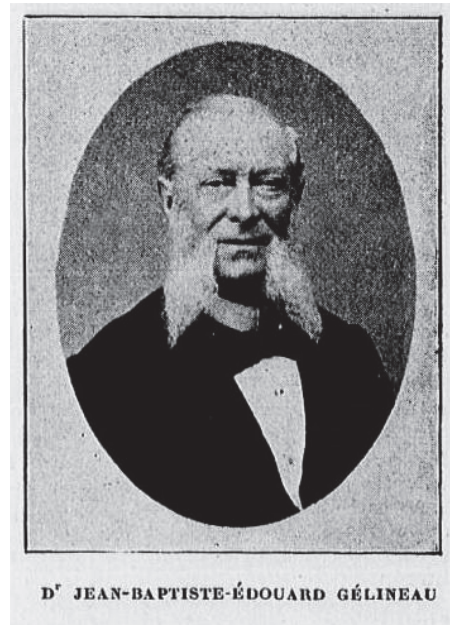
Figure 2. Jean Baptiste Edouard Gélineau (1828-1906)[b]

The term "cataplexy" for a cataplectic attack as it is known today was coined by Leopold Löwenfeld in 1902.[31] It originates from a Greek word *kataplexis, kata-* meaning "down" and *-plexis* or *-plexy* meaning "to strike". During the following decades accumulating case reports and case series of narcoleptic patients were published, which eventually led to William John Adie's conclusion of narcolepsy as its own disease, *sui generis*, without attribution to epilepsy or any other neurological disease.[32] Adie also suggested that the word narcolepsy should be reserved only for the narcolepsy syndrome, whether idiopathic or secondary, not to describe sudden sleepiness in general. Hypnagogic (during the transition from wake to sleep) and hypnopompic (during the transition from sleep to wake) hallucinations, ocular symptoms (ptosis, diplopia), amnesic states, disturbed nocturnal sleep, vivid dreaming, and weight gain were described by e.g. Daniels in his comprehensive review.[33] Based on these earlier findings, Yoss and Daly defined the classic full tetrad of narcolepsy, including cataplexy, sleepiness, hypnagogic hallucinations, and sleep paralysis, in 1957.[34]

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a   Retrieved from http://www.sammlungen.hu-berlin.de/dokumente/13677/ 16.10.2018. Public Domain.

b   Retrieved from https://en.wikipedia.org/wiki/Jean-Baptiste-%C3%89douard_G%C3%A9lineau. 16.10.2018. Public Domain.

History of narcolepsy research in Finland can be divided into three different time periods. First, there are only a few published reports before the 1990s; these have focused mainly on case descriptions or anti-cataplectic medication trials with e.g. imipramine.[35-37] In the 1990s, Christer Hublin, Markku Partinen, and coworkers conducted seminal work, especially on the epidemiology and familial aspects of narcolepsy utilizing, for instance, Finnish Twin Cohorts.[27,38,39] They also studied the effect of selegiline on the treatment of narcolepsy.[40] The third period of intensive research started after the H1N1-vaccine-associated cases in the 2010s.

2.1.2 Encephalitis lethargica and regulation of sleep

After the First World War in 1917, Viennese neurologist Baron Constantin von Economo described a severe epidemic encephalitis that impacted tens of thousands of people worldwide over the following 10 years.[41,42] He named the disease "encephalitis lethargica" and recognized three different phenotypes: somnolent-opthalmoplegic, hyperkinetic, and amyostatic-akinetic syndromes. The latter was investigated and treated with L-dopa in the 1960s by, among others, Olivier Sacks, who described his findings in the book "Awakenings". Based on thorough clinical and pathological studies of these subjects, von Economo localized the disease to the posterior hypothalamus and suggested it accurately also as the origin of narcolepsy (Figure 2.3).[43] Intriguingly, the severe H1N1 influenza pandemic ("Spanish flu") raged almost concurrently or before the peak of epidemic encephalitis in 1918-1919.[44] However, the first descriptions of encephalitis lethargica, before the massive increase in the number of cases, were recorded already in 1916, before the Spanish flu.

Rapid eye movement (REM) sleep was discovered in the 1950s, and its involvement in narcolepsy and cataplexy was established soon after.[45,46] The following development of the Multiple Sleep Latency Test (MSLT) to detect sleep latency and REM sleep latency in order to diagnose narcolepsy led to the clinical use of the test in the 1970s. Interestingly, already in the first reports a mean sleep latency (MSL) shorter than 5 minutes was considered pathological, while the current diagnostic criterion for narcolepsy is 8 minutes.[47,48]

Canine narcolepsy was found in more than 10 different breeds in the 1970s. Consequently, an animal model of heritable narcolepsy in Dobermans and Labradors was developed at the Stanford Narcolepsy Center.[49] The phenotype of narcolepsy in dogs strikingly resembles human narcolepsy with cataplexy and short sleep latency. Later, in 1999, the pedigree analysis and positive cloning of the canine narcolepsy gene linked the disease to a mutation in a single recessive *hypocretin/orexin receptor 2* gene (Hcrt2).[49,50] Chemelli and coworkers reported almost simultaneously a similar phenotype with REM sleep dysregulation in hypocretin knockout mice.[51] Novel neuropeptides, hypocretins/orexins, were thus associated with sleep regulation and narcolepsy syndrome for the first time.
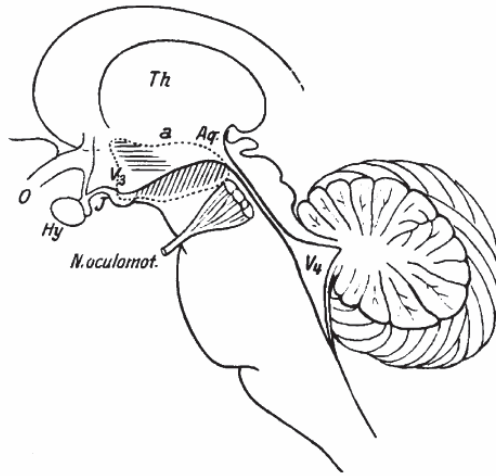
Figure 3. An illustration by von Economo of the hypothalamic area involved in regulation of sleep-wake control (dotted line). Aq, aqueduct; Hy, hypophysis; J, infundibulum; O, optic chiasm; Th, optical thalamus; V3, third ventricle; V4, fourth ventricle.[c]

2.1.3 Discovery of hypocretins/orexins

Hypocretins/orexins are small neuropeptides that were discovered almost concurrently by two different research groups in 1998. Luis de Lecea and coworkers found two molecules derived from the same precursor (preprohypocretin) and expressed in the hypothalamus that resembled structurally secretin, therefore naming these molecules hypocretin 1 and 2.[52] They had actually published a paper in 1996 in which they detailed finding a hypothalamus-specific neuropeptide using in situ hybridization, but did not yet assign the name hypocretin.[53] Takeshi Sakurai's group made a similar finding in mice, but focused also on the food consumption and feeding behavior modulating effects of the neuropeptides, naming the same molecules orexin-A and -B.[53] The two names are still used interchangeably. As the studies on which this thesis is based use the term hypocretin, this term is used throughout the thesis. The role of hypocretin was initially thought to be mainly in energy homeostasis. As mentioned earlier, Chemelli and coworkers showed in their extensive work that hypocretin knockout mice exhibited behavior similar to humans with narcolepsy such as sleep attacks and hypersomnolence.[51] They also observed fragmented sleep and REM onset sleep periods in these rodents. Moreover, they found that modafinil, a drug used to treat excessive daytime sleepiness, activated

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c   Shared under Creative Commons license. Originally published in von Economo C. Sleep as a problem of localization. The Journal of Nervous and Mental Disease. 1930; 71: 249-259

the orexin neurons.[51] They proposed that the hypocretins participated in sleep/wake regulation and were involved in the pathogenesis of narcolepsy causing REM sleep dysregulation. Involvement of hypocretin in the pathogenesis of narcolepsy was confirmed in histopathological studies where distinctive loss of orexin-producing neurons in the perifornical area of the hypothalamus was seen.[54,55] At the same time, decreased or undetectable levels of orexin-A levels in cerebrospinal fluid (CSF) of narcoleptic patients were reported.[56]
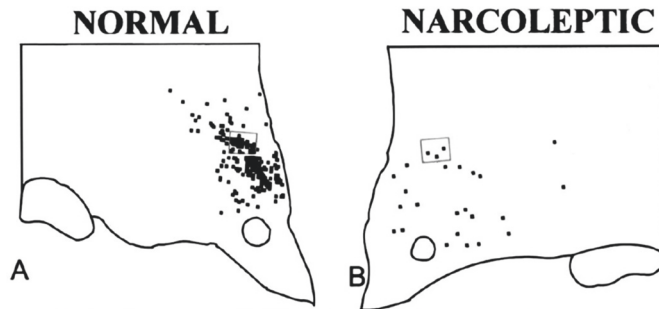
Figure 4. Distribution of orexin neurons in perifornical and dorsomedial regions of normal and narcoleptic humans.[d]

2.1.4 Early genetic studies

Despite the Hcrt2 gene finding in canine narcolepsy, familial narcolepsy in humans is rather rare and genetic mutations do not explain the vast majority of cases. A tight association between Human Leukocyte Antigen (HLA) class II antigen DR2 and narcolepsy in the Japanese was reported by Honda and coworkers in 1982.[57] In their landmark study, Honda's group serologically typed 40 Japanese narcolepsy patients for HLA DR2, all of whom were positive for the antigen, while in the randomly selected control sample the prevalence was 49%. The association was soon confirmed in other ethnic groups.[57-63] The observed HLA association with narcolepsy was stronger than with other diseases, a finding that has not changed in the decades to follow.

In African Americans, the association between DR2 haplotype and narcolepsy was lower, around 60%, which implied that in this population some other genetic interactions contribute to the narcolepsy risk.[64] Developing high-resolution sequencing techniques revealed that in European and Japanese populations DR2

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d   Adapted with permission from Thannickal et al. Reduced number of hypocretin neurons in human narcolepsy. Neuron. 2000 27:469-74.

consisted of DQB1*06:02 allele occurring in tight linkage disequilibrium with DQA1*01:02 and DRB1*15:01 (the alleles almost invariably coexist in a person). However, in African Americans, DRB1 alleles linked with DQB1*06:02 were more variable, and almost 30% express DR2 without DQB1*06:02. Work by Matsuki and the Stanford research group led to a finding that DQB1*06:02 allele was the most specific marker for narcolepsy across the different ethnic groups.[65] The HLA DQB1*06:02 allele, unsurprisingly, turned out to be a better and more accurate marker in narcolepsy than DR2 also in Caucasian and Japanese populations.[66-68] Currently, it is known that nearly 100% of patients with narcolepsy and cataplexy are positive for DQB1*06:02, while the prevalence of the allele in the general population is around 12-38%.[67]

2.1.5 Emergence of the autoimmune hypothesis

The main hypothesis in development of an autoimmune disease is an environmental immunological attack that acts as a trigger in a genetically predisposed individual. HLA molecules have been associated with various autoimmune diseases such as multiple sclerosis, rheumatoid arthritis, and spondyloarthritis. The strong association of narcolepsy with a certain HLA type led to supposition that narcolepsy would also have an autoimmune background already in the 1980s. Other circumstantial evidence of autoimmunity in narcolepsy was found after the HLA association. For instance, during the past decade, polymorphisms in other immune system-related genes, especially in the T-cell receptor alpha locus, have been associated with narcolepsy.[69] Age of onset of narcolepsy in the 2nd or 3rd decade of life is also similar to other autoimmune diseases.[70] Moreover, studies in monozygotic twins pointed more to an environmental trigger than to a direct genetic cause since the disease concordance was only 20-35%.[71]

The loss of hypocretin-producing neurons is also highly selective with e.g. intermingled melanin-concentrating hormone (MCH) spared, which implies also a very specific disturbance or attack that would be best explained by an immunological mechanism.[54]

A few reports of possible environmental triggers, including streptococcal infections, flu infections, smoking, and toxins, were also published, but the year 2010 turned a new page in narcolepsy research.[72-75] After the H1N1 pandemic and the related vaccination campaign, the incidence of narcolepsy increased remarkably, but fortunately temporarily, in countries where the Pandemrix vaccination was used. The first reports came from Finland and Sweden, followed by Norway, France, England, and Ireland.[6,7,9,10,12,23] At least in part, the outbreak of narcolepsy after the H1N1 pandemic resembled the emergence of encephalitis lethargica after the earlier H1N1 pandemic in 1918, although the two diseases are completely different.

While being a terrible tragedy for the children and families involved, the outbreak started a new chapter in narcolepsy research, which is discussed in the next sections.

2.2 Etiology of narcolepsy

Before delving deeper into the etiology of narcolepsy, two important clarifications must be made. Firstly, narcolepsy is divided into two main subcategories, narcolepsy type 1 (NT1) and narcolepsy type 2 (NT2). NT1 and NT2 are fundamentally different diseases, even though they share the same core symptoms of excessive daytime sleepiness (EDS). NT1 is usually accompanied by cataplexy, which is absent in NT2, but, more importantly, the hypocretin loss is characteristic only for NT1. Secondly, when speaking about the etiology of narcolepsy, it is commonly considered as the etiology of destruction of hypocretin-producing neurons, and therefore, the etiology of NT1.

2.2.1 HLA association

The close association with HLA class II allele DQB1*06:02 implies an autoimmune background in the etiology of NT1, but specific mechanisms have remained elusive. Genome-wide association studies have linked polymorphisms in T cell receptor alpha (TCRA) locus and also in other genes associated with immune regulation such as cathepsin H (CTSH), tumor necrosis factor (ligand) superfamily member 4 (OX40L), and purinergic receptor subtype $P2RY_{11}$ (P2RY11) with narcolepsy.[69,76,77] P2RY11 is abundant in CD8^{+} T cells, where it possibly regulates their survival and function by modifying cell energy metabolism.[78] OX40L is, for example, involved in clonal expansion of CD4 and CD8 T cells and has been associated with two other autoimmune diseases as well, namely systemic lupus erythematosus and Sjögren syndrome.[79] OX40L is also expressed on antigen-presenting cells. Cathepsins that are highly expressed in CD8^{+} T cells, participate e.g. in apoptosis, neurodegeneration, cellular protein degradation, and loading of protein particles to HLA class II molecules.[80]

Carriers of DQB1*06:02 are at 251-fold increased risk for narcolepsy, while other HLA DQ alleles provide either protection against or susceptibility for the disease.[81] HLA DQB1*06:02 is in tight linkage disequilibrium with HLA DQB1*01:02 gene, occurring almost always together and producing a heterodimer molecule expressed on the surface of antigen-presenting cells (APCs). APCs introduce exogenous antigens through HLA class II to T cell receptors on CD4^{+} T cells which, in turn, activate naïve B cells e.g. through cytokines to secrete antibodies, and help in macrophage recruitment.[82] In contrast, cytotoxic CD8^{+} T cells do not bind to HLA

class II but to class I complex on the cell surface. CD8^{+} T cell activation is, however, regulated by the CD4^{+} helper T cells.

Interestingly, despite the intensive, multidisciplinary research, the exact mechanisms of HLA - disease interaction in narcolepsy remain unknown. Several hypotheses have been proposed, all of which are linked to antigen presentation by HLA molecules and aberrant immune response towards foreign or putative self-antigens.[82,83] These include incomplete tolerance in thymus, abnormal recruitment of autoreactive or regulatory T cells, promiscuous interaction of T cells with foreign or self-peptides, epitope stealing by one HLA molecule over another, or presentation of endogenous antigens by class II HLA molecules, which is usually conducted by HLA class I molecules.[82]

2.2.2 Autoimmune hypothesis

There are several theories on how hypocretin neurons are destroyed (Figure 2.5). These include molecular mimicry, local cytotoxic reaction mediated by autoantibodies or cytotoxic CD8^{+} T cells, and bystander activation. According to the molecular mimicry hypothesis, the T cell recognizes antigens from virus or bacteria (or vaccine particles) that are presented by HLA on APCs. An activated T cell then migrates to the brain where it mistakenly recognizes a hypocretin-producing cell and induces an autoimmune reaction.[3,4] However, neurons are thought not to express HLA class II molecules. T cells would, therefore, not be able to attach to the hypocretin neurons through the HLA complex. It has been speculated that T cells could interact with neurons through surface adhesion molecules.[84] Different expression of adhesion molecules could explain why only hypocretin neurons are destroyed and e.g. co-localized MCH neurons are spared.

Activated T cells could also activate autoreactive B cells. These would then recognize autoantigen presented by HLA class I molecules, which are expressed on all cells, also on hypocretin neurons. B cells could then trigger a local cytotoxic reaction. In addition, other agents, such as streptococcal infections, could function as superantigens, activating autoreactive T cells and modulating immune response during the antigen presentation of an actual antigen. Bystander activation is another proposed mechanism in which autoreactive T cells are activated as a result of more general immune response.[4]

However, recent evidence implies that cytotoxic CD8^{+} cells contribute significantly to the pathogenesis of narcolepsy. First, antigen-specific cytotoxic T cells are able to trigger destruction of hypocretin-producing neurons in a mouse model.[85] Second, infiltration of cytotoxic CD8^{+} T cells in the hypothalamus has been encountered in a post-mortem subject with anti-Ma2 encephalitis that causes symptomatic narcolepsy.[86] Third, autoreactive CD8^{+} T cells are encountered in some narcoleptics (see also Section 2.2.3).[87] In one such case, NT2 with normal

hypocretin levels but autoreactive cytotoxic CD8^{+} cells, conversion to NT1 and hypocretin deficiency were reported.[87]

2.2.3 Autoreactive T cells

A landmark study on autoreactive T cells in narcolepsy was published in Nature in September 2018.[87] In the study, the researchers used highly sensitive methods and detected hypocretin-specific CD4^{+} and CD8^{+} T cells in all 19 tested patients. TRIB2-specific T cells were also found, but both in narcoleptics (8 out of 13) and in healthy controls (8 out of 12). However, the proliferation response in narcoleptics was significantly higher. Interestingly, they also detected high levels of autoreactive CD4^{+} and CD8^{+} T cells in some NT2 subjects, one of whom actually developed cataplexy later. This could imply that there was already an ongoing autoimmune attack, but the destruction was not widespread enough to cause a full-blown NT1 phenotype. Consequently, this raises the question of whether there is a way to recognize these patients and perhaps a method to halt the autoimmune reaction by immunotherapy. Furthermore, screening for autoreactive T cells in a blood sample from subjects with suspicion of narcolepsy could have diagnostic value if the specificity for narcolepsy in future studies is sufficiently high.

This finding of autoreactive T cells was supported by the Stanford research group through a study published shortly after the Nature paper in December 2018.[89] They found strong T cell reactivity to an amidated C-terminal end of hypocretin-1 and -2 in conjunction with HLA DQB1*06:02 in NT1 subjects, which implies that these amidated ends of the hypocretin molecules are the major autoantigens in narcolepsy. They also discovered that NT1 is associated with increased reactivity to specific HA and NP peptides from the reassortant influenza virus strain used in Pandemrix. Of these, HA has homology with two hypocretin residue sequences, suggesting molecular mimicry in the disease process. Cross-reactivity between HA and the C-terminal end of hypocretin molecules could also be demonstrated in the study.
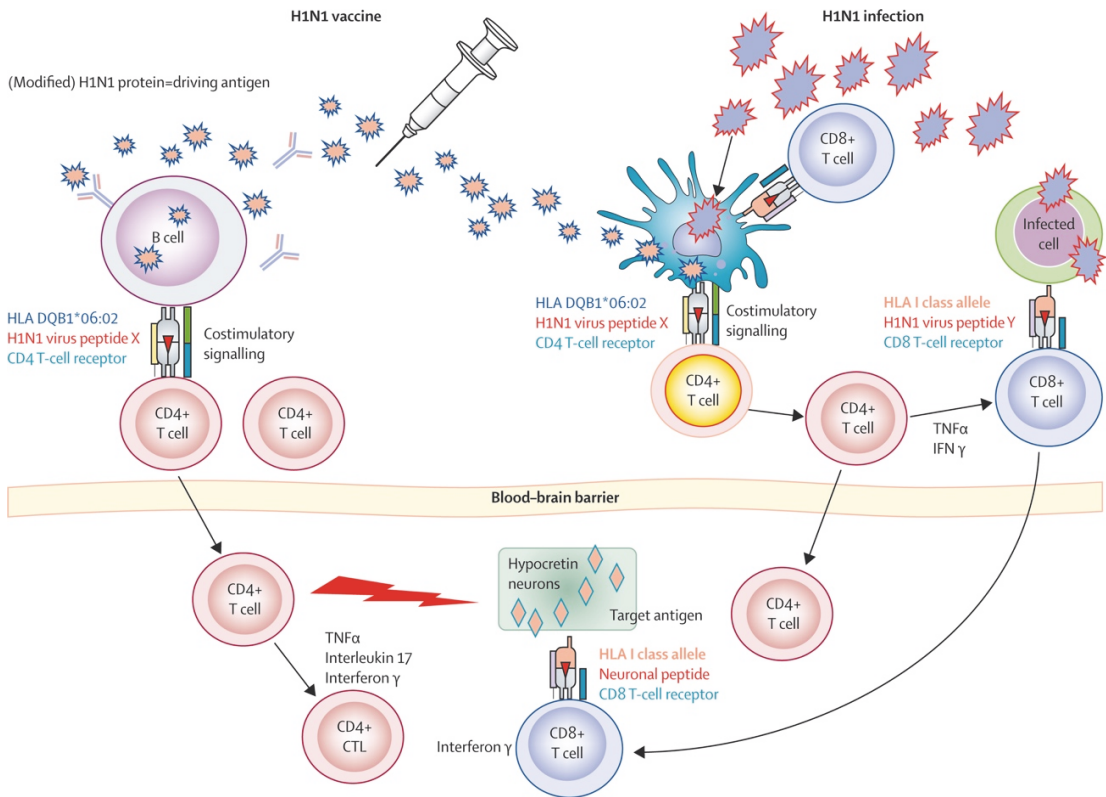
Figure 5. Hypothetical model of pathogenesis of narcolepsy caused by H1N1 vaccination or H1N1 infection. In this model published in 2014, Partinen and coworkers suggested that H1N1 peptide either from Pandemrix vaccine or H1N1 virus is presented in the HLA DQB1*06:02 complex on antigen-presenting cells to CD4+ T cells. These activated T cells could then either cross the blood-brain barrier and travel to the central nervous system (CNS) and hypothalamus or further activate cytotoxic CD8+ T cells, which could now also travel to the CNS. The destruction of hypocretin-producing neurons in the hypothalamus could be mediated by inflammatory cytokines secreted by CD4+ T cells or by cytotoxic CD8+ T cells.[e]

2.2.4 Autoantibodies

Autoantibodies involved in the pathogenesis of narcolepsy have been searched for since the association with HLA was established. The results have been limited, as described in an earlier editorial in Sleep.[88] Nonetheless, antibodies against anti-Tribbles homolog 2 (TRIB2) have been found in around 14% to 40% of narcolepsy patients, but they are not specific, as they are found also in other disorders and in healthy controls.[89-92] However, transfer of immunoglobulins from

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narcolepsy patients positive for TRIB2 antibodies causes a loss of hypothalamic hypocretin neurons and results in sleep disturbances when injected into mice.[93] Other antibodies, such as those against hypothalamic glutamic acid–isoleucine/ α-melanocyte-stimulating hormone (NEI/αMSH) neurons, GABAergic cortical interneurons, globus pallidus neurons, gangliosides, and prostaglandin D2 receptor DP1 (DP1), have been found in some narcolepsy patients, but their role in the disease process remains open.[91,94,95] It is unclear whether some of these are actually involved in the disease pathogenesis or whether they are merely side products of the neuron destruction.[88,96] DP1 antibodies are particularly interesting since prostaglandins play a key role in mediating immune response and cells expressing DP1 are linked to mast cell activation and histamine secretion (see Section 2.2.5).

Ahmed et al. found hypocretin receptor 2 antibodies in 85% of post-Pandemrix narcoleptic subjects compared with 35% in healthy controls.[99] They also showed that H1N1 influenza nucleoprotein A structurally resembles part of the hypocretin receptor 2, indicating a possibility for molecular mimicry. However, the autoimmune reaction in narcolepsy is likely towards hypocretin-producing cells, not hypocretin receptors. Moreover, the results could not be reproduced in two other comprehensive studies.[100,101]

We have screened narcolepsy patients for conventional antineuronal antibodies (antibodies against N-methyl-D-aspartate, gamma-aminobutyric acid B, AMPA, and glycine receptors, myelin, myelin-associated glycoprotein, aquaporin-4, contact-associated protein-like 2, amphiphysin, glutamic acid decarboxylase, and anti-Hu, anti-Ri, anti-Yo, anti-Tr, and anti-Ma/Ta antibodies) without any remarkable findings.[97]

2.2.5 Histamine

The role of histamine in narcolepsy is controversial, although it is the major promoter of wakefulness. The effect of hypocretin on wakefulness is mediated, among other routes, by histaminergic pathway. H1 receptor knockout mice, for example, do not gain a wake-promoting effect from hypocretin.[98] Post-mortem studies in human narcoleptics have shown a 64% to 93% increase in histaminergic neurons in the tuberomammillary region of the brain.[99,100] The increase of histaminergic neurons could be a compensatory mechanism for the reduced excitatory effect on histamine receptors. Low histamine levels in human narcolepsy have actually been observed, but the finding is not specific for narcolepsy since histamine levels are lowered in other conditions with sleepiness as well.[101-104] Human histamine H3 receptor inverse agonist pitolisant is an effective drug in narcolepsy.[105]

2.2.6 Vitamin D

Vitamin D has an important role in the regulation of both adaptive and innate immune systems.[106] Activated T and B cells express vitamin D receptors, as well as macrophages and dendrite cells in the brain. In addition, vitamin D affects HLA gene expression, especially HLA DRB1*15:01, which is in tight linkage disequilibrium with HLA DQB1*06:02.[107] Vitamin D could also be involved in the selection and escape of autoreactive T cells during their maturation in the thymus.[108] However, the evidence of the impact of vitamin D or vitamin D deficiency in the development of narcolepsy is limited and controversial. Carlander and coworkers reported previously that vitamin D deficiency is more common in narcolepsy patients than in controls, but they could not replicate the results in larger sample in crude comparison or when potential confounders (age, BMI, and season of blood sampling) were taken into account.[109,110] In these studies, the disease duration was 0 to over 50 years, in the latter on average 10.5 years. We do not have any information on the vitamin D levels at the onset of the symptoms of narcolepsy or during the process of development of autoimmunity leading to the destruction of hypocretin cells. For example, in multiple sclerosis it has been suggested that a maternal vitamin D deficiency during the first semester of pregnancy increases the risk of the disease in offspring.[111]

2.3 Neurobiology of narcolepsy and narcolepsy as a disorder of state dissociation

2.3.1 Neurobiology of sleepiness

The hypocretin neurons are highly active during active wakefulness. Activity decreases in quiet wakefulness and is the lowest in slow wave sleep and tonic REM sleep. Hypocretin neurons might fire in short bursts in the phasic phase and a the end of the REM sleep period.[112] Hypocretin neurons have an excitatory effect on wake-promoting and sleep-inhibiting systems, including noradrenergic, serotonergic, cholinergic, and histaminergic neurons in pontine nuclei and basal forebrain.[113]

GABAergic neurons in ventrolateral periaqueductal gray matter (vlPAG) and adjacent lateral pontine tectum (LPT) fire during NREM sleep to inhibit REM sleep. vlPAG neurons, in turn, are inhibited by neurons in the sublaterodorsal region (SLD) that fire during REM sleep. This interaction results in a model of a NREM-REM "flip-flop" switch that regulates transition between the states.[114] Here, hypocretin steps in. Hypocretin neurons inhibit REM sleep by activating REM-off neurons.[115]

Hypocretin deficiency in narcolepsy results in an impaired control of the switch between the different behavioral states, causing state instability and rapid transitions from one state to another.[116] Furthermore, the states can even intermingle, resulting in state dissociation. Accordingly, a distinctive characteristic for narcolepsy is that features and phenomena typical for REM sleep intrude into wakefulness causing unique and peculiar symptoms as described in Section 2.4. Lowered threshold to transition from wakefulness to sleep during daytime is probably responsible for excessive daytime sleepiness and sleep attacks encountered by narcoleptic patients. This threshold instability could account also for disturbed nocturnal sleep.

2.3.2 Neurobiology of cataplexy

Cataplexy and REM sleep share many common aspects, despite that during a cataplectic attack the person is awake. In REM sleep, similar muscle atonia is present in almost all muscles, except for those responsible for respiration and eye movements. This atonia is caused by inhibitory glycinergic spinal interneurons and neurons of the medial medulla, which, in turn, are under excitatory control from SLD near the locus coeruleus in the pons. During wakefulness this system is inhibited by vlPAG and other monoaminergic neurons.[117,118] As the hypocretinergic excitatory effect via other neurotransmitters on this system is impaired, the control of the inhibitory neurons further downstream of the regulatory pathway is lost, causing undesirable muscle atonia in wakefulness.[119,120] The neurotransmitter cascade leading to cataplexy would start in the amygdala where emotional trigger is processed and a signal transmitted to SLD neurons.[119,121] Conversely, this also causes excess muscle tone in REM sleep (REM sleep without atonia), leading to REM sleep behavior disorder.[119]

2.4   Clinical characteristics of narcolepsy

In the current 3rd revision of the International Classification of Sleep Disorders (ICSD-3),[122] narcolepsy is divided into two main subcategories: narcolepsy type 1 (NT1) and type 2 (NT2), as previously mentioned. The hallmark symptom of narcolepsy, regardless of the subtype, is excessive daytime sleepiness (EDS), defined as an irresistible need to sleep or lapses into sleep during the major waking episode of the day. This has to occur for at least three months. In young children, EDS may present only as excessively long nighttime sleep or as extensive napping. The main difference in NT1 and NT2 is the presence of cataplexy and hypocretin deficiency. In NT1, CSF hypocretin-1 levels are low (≤ 110 pg/mL or < 1/3 of mean values of healthy controls) or undetectable, while in NT2 hypocretin-1 levels are normal. If CSF hypocretin has not been measured, NT1 can be diagnosed if the subject

has cataplexy and positive MSLT for narcolepsy. In NT2 cataplexy is absent. The problem is that in hypocretin-deficient patients, cataplexy does not necessarily develop at the same time as EDS. Usually, it appears during the following months, but the delay can be years or even decades. These cases, whose CSF hypocretin has not been measured, are initially diagnosed as NT2, but should be reclassified as NT1 if cataplexy appears (and hypocretin is still not measured).

2.4.1 Excessive daytime sleepiness

Defining excessive sleepiness is not as easy a task as it may first seem. Is subjective complaint accompanied by a validated questionnaire adequate or do we need objective measures such as MSLT? This difficulty is reflected also in epidemiological studies of sleepiness, where the prevalence varies, based on definition and study population, somewhere between 2% and 50%, averaging around 20-25%.[123]

After a typical night, narcoleptic patients usually wake up at least somewhat refreshed unless the nocturnal sleep is very fragmented. During the day they start to feel sleepy after variable time has elapsed. Sleepiness is exaggerated after a meal, particularly during ingestion of carbohydrate-rich foods. Exercise and daytime naps might alleviate sleepiness for a certain amount of time. The threshold to fall asleep in monotonous situations is lowered, and some narcoleptics experience sleep attacks also in active situations such as eating and walking. Especially in children, sleepiness may manifest as full-day drowsiness, multiple napping, lengthened nighttime sleep, or paradoxical hyperactivity.[124]

2.4.2 Cataplexy

Cataplexy is a phenomenon of sudden loss of muscle tone triggered by emotions such as anger, excitement, or most commonly joy, possibly accompanied by laughter. When appearing regularly, it is considered almost pathognomonic for narcolepsy, but mild cataplectic features are sometimes seen in healthy individuals, e.g. when laughing heartily.[125,126]

The frequency and severity of cataplexy vary individually. Knee buckling and neck weakness causing a head drop are common features. Generalized cataplexy causing falls with inability to rise occurs less frequently. More subtle symptoms, such as ptosis (dropping of eyelids), tongue protrusion, and facial hypotonia, are common in children. Some patients experience cataplexy several times daily, while others may have cataplexy only weekly or monthly or even be in full remission with medication.

2.4.3 Other features

Disturbed nighttime sleep (DNS), characterized by fragmented sleep architecture and brief, frequent arousals and awakenings, is common in narcolepsy with a prevalence of 30% to 95% depending on the definition (Figure 6).[127] It is important to determine whether DNS in narcolepsy occurs as a secondary symptom caused by such disorders as periodic limb movements in sleep (PLMS) or obstructive sleep apnea (OSA). On the other hand, hypocretin deficiency could cause PLMS itself.[133] In patients with severe primary DNS caused by the narcolepsy syndrome itself, a treatment focused on improving quality of sleep might be the most efficient.
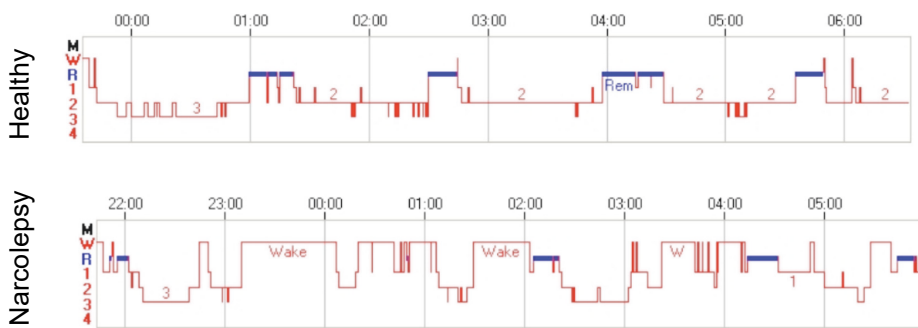
Figure 6. Hypnograms of a healthy individual and a narcoleptic patient. Notice the early REM sleep and the disturbed and fragmented sleep and wake periods of the narcoleptic patient. R, REM sleep; W, wake.

An association between **sleep paralysis (SP)** and narcolepsy was first recognized by Adie in 1926.[32] SPs are encountered in around 60-90% of narcoleptic patients, but are sometimes experienced by healthy people as well, with a lifetime prevalence of 6.7% in the general population and up to 34.6% in patients with panic disorder.[128,129] SPs can occur while falling asleep or more commonly when waking up. During a SP the person is awake, but cannot move his/her body except for the eyes and is unable to speak. The experience is often accompanied by hallucinations and is usually frightening. This is well described e.g. in stories from St. Lucia island in the Caribbean:

> "The attack comes at the time that an individual is just failing asleep or just waking up, and the individual's sensations include a pressure on the chest, inability to move, and anxiety... (the experience) is referred to as kokma. A kokma is the spirit of a dead baby that haunts an area, and will attack people in bed. They jump on your chest and clutch at your throat. To get rid of them the attacked person struggles to cry out, or in

some way gets another person's attention, who will scare off the kokma ....
The informants who have given me a description of kokma have always
talked about the babies actually clutching at their throats..., the attacks
are always by dead, unbaptized babies. The kokma cannot be controlled,
they 'grab' people just for the hell of it."[130]

Hypnagogic (while falling asleep) and **hypnopompic** (on awakening) **hallucinations (HH)** are the fourth classic symptom in narcolepsy.[34] They are characteristically multimodal comprising auditory, visual, and tactile phenomena.[131,132] Thus, they usually differ from hallucinations in schizophrenia, in which hallucinations tend to be only auditory and also accusative in tone. Insight into the unreal essence of the hallucinations is also better in narcolepsy. Nonetheless, dual cases of narcolepsy and schizophrenia have been reported.[133-136]

Narcolepsy is associated with **weight gain,** especially in children, near onset of the disease.[32,124,137,138] **Precocious puberty** is also common (16-17%).[137,138] **Behavioral problems**, overlapping with attention-deficit/hyperactivity disorder (ADHD), anxiety disorder, and mood disorder are also seen in narcoleptic children and adults.[124] For example, in H1N1-vaccine-associated narcoleptic children, the reported prevalence of behavioral problems is 38-48%.[23,24] Lecendreux and coworkers found clinically significant ADHD symptoms in 19.7% to 35% of children with narcolepsy.[139]

The prevalence of **REM sleep behavior disorder (RBD)** in narcolepsy is 12-36% based on interviews and questionnaires.[140,141] REM sleep without atonia can be seen in 50% of patients in polysomnographic recordings.[142] **Automatic behavior** is also common in narcolepsy. It is semi-purposeful behavior, such as writing lecture notes with handwriting that the subject cannot him-/herself later interpret and without awareness of the activity afterwards.

2.5   Diagnostics of narcolepsy syndrome

Diagnostic criteria and classification of narcolepsy have evolved as understanding of the disease pathophysiology has increased. In the Diagnostic Classification of Sleep and Arousal Disorders (DCSAD) from 1979 and the 1st version of the International Classification of Sleep Disorders (ICSD-1) published in 1990, there is only one form of narcolepsy.[143] In DCSAD, the diagnosis of narcolepsy is based on symptoms and on the appearance of REM sleep within 10 minutes of sleep onset.

In ICSD-1, the essential features of narcolepsy are as described in the following: "Narcolepsy is a disorder of unknown origin that is characterized by excessive sleepiness that typically is associated with cataplexy and other REM

sleep phenomena, such as sleep paralysis and hypnagogic hallucinations."[144] The diagnostic criteria require:

1) **cataplexy** combined with **recurrent napping** or **lapses into sleep**, or alternatively,
2) **EDS** combined with **associated features** (SP, HH, automatic behavior, DNS), **positive polysomnography** (sleep latency less than 10 minutes, or REM sleep latency less than 20 minutes, or MSLT with < 5 minutes sleep latency, or ≥ 2 sleep-onset REM periods), **HLA DQB1*06:02 or DR2**, and **no other disorder accounting for the symptoms**.

The second edition of the International Classification of Sleep Disorders (ICSD-2) lists already four different subtypes of narcolepsy: narcolepsy with cataplexy, narcolepsy without cataplexy, narcolepsy due to medical condition, and unspecified narcolepsy.[145]

Instead of phenotype-based categories, the classification was changed in ICSD-3 to NT1 and NT2 to better reflect the pathophysiology of the disease, NT1 being hypocretin-deficient narcolepsy and NT2 narcolepsy with normal hypocretin levels (Table 1).[122]

While HLA typing was included in ICSD-2, it is not listed in ICSD-3. HLA DQB1*06:02 prevalence in the general population is 12-38%, but it has extremely high negative predictive value for NT1. Therefore, it could be used in e.g. situations where diagnosis of narcolepsy is unclear after MSLT and lumbar puncture is considered. In a large multinational study involving almost 700 narcolepsy cases, only 1.8% were HLA DQB1*06:02 negative and hypocretin deficient.[152] Negative HLA typing practically excludes hypocretin deficiency so lumbar puncture in these patients is not needed.

2.5.1 Pitfalls in diagnostics

Diagnostics of NT1 is straightforward when EDS and typical cataplexy are present and MSLT diagnostic criteria are fulfilled. Unfortunately, the sensitivity and specificity of MSLT are far from perfect. Two or more sleep onset REM periods (SOREMP) and MSL < 8 minutes in MSLT yielded sensitivity of 78% and specificity of 95% in a study by Aldrich and coworkers.[146]

There have been some suggestions to improve the specificity of MSLT by e.g. analyzing the amount of REM sleep in naps in MSLT and analyzing the sequence of the states; occurrence of REM sleep before N2 is typical for narcolepsy. However, this would further compromise the sensitivity.[147,148] Test-retest reliability of MSLT is also limited, especially in diseases other than NT1.[25]

Table 1. Diagnostic criteria of type 1 and 2 narcolepsies (ICSD-3).[f]

NARCOLEPSY TYPE 1

Criteria A and B must be met

A. The patient has daily periods of irrepressible need to sleep or daytime lapses into sleep occurring for at least three months.[1]

B. The presence of one or both of the following:

1. Cataplexy (as defined under Essential Features) and a mean sleep latency of ≤ 8 minutes and two or more sleep onset REM periods (SOREMPs) on an MSLT performed according to standard techniques. A SOREMP (within 15 minutes of sleep onset) on the preceding nocturnal polysomnography may replace one of the SOREMPs on the MSLT.[2]

2. CSF hypocretin-1 concentration, measured by immunoreactivity, is either ≤ 110 pg/mL or <1/3 of mean values obtained in normal subjects with the same standardized assay.

NARCOLEPSY TYPE 2

Criteria A-E must be met

A. The patient has daily periods of irrepressible need to sleep or daytime lapses into sleep occurring for at least three months.

B. A mean sleep latency of ≤ 8 minutes and two or more sleep onset REM periods (SOREMPs) are found on a MSLT performed according to standard techniques. A SOREMP (within 15 minutes of sleep onset) on the preceding nocturnal polysomnography may replace one of the SOREMPs on the MSLT.

C. Cataplexy is absent.[3]

D. Either CSF hypocretin-1 concentration has not been measured or CSF hypocretin-1 concentration measured by immunoreactivity is either > 110 pg/mL or > 1/3 of mean values obtained in normal subjects with the same standardized assay.[4]

E. The hypersomnolence and/or MSLT findings are not better explained by other causes, such as insufficient sleep, obstructive sleep apnea, delayed sleep phase disorder, or the effect of medication or substances or their withdrawal.

[1.] In young children, narcolepsy may sometimes present as excessively long night sleep or as resumption of previously discontinued daytime napping.

[2.] If narcolepsy type I is strongly suspected clinically but the MSLT criteria of B1 are not met, a possible strategy is to repeat the MSLT.

[3.] If cataplexy develops later, then the disorder should be reclassified as narcolepsy type 1.

[4.] If the CSF Hcrt-1 concentration is tested at a later stage and found to be either ≤ 110 pg/mL or < 1/3 of mean values obtained in normal subjects with the same assay, then the disorder should be reclassified as narcolepsy type 1.

ICSD-3, International Classification of Sleep Disorders, 3rd edition.

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f   Adapted with permission from International Classification of Sleep Disorders, 3rd edition. American Academy of Sleep Medicine. Darien, IL, USA.

2.5.2 Questionnaires

Excessive daytime sleepiness is very common, but finding those patients with an actual central disorder of hypersomnolence among these subjects can be challenging. It is not feasible to conduct a comprehensive diagnostic work-up, such as full-night polysomnography, MSLT, and lumbar puncture, for all of these subjects. Clinical history and physical examination are the cornerstones of patient selection for these examinations, but questionnaires, either self-administered or filled by the examiner, provide quick and valuable information on the symptoms. One advantage is also their validation in larger population so that the decisions could be based on something other than a subjective general impression.

2.5.2.1 Ullanlinna Narcolepsy Scale

The Ullanlinna Narcolepsy Scale (UNS) was published by Hublin and coworkers in 1994 as a screening tool for narcolepsy in the general population (Table 2).[27] The UNS is calculated from 11 questions scored from 0 to 4, yielding a maximum sum of 44, with higher score reflecting more narcolepsy symptoms. The first validation study showed a sensitivity of 100% and sensitivity of 98.8% in a sleep clinic population with a cut-off point of 14.[27] However, the study was limited in size, and used the ICSD-1. The UNS has been used successfully in, for instance, Norway, Hong Kong, and South Korea.[149-151]

Advantages of the UNS include capturing also mild cataplexy e.g. in children (questions on mouth opening, head nodding). Answer choices are explicitly defined as opposed to e.g. choices of "rarely", "sometimes", or "often", which could be interpreted differently by each respondent.

Table 2. Ullanlinna Narcolepsy Scale

1 When laughing, becoming glad or angry, or in an exciting situation, have the following symptoms suddenly occurred?

	Never	1-5 times during lifetime	Monthly	Weekly	Daily or almost daily
Knees unlocking	0	1	2	3	4
Mouth opening	0	1	2	3	4
Head nodding	0	1	2	3	4
Falling down	0	1	2	3	4

2 How fast do you usually fall asleep in the evening?

>40 min	31-40 min	21-30 min	10-20 min	<10 min
0	1	2	3	4

3 Do you sleep during the day (take naps)?

No need	I wanted but cannot sleep	Twice weekly or less	On 3-5 days weekly	Daily or almost daily
0	1	2	3	4

4 Do you fall asleep unintentionally during the day?

Situation	Never	Monthly or less	Weekly	Daily	Several times daily
Reading	0	1	2	3	4
Travelling	0	1	2	3	4
Standing	0	1	2	3	4
Eating	0	1	2	3	4
Other unusual activity	0	1	2	3	4

2.5.2.2 Swiss Narcolepsy Scale

The Swiss Narcolepsy Scale (SNS) was introduced in 2004 (Table 3).[152] Calculation for SNS score is bit more complicated than in the UNS. The SNS has five questions that are answered on a 5-point scale. The scores are then summed after multiplying different questions with a certain factor from -13 to +9. Finally, 20 points is added to the final figure. Therefore, the SNS has values from -110 to +66, and scores below 0 indicate possible narcolepsy.

The first SNS validation study included 62 patients with narcolepsy with cataplexy (NC), 56 hypersomnolent patients, and 40 subjects without sleep complaints as controls. The hypersomnolent group included patients with sleep disordered breathing (SDB, $n = 30$), restless legs syndrome (RLS, $n = 10$), periodic limb movement disorder (PLMD, $n = 8$), and behaviorally induced insufficient sleep (BIIS, $n = 8$). In that population, the specificity and sensitivity of the SNS were 96% and 98%, respectively. Unfortunately, it is not clearly reported whether these values were relative to hypersomnolent patients, healthy controls, or both. Also, in that study only 92.5% of 57 narcolepsy patients were positive for HLA DQB1*06:02, cataplexy was classified as definite in only 41 and "possible" in 16 subjects, polysomnography was performed in 35 subjects, and hypocretin was measured in 12 subjects. It remains unclear how many of the 21 subjects without definite cataplexy could have been diagnosed as NT1 or NT2 according to the current diagnostic criteria.

The SNS has been validated in three other studies by the same research group. Unfortunately, the first two studies are published only as conference abstracts, and therefore, case definition and methods are incompletely reported.[153,154] Moreover, in the first study there are a few controversies on narcolepsy diagnoses that could alter the results. Nonetheless, these studies showed sensitivity of 85-98% and specificity of 96-89% in differentiating narcolepsy with cataplexy from other sleep-related disorders and hypersomnias.

Fortunately, we now have more information on the SNS as another study, using methods similar to Study IV, was published in a Swiss-based open-access journal during the review process of Study IV.[155] In that study, the sensitivity and specificity of the SNS were 89% and 88%, respectively.

Table 3. The five questions forming the Swiss Narcolepsy Scale (in the English translation, German original text in parentheses, with the corresponding values) and the calculation and evaluation.[g]

Q1 How often are you unable to fall asleep? (Wie oft können Sie nicht einschlafen?)

[1] never, [2] rarely (less than once a month), [3] sometimes (1–3 times a month), [4] often (1–2 times a week), and [5] almost always

Q2 How often do you feel bad or not well rested in the morning? (Wie oft fühlen Sie sich am Morgen schlecht/unausgeschlafen?)

[1] never, [2] rarely (less than once a month), [3] sometimes (1–3 times a month), [4] often (1–2 times a week), and [5] almost always

Q3 How often do you take a nap during the day? (Wie oft machen Sie einen Mittagsschlaf/ Nickerchen?)

[1] never, [2] Would like to, but cannot, [3] 1–2 times a week, [4] 3–5 times a week, and [5] almost daily

Q4 How often have you experienced weak knees/buckling of the knees during emotions like laughing, happiness, or anger? (Wie oft haben Sie weiche Knie/Knieschlottern bei Emotionen wie Lachen, Freude oder Wut empfunden?)

[1] never, [2] rarely (less than once a month), [3] sometimes (1–3 times a month), [4] often (1–2 times a week), and [5] almost always

Q5 How often have you experienced sagging of the jaw during emotions like laughing, happiness, or anger? (Wie oft haben Sie ein Absinken des Unterkiefers bei Emotionen wie Lachen, Freude oder Wut empfunden?)

[1] never, [2] rarely (less than once a month), [3] sometimes (1–3 times a month), [4] often (1–2 times a week), and [5] almost always

Calculation Narcolepsy score: 6 x Q1 + 9 x Q2 + 5 x Q3 - 11 x Q4 - 13 x Q5 + 20

Narcolepsy score < 0: narcoleptic

Narcolepsy score > 0: non-narcoleptic hypersomniac

2.5.2.3 Other narcolepsy scales

There is an entire book dedicated to different scales and questionnaires in sleep medicine, but still the questionnaires to quantify narcolepsy symptoms and severity are very limited.[156,157] The only scale aimed for this purpose, the Narcolepsy Severity Scale (NSS), was published in 2014, after the collection of samples for the studies in this thesis.[158] The NSS is, however, validated only in adult NT1 subjects in one study in one clinic, and in French, so further studies are needed to examine its validity in other populations and languages.

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g   Shared under Creative Commons Non-Commercial 4.0 License. from 155. Sturzenegger C, Baumann C, Lammers GJ, Kallweit U, van der Zande WL, Bassetti C. Swiss Narcolepsy Scale: A simple screening tool for hypocretin-deficient narcolepsy with cataplexy. Clinical & Translational Neuroscience. 2018:1-5.

One fundamental problem in the quality of life and depression questionnaires for narcoleptic patients is that they actually include many questions on sleep, sleep problems, and sleepiness. This can be problematic since in narcolepsy, sleep problems are not caused mainly by e.g. depression, and therefore, the questionnaire might result in false-positive findings. Rimon's Brief Depression Scale does not use any questions directly related to sleep, although two items enquire about general health and tiredness.[159]

Generating a completely new questionnaire is a time-consuming and laborious task. Therefore, it is advisable to determine whether an existing questionnaire is adequate for the question of interest.

2.5.3 Treatment of narcolepsy with a focus on autoimmune background and immunotherapy

Excessive daytime sleepiness in narcolepsy is treated with modafinil, psychostimulants (methylphenidate, rarely amphetamines), or novel wake-promoting agents such as the selective histamine-3 receptor inverse agonist pitolisant. The selective noradrenaline-dopamine receptor inhibitor solriamfetol, which is in phase 3 trials, is a possible future option. Treatment options for cataplexy include sodium oxybate, which has also effect on EDS, the selective serotonin and noradrenaline uptake inhibitor venlafaxine, tricyclic antidepressants, and e.g. atomoxetine. Hypocretin agonists or analogs are being developed, but their delivery to the central nervous system and the short half-life are major obstacles for clinical use.

Autoimmune-mediated pathophysiology in narcolepsy has stimulated many groups to attempt immunomodulatory treatment for the disorder. Unfortunately, the results in general have been rather disappointing, although there are single reports with hope-inspiring beneficial effects. We have reported a remarkable but short-lasting effect of rituximab in a patient with severe narcolepsy and psychiatric symptoms.[160] We have also treated five Finnish patients with intravenous immunoglobulin (IVIG) without any long-standing effect (Sarkanen et al., unpublished results). The shortest interval from symptom onset to treatment in our sample was 14 days, but from vaccination with Pandemrix, the presumed trigger of the disease, more than one year. Knudsen and coworkers have reported similar results 19 days after symptom onset, but also more than a year after Pandemrix.[161] No effect or a mild to moderate short-lasting effect on EDS and CPL by IVIG has been described in some case reports, but without improvement in CSF hypocretin levels.[162-168] The treatment administration in these case reports has also been open-label, except for one report where there was no difference between IVIG and placebo.[166] Conversely, Dauvilliers and coworkers reported a normalization of low hypocretin-1 levels and alleviation of symptoms after IVIG

in one patient.[169] Unfortunately, also in this case the symptoms returned after a couple of months and the patient refused further interventions.

Immunoadsorption and plasmapheresis have also been administered without any effects or with very complicated clinical phenotype involving most probably some other underlying conditions.[170,171] Interestingly, in one case, alemtuzumab treatment used for multiple sclerosis alleviated symptoms of comorbid narcolepsy as well.[172]

Taking into account the involvement of autoreactive T cells in the background of narcolepsy and the heterogeneous and often delayed timing of the published results of immunomodulatory treatment attempts, it cannot be excluded that proper intervention, initiated shortly after the triggering factor or symptom onset, could halt the autoimmune process of hypocretin-producing cell destruction.

2.6 Epidemiology of narcolepsy

2.6.1 Prevalence

The prevalence of narcolepsy in different studies has varied from 5.2 to 70 per 100,000 persons.[39,173] Although genetic predisposition differs in different populations, with 12-38% of the Western population carrying the HLA DQB1*06:02 allele, it is clear that the methodological differences in case collection and verification affect the figure greatly.

Clinical confirmation of the cases is important for accurate numbers. The methods used in prevalence studies have been structured telephone interviews, mailed questionnaires usually followed by clinical confirmation, and chart reviews of already diagnosed cases. Each of these methods has its flaws. It is obvious that by telephone interview solely the diagnosis of narcolepsy remains uncertain especially if the disease has not been diagnosed before. On the other hand, this method might help to recognize an underdiagnosed disease. In narcolepsy, the diagnostic delay especially in the older studies has been on average more than 10 years, implying low awareness of the disease.[21,174] Questionnaires have similar problems, but are probably even more prone to false-positive cases, as they are usually tailored to have high sensitivity, possibly compromising specificity. Therefore, these cases have to always be confirmed clinically. For instance, in a study by Hublin and coworkers, 75 of 12,504 respondents from the Finnish Twin Cohort had UNS ≥ 14, and only three of them had actual narcolepsy.[38] As narcolepsy is a rare disease, very large study samples are needed in research.

A chart review of diagnosed cases provides accurate diagnostic data, but these studies are prone to underestimate the true prevalence of narcolepsy as those subjects who have not sought medical advice are left out of the study and remain undiagnosed. Also diagnosed cases have to be confirmed since for e.g. ICD-10

diagnosis code G47.4 can be used mistakenly. In a recent study from Catalonia, 72.7% of ICD-10 G47.4 (narcolepsy and/or cataplexy) coded patients in primary care were not true narcolepsy.[175] In the same study, 23 of 373 (6%) diagnosed possible narcolepsy cases were classified by Brighton criteria as level 5, meaning no narcolepsy, and 14 (4%) as level 4 (doubtful narcolepsy).

Another problem in the older studies is the classification of narcolepsy. NT1 versus NT2 classification was presented in 2014, and in many studies before that time, especially prior to ICSD-2, even narcolepsy with and narcolepsy without cataplexy were not separated from each other. Currently, we know that these should be considered as different diseases on a pathophysiological level and separated from each other in epidemiological studies, although some NT2 subjects are probably in earlier stages of the pathophysiological process of developing autoimmune NT1. Diagnostic criteria for narcolepsy have also changed as discussed in the previous section. In ICSD-1, the diagnosis of narcolepsy could be made solely based on clinical history.

In questionnaire-based studies, where diagnosis is confirmed by PSG and MSLT, a potential cause of bias is also the moderate sensitivity of MSLT and the possibility for false negatives. Subjects selected from the general population who have not been diagnosed previously might have mild symptoms and perform well in MSLT, but they might still have hypocretin deficiency. Hypocretin measurements are not carried out in prevalence studies.

Due to these methodological differences, the true prevalence of narcolepsy, especially when NT1 and NT2 are discussed separately (as they should be), is unknown, but probably lies somewhere between 5 and 50 per 100,000 inhabitants if the two diseases are combined. In our data, around 10-20% of all narcoleptic patients are NT2 after a careful exclusion of other contributing factors (Sarkanen et al., unpublished results).

Table 4. Some prevalence studies of narcolepsy.

1st author, publication year	Area	Study years	Prevalence / 100,000	Case ascertainment	Diagnostic classification
Hublin, 1994 [38]	Finland	1990	26.0	Questionnaires., telephone interviews, clinical interviews	ICSD-1
Silber, 2002 [176]	Olmsted County, MI	1985	56.3	Record linkage	Mayo Classification
Ohayon, 2002 [177]	Europe (the United Kingdom, Germany, Italy, Portugal, Spain)	1994 - 1999	47.0	Telephone interviews, clinical interviews	Clinical symptoms based on ICSD-1 (w/o sleep studies)
Wing, 2002 [150]	Hong Kong, China		34	Questionnaires., clinical interviews	
Shin, 2008 [151]	South Korea		15	Questionnaires, telephone interviews., clinical interviews	ICSD-2
Longsreth, 2009 [173]	King County, WA	2001	21.8	Physician-diagnosed	
Heier, 2009 [149]	Norway	2006	22	Questionnaires., clinical interviews	ICSD-2
Tió, 2017 [175]	Catalonia, Spain	2015	5.2	Chart review	ICSD-3

ICSD-1, International Classification of Sleep Disorders, 1st edition; ICSD-2, International Classification of Sleep Disorders, 2nd edition, ICSD-3, International Classification of Sleep Disorders, 3rd edition.

2.6.2 Incidence before 2009

Interpretation of incidence studies in narcolepsy is likewise challenging because of the incomplete case ascertainment in some studies and the changing diagnostic classification. These issues are partly tackled by e.g. recent development of case definition and guidelines by the Brighton Collaboration Narcolepsy Working Group.[178] According to these Brighton Collaboration Criteria (BCC), level 1 diagnostic certainty in narcolepsy is achieved if either EDS or CPL is present, and CSF hypocretin level is below 110 pg/mL. Level 2 requires both EDS and unambiguous CPL in addition to MSLT criteria, which must be fulfilled only partly (MSL ≤ 8 minutes in adults or MSL ≤ 12 minutes in children and adolescents or 2 or more SOREMPs). Level 3 is identical to level 2, but in the absence of CPL MSLT criteria must be fulfilled completely (MSL ≤ 8 minutes in adults or MSL ≤ 12 minutes in children and adolescents AND 2 or more SOREMPs). Level 4 is

reported narcolepsy with insufficient evidence to meet the case definition. Level 5 is no narcolepsy.

In the highly cited study conducted in Olmsted County, Minnesota, a records linkage system was used to identify all new narcolepsy cases between 1960 and 1989.[176] The authors used their own Mayo Classification of narcolepsy for case ascertainment. The main differences to the current classification is that they included also cases with 1 ≤ SOREMP if cataplexy was present, and also cases with EDS and CPL but without proper sleep studies performed. They also excluded cases with apnea-hypopnea index ≥ 10 to avoid false positives, even though we now know that sleep apnea is a common comorbidity in narcoleptics.[179] They found an annual incidence rate of 0.74 per 100,000 persons for narcolepsy with cataplexy and 1.37 per 100,000 persons for narcolepsy in total.

The results in the Olmsted County study were based on only 35 patients. A larger study in six European countries using automated linked databases provided 280 million person-years of observation time and 2608 narcolepsy cases.[180] Only cases from the Netherlands were confirmed. This resulted in an incidence reduction from 1.26 to 0.19 per 100,000 person-years, highlighting the need for case ascertainment. Nevertheless, they also found the incidence to be around 1 per 100,000 person-years before September 2009.

2.6.3 Incidence after 2009 and the H1N1 pandemic vaccination campaign

In March 2009, a new reassortant H1N1-type influenza A virus (H1Npdm09) appeared first in Mexico and the USA. The spreading of the virus was rapid and the number of laboratory-confirmed cases increased soon to an amount that warranted WHO to declare a H1N1 pandemic by June 2009. Early observational studies from Mexico showed a high proportion of hospitalized and critically ill patients and high mortality especially in children, young adults, and pregnant woman, contrary to ordinary seasonal influenza epidemics that mainly pose a threat to the elderly and people with other severe diseases.[181] This resembled an earlier 1918-1919 H1N1 pandemic ("Spanish flu") that had spread worldwide, resulting in 50 to 100 million deaths.

Several pandemic vaccines were soon introduced. Vaccine coverage in Europe was at least 46 million and in the USA over 90 million people. Of the eight different vaccines used in Europe, three included an adjuvant; MF59 in two vaccines and AS03 in one vaccine (Pandemrix). Arepanrix, another vaccine with an AS03 adjuvant, was used in Canada. No adjuvanted vaccines were used in the USA.

An increasing number of children and adolescents with narcolepsy started to appear in 2010.[23] Before 2010, narcolepsy in young children was a rather rare disease in Finland. A 7-year-old Finnish boy who had been recently vaccinated with

Pandemrix and started to express EDS soon after was diagnosed with narcolepsy in February 2010.[23] During the following months several new cases with a recent onset of narcolepsy appeared, and a total of 14 children with narcolepsy were diagnosed in Finland by August 2010. Similar observations were made in Sweden. The first reports from Finland and Sweden were followed by France, Norway, the UK, Ireland, and Germany.[5-8,10,12,182] Vaccination coverage with Pandemrix vaccine was high, especially in Scandinavia. Most of the subjects had their first symptoms of narcolepsy during spring 2010 (Figure 7).[183]
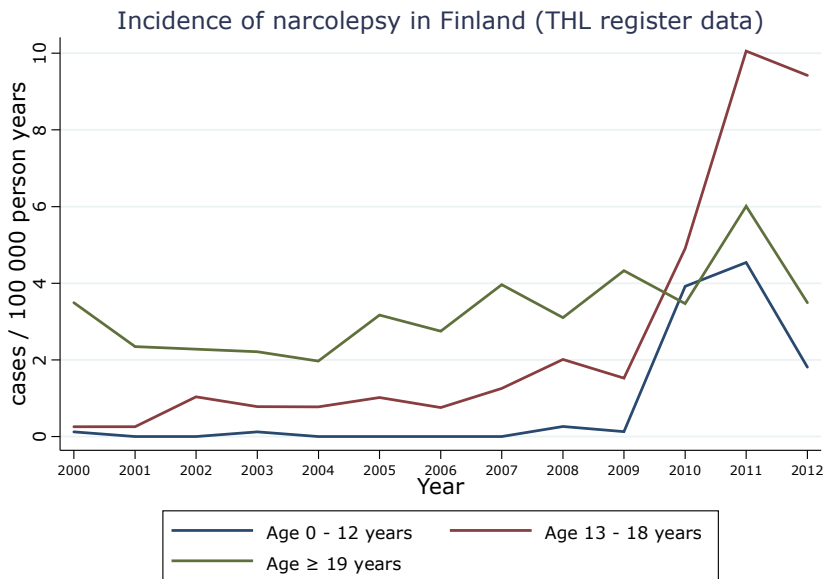
Figure 7. Incidence of narcolepsy in Finland according to verified ICD-10 diagnosis codes in the Finnish National Institute of Health Register (Hoitoilmoitusrekisteri) (Sarkanen et al., unpublished results).

The findings came from observational studies applying different methods e.g. case-control, case-coverage, or cohort designs. Observational studies are also prone to various biases, such as ascertainment and recall bias, and confounding. Simulations of the potential role of bias in narcolepsy and H1N1 vaccination studies displayed somewhat reduced risk, but could not completely explain the increased risk.[13-15] Interestingly, during the post-pandemic period a 3-fold increase in risk of narcolepsy and seasonal variation in narcolepsy incidence were reported from the Beijing and Shanghai areas in China, where vaccination coverage was low.[17,184,185] This incited discussion about whether the association between narcolepsy and H1N1 vaccination was true.

3   HYPOTHESES AND AIMS OF THE STUDIES

This thesis is based on Studies I-IV designed to test the following three hypotheses. The background and rationale behind the hypotheses are outlined below.

1. Incidence of narcolepsy increased after pandemic H1N1 vaccination.
Several observational studies reported an increased incidence of narcolepsy with variable risk ratios after immunization with H1N1 pandemic vaccine in 2009-2010. These studies are prone to various biases that could affect the results. In addition, seasonal variation and an increased incidence were reported also in China, where vaccination coverage was low. To analyze the actual risk of narcolepsy associated with H1N1 vaccination, we conducted a meta-analysis and systematic comprehensive review of the epidemiological studies (Study I).

2. Narcolepsy associated with H1N1 vaccination is a more severe disease than sporadic narcolepsy.
Abrupt, severe onset and short delay in diagnoses suggest differences in H1N1-vaccine-related narcolepsy and sporadic narcolepsy without an association with vaccination. The latter has traditionally been associated with a long diagnostic delay. Differences in polysomnographic and actigraphic characteristics might explain the observed abrupt onset, severe phenotype, and short diagnostic delay of H1N1-vaccination-associated narcolepsy. Autoimmune background might also suggest ongoing hypocretin cell destruction, reflected by a deteriorating clinical picture. Phenotype and clinical course of narcolepsy associated with H1N1 vaccination are unknown. Studies II and III were conducted to analyze the phenotype of vaccine-associated narcolepsy and to compare it with sporadic narcolepsy.

3. The Ullanlinna Narcolepsy Scale can be used in the clinic as a diagnostic tool for narcolepsy.
The Ullanlinna Narcolepsy Scale was developed to screen for narcolepsy in epidemiological studies. Feasibility of the UNS in a clinical population has not been systematically evaluated. In Study IV, it was validated in a clinical population with different sleep disorders.

4   METHODS AND SUBJECTS

4.1   Study I

To identify all articles reporting the incidence and the risk of H1N1-vaccination-associated narcolepsy we searched PubMed, Web of Science, and Scopus without language restriction using the following string: "(narcolepsy[MeSH] or narcolepsy) AND (vaccines[MeSH] OR vaccination[MeSH] OR influenza[MeSH] or Pandemrix OR vaccines OR vaccination)". We examined the references of returned articles to find additional sources. We also checked the web pages of the relevant health authorities (National Institute for Health and Welfare (THL) in Finland, Medical Products Agency (MPA) in Sweden, Norwegian Medicines Agency, Health Protection Surveillance Centre (HPSC) in Ireland, Public Health England, Agence Nationale de Sécurité des Médicaments et des Produits de Santé (ANSM) in France, and European Centre for Disease Control (ECDC)) for their reports on narcolepsy after 2009-2010. The search was conducted in November 2016.

Eligibility of the articles was assessed and data extraction conducted independently by two of the authors, Tomi Sarkanen and Professor Markku Partinen. Any disagreement was resolved by discussion. Eligibility assessment was based first on the title, then the abstract, and finally the full text. We included all studies and reports that evaluated the risk of narcolepsy or the number of narcolepsy cases after the Pandemrix vaccination. We also included studies assessing the risk of narcolepsy after other A(H1N1)pdm09 vaccines in a qualitative synthesis.

We included data of country or study area, author, publication year, study type, age of subjects, duration of follow-up or collection period, number of vaccinated and unvaccinated subjects, vaccination coverage, reported country-specific narcolepsy incidence rate before and after the 2009 influenza A H1N1 vaccination campaign in vaccinated and unvaccinated subjects, relative risk (RR) in cohort studies or odds ratio (OR) in case-control studies, 95% confidence interval (CI), and vaccine-attributable risk of narcolepsy. In the analyses, RRs and ORs were used interchangeably because narcolepsy is a rare disease and in such cases these two ratios are very close to each other. Only cases fulfilling BCC 1-3 or ICSD-2 criteria for narcolepsy (EDS and low CSF hypocretin or EDS, CPL, and positive MSLT) were analyzed.

Dutch population data were retrieved from Statistics Netherlands and Swiss population data from the Swiss Federal Statistics Office for calculations of RR in these countries. There is limited information on the incidence of narcolepsy in toddlers, but for the Dutch reports the background incidence was estimated to be

approximately 1 per 800,000 children aged less than five years based on Vaccine Adverse Event Surveillance & Communication (VAESCO) report data.[186]

We reviewed potential biases for all the post-Pandemrix narcolepsy studies. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline was followed throughout the study.

4.2 Studies II, III, and IV

In Study II, PSG and ACT characteristics of 69 Pandemrix vaccine-associated (pNC) and 57 non-vaccine-associated or sporadic (sNC) narcoleptic subjects were analyzed and compared. The diagnoses of pNC and sNC subjects were made in 2010-2012 and 2002-2012, respectively. The subjects were unmedicated.

In Study III, 26 patients with H1N1-vaccine-associated narcolepsy type 1 (pNT1) completed the modified Basic Nordic Sleep Questionnaire (mBNSQ) near onset of the disease and at the follow-up at least two years later. The first visit was during 2010-2012, and the follow-up during 2012-2014. The subjects were unmedicated at their first visit. During the follow-up the treating physician made treatment choices independently of this study. Follow-up results were compared with 25 subjects with sporadic type 1 narcolepsy (sNT1) who filled out similar questionnaires in 2014. The subjects were recruited from the Helsinki Sleep Clinic, Vitalmed Research Center (HSCVRC). All subjects had confirmed type 1 narcolepsy. Twenty-one of 26 pNT1 patients and 18 of 25 sNT1 patients had CSF hypocretin levels measured, all of which were below 110 pg/mL. The remaining 5 and 7 subjects had unambiguous CPL, carried the HLA DQB1*06:02 haplotype, and had a positive MSLT.

In Study IV, we reviewed sleep questionnaires of 267 subjects seen at the HSCVRS between April 15, 2004, and December 2, 2015. The subjects were either part of the NARPANord narcolepsy project or represented a sample of consecutive sleep disorder patients admitted to full-night polysomnography. NARPANord was a Finnish-Swedish consortium project examining the etiology and pathogenesis of H1N1-vaccine-associated narcolepsy. In Study IV, we included 89 patients with NT1, 10 with NT2, 37 with OSA, 56 with restless legs syndrome and/or periodic limb movement disorder (RLS/PLMD), 51 with other sleep-related disorders (OSRD), 7 with idiopathic hypersomnia (IH), 3 with Kleine-Levin syndrome (KLS), and 14 with other hypersomnia syndrome (ICD-10 code G47.1).

We combined the subjects with IH, KLS, and other hypersomnias and defined that entity as "hypersomnia" (HS). A case was classified as HS if NT1 and NT2 were excluded, and if he/she had severe daytime somnolence without other explaining factors (such as circadian rhythm disorder, insufficient sleep, or sleep disordered breathing). The OSA group was diagnosed with mild to severe sleep apnea. The

OSRD group consisted of insomnia ($n = 16$), delayed sleep phase syndrome ($n = 11$), depression ($n = 10$), attention-deficit/hyperactivity syndrome ($n = 4$), behaviorally induced insufficient sleep ($n = 3$), fatigue ($n = 3$), parasomnias ($n = 2$), pediatric autoimmune neuropsychiatric syndrome ($n = 1$), and REM sleep behavior disorder ($n = 1$). In addition, sleep questionnaires of 85 relatives of narcoleptic subjects were analyzed. Relatives were siblings or parents of NT1 subjects and were not diagnosed with sleep-related disorders, although a few of them reported mild and occasional parasomnias or some symptoms of restless legs in sleep questionnaires. Relatives did not answer the SNS questions.

Only subjects with complete UNS data were included. In analyses where also SNS and ESS were compared with UNS, only a dataset without any missing values in any scales was used, whereas in analyses including only UNS, subjects with missing values in SNS or ESS were also accepted. The dataset without missing values included 79 NT1, 9 NT2, 22 OSA, 12 RLS/PLMD, and 13 HS subjects.

Subjects in Study II were diagnosed according to ICSD-2 criteria, subjects in Study III according to ICSD-3 criteria, and subjects in Study IV according to both ICSD-2 and ICSD-3 criteria.

In all three studies, classification as H1N1-vaccine-associated narcolepsy was made if the subject had been immunized with Pandemrix vaccination during autumn-winter 2009-2010 and the symptoms of narcolepsy manifested within two years (Study III) or in less than 550 days (Study II) from the vaccination. The majority of sporadic narcolepsy patients had disease onset before the a(H1N1)pdm09 pandemic. Four sNT1 subjects in Study III had disease onset during 2009 or 2010, but none of them were vaccinated before disease onset and none had influenza-like illness. Vaccination data were missing for three subjects, all of whom had disease onset several years before the vaccination campaign.

Analysis of hypocretin-1 concentrations was performed at the Rinnekoti Research Centre using a Stanford reference sample (Orexin A RIA kit, Phoenix Pharmaceuticals, San Mateo, CA, USA).

4.2.1 Questionnaires

mBNSQ is a questionnaire including multiple standardized tools for the assessment of sleep-related symptoms.[187] Questionnaires used in Study III were ESS, UNS, SNS, 5-item World Health Organization Well-Being Scale (WHO5), and Rimon's Brief Depression Scale (RDS), and in Study IV ESS, UNS, and SNS.[27,152,159,187,188]

A separate questionnaire for disability caused by the disease was also used in Study III. This questionnaire included the main symptoms of narcolepsy and the disability caused by these symptoms on a scale from zero (subject does not have this symptom) to four (severe disability). Weight and height were asked to calculate body mass index (BMI). The number of cataplectic attacks per week (CPL/week)

was also ascertained. If questionnaires were partially completed, one follow-up phone call was made or a new questionnaire was sent.

For our studies, SNS was estimated and calculated with some adjustments from mBNSQ, UNS, and WHO5. The first and third questions in SNS are practically identical to the mBNSQ questions of inability to fall asleep and frequency of daytime napping. In mBNSQ, answer choices are "never or less than once per month", "less than once per week", "on 1-2 days per week", "on 3-5 days per week", and "daily or almost daily". These were changed directly to match the SNS scale. The second question in SNS is the opposite of WHO5 question four: "I woke up feeling fresh and rested". Therefore, the WHO5 scale was reversed and since WHO5 has response choices from 0 to 5, the "all of the time" and "most of the time" choices were combined and changed to a scale from 1 to 5. The last two questions in SNS regarding cataplexy are identical to UNS cataplexy questions after changing the scale from UNS 0 to 4 to SNS 1 to 5.

4.2.2 Parameters in polysomnography, mSLT, and actigraphy

The majority of PSG, MSLT, and ACT recordings in Study II were conducted during the normal diagnostic procedure. Sleep stages, breathing, and leg movements were scored manually according to international criteria.[189,190] The ACT recordings lasted for 1-2 weeks and were accompanied by a sleep log kept by the patients or the parents of under-aged subjects. Analyses were done using Actiwatch® (Cambridge Neurotechnology Ltd., Cambridgeshire, UK). The wake threshold algorithm sensitivity was set to a medium level. The epoch length was one minute.

The definitions of the ACT parameters used were as follows (from Alakuijala et al.[191]):

> **Sleep latency**: the difference between bedtime and sleep start (as set by the researcher or derived automatically from a marked event).

> **Actual sleep time**: the amount of sleep between sleep start and sleep end, wake time excluded.

> **Sleep efficiency**: the percentage of time spent asleep between bedtime and time getting up.

> **Number of immobile phases of 1 min**: the number of immobile phases during the sleep period (the epochs where activity scores of 0 were recorded) where the duration of the immobile phase was only 1 min; this parameter describes the fragmentation of sleep.

Movement and fragmentation index: the percentage of time spent moving (the epochs where activity scores greater than zero were recorded) plus the percentage of immobility phases of 1 min as a proportion of the total number of immobility phases during the sleep period.

Cosine peak: the time of the day when the parametric 24-h fixed period cosinor model of the subject's average diurnal activity profile peaks.

Light:dark ratio: the ratio between average activity count during daylight (set at 06:00–18:00) and during 'darkness' (18:00–06:00). The higher the ratio, the more of a morning person a subject tends to be.

L5 onset: the start time of the sequence of the five least active hours in the 24-h average activity profile.

M10 onset: the start time of the sequence of the ten most active hours in the 24-h average activity profile.

Relative amplitude: the normalized difference between the most active 10-h period and the least active 5-h period in an average 24-h pattern; higher values indicate a stronger rhythm.

The three last parameters are part of nonparametric variables for actigraphic data and are designed for more accurate descriptions of sleep–wake rhythms, which are actually not sinusoidal. Median values are used to describe the ACT parameters, as they are less sensitive to extreme results than mean values.

4.3 Ethics

Studies II-IV were approved by the Helsinki and Uusimaa Ethics Committee (NarpaNord #214/13/03/00/2011). Written informed consent was received from all patients. Parents signed the informed consent on behalf of the children involved in the studies. Study I included only anonymous data published in previous reports, and therefore, an ethics committee statement was not requested.

4.4 Statistical methods

Statistical analyses were performed using SPSS (IBM SPSS, Statistics 19.0, Armonk, NY, USA) and STATA for Mac version 13.1-14.1 (Stata Corporation, TX, USA). Graphs in Study III were made using R software (R development core team, 2014) and ggplot2 package (H. Wickham. ggplot2: elegant graphics for data analysis, Springer New York, 2009) or Excel. Relative risks or odds ratios were used for pooled analysis in Study I. The results in Study I are presented with 95% CIs using random effects model, and heterogeneity was analyzed using I^2 statistics. For statistical comparisons in Studies II, III, and IV, continuous, parametric, and nonparametric methods were used according to the normality of distributions as verified by skewness, kurtosis, Kolmogorov–Smirnov, and Shapiro–Wilkinson tests. Mean (*M*) and standard deviation (*SD*) are reported for variables following normal distribution, and median (*Mdn*) and range for variables with nonparametric distribution. Multifactorial analyses were performed with linear regression. Due to missing data, the number of subjects differs in some of the analyses in Study IV, as described in the results section. Missing data imputation methods were not used. All P values were two-sided and the significance level was set at .05 throughout.

5   RESULTS

5.1   Study I – Incidence of narcolepsy after H1N1 influenza and vaccinations: systematic review and meta-analysis

The database search and screening for other sources as explained in the methods section resulted in a total of 315 records after removal of duplicates. The full search protocol is shown in detail in Figure 8. Eleven studies or reports were included in the final quantitative meta-analysis.[5,7,9-12,192-195] Eighteen additional studies or reports were included in the qualitative synthesis. Of these 18 papers, 3 came from countries where Pandemrix vaccination was used and 15 from countries where it was not.[8,17,18,184,196-209] Summaries of the included studies are given in Tables 7 and 8.
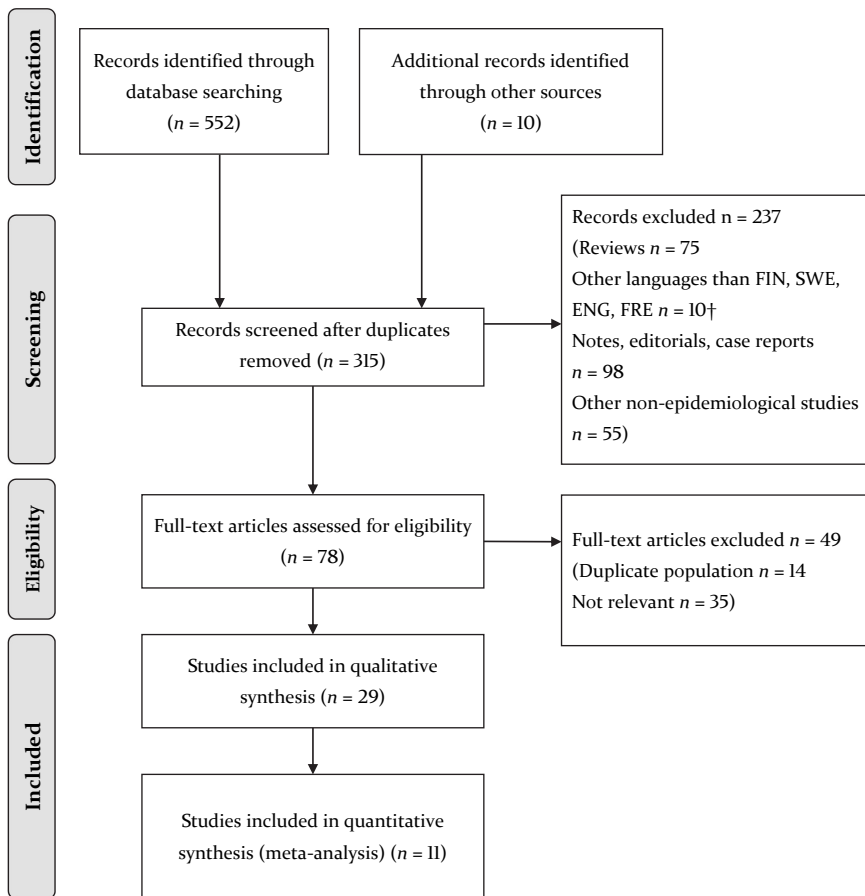
Figure 8. PRISMA flow diagram of included studies. FIN, Finnish; SWE, Swedish; ENG, English; FRE, French.†
2 Norwegian, 4 Danish, 1 German, 1 Portuguese, 1 Russian, 1 Turkish. English titles and/or abstracts screened for relevant information. Reproduced with permission from Sarkanen et al. 2018.[210]

The meta-analysis included either register cohort ($n = 8$), case-coverage ($n = 2$), or case-control studies ($n = 1$). A paper from the Netherlands Pharmacovigilance Centre reporting cases of narcolepsy in children aged six months to five years after Pandemrix vaccination was also included.[194] From this paper, only children aged under five years with confirmed narcolepsy (BCC 1-3) and onset after Pandemrix vaccination were included in the meta-analysis (7 of 20 reported cases). We analyzed the studies, which were divided into two subgroups: (1) children and adolescents, and (2) adults (Figures 9 and 10).

5.1.1 Index dates and study period

Different study periods with termination point ranging from August 2010 to the end of the year 2012 were used. Five different index dates were used in the studies:

1. Onset of symptoms (primary index date in the studies from Norway and the UK).
   - The most obvious index date, but difficult to assess objectively in retrospect. Also, prone to recall bias.
2. First healthcare contact (primary index date in the studies from Finland and Ireland).
   - The earliest objective time point.
3. Referral to specialist.
4. Referral to MSLT (used only in the study from France).
5. Final diagnosis (primary index date in the studies from Sweden)

5.1.2 Actual risk of post-vaccination narcolepsy

The total number of narcolepsy cases and follow-up years both vaccinated and not vaccinated are given in Table 5.

Table 5. Number of narcolepsy cases and follow-up years by age groups in the studies included in the meta-analysis.

Age group	Narcolepsy cases		Follow-up years	
	Vaccinated	Non-vaccinated	Vaccinated	Non-vaccinated
Children, adolescents	376	95	5,100,000	11,300,000
Adults	133	59	9,000,000	12,000,000

Reproduced with permission from Sarkanen et al. 2018.[210]

Using the onset of symptoms as an index date resulted in a RR of 14.32 (95% CI 8.92 to 22.99) in children and adolescents during the primary study period. Corresponding figures were 9.68 (4.88, 19.23) if the first healthcare contact, and 5.02 (3.36, 7.51) if the diagnosis was used as the index date (Figure 9).

In adults, RR was 7.01 (3.40, 14.46) using symptom onset, 8.08 (3.86, 16.89) using healthcare contact, and 2.95 (1.88, 4.62) using diagnosis as the index date (Figure 10). Heterogeneity measured by I^2 statistic was very low, 0% in all subgroups, except for healthcare contact in children and adolescents, where I^2 = 44.1%, nevertheless, it was not statistically significant (P = .167).

We were able to calculate the vaccine-attributable risk approximately one year after the vaccination from five studies in children and adolescents. We approximated that the vaccine-attributable risk in this age group was 1 case per 18,400 (95% CI 1/16,700 to 1/20,400) or 5.4 per 100,000 vaccinations. However, this is only a crude estimate since the follow-up periods and index dates were variable.

In adults, the smaller number of cases provides less robust calculations. Based on studies by Jokinen et al., Stowe et al., and O'Flanagan et al. the vaccine-attributable risk in adults was 1 per 181,000 (95% CI 1/141,000 to 1/254,000), using healthcare contact as the index date. These calculations were made from primary follow-up periods, which ended from August 2010 to April 2011.

Narcolepsy risk estimates more than one year after the initial vaccination were available only from Finnish and Swedish register studies. In Finland, RR based on healthcare contact one to two years after the vaccination was 4.7 (95% CI 2.2, 11.7) in children and adolescents, and 6.8 (95% CI 2.8, 17.7) in adults, returning to baseline within two years of the vaccination. In Sweden, RR based on diagnosis one year or more after the vaccination was 2.66 (95% CI 1.50, 4.72) in subjects aged less than 20 years. In 21–30-year-olds, the RR was 2.24 (95% CI 0.86, 5.85). Still, onset of symptoms occurred most often during the first three to six months following vaccination.

Due to the small number of studies, funnel plots and Egger and Beggs tests to analyze the publication bias are inaccurate and therefore not shown. We searched thoroughly all available evidence without language restriction, including also reports not listed in public databases, to minimize publication bias.

Study	Country	Age		ES (95% CI)	% Weight
Onset					
Nohynek et al. 2012	Finland	4 to 19		20.00 (9.00, 80.00)	18.78
O'Flanagan et al. 2013	Ireland	5 to 19		13.40 (5.40, 32.00)	28.31
Miller et al. 2013	England	4 to 18		14.40 (4.30, 48.50)	15.27
Dauvilliers et al. 2013	France	< 18		21.50 (2.80, 166.60)	5.37
Heier et al. 2013	Norway	4 to 19		11.60 (4.70, 28.90)	27.17
Lareb 2015	Netherlands	0.5 to 5		11.94 (1.46, 96.54)	5.11
Subtotal (I–squared = 0.0%, P = .979)				14.32 (8.92, 22.99)	100.00
Healthcare contact					
O'Flanagan et al. 2013	Ireland	5 to 19		13.87 (5.21, 37.34)	29.55
Miller et al. 2013	England	4 to 18		4.71 (1.90, 11.82)	32.23
Jokinen et al. 2014	Finland	4 to 19		13.46 (6.69, 31.82)	38.22
Subtotal (I–squared = 44.1%, P = .167)				9.68 (4.88, 19.23)	100.00
Diagnosis					
O'Flanagan et al. 2013	Ireland	5 to 19		6.10 (1.50, 25.00)	8.21
Nohynek et al. 2012	Finland	4 to 19		6.00 (4.00, 20.00)	25.09
Miller et al. 2013	England	4 to 18		3.30 (1.50, 7.40)	25.52
MPA 2011	Sweden	< 20		6.60 (3.10, 14.50)	27.31
Dauvilliers et al. 2013	France	< 18		4.10 (1.40, 12.20)	13.87
Subtotal (I–squared = 0.0%, P = .746)				5.02 (3.36, 7.51)	100.00

NOTE: Weights are from random effects analysis
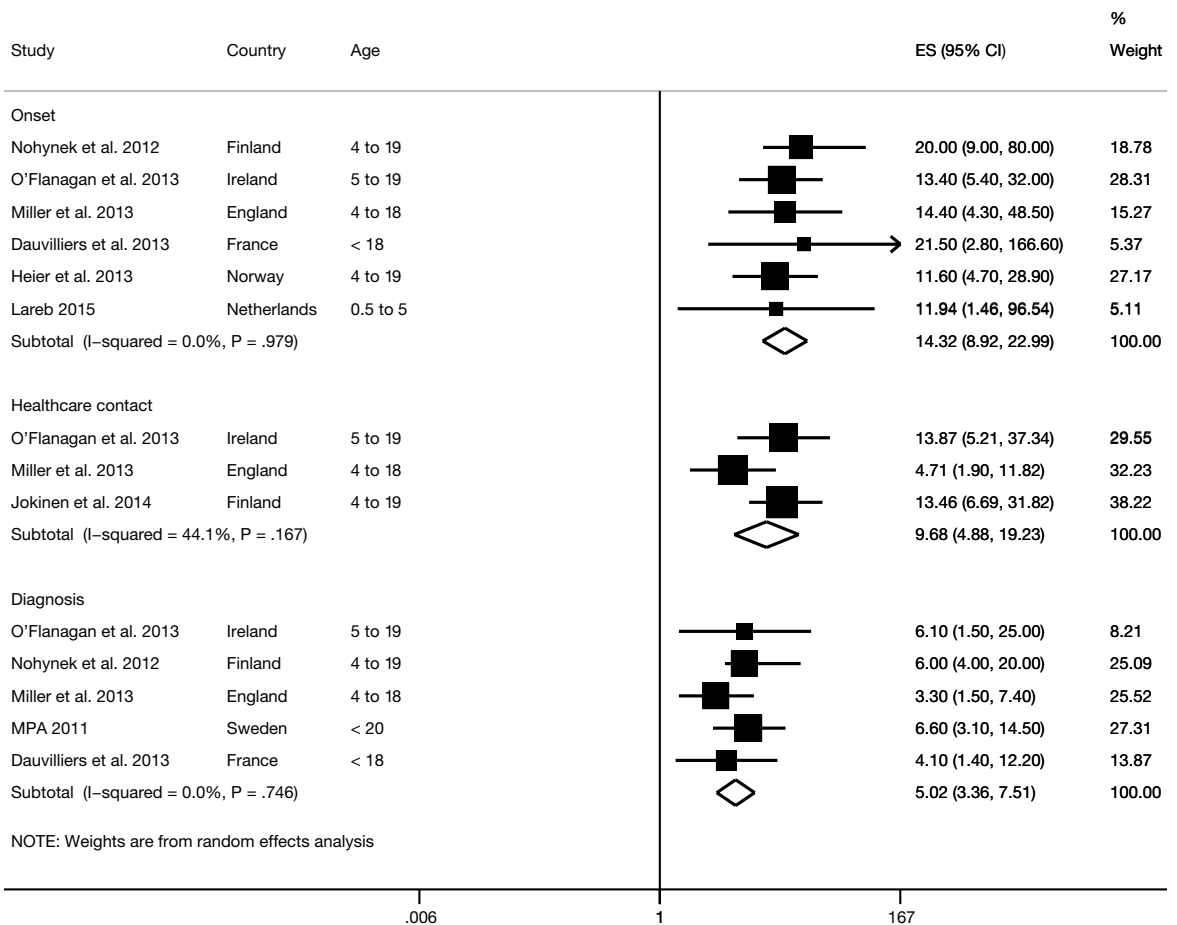
.006   1   167

Figure 9. Forest plot of studies assessing the risk of narcolepsy related to Pandemrix vaccine in adults. ES, Effect size; CI, Confidence interval. Reproduced with permission from Sarkanen et al. 2018.[210]
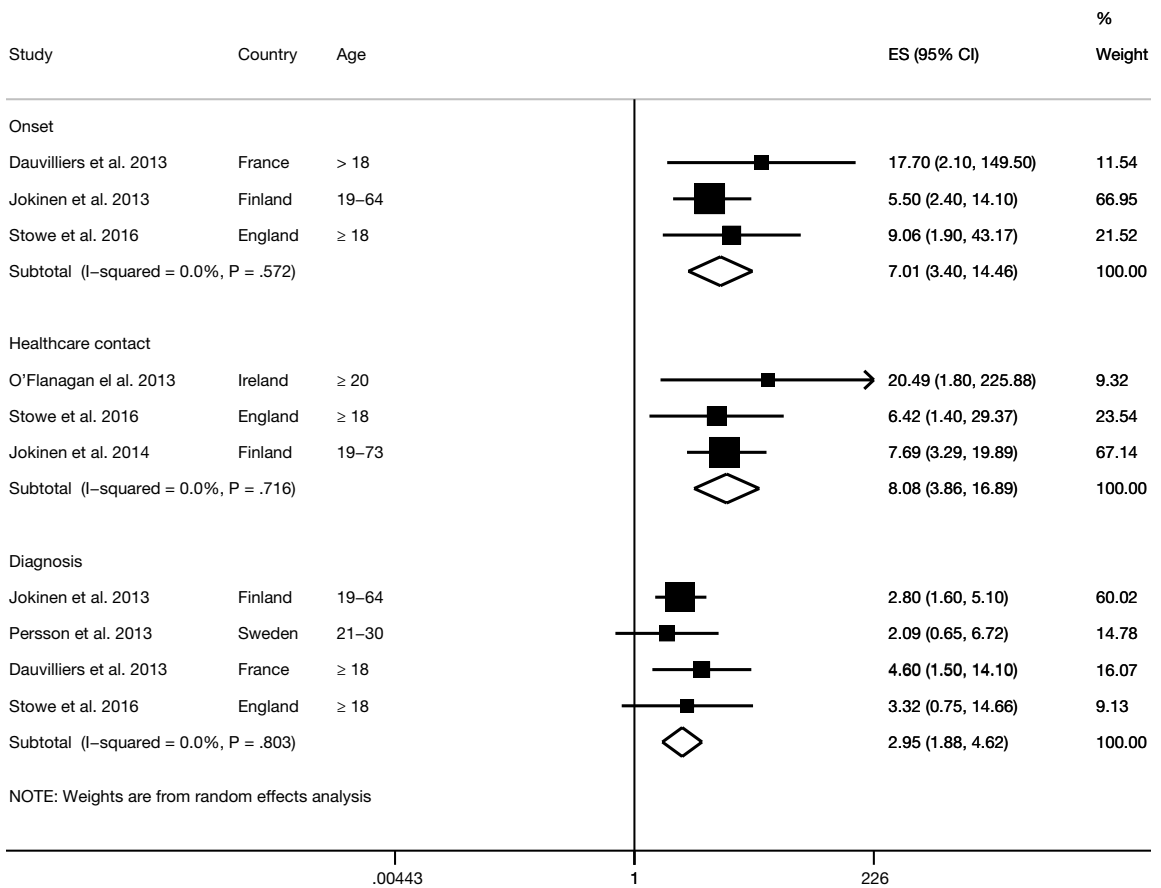
Study	Country	Age		ES (95% CI)	% Weight

Onset

Dauvilliers et al. 2013	France	> 18		17.70 (2.10, 149.50)	11.54
Jokinen et al. 2013	Finland	19–64		5.50 (2.40, 14.10)	66.95
Stowe et al. 2016	England	≥ 18		9.06 (1.90, 43.17)	21.52
Subtotal (I–squared = 0.0%, P = .572)				7.01 (3.40, 14.46)	100.00

Healthcare contact

O'Flanagan el al. 2013	Ireland	≥ 20		20.49 (1.80, 225.88)	9.32
Stowe et al. 2016	England	≥ 18		6.42 (1.40, 29.37)	23.54
Jokinen et al. 2014	Finland	19–73		7.69 (3.29, 19.89)	67.14
Subtotal (I–squared = 0.0%, P = .716)				8.08 (3.86, 16.89)	100.00

Diagnosis

Jokinen et al. 2013	Finland	19–64		2.80 (1.60, 5.10)	60.02
Persson et al. 2013	Sweden	21–30		2.09 (0.65, 6.72)	14.78
Dauvilliers et al. 2013	France	≥ 18		4.60 (1.50, 14.10)	16.07
Stowe et al. 2016	England	≥ 18		3.32 (0.75, 14.66)	9.13
Subtotal (I–squared = 0.0%, P = .803)				2.95 (1.88, 4.62)	100.00

NOTE: Weights are from random effects analysis

.00443   1   226

Figure 10. Forest plot of studies assessing the risk of narcolepsy related to Pandemrix vaccine in children and adolescents using different index dates. ES, Effect size; CI, Confidence interval. Reproduced with permission from Sarkanen et al. 2018.[210]

5.1.3 Synthesis of non-Pandemrix studies

A clear association with any vaccine other than Pandemrix was not seen. Reported RRs in Québec, Canada, where Arepanrix vaccine was used, 16 weeks after vaccination were based on different methods as follows:[18]

case-control method: 1.48 (0.37-7.03),
self-controlled case-series: 2.96 (0.71-12.39), and
cohort method 4.32 (1.50-11.12).

Yet, the vaccine attributable risk was only 1 per 1,000,000 people, which is significantly lower than in European countries and compared with Pandemrix. Data in a post-marketing safety surveillance study do not support a risk association

with Arepanrix and narcolepsy.[203] In the USA and South Korea, Pandemrix was not used and no increased risk was observed.[198,200,201,204] Pharmacovigilance database explorations or spontaneous reports have not shown an increased risk of narcolepsy associated with MF59-adjuvanted vaccines with over 23 million administered doses.[197,207]

In Beijing and Shanghai, China, a 3-fold increase in the incidence of narcolepsy was observed during the post-pandemic period, peaking 3-6 months after the H1N1 pandemic and returning to baseline two years later. [17,184,196] The vaccine coverage in China was very low and this increase was not related to any vaccine, which implies seasonal variation and a role of influenza virus in the development of narcolepsy.

Also in Germany, the age-standardized adjusted incidence rate increased more than 3-fold between the pre- and post-pandemic periods.[206] The increase started already in spring 2009, and the incidence density ratio was 3.57 (95% CI 1.94, 7.00). The study was, however, based only on non-validated register diagnoses and no vaccine history was available. The overall vaccine coverage in Germany was 4-8%, mostly with Pandemrix.

Clinical characteristics of the patients were reported in four risk association studies and four separate papers (Table 6).[7,9,12,23,24,211]. Vaccinated and unvaccinated subjects described in the studies differed e.g. in terms of diagnostic delay and time from EDS to CPL (shorter in vaccinated), and prevalence of CPL or other symptoms near onset (more common in vaccinated). All of these slight differences in clinical and polysomnographic features are linked to disease duration and make it difficult to determine the direct effect of Pandemrix.

Table 6. Comparison of demographic, clinical characteristics, and HLA typing between the total number of vaccinated and unvaccinated child and adolescent narcolepsy cases.[7,9,12,23,24,192,211]

	Vaccinated	Unvaccinated	P
Females	129/244 (53%)	53/113 (47%)	.294
Cataplexy	**194/206 (94%)**	**63/80 (79%)**	**<.001**
Hypnagogic hallucinations	**65/178 (37%)**	**59/102 (58%)**	**.001**
Sleep paralysis	31/158 (20%)	28/102 (27%)	.141
Disturbed sleep	87/121 (72%)	63/102 (62%)	.108
Behavioral problems	52/147 (35%)	23/72 (32%)	.615
Rapid weight gain near onset	**89/170 (52%)**	**39/102 (38%)**	**.024**
HLA DQB1*06:02 positive	**144/147 (98%)**	**58/65 (89%)**	**.006**
CSF hypocretin < 110 pg/mL	**108/110 (98%)[a]**	**19/22 (86%)**	**.008**

CSF, cerebrospinal fluid. [a] In the vaccinated group, one subject with hypocretin level of 121 pg/mL and one "borderline". Reproduced with permission from Sarkanen et al. 2018.[210]

Table 7. Summary of included Pandemrix studies in the meta-analysis. Reproduced with permission from Sarkanen et al. 2018.[210]

Authors, reference number	Country	Type	Age group (years)	Follow-up period Start	Follow-up period End	NC	Vaccinated Healthy	Vaccinated NC	Unvaccinated Healthy	Unvaccinated NC
Children and adolescents										
Dauvilliers et al. 2013 [9]	France	CC	< 18	Apr 1, 2009	Apr 30, 2011		21	N/A[a]	14	N/A[a]
Heier et al. 2013 [7]	Norway	RC	4 - 19	Nov 1, 2009	Nov 1, 2011	1st y	48	470,000	3	470,000
						2nd y	5	470,000	2	470,000
Jokinen et al. 2014 [183]	Finland	RC	4 - 19	Jan 1, 2009	Dec 31, 2012	1st y	93	687,000	7[b]	692,000[b]
						1-2 yrs	33	687,000	"	"
						> 2 yrs	13	751,000	"	"
Lareb 2015 [194]	Netherlands	Report	0.5 - 5				7	589,000	NA	450,000[c]
Miller et al. 2013 [10]	England	CC	4 - 18	Jan 1, 2008	Jul 31, 2011		11	360,000	64	8,130,000
MPA 2011 [192]	Sweden	RC	< 20	Jan 1, 2009	Dec 31, 2010		69	1,624,000	7	1,093,000
Nohynek et al. 2012 [5]	Finland	RC	4 - 19	Jan 1, 2009	Aug 15, 2010		46	511,000	7	986,000
O'Flanagan et al. 2014 [12]	Ireland	RC	5 - 19	Apr 1, 2009	Dec 31, 2010		19	160,000	5	620,000
Persson et al. 2013 [195]	Sweden	RC	0 - 20	Oct 1, 2009	Oct 1, 2011		126	966,000		425,000
Adults										
Dauvilliers et al. 2013 [9]	France	CC	≥ 18	Apr 1, 2009	Apr 30, 2011		11	N/A	16	N/A
Jokinen et al. 2013 [193]	Finland	RC	20 - 65	Jan 1, 2009	Jan 1, 2011		24	3,151,477	14	6,705,173
Jokinen et al. 2014 [183]	Finland	RC	20 - 74	Jan 1, 2009	Dec 31, 2012	1st yr	17	1,841,000	7[b]	5,802,000[b]
						1-2 yrs	15	1,841,000	"	"
						> 2 yrs	2	1,825,000	"	"
O'Flanagan et al. 2014 [12]	Ireland	RC	≥ 20	Apr 1, 2009	Dec 31, 2010		2	470,000	1	2,850,000
Persson et al. 2013 [195]	Sweden	RC	21- 30	Oct 1, 2009	Dec 31, 2011		23	310,000	NA	428,000
			31- 40				18	445,000	NA	361,000
			> 40				40	1,627,000	NA	1,283,000
Stowe et al. 2016 [11]	England	CCov	≥ 18	Sep 1, 2009	Oct 30, 2011		5	650,000	35	1,340,000

NC, narcolepsy; CC, case-control; N/C, not applicable; RC, register cohort; NA, not available; CCov, case-coverage

a Number of vaccinated and unvaccinated subjects cannot be given because of the case-control design.
b Number of unvaccinated cases reported for the full follow-up period until the end of 2012.
c Estimated from Statistic Netherlands.

Table 8. Summary of incidence studies from countries where Pandemrix vaccination was not used. Reproduced with permission from Sarkanen et al. 2018.[210]

Authors, reference number	Area	Study type	Collection period	Pandemic vaccine	Vaccine coverage	Vaccination-associated risk of narcolepsy
Choe et al. 2012 [198]	South Korea	Ecological	Jul 2006 - Jun 2011	MF59-ADJ, non-ADJ	26.10%[a]	Not increased
Duffy et al. 2014 [200]	USA	Register cohort	Oct 2009 - Dec 2011	Non-ADJ[b]	~10%	Not increased (OR ~ 1 in different age groups)
Han et al. 2011 [17]	Beijing, China	Case series	1996 - 2011	Non-ADJ	5.6%	3-fold increase, not associated with vaccination
Han et al. 2013 [196]	Beijing, China	Case series	1998 - Sep 2012	Non-ADJ	5.6%	Risk reduced to normal after 2 years
Harris et al. 2014 [203]	Ontario, Canada	Reported adverse effects[c]	Until Apr 2013	AS03-ADJ[d]	37%[e]	Not increased
Kim et al. 2015 [204]	South Korea[f]	Ecological	2007 - 2013	MF59-ADJ, non-ADJ	NR	Not increased
Montplaisir et al. 2014 [18]	Québec, Canada	CC, S-CCS, RC	Jan 2009 - Dec 2010	AS03-ADJ[d]	57%	Small increase possible[g]
Tsai et al. 2011 [207]	Worldwide	Reported adverse effects	Until Jul 2010	MF59-ADJ	N/A	Not increased
Wu et al. 2014 [184]	Shanghai, China	Case series	2003 - 2012	Non-ADJ	NR	x3-fold increase, not associated with vaccination

ADJ, adjuvanted; OR, odds ratio; CC, case control, S-CCS, self-controlled case series; RC, register cohort; RR, relative risk; NR, not reported, N/A, not applicable.
[a] 87% in age group under 18 years.
[b] Monovalent inactivated vaccine and monovalent live attenuated influenza vaccine during the pandemic period in 2009, and trivalent inactivated vaccine and live attenuated influenza vaccine during the seasonal influenza vaccination campaign in 2010-2011.
[c] From Pharmacovigilance databases and clinical trials.
[d] Arepanrix (GlaxoSmithKline Inc., Mississauga, Ontario, Canada; GlaxoSmithKline Biologicals, Wavre, Belgium).
[e] Based on the number of distributed vaccines, not administered.
[f] Active duty soldiers and military officers in the Korean military.
[g] RR 16 weeks after vaccination CC 1.48 (95% CI 0.37, 7.03), S-CCS 2.96 (0.71, 12.39), RC 4.32 (1.50, 11.12). Attributable risk 1 per million vaccine doses.

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5.2 Study II – Polysomnographic and actigraphic characteristics of patients with H1N1-vaccine-related and sporadic narcolepsy

Characteristics of the study population are given in Table 9. Females accounted for 52% of pNC and 45% of sNC patients. All pNC subjects were HLA DQB1*06:02 positive and 89.7% had cataplexy. In sNC patients, the corresponding figures were 96% and 79.6%. The diagnostic delay was substantially longer in sNC than in pNC, resulting also in significantly higher age at diagnosis. pNC patients were also younger at the age of onset of the disease. No differences were present in male:female ratio, BMI, or ESS results when adjusted for age at time of examination.

Table 9. Basic and laboratory characteristics of all narcolepsy patients in Study II.

	Vaccine-associated (n = 69)		Non-associated (n = 59)			
	M	SD	M	SD	P	P (adj.)
Age at onset, years	13.67	8.17	15.50	6.53	.191	<.001
Age, years	14.61	8.37	21.48	9.47	<.001	
Diagnostic delay, days	308.15	142.03	2187.87	2504.66	<.001	<.001
BMI	22.04	4.19	25.00	6.39	.011	.185
ESS	16.04	3.49	16.48	3.5	.653	.860
CSF orexin-A, pg/mL	36.96	67.61	88.72	113.3	.058	.064

M, mean; *SD*, standard deviation; P (adj.), P adjusted for age at diagnosis (at the time of study). BMI, body mass index; ESS, Epworth Sleepiness Scale; HLA, human leukocyte antigen; CSF, cerebrospinal fluid. Reproduced and modified with permission from Alakuijala et al. 2015.[191]

The main results are shown in Tables 5.6a and 5.6b. pNC subjects had 0.74 minute shorter mean sleep latency and more SOREMPs in MSLT than subjects with sNC, but the differences were no longer significant after adjustment for age. However, some gender differences emerged. Female subjects with pNC had higher percentage of SOREMPs than female sNC subjects (*M* = 82.14 *SD* 25.90 vs. *M* = 65.20 *SD* 29.20, age-adjusted P = .029). In males, mean sleep latency was significantly shorter in the pNC group than in the sNC group (*M* = 1.69 *SD* 1.16 vs. *M* = 2.87 *SD* 2.46 minutes, age-adjusted P = .042)

In the PSG, sleep efficiencies were within the normal range, as were the total sleep times. Sleep latency was short in both groups and REM sleep latency was on average rather short but with wide variation. Nocturnal SOREMP (REM latency less than 15 minutes) was observed in 61% in the pNC group and in 46% in the sNC

group (P = NS). SOREMP in PSG correlated positively with number of SOREMPs in MSLT and negatively with sleep latency in both MSLT and PSG.

PLMSI was 4.34/h (95% CI 1.85, 6.83) higher in sNC than in pNC (P = .018), but after adjustment for gender, the difference was significant only in females. Serum ferritin concentration was analyzed in only 14 patients, and no differences emerged between the groups. Neither were there differences in reported symptoms of RLS.

Regarding the actigraphic parameters, sNC patients had delayed cosine peak and reduced light:dark ratio compared with pNC. The differences remained significant after adjustment for age.

Table 10a. MSLT, polysomnographic, and actigraphic characteristics of all patients.

	Vaccine-associated (*n* = 69)		Non-associated (*n* = 59)			
	M	*SD*	*M*	*SD*	P	P (adj.)
MSLT						
Mean sleep latency, min	2.10	1.98	2.84	2.22	.049	.136
SOREMP, %[†]	82.01	26.30	68.57	28.65	.008	.056
PSG						
REM latency, min	40.23	51.44	58.38	67.22	.209	.251
Sleep latency, min	5.81	7.94	5.43	4.69	.814	.957
Total sleep time, h	7.56	1.12	7.64	1.16	.747	.204
Sleep efficiency, %	87.92	7.48	85.87	10.55	.271	.624
AHI, /h	1.05	1.95	1.69	3.31	.208	.510
PLMSI /h	**1.92**	**3.31**	**6.26**	**9.81**	**.004**	**.018**
Actigraphy						
Sleep latency, min	8.94	6.96	16.45	21.17	.173	.213
Actual sleep time, min	7.00	1.94	6.39	1.09	.351	.547
Sleep efficiency, %	71.41	8.75	74.38	9.39	.383	.510
No. of immobile phases in 1 min	15.06	6.91	14.73	7.82	.907	.946
FRI	46.23	13.34	45.48	14.37	.884	.725
Cosine peak, hh:mm	**14:16**	**01:16**	**16:11**	**01:47**	**.002**	**.013**
Light:dark ratio	**2.60**	**0.82**	**1.59**	**0.74**	**.002**	**.001**
L5 onset, hh:mm	00:44	01:44	02:22	02:44	.045	.100
M10 onset, hh:mm	09:32	01:57	10:55	01:48	.049	.067

M, mean; *SD*, standard deviation; P (adj.), P adjusted for age at diagnosis (at the time of study). MSLT, Multiple Sleep Latency Test; SOREMP, sleep onset REM period; PSG, polysomnography; AHI, apnea–hypopnea index; PLMSI, periodic leg movement index during sleep; FRI, Movement and fragmentation index; L5, lowest 5 [hours of activity, see methods for more details]; M10, maximal 10 [hours of activity]. [†] Percentage of MSLT sessions containing SOREMP. Reproduced and modified with permission from Alakuijala et al. 2015.[191]

Table 10b. Comparison between male and female patients in pNC (data presented as means (SD)).

	Male	Female	P
n	33	36	
Age at onset, years	12.65	14.68	.277
Diagnostic delay, days	322.13	294.61	.632
Age at diagnosis, years	13.67	15.51	.329
BMI kgm^{-2}	21.79	22.36	.634
ESS at diagnosis	**14.64 (3.92)**	**17.82 (1.78)**	**.021**
Cataplexy, %	84.8	94.3	.187
HLA DQB*0602 positivity, %	100	100	1.000
CSF hypocretin-1, pg/mL	29.39	45.91	.332
Sleep latency in MSLT, min	1.69	2.45	.129
SOREMPs	3.53	3.57	.931
Percentage of SOREMPs in MSLT	81.88	82.14	.991
REM latency in PSG, min	40.64	39.80	.959
Sleep latency in PSG, min	7.34	4.19	.208
Total sleep time in PSG, h	7.87	7.31	.067
Sleep efficiency in PSG, %	88.65	87.35	.528
AHI in PSG, /h	1.19	0.93	.610
PLMSI in PSG, /h	2.24	1.65	.539
Sleep latency in ACT, min	9.89	8.00	.580
Actual sleep time in ACT, h	7.29	6.71	.544
Sleep efficiency in ACT, %	68.70	74.12	.197
Number of immobile phases in 1 min in ACT	17.22	12.89	.191
Movement and fragmentation index in ACT	49.49	42.97	.314
Cosine peak in ACT, hh:mm	**13:27 (1:19)**	**15:04 (0:29)**	**.003**
Light-dark ratio in ACT	2.82	2.38	.268
L5 onset in ACT, hh:mm	0:18	1:13	.256
M10 onset in ACT, hh:mm	**8:42 (1:42)**	**10:26 (1:53)**	**.049**

SD, standard deviation; BMI, body mass index; ESS, Epworth Sleepiness Scale, HLA, human leukocyte antigen, CSF, cerebrospinal fluid; MSLT, Multiple sleep latency test; SOREMP, sleep onset REM sleep period; PSG, polysomnography, AHI, apnea-hypopnea index; PLMSI, periodic limb movements in sleep index; ACT, actigraphy. Reproduced and modified with permission from Alakuijala et al. 2015.[191]

5.3 Study III – Clinical course of H1N1-vaccine-related narcolepsy

5.3.1 Baseline characteristics

There were no differences in the medians of age at symptom onset in pNT1 and sNT1 patients (Table 11). H1N1-vaccine-related subjects had shorter diagnostic delay, had shorter disease duration, and were therefore younger at the follow-up visit. Median time from vaccination to disease onset was 4.5 months. Hypocretin-1 concentration in CSF was low in all tested 19 pNT1 and 15 sNT1 subjects. MSLT results were similar between the two groups.

BMI was higher in sNT1 (*Mdn* = 26.4, range 20.2 - 47.5) than in pNT1 (*Mdn* = 23.3, range 16.6 - 38.3, U = 166.0, P = .02). pNT1 subjects had more weekly cataplectic attacks than sNT1 subjects (*Mdn* = 10.5, range 0 - 210 vs. *Mdn* = 3.0, range 0 - 50, U = 457.0, P = .018). No significant differences were present in UNS, ESS, SNS, WHO5, and RDS. Three of 10 vaccinated sNT1 subjects experienced some worsening of symptoms after a(H1N1)pdm09 vaccination.

There was a strong correlation between the age at symptom onset and MSL (r_s = .560, P = .005) in pNT1 patients, but not in sNT1 patients (r_s = -.309, P = .161). Onset age correlated also with number of SOREMPs in MSLT in both pNT1 (r_s = -.482, P = .020) and sNT1 (r_s = -.480, P = .02) in MSLT. No correlation existed between onset age and questionnaire scores.

Table 11. Characteristics of Study III subjects.

	pNT1 (*n* = 26)		sNT1 (*n* = 25)		
	Mdn	Range	*Mdn*	Range	P
Age at onset, years	15.2	6.0 - 46.5	14.5	6.26 - 31.8	.749
Age at diagnosis, years	**15.9**	**6.4 - 48.5**	**18.5**	**11.6 - 39.0**	**.034**
Age at follow-up visit, years	**18.5**	**8.2 - 49.9**	**28.2**	**12.7 - 51.9**	**.004**
Disease duration (follow-up), years	**3.3**	**2.1 - 4.6**	**10.2**	**2.3 - 31.4**	**<.001**
Vaccination to disease onset, days	145	6 - 487	NA	NA	NA
CSF hypocretin, pg/mL	8	0 - 108	20	0 - 79	.737
MSLT sleep latency, min	2.25	0.4 - 10.0	3.10	0.1 - 6.9	.211
SOREMPs	3	0 - 5	3	0 - 5	.622

pNT1, Pandemrix-related type 1 narcolepsy; sNT1, sporadic type 1 narcolepsy; *Mdn*, median; MSLT, Multiple Sleep Latency Test; SOREMPs, Sleep Onset REM-sleep Periods in MSLT; NA, not applicable; Hypocretin-1 measured in 19/24 Pandemrix H1N1-vaccine-related subjects and in 15/23 sporadic cases. P, Mann-Whitney U Test P value. Reproduced with permission from Sarkanen et al. 2016.[212]

5.3.2 Longitudinal follow-up of H1N1-vaccine-related narcolepsy patients

RDS scores decreased significantly between the visits, implying less symptoms of depression (Table 12). Mean difference in RDS score was -3.44 at the follow-up visit, being the only statistically significant difference in the questionnaire scores between the visits (paired samples t-test t(24) = 3.37, P = .003). No significant changes occurred in ESS (-0.63), UNS (-1.68), SNS (7.88), or WHO5 (2.54) points. However, wide CIs imply that there were also individual patients with marked changes in follow-up. As seen in Figure 5.4, these changes were mostly amelioration of symptoms, but some patients had worse scores (e.g. in UNS higher values) than on the first visit (Figure 11). Median BMI increased between the visits by 2.3 kgm^{-2} (Z = 3.8, P = .001). Number of cataplectic attacks per week did not change significantly (Table 12.).

Table 12. Symptoms of H1N1-vaccine-related narcolepsy at the first visit and at the follow-up visit.

	1st visit		2nd visit		95% CI for mean difference	P
	M	*SD*	*M*	*SD*		
ESS	15.8	6.2	14.9	5.0	-3.3, 2,0	.631
UNS	23.3	7.3	21.1	6.9	-5.3, 1.9	.352
SNS	-40.4	38.3	-32.0	29.7	-9.8, 25.6	.368
RDS	**10.2**	**4.7**	**6.7**	**4.5**	**-5.5, -1.3**	**.003**
WHO5	45.5	24.8	48.0	19.3	-10.4, 15.4	.688
	Mdn	range	*Mdn*	range		
CPL / week	15	0-210	10.5	0-210	NA	.281
BMI, kgm⁻²	**20.8**	**14.4-35.1**	**23.4**	**16.6-38.3**	**NA**	**<.001**

M, mean; *SD*, standard deviation; CI, confidence interval; ESS, Epworth Sleepiness Scale; UNS, Ullanlinna Narcolepsy Scale; SNS, Swiss Narcolepsy Scale; RDS, Rimon's Brief Depression Scale, WHO5, WHO-Five Well-Being Index; CPL / week, number of cataplectic attacks per week; BMI, Body Mass Index. *M*, *SD*, and 95% CI for mean difference is reported for parametric and median (range) for nonparametric variables. Paired samples t-test was used to compare parametric variables and Wilcoxon signed-rank test to compare nonparametric variables between different visits in pNT1. Reproduced with permission from Sarkanen et al. 2016.[212]
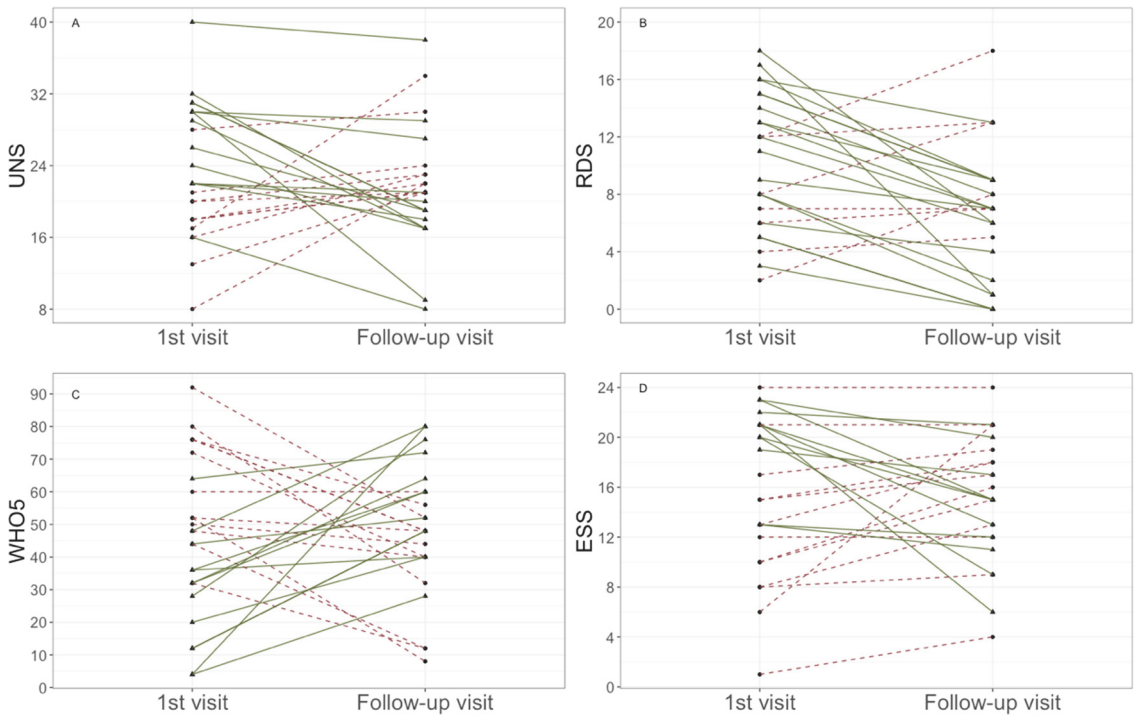
Figure 11. Changes in questionnaire scores between the visits in Pandemrix-related narcolepsy. Solid green line: better. Dotted red line: worse. UNS, Ullanlinna Narcolepsy Scale; RDS, Rimon's Brief Depression Scale; WHO5, WHO-Five Well-Being Index; ESS, Epworth Sleepiness Scale. Reproduced and modified with permission from Sarkanen et al. 2016.[212]

5.3.3 Results in relation to hypocretin levels in H1N1-vaccine-related narcolepsy

Hypocretin-1 levels, mean sleep latency, or SOREMPS in MSLT did not explain any changes in sleep questionnaire scores when analyzed in a linear regression model. Next, we divided the subjects into two subgroups based on hypocretin levels (< 20 pg/mL (below detection limit) and 20 to 110 pg/mL). pNT1 patients with lower hypocretin levels had higher UNS scores (M = 24.4, 95% CI 20.4, 28.4) than subjects with higher hypocretin levels (M = 18.8, 95% CI 15.0, 22.5, t(19) = −3.1, P = .048) at the follow-up visit (Figure 12).

pNT1 patients with lower hypocretin levels also had higher ESS points at the follow-up (M = 17.2, 95% CI 14.4, 20.3 vs. M = 13.1, 95% CI 11.4, 14.9, t(18) = −2.1, P = .040. Moreover, the delay to diagnosis in this group was shorter than in patients with higher hypocretin (M = 207 days, 95% CI 99, 316 vs. M = 803 days, 95% CI 587, 1017, t(17) = −6.2, P < .005).

There questionnaire scores at the first visit or the difference in questionnaire scores between the first and the second visit did not differ between the two hypocretin subgroups.
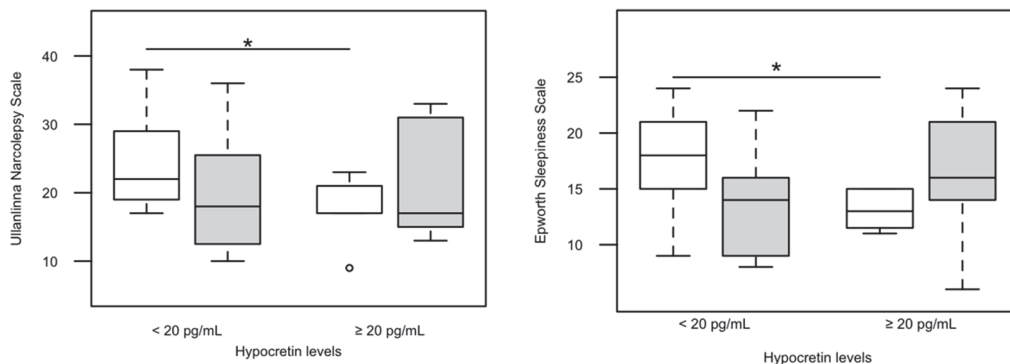
Figure 12. Ullanlinna Narcolepsy Scale and Epworth Sleepiness Scale scores at the follow-up visit according to hypocretin level and narcolepsy type. White boxplot, H1N1-vaccine-related narcolepsy; gray boxplot, sporadic narcolepsy; asterisk, independent samples t-test. *P < .05. Reproduced with permission from Sarkanen et al. 2016.[212]

We also analyzed different UNS questions or items in relation to hypocretin levels. The very low hypocretin pNT1 subgroup reported head nods more often than those with hypocretin concentration of 20 to 110 pg/mL (Mdn = 4, M = 3.18, 95% CI 2.24, 4.12 vs. Mdn = 1.5, M = 1.63, 95% CI 0.29, 2.96, P = .033). No differences were present in other UNS cataplexy items.

5.3.4 Comparison of H1N1-vaccine-related narcolepsy to sporadic narcolepsy

Differences in BMI and cataplexy between pNT1 and sNT1 were analyzed by Mann-Whitney U test. pNT1 subjects had lower BMI than sNT1 subjects (Mdn =26.4, range 20.2 - 47.5 vs. Mdn = 23.3, range 16.6 - 38.3), U = 166.0, P = .02). Cataplexy was more frequent in pNT1 than in sNT1 (Mdn = 10.5, range 0 - 210, vs. Mdn = 3.0, range 0 - 50), U = 457.0, P = .018.

Differences in UNS, ESS, SNS, and WHO5 were analyzed by independent samples t test. No significant differences emerged in these questionnaire scores between the groups (Table 13). We also did not see any differences in the correlation between hypocretin levels and questionnaire scores in sNT1 (Figure 12).

Table 13. Comparison of pNT1 at the follow-up visit and sNT1

	pNT1		sNT1		P
	M	95% CI	*M*	95% CI	
ESS	14.9	12.8, 16.9	15.6	13.6, 17.7	.587
UNS	21.1	18.3, 23.9	21.4	17.5, 25.3	.896
SNS	-32.0	-44.2, -19.7	-31.2	-46.1, -16.3	.933
RDS	6.7	4.9, 8.5	6.7	4.4	.980
WHO5	48	40.2, 55.8	55.4	47.1, 63.6	.187

pNT1, Pandemrix-related type 1 narcolepsy; sNT1 sporadic type 1 narcolepsy*; M*, mean; CI, confidence interval; ESS, Epworth Sleepiness Scale; UNS, Ullanlinna Narcolepsy Scale; SNS, Swiss Narcolepsy Scale; RDS, Rimon's Brief Depression Scale, WHO5, WHO-Five Well-Being Index.

5.3.5 Medications used

pNT1 subjects were free of any narcolepsy medication on the first visit. Medications used by pNT1 subjects on the second visit are shown in Table 14. Modafinil - sodium oxybate combination was used by 10 subjects. Two subjects with somewhat average questionnaire scores and no change between the visits were still without medication on the second visit.

Table 14. Medications of subjects at follow-up.

Modafinil	16
Sodium oxybate	12
Methylphenidate	3
Venlafaxine	3
Clomipramine	2
MAO-B inhibitors	1
Other narcolepsy medications (e.g. SSRI)	3
Other medications (incl. melatonin)	8
No medication	2

MAO-B, monoamineoxidase; SSRI, selective serotonin reuptake inhibitor. Reproduced with permission from Sarkanen et al. 2016.[212]

5.3.6 Disability caused by narcolepsy

Excessive daytime sleepiness was experienced as the most disabling symptom in pNT1, causing moderate to severe harm or disability to 84% of study subjects (Figure 13). Disturbed sleep caused similar harm to 64%, partial cataplexies to

37%, and generalized cataplexies to 32% of respondents. Moderate medication side effects were reported by 27%. Adverse effects were as follows: weight increase 21%, hypnagogic or hypnopompic hallucinations 26%, other hallucinations 16%, and sleep paralyses 16%.
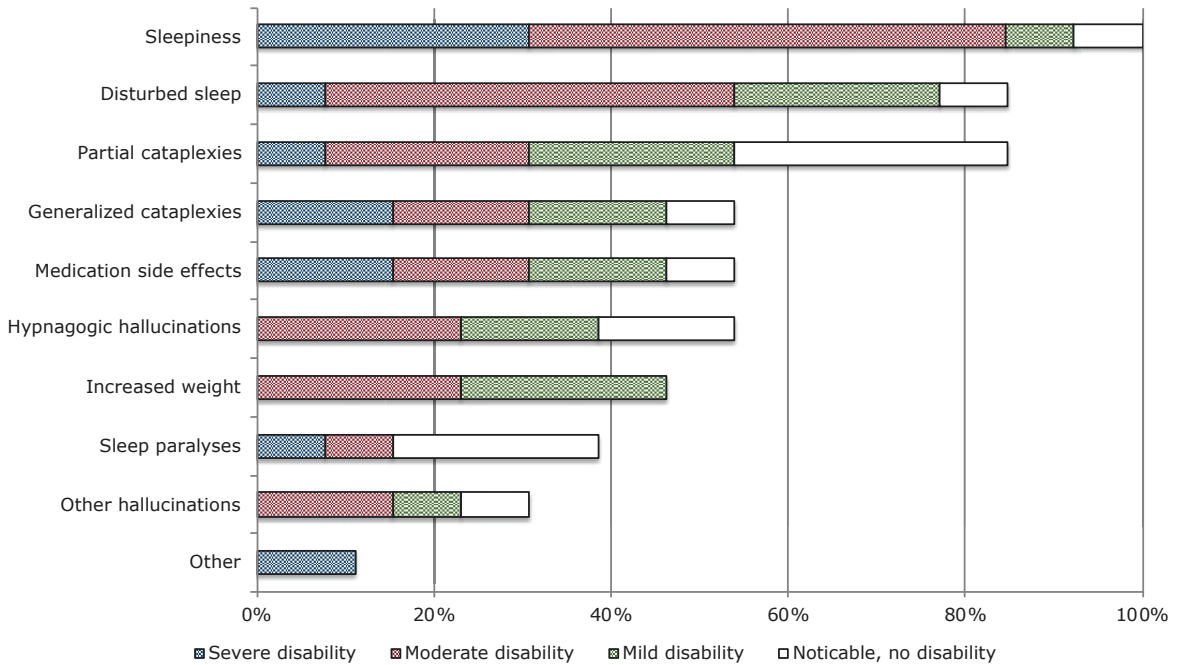

Figure 13. Disability caused by symptoms of Pandemrix H1N1-vaccine-related narcolepsy at the follow-up visit (n = 19/26). Sorted by severity of disability. Reproduced and modified with permission from Sarkanen et al. 2016.[212]

5.4 Study IV – Ullanlinna Narcolepsy Scale in diagnosis of narcolepsy

5.4.1 Characteristics

The mean age of NT1 patients in Study IV was 23.7 years (*SD* 11.9). They were younger than HS, OSA, OSRD, RLSD/PLMS patients or healthy controls, but compared with NT2 patients, the mean age was similar (Table 15). Hypocretin-1 levels were below 150 pg/mL in all 69 tested NT1 patients and below 110 pg/mL in 65 of these patients. The four patients with hypocretin of 110 to 149 pg/mL had unambiguous cataplexy and positive MSLT. They were also HLA DQB1*06:02-positive, as were all NT1 patients, excluding one patient with missing HLA data. Of NT1 patients, 64% had Pandemrix-associated narcolepsy.

By definition, all NT2 patients fulfilled the MSLT criteria for narcolepsy. Conversely, in NT1, only 74% had two or more SOREMPs and 89% had MSL less than eight minutes. MSLT data were missing for NT1 subjects who had their MSLT done in another clinic, but all of these subjects had hypocretin-1 below 110 pg/mL. In OSA, HS, and ORSD groups, 22-33% of subjects had two or more SOREMPs, and three subjects in these groups fulfilled MSLT criteria for narcolepsy (one subject with Kleine-Levin syndrome, one subject with severe delayed sleep phase syndrome, and one subject with severe OSA).

5.4.2 Analyzed scales in different diseases

The results of UNS were similar in all age groups (Figure 14). Mean UNS score in NT1 was 22.0 (95% CI 20.4, 23.6), which was higher than in any other group (P < .001 in all comparisons). Intriguingly, mean UNS score in NT2 was also less than 14 and significantly lower than inNT1 (M = 13.7, 95% CI 10.3, 17.1, P = .0013). If the cataplexy questions were omitted, there was only a minor reduction in UNS scores in NT2 (M = 12.8, 95% CI 9.0, 16.6), while NT1 scores were significantly lower (M = 14.6, 95% CI 13.6, 15.6) and no longer differed from NT2 scores (P = .269). Thirteen NT1 patients did not have cataplexy. Their UNS scores did not differ from NT2 patients (M = 14.2, 95% CI 11.9, 16.5, P =.476).
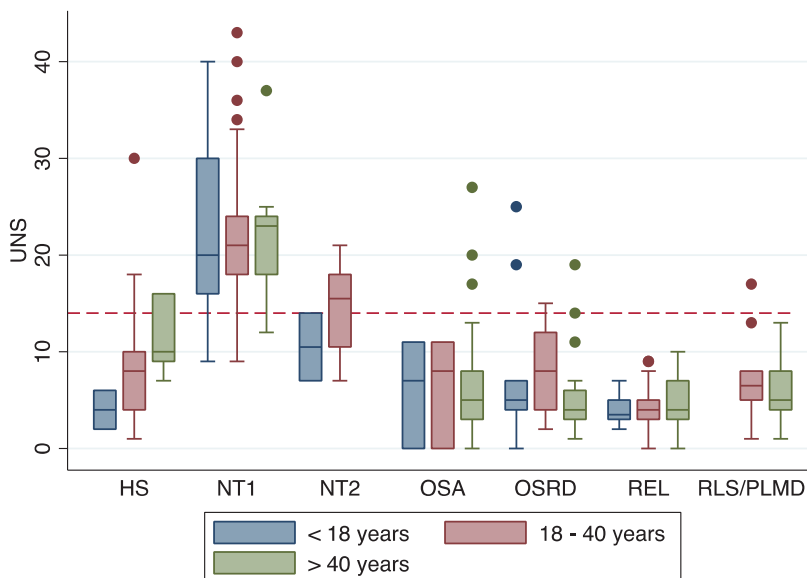
Figure 14. Ullanlinna Narcolepsy Scale by age group (< 18 years, 18 to 40 years, > 40 years). NT1, Narcolepsy type 1; NT2, Narcolepsy type 2; HS, Hypersomnia; OSA, Obstructive sleep apnea; OSRD, Other sleep-related disorders, REL, Relatives; RLS, Restless legs or periodic limb movement disorder. Dots indicate outliers. The dashed line indicates Ullanlinna Narcolepsy Scale score of 14.

Mean SNS score in NT1 was -32.4 (95% CI -40.2, -24.6). SNS scores were also lower in NT1 than in any other group. In NT2, scores were on average above zero (*M* = 16.0, 95% CI -4.0, 36.0), which is the cut-off point for SNS. Conversely, ESS did not distinguish NT1 and NT2 from each other, but resulted in higher points in these two syndromes than in HS, OSA, or any other group (Table 15).

Table 15. Characteristics of study population in Study IV.

	NT1	NT2	HS	OSA	OSRD	RLS/ PLMD	RELATIVES[†]
	(*n* = 89)	(*n* = 10)	(*n* = 24)	(*n* = 37)	(*n* = 51)	(*n* = 56)	(*n* = 85)
Age *M* (SD), years	23.7 (11.9)	23,6 (8.1)	35 (15.9)*	52.7 (17.3)*	32.1 (13.8)*	54,5 (15.3)*	39.1 (17.8)*
Age < 18, *n*	38	2	2	3	9	0	14
Age 18-40, *n*	41	8	13	3	25	10	25
Age > 40, *n*	10	0	9	31	17	46	46
Age at onset *M* (*SD*), years	18.7 (10.6)	21.6 (7.7)	15.4 (5.5)	UK	18.4 (8.6)	UK	N/A
UNS *M* (*SD*) min, max	22.0 (7.6) 9, 43	13.7* (4.8) 7, 21	9.7* (6.5) 1, 30	6.9* (5.7) 0, 27	7.2* (5.4) 0, 25	6.0* (3.4) 1, 17	4.3* (2.4) 0, 10
ESS *M* (*SD*) min, max	16.2 (4.7) 0, 24	14 (6.1) 2, 20	12.2* (5.3) 0, 20	7.8* (6.1) 0, 20	9.2* (6.3) 0, 21	8.4* (5.4) 1, 17	4.3* (3.1) 0, 12
SNS *M* (*SD*) min, max	-32.4 (35.6) -110, 45	16.0* (26.1) -18, 48	23.2* (14.8) -12, 42	38.1* (16.8) 6, 61	28.5* (26.3) -51, 66	26.1* (16.9) 1, 56	N/A
HLA DQB1*06:02 positive *n/n* studied	88/88 (100 %)	3/7 (43%)*	7/17 (41%)*	1/8 (13%)*	9/20 (45%)*	1/6 (17%)*	53/79 (67%)*
Hypocretin-1 *M* (*SD*), pg/mL	36.7 (38.2)	248.6* (58.0)	272.5* (57.6)	320.5* (38.9)	240.6* (56.1)	297.3* (61.1)	N/A
Hypocretin-1 <110, pg/mL *n/n* studied	65/69‡	0/8	0/11	0/2	0/12	0/3	N/A
MSL in MSLT *M* (*SD*), minutes	3.7 (3.5)	4.1 (2.3)	8.7 (5.6)	9.8 (3.7)	13.1 (3.5)	12.5 (5.5)	N/A
SOREMPS in MSLT *M* (SD)	2.7 (1.7)	3.4 (1.3)	0.4 (0.8)	1.2 (1.5)	1.1 (1.7)	0 (0)	N/A
SOREMP ≥2 n/n studied	55/74 (74%)	9/9 (100%)	2/19 (22%)	2/6 (33%)	7/28 (25%)	0/9 (0%)	N/A

NT1, narcolepsy type 1; NT2, narcolepsy type 2; HS, hypersomnia; RLS, restless legs syndrome; PLMD, periodic limb movement disorder; OSA, obstructive sleep apnea; OSRD, other sleep-related disorders; *M mean;* SD, standard deviation; UNS, Ullanlinna Narcolepsy Scale; ESS, Epworth Sleepiness Scale; SNS, Swiss Narcolepsy Scale; MSLT, multiple sleep latency test; SL, mean sleep latency; SOREMPS, sleep onset REM sleep periods; N/A, not applicable; UK, unknown. * P < .05 compared with NT1. † Relatives of NT1 patients. ‡ All four had values between 110 and 150 pg/mL.

5.4.3 Sensitivity, specificity, and predictive values

Using the whole dataset, UNS cut-off point 14 separated NT1 from other syndromes (HS, OSA, OSRD, RLS/PLMD, NT2) with 85.4% sensitivity and 87.6% specificity (Table 16). If NT1 and NT2 were combined, the figures were 82.8% and 90.5, respectively. The lowest UNS score in NT1 was 9, yielding 100% sensitivity, and the highest score in other diseases was 30, resulting in 100% specificity for 31 points. Positive predictive value (PPV) of UNS ≥ 14 was 77.6% for NT1. Negative predictive value (NPV) was 92.3%. If the cut-off point was lowered to 13, the PPV and NPV were 73.2% and 93.7%, respectively.

Table 16. Detailed report of sensitivity and specificity of UNS with different cut-off points in NT1 (*n* = 89) versus NT2, HS, RLS/PLMD, OSA, and OSRD (n = 178) combined.

Cut-off point	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Correctly classified (%)	LR+	LR-
≥ 9	100	69.1	61.8	100	79.4	3.24	0
≥10	96.6	73.6	64.7	97.8	81.3	3.66	0.05
≥11	92.1	78.7	68.3	95.2	83.2	3.47	0.1
≥12	91	80.9	70.4	94.7	84.3	4.32	0.11
≥13	88.8	83.7	73.2	93.7	85.4	5.45	0.13
≥14	85.4	87.6	77.6	92.3	86.9	6.91	0.17
≥15	84.3	88.8	79	91.9	87.3	7.5	0.18
≥16	80.9	91	81.8	90.5	87.6	9	0.21
≥17	78.7	93.3	85.4	89.7	88.4	11.67	0.23
≥18	74.2	94.4	86.8	88	87.6	13.2	0.27
≥19	70.8	96.1	90	86.8	87.6	18	0.3
≥20	64	97.2	91.9	84.4	86.1	22.8	0.37
...	...	...	...	...	...	...	...
≥ 31	13.5	100	100	69.8	71.2	N/A	0.86

UNS, Ullanlinna Narcolepsy Scale; NT1, narcolepsy type 1; NT2, narcolepsy type 2; HS, hypersomnia; RLS/PLMD, restless legs syndrome or periodic limb movement disorder; OSA, obstructive sleep apnea; OSRD, other sleep-related disorders; PPV, positive predictive value; NPV, negative predictive value. N/A, not applicable. LR+, positive likelihood ratio; LR-, negative likelihood ratio. LR+ ≥ 5 is considered a moderate increase in the likelihood of disease.

In the dataset without cases with any missing values in SNS and ESS (*n = 167*), the sensitivity and specificity of UNS were 83.5% and 84.1%, respectively (Table 17). Sensitivity and specificity of SNS in separating NT1 from other disorders were 77.2% and 88.6%, and PPV and NPV were 85.9% and 81.3%, respectively (Table 15). If NT1 and NT2 were combined, the sensitivity was 72.7% and specificity 91.1%. Positive and negative predictive values were 81.8%, and 81.3%, respectively.

ESS ≥ 11 showed 88.6% sensitivity, 45.5% specificity, 59.3% PPV, and 81.6% NPV for NT1 (Table 17). NT1 combined with NT2 resulted in 87.5% sensitivity, 48.1% specificity, 65.3% PPV, and 77.6% NPV for ESS.

Table 17. Sensitivity and specificity of cut-off points of UNS, SNS, and ESS in separating different disorders. Data are presented as percentages. Subjects with any missing values in SNS or ESS were excluded (n = 167).

	UNS ≥ 13	UNS ≥ 14	UNS ≥ 17	SNS < 0	ESS ≥ 11	
	Sensitivity	Sensitivity	Sensitivity	Sensitivity	Sensitivity	n
NT1 vs.	87.3	83.5	76.0	77.2	88.6	79
	Specificity	Specificity	Specificity	Specificity	Specificity	n
OSA	86.4	95.5	95.5	100.0	63.6	22
RLS/PLMD	66.7	91.7	91.7	100.0	33.3	12
OSRD	90.6	90.6	93.8	84.4	50.0	32
HS	61.5	69.2	92.9	84.6	30.8	13
NT2	44.4	44.4	66.7	66.7	22.2	9
ALL	77.3	84.1	90.9	88.6	45.5	88
	Sensitivity	Sensitivity	Sensitivity	Sensitivity	Sensitivity	n
NT1 and NT2 vs.	84.1	80.7	71.6	72.7	87.5	88
	Specificity	Specificity	Specificity	Specificity	Specificity	n
HS	61.5	69.1	92.3	84.6	30.8	13
ALL (without NT2)	81.0	88.6	93.7	91.1	48.1	79

UNS, Ullanlinna Narcolepsy Scale; SNS, Swiss Narcolepsy Scale; ESS, Epworth Sleepiness Scale; NT1, narcolepsy type 1; NT2, narcolepsy type 2; RLS/PLMD, restless legs syndrome or periodic limb movement disorder; OSA, obstructive sleep apnea; OSRD, other sleep-related disorders; HS, hypersomnia; ALL = HS, OSA, OSRD, RLS/PLMD.

False positives for NT1 in UNS were caused mostly by other hypersomnias, including NT2, and also by a few OSA and OSRD cases and a single RLS/PLMD case (Table 18).

Table 18. False positives (for narcoleps type 1) in the Ullanlinna Narcolepsy Scale.

HS	6
OSA	3
OSRD	6
RLS/PLMD	1
NT2	6

HS, hypersomnia; OSA, obstructive sleep apnea; OSRD, other sleep disorders; RLS/PLMD, restless legs or periodic limb movement disorder; NT2, narcolepsy type 2

5.4.4 Area under curve analysis, correlation with hypocretin, and effect of medication

A large area under the receiver operating characteristic curve (ROC AUC) was observed in both UNS (.928, 95% CI .891, .963) and SNS (.921, 95% CI .887, .964), without statistically significant differences (X^2 (1, n = 167) = .01, P = .921). The ROC AUC was significantly smaller in ESS (.784, 95% CI .704, 843) than in UNS (X^2 = 25.58, P < .001) or SNS (X^2 = 14.54, P < .001) (Figure 15).
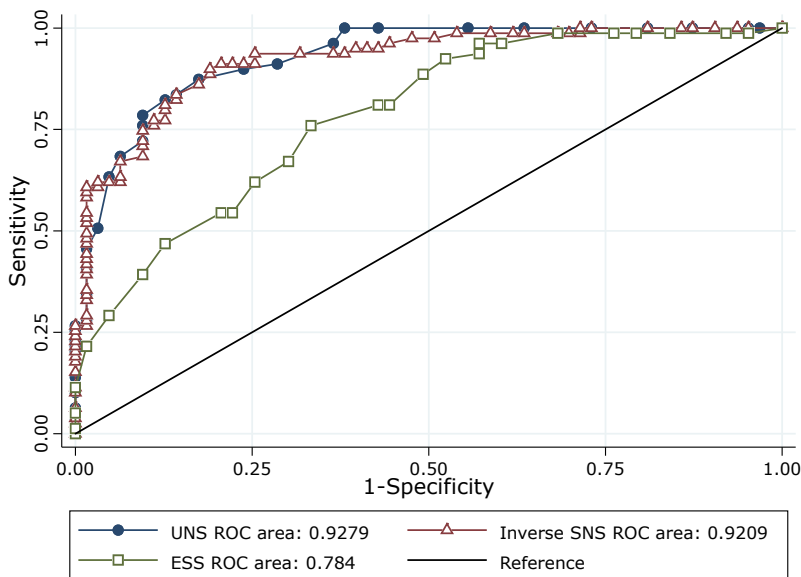
Figure 15. Performance of UNS, SNS, and ESS in differentiating narcolepsy type 1 from narcolepsy type 2, other hypersomnias, restless legs and periodic limb movement disorder, obstructive sleep apnea, and other sleep-related disorders combined. Subjects with any missing values in SNS or ESS were excluded (n = 167). UNS, Ullanlinna Narcolepsy Scale; SNS, Swiss Narcolepsy Scale; ESS, Epworth Sleepiness Scale; ROC, receiver operating characteristic. With SNS, opposite values (e.g. 30 to -30) were used in comparison.

UNS correlated negatively with CSF hypocretin-1 levels (r_s =. -564, P < .001) (Figure 16). A similar negative correlation was seen with MSL in MSLT (r_s = -.608, P < . 001).

Cronbach's alpha for the UNS was .898, indicating high internal consistency of the scale.
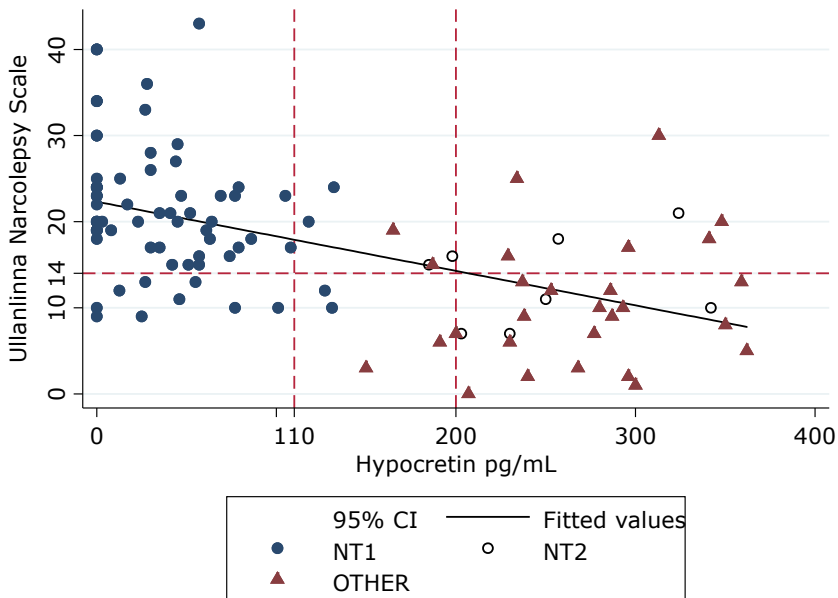
Figure 16. Correlation between Ullanlinna Narcolepsy Scale and hypocretin-1 levels (n = 89). NT1, narcolepsy type 1; NT2, narcolepsy type 2; OTHER, other sleep disorders.

Twenty-three NT1 subjects were unmedicated. UNS scores did not differ between medicated and unmedicated subjects (*M* = 22.2, 95% CI 20.5, 24.0 vs. *M* = 21.8, 95% CI 17.9, 25.7, P = .083).

6   DISCUSSION

6.1   Epidemiology of narcolepsy associated with H1N1 vaccine

Our main goal in Study I was to assess the magnitude of the risk of narcolepsy after pandemic H1N1 vaccination with Pandemrix. Furthermore, we examined whether a connection existed between narcolepsy and any other influenza vaccine, or whether there was an epidemiological connection with an influenza infection per se. We found a clear and consistent increase in the incidence of narcolepsy across multiple studies after the Pandemrix vaccination. Conversely, there was no indication of a risk association with any other vaccine. In children and adolescents, the increase was 5- to 14-fold in all countries where vaccine coverage with Pandemrix was high (Finland, Sweden, Norway, France, England, Ireland, and the Netherlands). Correspondingly, the vaccine-attributable risk was high in this age group, 1 per 18,400 doses. In HLA DQB1*06:02 carriers, this means an individual risk of 1 case of narcolepsy per 4,500 vaccines administered (0.022%), assuming that the prevalence of HLA DQB1*06:02 in the general population is around 25%.

In adults, the risk was smaller, but still 3- to 7-fold compared with unvaccinated subjects. The risk window for an increased risk of narcolepsy post-vaccination seemed to be as long as two years in children and adolescents, and also in adults, but this finding must be considered tentative because of possible biases and lack of confirmation other than two studies from Finland and Sweden.[183,195]

A recent multinational SOMNIA study also aimed to assess the association between narcolepsy and the pandemic H1N1 vaccine.[213] Unfortunately, it failed in its primary aim due to lack of power to show any connection with Pandemrix and narcolepsy. Furthermore, it focused on vaccine adjuvants, but recent evidence indicates that the virus component and especially viral nucleoprotein was the main causative factor in the pathogenesis of H1N1-vaccine-associated narcolepsy (see Section 2).[214,215] Moreover, since no increase in narcolepsy incidence rate was observed in the SOMNIA study in areas without large-scale use of Pandemrix the study provides some evidence against the role of H1N1 infection in the increased disease risk, which could have been a confounder in the Pandemrix studies (see later). This further supports the role of Pandemrix in the development of narcolepsy.

Reliability of our results is supported by very low heterogeneity in Study I. The only exception is the subgroup with the first healthcare contact as an index date in children and adolescents, although the P value was still insignificant ($I^2 =$

44%, P = .167), implying no true heterogeneity. The study by Miller et al. could have caused some heterogeneity due to case-coverage design, which differs from the other studies that used a cohort or a case-control setting.[10] Miller et al. also applied a different case collection method (contact with sleep centers vs. national or regional registries).

6.1.1 Possible biases in observational studies

The meta-analysis of observational studies is based on multiple individual studies with variable methods. Internal validity of these studies can be affected by numerous biases such as confounding, selection bias, and ascertainment bias.

6.1.1.1 Confounding

Confounding is probably the most interesting potential source of bias in the case of Pandemrix-associated narcolepsy since it relates directly to the question of whether some exposure other than the H1N1 vaccine either caused narcolepsy or made a narcoleptic subject (or more accurately, a subject susceptible to development of narcolepsy) more likely to be vaccinated in the first place. The most obvious source for confounding would be the A(H1N1)pdm09 infection itself. In many European countries, the H1N1 vaccination campaign was conducted almost simultaneously or even after the onset of regional epidemic or its peak, making concurrent H1N1 virus infection a tempting alternative explanation for the increased risk of narcolepsy.[216,217] Confounding by H1N1 infection is supported by limited epidemiological data, interestingly only from China. Seasonal variation and a post-pandemic 3-fold increase in the incidence of narcolepsy were observed in the Beijing and Shanghai areas following the H1N1 pandemic in 2010.[17,184] Recently, an increased incidence of narcolepsy was reported also in Taiwan.[213] In Germany, a modest increase in the incidence of narcolepsy was seen already from spring 2009 onwards, but in a more recent study (published after Study I) an association with Pandemrix and narcolepsy was observed in Germany as well.[182,218,219]

In summary, except for China, there is no epidemiological evidence of an increase in the incidence of narcolepsy anywhere else, which alone makes confounding and H1N1 infection a very unlikely cause for the observed increase in the incidence.[213] In addition, in a Finnish study, H1N-vaccine-related narcolepsy patients did not seem to have any serological evidence of a recent H1N1 infection.[220] Reasons for the increase in the number of narcolepsy cases in China after A(H1N1)pdm09 are unclear, but may include subtle alterations in the circulating virus or different susceptibility of the Chinese to narcolepsy after H1N1 virus infection. Also, other environmental factors unique to China could act as superantigens.

6.1.1.2 Recall bias

Narcoleptic subjects could falsely claim that they had been vaccinated in the hope of reimbursement or the mistake could be unintentional. They might also remember the vaccination and symptom onset date erroneously. To eliminate this recall bias, vaccination registries were used in most of the studies. Using the first healthcare contact as an index date also provides more reliable results than using patient-reported date of onset of symptoms. Therefore, we have presented three different index dates: the onset of symptoms, the first healthcare contact, and the date of diagnosis. However, using the date of diagnosis, referral to MSLT, or referral specialist as an index date may cause exposure misclassification if the subject was vaccinated after symptom onset but before these dates. Diagnosis date is probably the easiest date to use, but the diagnostic procedure may take months, excluding some subjects from the studies. There could also be variability in access to PSG and MSLT across countries. First healthcare contact is more reliable also because the onset of narcolepsy can be rather variable. Some patients experience a sudden full-blown or nearly complete narcolepsy phenotype with severe EDS and CPL from the beginning, while others may have a subtler and slower progressive course, with EDS or e.g. parasomnias appearing first, followed by CPL.
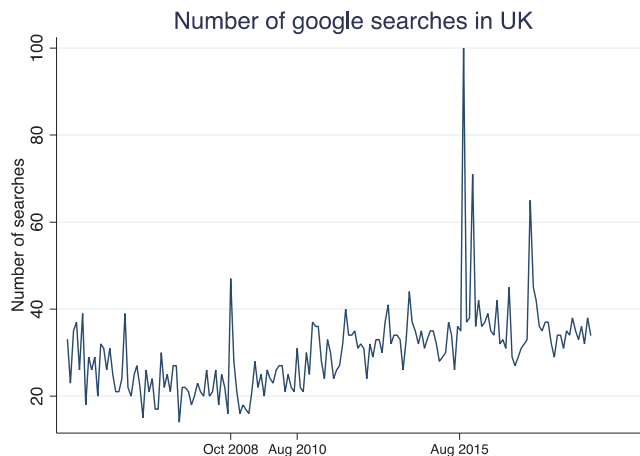
Figure 17. Number of Google searches with the search term "narcolepsy" by year and month.

6.1.1.3 Ascertainment bias

Ascertainment bias is caused by an imbalanced collection of subjects to a study. In narcolepsy incidence studies, this bias could arise if vaccinated subjects were more likely screened or diagnosed for narcolepsy, which could have been caused by increased media awareness. Vaccinated narcoleptic subjects may have more readily

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suspected that they had narcolepsy than unvaccinated subjects. They might also have sought care earlier or more often, and the threshold may have been lower for the doctors attending to these patients to refer them to a sleep specialist or to diagnostic studies. The process of case confirmation might also be a source of ascertainment bias if cases were not properly validated or vaccination status was not confirmed. The easiest way to reduce the bias caused by heightened media awareness is to analyze only those cases that appeared before increased public attention. In the study by Nohynek et al., the follow-up period ended already in August 2010, which was the date of the first reports in the media.[5] However, if the risk ratio was extended to December 2010, the decrease in the risk ratio was rather modest, from 12.7 (95% CI 6.1, 30.8) to 11.4 (95% CI 5.6, 27.5), rendering it unlikely that the increased risk is explained mainly by media attention.[5] On the other hand, the follow-up period in the Swedish, Norwegian, and French reports lasted until the end of 2010 or 2011, and therefore, a small bias due to the increased awareness cannot be completely ruled out in these studies.[7,9,221] The media awareness could be assessed e.g. by examining internet searches on a rare disease such as narcolepsy through Google trends, which probably reflects the media attention but not the actual disease epidemiology.[222] Google trends did not show any significant increase in the UK or Ireland prior to the collection period in these studies (Figure 6.1).[10,12]

Narcolepsy cases were verified using a previous ICSD-2 classification and also Brighton collaboration criteria (BCC) for narcolepsy. BCC level 1 denotes narcolepsy with proven hypocretin deficiency and level 2 cases with unambiguous cataplexy and positive MSLT, although only one of the two criteria has to be met (either < 8 minutes MSL or ≥ 2 SOREMPs). In level 3, cataplexy is not required, but both the MSLT criteria must be met and possible mimics excluded. Using ICSD-2 and BCC reduces the risk of misclassification of cases since the diagnosis is based on objective measures rather than subjective assessment. Especially BCC levels 1 and 2 can be considered accurate and reliable if the history on cataplexy is properly evaluated. BCC and ICSD-2 were used in all studies, except for the register study by Persson et al., but most diagnoses in the register were validated in the previous MPA study.[221,223] During the chart review, however, blinding to vaccination status for case confirmation can be challenging. Here, bias is also possible if the reviewers classify vaccinated cases more often as narcolepsy than unvaccinated cases, but objective criteria reduce this possibility. For the Dutch data, we chose only those cases fulfilling BCC 1 to 3 criteria, resulting in 7 of 20 cases; the rise in incidence was significant. Moreover, excluding BCC 2-3 did not change the results.

6.1.2 Bias analysis

Unfortunately, a quantitative bias analysis was beyond the scope of our study, which could be considered as a weakness. Greenland and coworkers state in their article focusing on bias analysis that: "Bias analysis may also be unnecessary when the observed associations are dramatic, consistent across studies and coherent to the point that bias claims appear unreasonable or motivated by obfuscation goals".[224] All of these points, possibly the last excluded, seem to apply to Pandemrix-associated narcolepsy, confirmed also by our meta-analysis. Nonetheless, two papers on bias analysis of the studies included in Study I have been published.[14,15] In these papers, the association could not be explained by biases only.

6.1.3 Further remarks and evidence published after the meta-analysis

Our study was comprehensive. We searched thoroughly all of the available sources on the risk of narcolepsy without language restriction, including also reports from health authorities not published in academic journals or collections.

Data on the clinical picture and differences in the clinical presentation of Pandemrix-associated and sporadic narcolepsies need to be interpreted with caution. It is possible that subjects with more severe symptoms are diagnosed earlier after the vaccination than those with a milder phenotype. In countries with many smaller centers (rather than a few central hospitals or sleep clinics), some narcolepsy patients may also be unrecognized or underreported for epidemiological studies. High frequencies of HLA DQB1*06:02 allele, cataplexy, and hypocretin deficiency are characteristic for vaccine-associated narcolepsy (and sNT1). Currently, there is no evidence of an increased risk of NT2 or other hypersomnias associated with vaccination.

It is estimated that the A(H1N1)pdm09 influenza virus caused over 12,000 deaths and 270,000 hospitalizations in the United States alone.[225] Globally, the pandemic may have caused more than 200,000 respiratory deaths and 80,000 cardiovascular deaths.[226] Mortality and morbidity were exceptionally high, especially in persons under 65 years of age. Even though the incidence of narcolepsy was markedly increased in the countries where Pandemrix vaccination was used, data on the benefits of the pandemic H1N1 vaccination clearly outweigh the vaccination-associated health risks.

After the publication of the meta-analysis, a report from Saudi Arabia noted that the incidence of narcolepsy had not increased even though Pandemrix was the only vaccination used in the country.[227] This could be explained by a number of factors. First, pandemic vaccine coverage in Saudi Arabia is completely unknown. Second, the HLA DQB1*06:02 prevalence in Saudi Arabia is low, around 3.8%, and the frequency of protective HLA types such as DQB1*02 is high (30-40%).[228] A

previous report has, however, claimed that narcolepsy prevalence in Saudi Arabia would be around 40 per 100,000 inhabitants.[229] The result should be interpreted with skepticism since the study was not focused on narcolepsy, and it was conducted using only a general neurologic symptom questionnaire accompanied by an interview without confirmation of the diagnosis. Furthermore, this study included only one patient with suspected narcolepsy (not revealed whether with or without cataplexy) in a population sample of around 23,000 people (95% CI not given, but if calculated it would be around 7.8 to 250 per 100,000 people). This one case could be a false positive, and if a true positive, explained by chance.

In Norway, some vaccine-related adult cases have also been reported after the pandemic vaccination.[208] The overall incidence in Norway returned to baseline in 2012-2013.[208] Seven post-Pandemrix narcolepsy cases and one after vaccination with Focetria were found in Switzerland, where approximately 400,000 to 500,000 subjects were vaccinated, but the vaccination was limited only to adults (aged 18 to 60 years).[209] Finally, in Germany an OR of 4.2 (95% CI 1.9, 9.5) to 5.5 (95% CI 2.2, 14.1) for narcolepsy after immunization with Pandemrix was reported in 2017.[182]

6.2 Sleep studies in vaccine-related and sporadic narcolepsy

6.2.1 Polysomnography and MSLT

To analyze possible differences between Pandemrix-associated and sporadic narcolepsy, we compared sleep recordings and actigraphic measurements in these two entities. In the crude comparison in Study II, pNC patients had more severe MSLT results than patients with sNC, but the differences diminished when adjusted for age. Sleep architecture was also similar between the groups and in line with previous reports of pNC or sNC.[9,10,230] In another report, the sleep latency was shorter in Finnish children with pNC than in Italian children with sNC.[231] Also, in this study, the pNC subjects had more frequently disturbed nocturnal sleep.

We found less periodic limb movements in pNC than in sNC. Ferritin levels and restless legs symptoms were similar and do not explain the finding. PLMSI in our patients was also lower than in previous reports, which might be explained by younger age and lack of any medication in our subjects.[174,232,233] There might also be some variability in equipment and methods in measuring PLMS across the groups. In this study, it was not possible to investigate nocturnal limb movements in more detail. Earlier reports indicate that periodic leg movements in narcolepsy differ from idiopathic RLS or PLMS in temporal distribution during the night. Specific high-frequency leg movements and fragmentary myoclonus are also prevalent in narcolepsy.[174,233]. Dopamine plays a crucial role in the development of RLS and PLMS, and changes in dopamine metabolism in narcolepsy have been

observed.[234] Direct measurement and comparison of dopamine transmission in pNC and sNC are difficult and were not possible in this study. It is not known whether these possible alterations in dopamine metabolism develop concurrently with hypocretin deficiency or whether they occur later as e.g. a compensatory mechanism. If differences are present in dopamine metabolism between pNC and sNC, they could potentially explain changes in periodic leg movements.

6.2.2 Actigraphy

One of the most interesting findings in our study was the clinically significant delay in sleep-wake rhythms in sNC, but not in pNC. Relative amplitude (normalized difference of activity levels) between day and night was similar between the groups, indicating equally stable sleep schedules. pNC patients might have suffered slightly more from EDS, taking into account the shorter sleep latency in MSLT, which could also urge them to bed earlier. However, no differences emerged in ESS scores and the subjective time in bed was similar.

Sleep efficiency in actigraphy was clearly reduced compared with normative values and sleep seemed to be rather fragmented. Conversely, in PSG sleep efficiency was at normal levels. This discrepancy between the methods could be explained by failure of actigraphy to detect REM sleep and REM sleep behavior disorder, which is common in narcolepsy. As actigraphy measures movement, not sleep stages, we could hypothesize that REM sleep without muscle atonia resulting in motor activity would be scored as wake in actigraphy but as sleep in PSG. There is some evidence to support this theory. At least two studies in Parkinson's disease and RBD have reported an increased number of wake bouts in subjects with RBD relative to subjects without RBD.[235,236] Also interesting is that we saw a longer sleep latency in actigraphy than in polysomnography, although this is commonly reported to be vice versa.[237]

6.2.3 Limitations

Study II has certain limitations. Hypocretin level was not analyzed in all patients because it was considered unnecessary for the diagnostic procedure if the criteria for narcolepsy were already clearly met. The number of MSLT sessions could have been only four in some cases if there had been already two or more SOREMPs in the first four sessions. Finally, the findings represent the situation at the time of diagnosis, but due to the long diagnostic delay in narcolepsy they cannot be generalized to the time near onset of the disease.

6.3 Clinical course of H1N1-vaccine-related narcolepsy

Short diagnostic delay and abrupt onset of symptoms suggested a more severe clinical phenotype of pNT1 than of sNT1. In our study, symptoms of most of our patients had ameliorated at least slightly at the follow-up visit, but there were a few patients who were clearly worse. Therefore, course of pNC seems highly variable, which is in line with previous reports of sNC.[238]

Average questionnaire scores in our study did not differ between pNC and sNC patients, although sNC subjects were older at the time of the visit. Nonetheless, this finding suggest that the phenotype of these diseases is similar and pNC is not a novel disease but is related to sNC. This in an important finding, especially when estimating the future disease course, e.g. when evaluating disability caused by narcolepsy for reimbursement or employment purposes.

Somewhat surprisingly, there were no differences in the questionnaire scores between adult and under-aged (< 18 years) subjects. Some evidence indicates unique features in childhood narcolepsy, such as facial and generalized hypotonia and increased sleep time, that gradually ameliorate over time.[238] Sensitivity of our questionnaires for differentiating adult and childhood narcolepsy may be inadequate since they were not developed for such a purpose. The shorter diagnostic delay in our adult subjects than that previously reported (more than 10 years) could also imply that our subjects have a more severe clinical picture. Alternatively, increased public awareness of narcolepsy could explain the shorter diagnostic delay. Type II error caused by the small sample size could also explain the lack of differences between adult and younger subjects.

The classic tetrad of narcolepsy described by Yoss and Daly included EDS, CPL, hypnagogic and/or hypnopompic hallucinations, and sleep paralysis.[239] Although these still are the most visible symptoms of narcolepsy, Mitler and coworkers suggested in 1990 that disturbed nocturnal sleep (DNS) is such a frequent symptom that it should be added to form a narcolepsy "pentad". We also found that DNS is very common and causes disability. Treatment of DNS (e.g. with sodium oxybate) may also improve daytime symptoms including alertness.[240,241]

Obesity and weight increase are common features in narcolepsy.[138] We observed a significant increase in BMI in our subjects, although the majority remained within the normal range. BMI was even higher in subjects with sNC. There are many possible explanations for the weight increase. Hypocretin neurons regulate energy metabolism and feeding behavior. Basic metabolic rate in obese narcoleptics is similar to BMI-matched controls, but in non-obese narcoleptics metabolic rate and energy expenditure are reduced, which could lead to increased BMI.[242] Transgenic hypocretin-ablated mice also gain weight despite eating less than controls.[243] Some medications used for narcolepsy, such as venlafaxine and clomipramine,

may increase weight, while others, e.g. sodium oxybate and methylphenidate, may decrease weight. This is important to keep in mind when choosing an individual narcolepsy therapy since weight gain increases the risk for comorbid sleep apnea and cardiovascular diseases, which in turn, might be a contraindication for wake-promoting medication.

6.3.1 Correlation with hypocretin levels

In Study III, we saw that subjects with very low or undetectable hypocretin levels had higher UNS and ESS scores at the follow-up visit than subjects without severe hypocretin deficiency (but still below 110 pg/mL). This implies that the degree of hypocretin loss could affect the disease course, which is supported by the earlier work of Bauman and coworkers.[244] They observed that patients with the lowest hypocretin levels had shorter sleep latency and more SOREMPs in MSLT. Conversely, they did not see differences in the same questionnaire scores (UNS, ESS, SNS) that we used, but their hypocretin level detection limit was set at 60 pg/mL, while ours was 20 pg/mL. It is worth noting that in our study no direct correlations existed between the clinical course or symptom severity and hypocretin levels or MSLT findings. In practice, patients who had undetectable hypocretin levels might still have had very mild symptoms. On the other hand, patients with hypocretin levels near 110 pg/mL could have had severe symptoms as well.

The fact that we did not see a direct correlation between hypocretin levels and symptom severity in this sample could imply that neural networks other than solely hypocretin contribute to symptom severity. Substantially increased numbers of histaminergic neurons in narcolepsy have been observed in recent studies.[100,245] CSF histamine levels also seem to be decreased in narcolepsy as well as in narcolepsy without hypocretin deficiency.[102,103,246] Histamine plays a crucial role in modulating wakefulness, as tuberomammillary histaminergic neurons project widely to the arousal-promoting network, which is active during wake. Accordingly, older anti-histamines that penetrated the blood-brain barrier involved sedating side effects modulated by histamine H1 receptors. Conversely, histamine H3 receptor inverse agonist pitolisant activates these neurons and is now used in the treatment of narcolepsy.[247] Interestingly, pitolisant seems to have also an anti-cataleptic effect unlike traditional stimulants such as modafinil and methylphenidate.[248] It is unclear when the changes in histaminergic signaling and detectable histamine cell count occur in narcolepsy, but one hypothesis is that they are a compensatory mechanism for hypocretin loss.[100]

Another possible compensatory mechanism could be related to the opioid system. A recent study demonstrated an increase in hypocretin neurons in heroin addicts. Furthermore, the same group found an increase in hypocretin neurons in mice administered morphine.[249] What is even more striking is that they found

hypocretin neurons in a narcoleptic patient with undetectable CSF hypocretin levels who had been treated with opioids over a long period.

6.3.2 Limitations

The study was conducted in a tertiary sleep clinic, which may cause selection bias. Our study population might have more severe symptoms than most narcoleptic patients, as some patients with mild symptoms may have visited our clinic only for diagnostic purposes and the follow-up might have occurred in primary or secondary healthcare. The finding that many patients in our cohort reported medication side effects supports this view. CSF hypocretin levels were not measured in all patients. However, patients were all HLA DQB1*06:02-positive, had unambiguous cataplexy, and positive MSLT, which implies that they were hypocretin-deficient. Theoretically, it would have been interesting to follow hypocretin levels at the second visit with another lumbar puncture, but we did not do so for ethical reasons, as it would not have any effect on treatment. There could also be some inter-assay variability in the hypocretin measurement kits used. Unfortunately, the Rinnekoti Research Laboratory, where the analyses were conducted, was shut down in 2017-2018, and therefore, analysis of this variability could not be performed afterwards.

Unfortunately, at the time of the study, no actual scales developed especially for the measurement of the severity of narcolepsy syndrome existed. The Narcolepsy Severity Scale was published in 2014, but to our knowledge, it has not been validated or used in any other published studies.[158] UNS has some face validity for severity assessment and UNS scores increase with more frequent cataplexy and more sleep attacks. It could also measure change in these symptoms, but validation studies are lacking. There is a clear need for better patient-reported outcome measures (PROMs) to assess the symptoms of narcolepsy for other than screening purposes. Objective measures such as MSLT and MWT are expensive and time-consuming, and we had no possibility to perform these repeatedly. Correlation of MSLT or MWT results to the severity of narcolepsy is also poor or at least questionable, although these are probably the best available measures.[250,251] PROMs also have a clear advantage over objective measures as they better reflect health-related quality of life and can be developed to gather comprehensive information about the disease. So far, there is no such tool for narcolepsy. Finally, although the results in general seem very similar between the visits and the groups, we cannot completely exclude type II error due to the limited sample size.

6.4 Ullanlinna Narcolepsy Scale in diagnosis of narcolepsy

The results of Study IV demonstrate that the Ullanlinna Narcolepsy Scale can be used as a screening tool for narcolepsy in a clinical population as well as in population screening. UNS proved to be especially useful in the diagnosis of NT1, demonstrating 84-85% sensitivity and 84-88% specificity against other sleep disorders, including NT2 and other hypersomnias. UNS is specific for narcolepsy since it separates both narcolepsy syndromes from other disorders with 80% sensitivity and 88% specificity. Sensitivity in differentiating NT1 and NT2 from HS is lower, 69%.

On average, UNS scores in NT2 were clearly lower than in NT1, but UNS still does not reliably distinguish NT1 from NT2, as the specificity in this comparison was only 44%. Mean UNS score in NT2 was almost 14 points and NT1 subjects without cataplexy had mean UNS scores just above 14, but the difference to NT2 was not statistically significant. This suggests that sleepiness in both diseases is similar in terms of UNS responses. However, the small number of NT2 and NT1 subjects without cataplexy does not allow strong conclusions to be drawn from this analysis.

The minimum UNS score in NT1 subjects in our study was nine. Considering our large total sample, we can state that if UNS is below nine then the diagnosis of NT1 is very unlikely. This finding could help in diagnostics. For instance, a sleepy patient with eight points in UNS probably has some disorder other than narcolepsy. In such a case, it might be feasible to screen for sleep-disordered breathing or circadian rhythm disorder by cardiorespiratory polygraphy or actigraphy first. Complementary laboratory diagnostics to rule out other diseases, such as hypothyroidism, may also be warranted, instead of admitting the patient directly to full-night polysomnography followed by MSLT.

Specificity of UNS can be increased with a higher cut-off point. All subjects with 31 or more points had NT1 (specificity 100%) and only one hypersomnia patient had 30 points. A cut-off point of 17 yielded over 90% specificity; the diagnosis of narcolepsy was very likely. Fourteen as a cut-off point showed a relatively good compromise, with few false negatives or false positives. UNS cut-off points of 13 and 14 result in similar positive likelihood ratios, both higher than 5. Sensitivity of the cut-off point of 13 is high, nearly 89%, without marked loss in specificity (83.7%). If validated in further studies, 13 might be a more feasible cut-off point for narcolepsy screening, especially in samples with a low pretest likelihood of narcolepsy.

6.4.1 Comparison of UNS to SNS and ESS

Performances of UNS and SNS were quite similar. ROC areas were almost equal. SNS demonstrated also an adequate sensitivity in predicting NT1, even though it had slightly lower sensitivity (78% vs. 85%). McNemar test did not show any differences in sensitivity (P = .285) and specificity (P = .197) of these two measures (Sarkanen et al., unpublished data). Since ESS has low specificity and low positive predictive value for hypersomnias, it is not a feasible screening tool for hypersomnia. ESS performed also poorly in ROC area comparison, with significantly lower AUC than UNS or SNS (Figure 5.8). Moreover, ESS does not correlate very well with MSLT. Chervin and coworkers have previously reported a moderate correlation (Spearman's r_s = -.37, P = .004) or no correlation at all between ESS and mean sleep latency in MSLT or severity of OSA.[251,252] In our study, the correlation was moderate (r_s = -.316, P < .001).

6.4.2 Correlation of UNS with hypocretin levels

In Study IV, we found a strong correlation between UNS and CSF hypocretin-1 level, as displayed in Figure 5.9. False negatives of UNS are those cases that are situated in the lower left rectangle of the figure and false positives in the upper right rectangle. The figure is, however, somewhat biased since lumbar puncture was performed mainly when narcolepsy was suspected or the clinician wanted to rule it out. Therefore, had we performed lumbar puncture and hypocretin measurements for the whole sample, presumably more cases would have been added to the lower right part of the figure, strengthening the association. The areas in the middle rectangles are intriguing since these subjects have hypocretin levels of 110-200 pg/mL, which can be considered a gray zone between clearly abnormal and normal levels. UNS scores also scatter almost equally in this area.

A rather strong correlation with UNS and hypocretin levels in Study IV might seem a bit contradictory to Study III, where the correlation was seen only if hypocretin levels in NT1 subjects were divided into two subgroups, below 20 pg/mL and 20-110 pg/mL. However, the sample in Study IV is completely different, as also subjects with hypocretin levels above 110 pg/mL and disorders other than NT1 are included. Nevertheless, if only subjects with hypocretin < 110 g/mL in Study IV are included, we see a weak correlation between UNS and hypocretin levels (r_s = -.276, P = .034) (Sarkanen et al., unpublished data).

6.4.3 Comparison to MSLT

In our study, only 74% of NT1 subjects had ≥ 2 SOREMPs in MSLT, suggesting that UNS is more sensitive for NT1 than SOREMPs in MSLT. On the other hand, 22-33% of HS, OSA, and OSRD subjects had ≥ 2 SOREMPs, which could lead to

false-positive narcolepsy diagnoses, especially in HS. These findings are supported by reports from other groups. Aldrich and coworkers noted in 1997 that in a sample of 2083 subjects with 170 narcoleptics 30% of all subjects with mean sleep latency < 5 minutes and ≥ 2 SOREMPs did not have narcolepsy.[146] With ≥ 3 SOREMPs, specificity for narcolepsy was 99.2%, but sensitivity only 46%. In the Zurich cohort, especially patients with insufficient sleep presented with SOREMPs and short sleep latency.[253] Johns suggested that ESS is more specific and sensitive for narcolepsy than MSLT or MWT, but this analysis might have been somewhat biased.[250,251]

Specificity of MSLT could be increased by reducing the mean sleep latency limit from 8 to 5 minutes. Another method is analyzing both the proportion of REM sleep of all naps and the sequence of the sleep stages, mainly if REM sleep occurs before N2, which is typical for narcolepsy.[147,148] These methods would, however, limit the sensitivity to around 50%. Test-retest reliability of MSLT is also limited, especially in diseases other than NT1.[25,254]

We must consider that UNS and MSLT were developed and meant for different purposes, and comparison between the methods is therefore a bit artificial. UNS is not supposed to replace MSLT. However, for a doubtful MSLT result, i.e. suspicion of a false negative or positive, UNS could help in deciding whether retesting or CSF hypocretin measurement is warranted. UNS may indicate that the MSLT result is inaccurate.

6.4.4 Effect of medication

We did not observe differences in UNS scores between medicated and unmedicated narcolepsy subjects, which could be explained by a number of facts. First, psychometric properties of UNS in measuring change over time have not been studied. Therefore, we do not know the test-retest reliability of UNS, i.e. how consistent UNS scores are at different time points without an actual change in health or symptoms of narcolepsy. Neither do we know the minimal important change in UNS points if general health or quality of life of the subject changes. In other words, if narcolepsy treatment is started and the symptoms of narcolepsy are alleviated, UNS scores might not reflect this improvement. Considering the characteristics of UNS, this seems unlikely. Nonetheless, we saw in Study III that there are vast individual differences and heterogeneity in the evolvement of the disease itself. Therefore, even if we start medication, and it would seem to the physician that the patient is doing better, he or she might still interpret/experience no change in the health-related quality of life.

6.4.5 Limitations

Some limitations regarding Study IV are noteworthy. First, as there is no official Finnish translation of SNS, the questions were derived from the Basic Nordic Sleep Questionnaire. The wording in these questions is very similar to SNS, but it is possible that there is some inaccuracy between the original version and our questions (see also Methods 4.2.1). Unfortunately, we had missing data on SNS, but to avoid possible biases, we used only data without any missing values to compare different scales. Our main aim, however, was not the comparison of UNS and SNS, but to demonstrate the feasibility of UNS in a clinical setting. Second, we used a Finnish version of UNS which might reduce the generalizability of our results to other populations. In theory, it is possible that the original phrasing in UNS does not translate accurately to other languages. Yet, UNS has been successfully used in different languages, e.g. in Chinese, Korean, and German, yielding credible and similar prevalence rates, which implies that the translation at least in these languages is adequate. UNS phrasing is also quite close to other scales such as SNS and NSS (Table 19).[158] Third, this study was conducted in a single specialized sleep clinic setting, which can cause a biased study population. In particular, our clinic acts as a national tertiary center for narcolepsy, and therefore, the most severely affected narcolepsy patients may be overrepresented in our sample.

Table 19. Comparison of phrasing of cataplexy questions in UNS, SNS, and NSS.

UNS	When laughing, becoming glad or angry, or in an exciting situation, have the following symptoms suddenly occurred?
SNS	How often have you experienced weak knees/buckling of the knees during emotions like laughing, happiness, or anger?
NSS	How frequently do you have episodes of generalized cataplexy when experiencing emotions (laughter, intense pleasure, surprise) (cataplexy = loss of muscle tone)?

UNS, Ullanlinna Narcolepsy Scale; SNS, Swiss Narcolepsy Scale; NSS, Narcolepsy Severity Scale.

7 SUMMARY

The incidence of narcolepsy increased significantly especially in children and adolescents but also in young adults after the pandemic H1N1 vaccination in Finland, Sweden, Norway, France, and UK and probably in small children in the Netherlands. The risk was associated only with Pandemrix, not any other pandemic vaccine. Further follow-up is needed to determine the time window during which the risk was increased. H1N1-vaccine-associated narcolepsy opened new doors in research of the etiology of narcolepsy and neuroimmunology, although intensive work is still required to shed light on the link between the vaccination and development of narcolepsy. Being such an extraordinary, unfortunate, and unexpected event, a thorough clinical analysis of the disease is also needed.

As seen in observational studies reporting the signal of increased risk of narcolepsy, diagnostics of narcolepsy syndrome can sometimes be challenging. Our diagnostic methods, especially multiple sleep latency test, clearly fall short of 100% accuracy. Patient-reported outcome measures and questionnaires cannot replace a proper clinical history and physical examination, but they can provide the clinician with a valuable tool to aid in the diagnosis of hypersomnia patients. Clear discrepancy between questionnaire results and sleep recordings should prompt further analysis of possible false positives and negatives.

Our study suggests that UNS could be used in the clinical setting to assess a priori probability of positive MSLT. In a subject with UNS < 9, looking for another reason for sleepiness than narcolepsy by e.g. laboratory tests, cardiorespiratory polygraphy, or actigraphy might be more feasible than full-night polysomnography and MSLT. On the other hand, subjects with UNS ≥ 13 or 14 could be sent directly to PSG and MSLT. Use of UNS post hoc is also possible. For instance, if a subject has positive MSLT for narcolepsy (mean sleep latency ≤ 8 minutes and 2 ≥ SOREMPs) but UNS < 9, MSLT findings should be interpreted with caution. In such a case, other factors that may cause false positives, such as behaviorally induced insufficient sleep, circadian rhythm sleep-wake disorders, sleep-disordered breathing, and other sleep disorders, need to be carefully excluded. UNS ≥ 31 with normal MSLT may suggest an incorrect negative MSLT. A following step to diagnose/exclude narcolepsy type 1 could be measuring CSF HCRT levels.

An actigraphy recording of 1−2 weeks is useful when studying nocturnal aspects of narcolepsy and, in clinical practice, to make the distinction between delayed sleep phase syndrome, behaviorally induced insufficient sleep syndrome, and narcolepsy. Bearing in mind that even mild chronic sleep debt can facilitate the transition from wakefulness to sleep in a sleep-conducive environment, a preceding

actigraphy recording should perhaps be added to the requirements for a reliable MSLT, in addition to PSG.

In polysomnographic, actigraphic, and clinical characteristics, there were no clinically significant differences between H1N1-vaccine-associated and sporadic narcolepsy cases. This implies that these two disease forms share the same biological background and their evolution is similar. However, the clinical course of H1N1-vaccine-associated narcolepsy seems very heterogeneous, although Study III was limited by the small sample size Most of our patients felt that their symptoms improved at least to some degree in the two-year follow-up. Improvement in depression scale points could also reflect adaptation to the disease and development of better coping mechanisms. As shown in our study, clinicians must also pay attention to symptoms not directly related to the traditional narcolepsy tetrad such as disturbed nocturnal sleep, weight gain, and cognitive issues. In the future, we need to learn how we can best help these patients alongside medical care by supporting e.g. lifestyle changes, coping skills, mindset, and other non-medical treatments.

ACKNOWLEDGMENTS

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