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Par Julie MOUCHET LE MOAL

VACCINATION ET RISQUE DE DEMYELINISATION : EXISTE-T-IL UN LIEN ?

EXEMPLES DES VACCINS ANTI-HEPATITE B ET ANTI-PAPILLOMAVIRUS

Sous la direction de : **Monsieur le Professeur Bernard Bégaud**

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TITRE : Vaccination et risque de démyélinisation : existe-t-il un lien ? Exemples des vaccins anti-hépatite B et anti-papillomavirus

RESUME (3,984 caractères)

Bien que les vaccins représentent une avancée majeure pour la santé publique, le risque d'effets secondaires constitue une menace réelle pour leur acceptation par le grand public et les professionnels de santé. La France se classe, d'ailleurs, comme le pays manifestant la plus grande défiance envers le vaccin. Cela s'est souvent traduit pas des couvertures vaccinales faibles. L'origine de cette perte de confiance est, entre autres, liée à la polémique intense autour du vaccin anti-hépatite B (HB) et le risque de sclérose en plaques dans les années 1990.

Le but de cette thèse est d'évaluer le lien potentiel entre vaccination et démyélinisation, en considérant deux exemples : les vaccins anti-VHB et anti-papillomavirus (HPV).

Une approche méthodologique, progressive, fondée sur les preuves a été utilisée pour les deux vaccins. La génération d'hypothèse a considéré la plausibilité biologique, les rapports de cas publiés, les analyses de disproportionnalité conduites dans le système américain de pharmacovigilance des vaccins (i.e*., Vaccine Adverse Event Reporting System* (VAERS)), et l'analyse des signaux détectés par la surveillance passive. Concernant la vaccination anti-VHB, des analyses attendu/observé ont également été menées à partir des cas confirmés rapportés à la pharmacovigilance française dans les années 1990. Des revues systématiques de toutes les études individuelles ayant évalué la plausibilité de l'association entre démyélinisation et les deux vaccins considérés ont été réalisées, tandis que des méta-analyses ont permis d'obtenir des estimations de risque « poolées » à partir des preuves accumulées à ce jour.

Les résultats restent mitigés pour les deux vaccins. Pour la vaccination anti-VHB, une plausibilité biologique faible et indirecte, l'analyse du signal français détecté en 1996 qui a révélé une disjonction complète entre les populations cible et rejointe, ainsi que les résultats des analyses de disproportionnalité dans VAERS sont des éléments en faveur d'une possible association entre démyélinisation centrale et vaccin anti-VHB. Cependant, ni la méta-analyse, ni les analyses attendu/observé (bien que leurs conclusions puissent être renversées par un facteur modéré de sous-notification), n'ont fourni de résultat statistiquement significatif. En tout état de cause, si un risque en excès existait, il serait faible et ne concernerait que l'adulte. Les recommandations actuelles qui minimisent la probabilité d'exposition à l'âge adulte, sont donc plus que justifiées. Pour la vaccination anti-HPV, le risque de démyélinisation centrale semble, à ce jour, écarté. Néanmoins, un doute subsiste concernant un possible risque en excès pour le syndrome de Guillain et Barré. Il serait nécessaire de conduire d'autres études, rendues difficiles par la rareté de l'événement, estimée à 1 cas pour 1,000,000 doses vendues.

En conclusion, une association forte avec un risque de démyélinisation semble à exclure pour les deux vaccins, rendant la balance bénéfice/risque largement positive pour ces produits, dès lors qu'ils sont utilisés dans leurs populations cibles. Dans ce contexte, une communication scientifique, indépendante et claire est la clé pour promouvoir les programmes de vaccination et créer la confiance et l'adhésion du grand public. Les décisions politiques ont aussi une lourde responsabilité. En effet, les suspensions des campagnes

nationales de vaccination peuvent avoir des conséquences délétères à long terme. Le futur de la pharmacovigilance des vaccins pourrait résider dans la mise en place d'un réseau collaboratif entre le patient et son médecin, via l'utilisation de SMS et smartphones, comme cela existe déjà en Australie. En plus de collecter les effets secondaires des vaccins, cela représenterait une opportunité unique de placer le patient au cœur du système de surveillance, lui offrant une voix et contribuant à restaurer sa confiance envers les vaccins, et même envers les décideurs de santé publique.

MOTS CLES : vaccin, démyélinisation, sclérose en plaques, risque, pharmaco-épidémiologie

TITLE : Vaccination and demyelination: Is there a link? Examples with anti-hepatitis B and papillomavirus vaccines

ABSTRACT (3,985 characters)

While vaccines represent a great achievement for public health, the risk of adverse effects is a real threat for vaccine acceptability by both the population and healthcare professionals. France still ranks as the country having the highest vaccine defiance. This often turned into poor vaccination coverages. This origin of this mistrust in vaccines is probably related to the intense polemic around anti-hepatitis B (HB) vaccination and the risk of multiple sclerosis in the 1990's. The main aim of this thesis was to assess the putative link between vaccination and demyelinating disorders by considering two examples: anti-HB and anti-papillomavirus (HPV) vaccines.

For both vaccines, methods adopted a stepwise evidence-based approach. Hypothesis generation was based on evidence regarding the biological plausibility, the published case reports, the disproportionality analyses conducted in the US Vaccine Adverse Event Reporting System (VAERS) and the analysis of signals detected by spontaneous reporting systems, if any. For the research question centered on the anti-HB vaccination, observed-to-expected analyses based on all confirmed cases reported to the French pharmacovigilance in the 1990's were also conducted. Systematic reviews of all individual studies having assessed the possible association between demyelination and either anti-HB or HPV vaccines were then conducted while meta-analyses brought pooled risk estimates of all evidence published so far.

Results were non-conclusive for both vaccines. For anti-HB vaccination, several elements could give credence to an association with central demyelination: a weak and indirect biological plausibility, the analysis of the French signal detected in the 1990's which revealed a complete disjunction between the target and the joint populations, and the results of the disproportionality analyses in VAERS. Nevertheless, neither the meta-analysis nor the observed-to-expected analyses (although might be easily reversed by a moderate degree of underreporting), provided statistically significant findings. If the excess risk actually existed, it would be weak and would be a concern for adults only. The current recommendations which are minimizing the probability of the French population to be exposed at an adult age, are therefore more than justified. For the anti-HPV vaccination, after reviewing all materials available, the risk of central demyelination seems, at this date, unlikely. Nevertheless, a doubt remains regarding a possible excess risk of Guillain Barré Syndrome (GBS) in the follow of an anti-HPV immunization. More specific studies would be needed, although the rarity of this event renders its evaluation difficult. From the studies already conducted, it was estimated that this excess risk, if any, would be lower than 1 per 1,000,000 doses sold.

To conclude, a strong association with a risk of central demyelination can be ruled out for both vaccines, making the benefit and risk balances still largely positive for both products if used in their current target populations. In that context, an independent, clear and scientifically-based communication is the key element to promote vaccination programmes and to generate the confidence and adherence of the general population. Political decisions also carry a heavy responsibility in ensuring trust towards vaccination programmes, as the suspension of national immunization campaigns which could have long-lasting deleterious

consequences. The future of vaccine pharmacovigilance could rely on the implementation of a collaborative GP-patient network-based solution using SMS and smartphones, as already experimented in Australia. While collecting potential adverse effects of vaccines, it would also be a unique opportunity to place the patients at the heart of the surveillance system, giving them a voice and potentially contributing to restore their confidence in vaccines and even, in the decision-makers in the field of public health.

KEYWORDS : vaccine, demyelination, multiple sclerosis, risk, pharmacoepidemiology

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List of Abbreviations

ACIP	Advisory Committee on Immunization Practices
ADEM	acute-disseminated encephalomyelitis
ADS	acute demyelinating syndromes
AEFI	adverse events following immunization
AEFI-CAN	Adverse Events Following Immunisation – Clinical Assessment Network
AIDS	acquired immune deficiency syndrome
AIR	Australian Immunisation Register
CANVAS	Canadian Vaccine Safety Network
CDC	Centers for Disease Control and Prevention
CHO	Chinese hamster ovary
CIS	clinical isolated syndrome
CISA	Clinical Immunization Safety Assessment
CNAMTS	Caisse Nationale d'Assurance Maladie des Travailleurs Salariés
CNS	central nervous system
CPCSSN	Canadian Primary Care Sentinel Surveillance Network database
CPRD	Clinical Practice Research Datalink
CSF	cerebrospinal fluid
DIS	dissemination in space
DIT	dissemination in time
DPA	disproportionality analyses
EBV	Epstein Barr virus
EGB	Echantillon Généraliste des Bénéficiaires
FDA	Food and Drug Administration
GBS	Guillain Barre Syndrome
GePaRD	German Pharmacoepidemiological Research Database
GMO	genetically modified organisms
GPRD	General Practice Research Database
HBsAg	hepatitis B surface antigen
HBV	hepatitis B virus

HES	Hospital Episode Statistics
HIV	human immunodeficiency virus
HMO	health maintenance organizations
HPV	human papillomavirus
InVS	Institut national de Veille Sanitaire
IPD	invasive pneumococcal disease
Max-SPRT	maximized sequential probability ratio test
MOOSE	Meta-analysis of Observational Studies in Epidemiology
MRI	magnetic resonance imaging
MS	multiple sclerosis
NASH	nonalcoholic steatohepatitis
NCIRS	National Center for Immunization Research & Surveillance
NMO	neuromyelitis optica
NPS	National Prescribing Service
OE	observed-to-expected
OFSEP	Observatoire Français de la Sclérose en Plaques
ON	optic neuritis
PGRx	Pharmacoepidemiologic General Research eXtension
PPMS	primary progressive multiple sclerosis
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-analyses
PRMS	progressive relapsing multiple sclerosis
ProMED	Program for Monitoring Emerging Diseases
PRR	proportional reporting ratio
RAMQ	Régie de l'Assurance Maladie du Québec
RNA	ribonucleic acid
ROR	reporting odds ratio
RRMS	relapsing remitting multiple sclerosis
SCCS	self-controlled case series
SD	standard deviation
SIDS	sudden infant death syndrome
SLR	systematic literature review

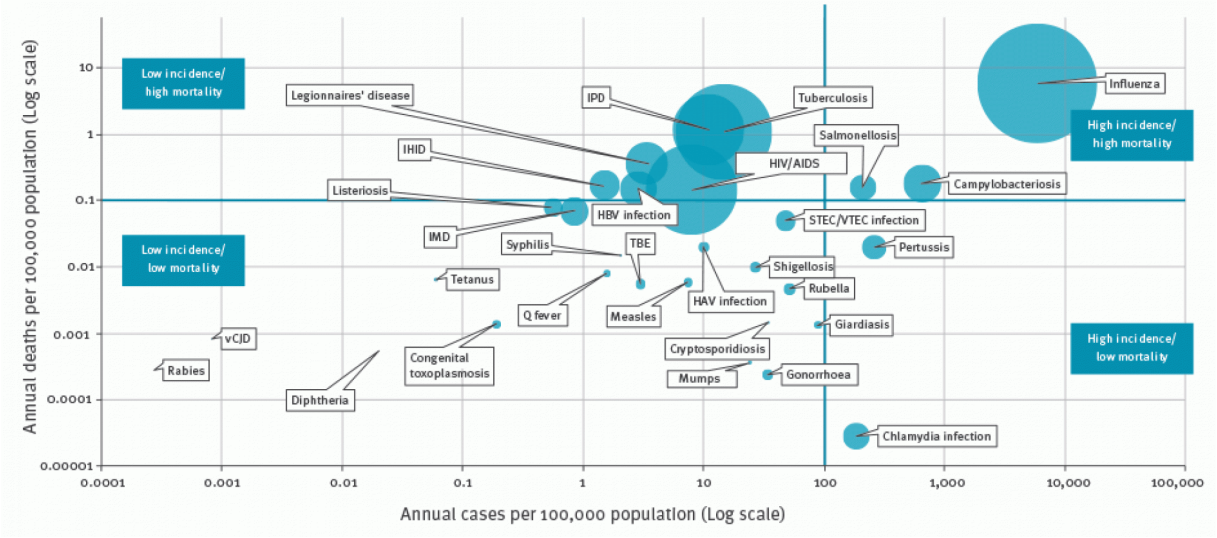
SNDS	Système National des Données de Santé
SNIIRAM	Système National d'Information Inter-Régimes de l'Assurance Maladie
SNP	single nucleotide polymorphism
SPSMS	secondary progressive multiple sclerosis
SRS	spontaneous reporting system
THIN	The Health Improvement Network database
TM	transverse myelitis
UNICEF	United Nations International Children's Emergency Fund
US	United States
VAERS	Vaccine Adverse Event Reporting System
VSD	Vaccine Safety Datalink
WHO	World Health Organization

1 Introduction to vaccinology

1.1 Infectious diseases: a huge burden for humans

Infectious diseases, caused by either bacteria or virus, have always been a major threat for humans. In 1900, the three leading causes of deaths in the United States (US) were pneumonia, tuberculosis, diarrhea and enteritis, which (together with diphtheria) caused one third of all deaths in this country, especially in children aged less than 5 years who accounted for 40% of these deaths. (Centers for Disease Control and prevention, 1999a). In 2015, heart diseases, malignant neoplasms and chronic lower respiratory diseases accounted for 51.5% of all deaths (Centers for Disease Control and prevention, 2016) and life-expectancy gained almost 30 years in one century in the US.(Centers for Disease Control and prevention, 1999a). Similar findings were observed in Europe where the average life expectancy peaked up to 77.9 years in 2015. (World Health Organization, 2018a) Vaccination and control of infectious diseases were among the ten reasons put forward to explain this tremendous public health improvements. (Centers for Disease Control and prevention, 1999b).

Nevertheless, infectious diseases still remain a public health concern for both developed and developing countries. Human migrations, resistance to antibiotics, emerging or reemerging infections, lack of vaccination coverage are direct or indirect factors contributing to the burden of infectious diseases. In Europe, communicable diseases accounted for 9% of the total disease burden in 2005. (World Health Organization, 2005) Between 2009 and 2013, it was estimated that one in 14 European inhabitants experienced an infectious disease episode. As shown in Figure 1 below, influenza had the highest burden in Europe (30% of the total burden), followed by tuberculosis, human immunodeficiency virus (HIV) infection/acquired immune deficiency syndrome (AIDS) and invasive pneumococcal disease (IPD). (Cassini et al., 2018)



EU/EEA: European Union/European Economic Area; HAV: Hepatitis A virus; HBV: Hepatitis B virus; HIV/AIDS: Human immunodeficiency virus infection; IHID: Invasive Haemophilus influenzae disease; IMD: Invasive meningococcal disease; IPD: Invasive pneumococcal disease; STEC/VTEC: Shiga toxin/verocytotoxin-producing Escherichia coli; TBE: Tick-borne encephalitis; vCJD: variant Creutzfeldt–Jakob disease

The diameter of the bubble reflects the number of DALYs per 100,000 population per year.

Figure 1 : Bubble chart of the burden of selected infectious diseases in terms of mortality and incidence, EU/EEA countries, 2009–2013 *(extracted from(Cassini et al., 2018))*

1.2 History of vaccine: from first experiments to modern vaccinology

As far we can know, the ancestry of vaccination is variolation which probably started around the 16th century in China by inserting smallpox into the skin producing an immunologic reaction against its viral agent: poxvirus. Initially, people were using pustules taken carefully from the body of other child or people having survived after infection and then transferred the viral agent to healthy people. People perceived that among those receiving variolation, none developed the infection twice.

Variolation was then progressively expanded to Europe, following the Silk Road and was observed and reported by Jesuits. In the late 18th century, Edward Jenner developed the first vaccination by using animal viruses. Jenner observed that milkmaids were immune to smallpox. He realized that they were immune to smallpox because they acquired cowpox during their work. He took pustules from cowpox/horsepox and used them as a human vaccine by inserting the cowpox into the skin of children and adults. Inoculated cowpox was therefore found to be a safe alternative to inoculated smallpox for the prevention of smallpox.(Baxby, 1999) This constitutes the first experiment of cross-immunization based on

the idea that an agent virulent for animals might be less aggressive for humans, although conferring a long-lasting protection. (Plotkin, 2014)

In 1879, the first laboratory vaccine against *Pasteurella multocida* was produced by Louis Pasteur and his colleagues to fight chicken cholera. Attenuation was used to weaken the pathological agent. The discovery of this vaccine happened by chance; when an assistant was asked to inject chicken with the live bacteria but forgot this order. One month later, when the assistant was back at work, he administered the culture to chickens, which developed only mild signs of the disease but survived. Upon recovery, Pasteur then injected them with fresh bacteria and chickens remain healthy. Pasteur considered that the exposition of fresh bacteria to oxygen had produced an attenuation of the infectivity of these agents. (The College of Physicians of Philadelphia, 2018a). Pasteur then applied this reasoning to rabies virus, which represented a major threat in late 1880's. By using heat or exposure to oxygen, he was able to attenuate the virus and make a vaccine that could protect against the disease, even after the bite by a rabid animal. These observations opened the way for the development attenuated vaccines such as Calmette and Guérin bacillus (tuberculosis) and yellow fever. (Plotkin, 2014)

Inactivation of virus or bacteria is also a common process to produce vaccine candidates. By the end of the 19th century, scientists understood that immunogenicity could be retained if pathological agents were carefully killed by heat or chemical treatment. Inactivation was first applied to pathogens such as the typhoid, plague, and cholera bacilli.

Empirical approach was progressively replaced by modern vaccinology which uses several sophisticated processes to produce vaccines. Amongst other, they include attenuation achieved by passage in abnormal hosts (e.g., rotavirus, measles, mumps), ribonucleic acid (RNA) reassortment (e.g., influenza, rotavirus), protein conjugation of polysaccharides (e.g., Hemophilus influenzae type b), isolation of purified proteins (e.g., acellular pertussis), genetic engineering (e.g., hepatitis B virus (HBV), human papillomavirus (HPV)) and reverse vaccinology (e.g., meningococcal group B). (Plotkin, 2014)

Table 1 outlines of the development of human vaccines over time.

Table 1: Development of vaccines over time (reproduced from (Plotkin, 2014))

Live attenuated	Killed whole organisms	Purified proteins or polysaccharides	Genetically engineered
18th Century			
Smallpox (1798)			
19th Century			
Rabies (1885)	Typhoid (1896) Cholera (1896) Plague (1897)		
Early 20th Century, first half			
Tuberculosis (Calmette–Guérin Bacillus) (1927)	Pertussis (1926)	Diphtheria toxoid (1923)	
Yellow fever (1935)	Influenza (1936)	Tetanus toxoid (1926)	
	Rickettsia (1938)		
20th Century, second half			
Polio (oral) (1963)	Polio (injected) (1955)	Anthrax secreted proteins (1970)	Hepatitis B surface antigen recombinant (1986)
Measles (1963)	Rabies (cell culture) (1980)	Meningococcus polysaccharide (1974)	Lyme OspA (1998)
Mumps (1967)	Japanese encephalitis (mouse brain) (1992)	Pneumococcus polysaccharide (1977)	Cholera (recombinant toxin B) (1993)
Rubella (1969)	Tick-borne encephalitis (1981)	Haemophilus influenzae type B polysaccharide (1985)	
Adenovirus (1980)	Hepatitis A (1996)	H.influenzae type b conjugate (1987)	
Typhoid (Salmonella TY21a) (1989)	Cholera (WC-rBS) (1991)	Typhoid (Vi) polysaccharide (1994)	
Varicella (1995)	Meningococcal conjugate (group C) (1999)	Acellular pertussis (1996)	
Rotavirus reassortants (1999)		Hepatitis B (plasma derived) (1981)	
Cholera (attenuated) (1994)			
Cold-adapted influenza (1999)			
21st Century			
Rotavirus (attenuated and new reassortants) (2006)	Japanese encephalitis (2009) (Vero cell)	Pneumococcal conjugates* (heptavalent) (2000)	Human papillomavirus recombinant (quadrivalent) (2006)
Zoster (2006)	Cholera (Whole Cells only) (2009)	Meningococcal conjugates* (quadrivalent) (2005)	Human papillomavirus recombinant (bivalent) (2009)
		Pneumococcal conjugates* (13-valent) (2010)	Meningococcal group B proteins (2013)

**Capsular polysaccharide conjugated to carrier proteins.*

1.3 Why are vaccines different?

1.3.1 Vaccines' characteristics

Vaccines are preparations of antigenic materials, which are administered with the objective of inducing in the recipient specific, active and long-term immunity against infectious agents or toxins produced by them.

Compared to “chemical” drugs, they present specific characteristics:

- They are used to prevent diseases;
- They are mainly administered to healthy populations, including children;
- They target large birth cohorts or groups at risk with administration at specific ages or in relation to special circumstances (e.g., outbreak or travel);
- They are delivered through public mass campaigns;
- They may be a pre-requisite for enrolment in school or some other public structures.

In addition, vaccines may have a rapid epidemiological impact and could save lives and costs, as highlighted by a panel of health economists who put expanded immunization coverage for children in fourth place on a list of 30 cost-effective ways of advancing global welfare. (World Health Organization, 2018b)

It should also be noted that vaccines carry a low acceptance of any potential risks related to the product. Therefore, they require extensive investigation of severe/serious adverse events following immunization (AEFIs) while monitoring of minor AEFIs is also mandatory to avoid any public rejection.

1.3.2 Herd effect

Vaccination protects individuals directly by inducing active immunity, but also offers indirect benefits in unvaccinated populations. This phenomenon is called “ herd effect”, which refers to the indirect protection of unvaccinated people, whereby an increase in the prevalence of vaccine immunity prevents circulations of infectious agents in unvaccinated susceptible populations. (Kim, Johnstone, & Loeb, 2011). This principle is illustrated in Figure 2.

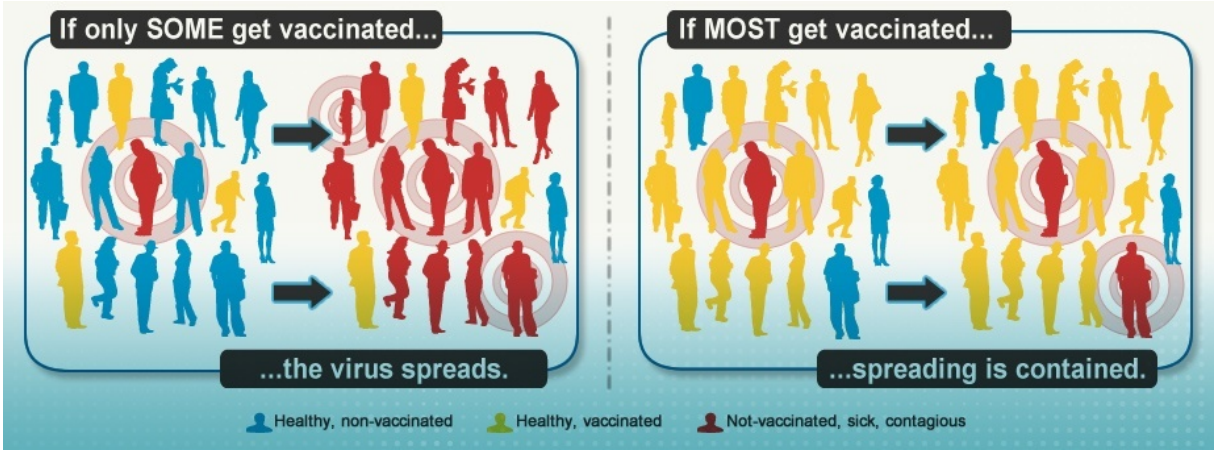


Figure 2: Herd Effect (extracted from ("Vaccines: beneficial to health," 2018))

The existence and magnitude of a herd effect both depend on the infectious agent, the degree of contagiousness, the geographic zone considered as well as the coverage and efficacy of the vaccine.(John & Samuel, 2000) Thus, herd effect thresholds have been defined for each type of vaccine and infectious disease. Measles and pertussis which are among the most contagious diseases with 12-18 secondary infectious cases produced by a single initial index case in a susceptible unvaccinated population, have a herd effect threshold about 94%. In other words, achieving a vaccination coverage of at least 94% in a given population would also protect individuals who are not vaccinated. These latter refer to the so-called “free-rider” paradox, which is the ideal strategy for an individual with respect to vaccination in a population where everybody else is vaccinated and the individual is not. The individual is thus protected from infection because of the herd effect, but suffers none of the potential adverse effects of vaccination.(Smith, 2010)

However, the herd effect may also have some deleterious effects. By reducing the risk of infection among susceptible people, the average age at infection onset will increase among those who are infected but not vaccinated. In that case, the clinical manifestations of the disease could be worse for people infected at older ages (e.g. poliomyelitis, rubella, varicella, measles, and hepatitis A). (Smith, 2010)

Additionally, the herd effect has limitations and, by definition, fails when the vaccination coverage is too low. In real-life settings, unvaccinated people are populations not homogeneously distributed in a given area but tend to be grouped together according to various socio-economic factors. (Fine, Eames, & Heymann, 2011) Thus, outbreaks can re-occur in specific regions as it was observed with pertussis in California in 2014 or mumps among students in the UK in 2009.(Centers for Disease Control and prevention, 2014; The National Archives, 2009)

1.4 Clinical development of a vaccine

Clinical development of vaccines is a complex and long process. Before initiating it, a specific clinical development plan should be prepared for each vaccine by outlining the following points:

1. Identification of the target population (mostly healthy people with particular demographic characteristics) and their sociocultural factors;
2. Risk assessment of the target disease and the vaccine itself;
3. Understanding of the incidence of the target disease and environmental factors;
4. Identification of the dose and route of administration;
5. Plans to induce herd immunity;
6. Regulatory strategies.

Once these preliminary elements are defined, the vaccine candidate follows a stepwise evaluation through clinical phase I to III.(Han, 2015)

Clinical Phase I Trial

Phase I aims at defining the safety and tolerability which are evaluated at both the local and systemic levels as the primary endpoint through dose-escalation and/or repeated-dose studies. Preliminary information on immunogenicity and efficacy may also be collected as secondary endpoints. The first-in-human phase usually involves a small sample of 20 to 80 healthy immunocompetent participants, making the statistical analysis essentially descriptive and exploratory. (Goetz, Pfleiderer, & Schneider, 2010; The College of Physicians of Philadelphia, 2018b). When the vaccine is targeted for children, researchers will first test adults, and then gradually step down the age of subjects until they reach their target population. (The College of Physicians of Philadelphia, 2018b). In the first-in-human setting, more attention should be brought to the safety of live attenuated vaccines because the risks tend to be higher than those of killed vaccines.(The College of Physicians of Philadelphia, 2018b)

Clinical Phase II Trial

While involving several hundreds of subjects, the main goal of the Phase II vaccine trials is to provide the “proof-of-concept””. This phase should document the immunogenicity of the relevant active component(s) and the safety profile of a candidate vaccine within the target population and to define the optimal dose and immunization schedule (i.e., number of doses, sequence/interval between doses, and route of administration). These trials are usually designed as randomized and controlled studies using either a placebo or active group. Phase IIA is usually an extended safety study while Phase IIB trials constitutes the

preliminary assessment of vaccine efficacy. Prospective and confirmatory statistical analyses should be conducted, and the percentage of responders should be defined and described based on predefined endpoints of an immune response (e.g., antibodies and/or cell-mediated immunity). Vaccine efficacy may also be assessed by using surrogate parameters. (Han, 2015)

Clinical Phase III Trial

Here, the objective is to confirm the safety profile and the efficacy of the vaccine before its market launch. These pivotal studies involving large sample sizes, from thousands to tens of thousands of people, are usually randomized, double-blind and controlled against a placebo which may be a saline solution, a vaccine for another disease, or another substance. Given the possibility of being administered with several vaccine valences, interactions or interferences with other vaccines should also be studied, when applicable. In addition, if relevant, bridging studies aiming at extrapolating existing efficacy, immunogenicity and safety to a different condition are also part of phase III trials. Even though large sample sizes are enrolled in these studies, they are often underpowered to detect rare adverse events (frequency ≤1 per 10,000). Consequently, the post-marketing period is crucial for the collection of real-life data on safety and effectiveness. (Han, 2015; World Health Organization, 2017b)

As vaccines are considered as a fixed combination of an antigen, adjuvant and device by regulators, several peculiarities should be pointed out between the clinical development of a chemical drug and a vaccine (Han, 2015):

- Sample sizes of vaccine trials are usually larger (around 5,000 participants) than those for “chemical” drugs.
- People enrolled are participants (free of the disease) instead of patients.
- The manufacturing process for vaccines under development is a true challenge, given that lot-to-lot comparisons are required to ensure the reproducibility of the process and the stability of the product.
- Benefit of chemical drugs are usually direct and do not include herd effect.
- In addition to national or European competent regulatory agencies, the World Health Organization (WHO) is also involved in the vaccine approval/recommendations.

Moreover, clinical development of vaccines should also cover extra safety issues such as the host-pathogen interactions, the risk of reversion to virulence and the risk of recombination with wild-type organisms. Given that vaccines are biologic products, extra legislation is also required for genetically modified organisms (GMO). The contained use of GMO covers any premises where GMO are cultured, stored, used, transported, destroyed or disposed of. Physical barriers, or a combination of physical, chemical and/or biological barriers, are used to limit their contact with people and the environment. In that context, risk of transmission for third parties should be assessed and risk management plans should include isolation or quarantine. (European Commission, 2018)

Conversely, deliberate use authorizes the intentional release of GMO into the environment. In that case, environmental safety should be investigated to ensure that no significant effects on animals, environment, virus/bacteria persistence or antibiotic resistance will occur when spreading the new vaccine.

1.5 Pharmacovigilance of vaccines

Pharmacovigilance of vaccines is defined as "*the science and activities relating to the detection, assessment, understanding and communication of AEFIs and other vaccine- or immunization-related issues, and to the prevention of untoward effects of the vaccine or immunization*".(Council for International Organizations of Medical Sciences (CIOMS) and World Health Organization, 2012)

Vaccine pharmacovigilance includes three main pillars ("Global safety of vaccines: strengthening systems for monitoring, management and the role of GACVS," 2009):

- Signal detection;
- Development of causality hypothesis;
- Testing of causality hypothesis.

Occurrence of AEFIs does not imply a causal relationship with the vaccine. Given the complex nature of vaccines (mixture of antigens, adjuvants, antibiotics, stabilizers, preservatives and a device), adverse events could be related to any of these elements, but also to the vaccine production, storage or administration. (World Health Organization, 2013)

They are classified into 5 main categories:

- **Vaccine product-related reaction:** An AEFI that is caused or precipitated by a vaccine due to one or more of the inherent properties of the vaccine product. **Example:** Extensive limb swelling following DTP vaccination.
- **Vaccine quality defect-related reaction:** An AEFI that is caused or precipitated by a vaccine that is due to one or more quality defects of the vaccine product including its administration device as provided by the manufacturer. **Example:** Failure by the manufacturer to completely inactivate a lot of inactivated polio vaccine leads to cases of paralytic poliomyelitis.
- **Immunization error-related reaction**: An AEFI that is caused by inappropriate vaccine handling, prescribing or administration and thus by its nature is preventable. **Example:** Transmission of infection by contaminated multidose vial.
- **Immunization anxiety-related reaction:** An AEFI arising from anxiety about the immunization. **Example:** Vasovagal syncope in an adolescent during/following vaccination.
- **Coincidental event:** An AEFI that is caused by something other than the vaccine product, immunization error or immunization anxiety. **Example:** A fever occurs at the time of the vaccination (temporal association) but is in fact caused by malaria. Coincidental events reflect the natural occurrence of health problems in the community with common problems being frequently reported.

1.5.1 Signal detection

Mass vaccination campaigns are a considerable challenge for vaccines' pharmacovigilance as these programmes lead to a massive exposition of a considerable population, usually in a short period of time. AEFIs may then be reported soon after the vaccine launch, sometimes producing safety signals when the number of case reports (for a given period of time) approaches or exceeds the number one could expect when considering the background rate of the considered disease, bearing in mind that a certain degree of underreporting is inescapable.

Figure 3 illustrates the relationship between the background rate in a given population, the observed and the vaccine-attributable rates.

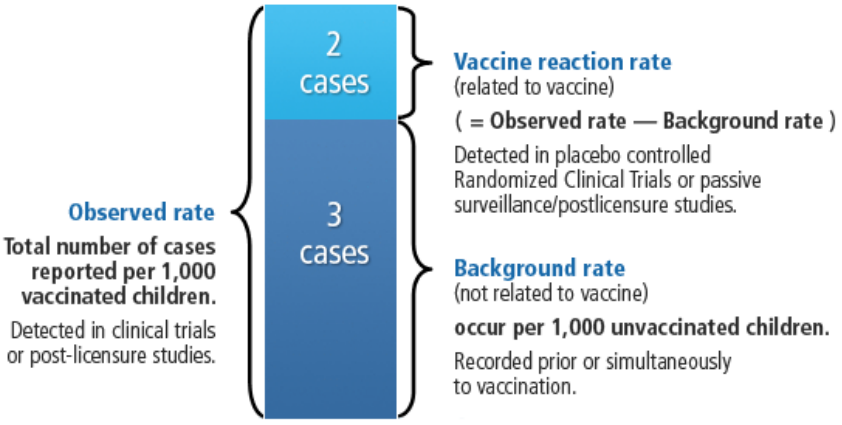


Figure 3: Relationship between the observed and background rates (extracted from (World Health Organization, 2013))

Nevertheless, when comparing the background and observed rates for a specific event, several confounding factors should be considered such as:

- The vaccine reactogenicity, which may be vary from one lot to another;
- The age groups targeted by the immunization campaign with specific events dependent of age (e.g. febrile convulsions after immunization may be observed in infants, but not in adolescents);
- The vaccine dose (e.g. the primary dose may have a different reactogenicity than boosters);
- The population characteristics (e.g. risk factors could predispose some people to experience the event).

Signal detection can be performed through passive and active surveillance systems:

- **Passive surveillance** relies on spontaneous reporting systems (e.g., national pharmacovigilance systems, Vigibase, Eudravigilance, Vaccine Adverse Event Reporting System (VAERS)). Easy to implement, they allow anyone (i.e., patients or healthcare professionals) to report an adverse event. As a result of the lack of precise clinical details and information about comorbidities, they are not suitable to evaluate the causal association between the event and a vaccine but are the cornerstone for safety signal generation.
- **Active surveillance** includes:
    - **Post-licensure clinical trials and Phase IV surveillance studies** to assess the effects of changes in vaccine formulation, vaccine strain, age at vaccination, number and timing of vaccine doses, simultaneous administration and interchangeability of

vaccines from different manufacturers on vaccine safety and immunogenicity; and to improve the ability to detect adverse events that are not detected during pre-licensure trials;

- **Large linked databases** which may allow to investigate causality. Vaccine Safety Datalink (VSD) project which was established in 1990 to monitor immunization safety and to address the gaps in scientific knowledge about rare and serious events following immunization, is an example of a linked database between the US Centers for Disease Control and Prevention and eight health maintenance organizations (HMO);
- **Clinical centres**, including the Clinical Immunization Safety Assessment (CISA) centres which are were established in 2001 to address the unmet vaccine safety clinical research needs of the US.

Moreover, review of all relevant data including case series, clinical data, literature, non-clinical data should also be performed to evaluate a safety signal.

1.5.2 Evaluating causality

Causality can be discussed both at an individual level (i.e., case by case causality assessment) or at the population level. While causal inference can be claimed in experimental trials, observational studies generally do not allow to draw direct, or at least clear-cut, conclusions. In that context, Sir Bradford Hill set out a list of criteria for establishing causality in the context of observation.

There are five principles that underpin the causality assessment of AEFIs (cf. Figure 4).(World Health Organization, 2001)

Consistency: The association of a purported AEFI with the administration of a vaccine should be consistent: the findings should be replicable in different localities, by different and independent investigators, and by different methods of investigation, all leading roughly to the same conclusion(s).

Strength of association: The larger an association between exposure and disease, the more likely it is to be causal. Low-level associations could more conceivably be attributed to other underlying contributors (including biases or confounding) and, therefore, are less supportive of causation.

Specificity: As defined by WHO, the association should be distinctive. The adverse event should be linked uniquely or specifically with the vaccine concerned rather than occurring frequently, spontaneously or commonly in association with other external stimuli or conditions. Nevertheless, the original criterion of specificity is widely considered weak or irrelevant from an epidemiologic standpoint. While some examples of highly specific agent-outcome associations exist, most exposure and health concerns at the forefront of research today center around complex chemical mixtures and low-dose environmental and occupational exposures made complex by a variety of risk factors.

Temporal relation: There should be a temporal relationship between the vaccine and the adverse event. For example, that receipt of the vaccine should precede the earliest manifestation of the event.

Biological plausibility: For WHO, the association should be coherent, that is, plausible and explicable according to established facts in the natural history and biology of the disease. For Sir Bradford Hill, the criterion of plausibility was satisfied if the relationship was consistent with the current body of knowledge regarding the etiology and the supposed pathogenetic mechanism of the disease; though, Hill admitted that this interpretation of biological plausibility was dependent on the current state of knowledge.

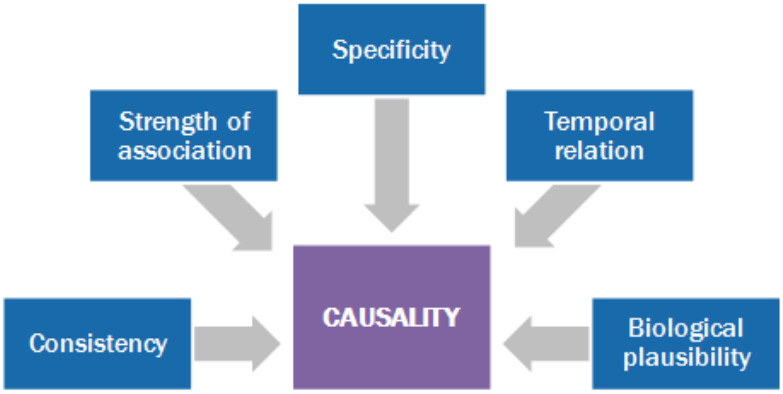


Figure 4: Principles of causality assessment (extracted from (World Health Organization, 2018b))

Unlike ‘’chemical” drugs, vaccine pharmacovigilance faces additional challenges given that information on dechallenge/rechallenge is often missing and several vaccines can be given at the same immunization visit. Additionally, vaccine storage, handling, transport and administration can also lead to safety issues if improper practices occurred.

1.5.3 Testing hypothesis

Putative association between an event and a vaccine can be assessed by well-designed post-marketing studies when comparing exposed *versus* unexposed (e.g., historical cohort) subjects.

Once causality was established between an AEFI and a vaccine, it is crucial to determine whether there is a predisposed set of subjects (e.g., age, ethnicity, comorbid conditions, social determinants, genetic/immunological factors, etc.). Besides, a geographical or time cluster of AEFIs could reveal inappropriate local administration practices or issues with the vaccine storage/transport.(World Health Organization, 2013)

1.6 Benefit and risk balances for vaccines

Premices of modern vaccinology principles can be found in very ancient books such as “Les opuscules mathématiques” written by Jean Le Rond d’Alembert in 1761 in response to a thesis about variolation prepared by Daniel Bernouilli.(D'Alembert, 1761) In this scientific document, d’Alembert conducted the first benefit and risk assessment of variolation by considering the probability of having a direct individual benefit from smallpox inoculation and the risk function of smallpox over age. Both scientists were estimating the benefits of variolation in terms of life years gained for inoculated children.

Assessing benefit and risk balances is a complex and dynamic process which requires superimposing evaluative judgments on scientific facts, such as the available efficacy and safety data, and scientifically acceptable hypotheses. Various parameters should be individually assessed to evaluate such a balance for a given vaccine.

Table 2 provides several points that should be raised when building benefit and risk balance for vaccines. (World Health Organization, 2013)

Table 2 : Elements to be considered when evaluating the benefits *versus* risks

BENEFITS	RISKS
Description of implicated vaccine and lots (incl. manufacturing process and assurance quality)	Weight of evidence for suspected risk (frequency and severity)
Indications for use (reduced risk of morbidity and mortality)	Detailed presentation and analysis of data on new suspected risk (results of case investigation, incidence in campaign)
Identification of alternative modalities	Probable and possible explanations
Brief description of safety of vaccine	Preventability, predictability and reversibility of new risk
Epidemiology and natural history of disease	Risks of alternate vaccines
Known efficacy of vaccine used	Review of complete safety profile of vaccine
Risks associated with not vaccinating, i.e. the risks arising from the infectious disease in unvaccinated individuals.	Estimation of excess incidence of any AEFI

While natural infection might provide long-term immunity, potential serious complications and/or long-term sequelae may be feared for unvaccinated people when they are exposed to a vaccine-preventable microorganism. Since, unlike “chemical” drugs, subjects exposed to vaccines do not endure the disease, immunization should be associated with a very low risk of adverse events. Fortunately, apart from very few exceptions, in any case, the risks associated with vaccination are insignificant compared to those associated to a natural infection. Nonetheless, benefits of immunization are hypothetical and could be delayed provided that an exposition to the microorganism is not guaranteed over life whereas exposition to the vaccine places the subject at potential immediate risks. (D'Alembert, 1761)

Besides, the benefit/risk modeling for vaccines carries additional specificities when compared to other drugs:

- Benefit/risk balance is a dynamic, i.e. not static, process that should be reiterated over time to consider temporal relationships (e.g., bacteria/virus strain replacement, changing vaccine effectiveness).
- Scenario of rare but serious AEFI are not unlikely.
- There may be different, if not opposite, perceptions and weightings of benefits and risks of vaccines between regulators and the population.
- It is crucial to perform iterative benefit/risk assessments to evaluate the post-marketing impact of vaccination.

Provided that vaccines are associated with a low level of acceptance of adverse events, conflicting perceptions about the benefit and risk balance can arise between the different stakeholders. While the national regulatory authorities evaluate benefits and risks at the **population level,** the physician appraises the benefits/risks for the **subject** based on his understanding and knowledge. On his/her own side, the subject assesses benefits/risks in terms of **personal value,** which could be in opposition to the societal expected benefit at the population level.

As mentioned in section 1.3 Why are vaccines different?, vaccines may be a pre-requisite for enrolment in schools or other public structures (e.g., army, hospitals). In several countries (mainly France), immunization schedules for infants aged below 24 months are no longer recommended but are becoming mandatory. In this context, populational perspective could be viewed as a barrier to individual rights, making the vaccine mistrust and defiance more prominent.

2 Designs to evaluate vaccine effectiveness and safety in a real-world setting

Observational studies of vaccines are usually conducted on large numbers of exposed/vaccinated people in a real-life setting, in order to study vaccine effectiveness or to identify rare events not captured during clinical development. The introduction of a new vaccination programme should be considered as an important opportunity to evaluate both effectiveness and safety. It could indeed provide the background rates of a given event in a contemporary unvaccinated population before the expansion of the mass immunization campaigns producing a rapid diminution of suitable unvaccinated controls. Vaccine exposition is relatively easy to determine with single or multiple dose injections scheduled according to a specific calendar. In addition, vaccination details (e.g., date of injection, vaccine lot, vaccine brand, etc.) are usually appropriately recorded in various data sources, including administrative claim databases, vaccine records/registries or electronic medical records.

For assessing the safety profile of a vaccine, it should also be noted that many designs proposed during the last decade(s) use subjects as their own controls, such as:

- Vaccinated subjects only;
- Cases only;
- Vaccinated and cases only.

2.1 Methods for signal detection

2.1.1 Disproportionality analyses

When an event is suspected to be linked to the administration of a health product, a case report should be sent to national regulatory authorities by the observer (physician, other health professional, patient, a patient association, etc.) and in most countries by the concerned manufacturer; they constitute the basis of all the spontaneous reporting systems (SRSs). These latter compile all case reports for either a given area (e.g., Eudravigilance in Europe) or a specific product type (e.g. VAERS in the US). Disproportionality analyses (DPA)

represent the primary class of analytic methods for analyzing data from SRSs from a drug safety surveillance perspective. However, considering its basic principle, spontaneous reporting can neither provide the total number of people having taken the drug of interest without presenting the event of interest nor the number of people having experienced the event without being exposed to the product of interest. In clear, spontaneous reporting provides information on exposed cases only and precludes computation of the classical rates used in pharmacoepidemiology. This feature leads to the major consequences that denominators chosen for DPA are limited and disproportionality measures are relative proportions conditional on what was reported to a given pharmacovigilance database. Their basic principle is that, under the null association, the number of reports of a given event implying a particular drug should reflect the weight of this drug, i.e. the proportion of reports implying this drug in the whole database. Conversely, for a given drug, the proportion of reports concerning a particular event not should significantly differ from the proportion of this event in the whole database (expected ratio or expected number). If a difference was observed and considered as statistically significant, one concludes that there is an association between the considered cases and event. To the extreme, considering the reporting ratios to be identical for the various drug-event pairs present in the database, these ratios could be viewed as proxies for what would have been observed in the general population, i.e. the source population from where the reports originated.

There are several statistical methods used for DPA, such as the proportional reporting ratio (PRR), the reporting odds ratio (ROR), the Bayesian approaches, and some others (e.g Empirical Bayes Gamma-Poisson Shrinker or GPS method). In their simplest form, they are based on two-by-two contingency tables (cf. Table 3). The principle is to calculate a ratio formed of the number of case reports for a given product considering a specific event of interest divided by the number of cases of this same event reported for other products.

Table 3 : Example of a two-by-two contingency table

	Event of interest	Other events	Total
Drug of interest	a	b	e (=a+b)
Other drugs except the one above	c	d	f (=c+d)

According to Table 3, PRR and ROR can be expressed according to the following formulas:

$$PRR = a/e * c/f \qquad \text{and} \qquad ROR = ad/bc$$

As mentioned above, it should be acknowledged that statistics produced may merely reflect a disproportionality of reporting, or the influence of numerous non-causal factors such as confounding, different coding practices or combination of the above. Therefore, a statistical association does not imply in any kind the existence of causal relationship between the administration of the drug (here, the vaccine) and the occurrence of the adverse event. (European Medicine Agency, 2016) As recommended by the EMA in its guideline on statistical signal detection methods (European Medicine Agency, 2006), PRR based on more than 3 individual cases, being equal to or greater than 2 and having a Chi square test statistic equal to or greater than 4 should be considered as a potential signal. For ROR, a cut-off value of 2 with a lower bound of the confidence interval at 95%CI greater to 1 is routinely used to identify signals (European Medicine Agency, 2006; Evans, Waller, & Davis, 2001).

As mentioned above, drug-event associations can be induced by confounding factors. Amongst others, we can list gender and age which could condition the predominant usage of a specific drug or a higher incidence of the event of interest in a given age group (e.g. infants and sudden infant death syndrome (SIDS)). Country of origin, time period and role of the reporter (e.g., manufacturer, physician, patient, etc.) are also common confounders.

Stratification and subgroup analyses can be used to minimize such methodological biases (European Medicine Agency, 2016):

- **Subgrouping**: different measures of disproportionality are computed, one within each of a number of subgroups defined by the covariates of interest.
- **Stratification**: a single measure of disproportionality is estimated by a weighted average across all the subgroups, using standard methods. Stratification is generally used in epidemiology to reduce confounding, when a third variable is associated both with the drug exposure and the event of interest, and may also be of benefit in signal detection algorithms.

While several articles have been published on the choice and impact of the method selected, on the use of subgrouping or stratification approaches, (Evans, 2008; Hopstadius, Noren, Bate, & Edwards, 2008; Seabroke et al., 2016; Woo, Ball, Burwen, & Braun, 2008), no consensus was established. Nevertheless, subgroup analyses tend to perform better than

stratified/adjusted analyses, and also achieved, at least for some specific variables, a better precision and sensitivity over crude analyses. (Seabroke et al., 2016)

In the context of vaccines, impact of age may be crucial for the interpretation of DPA results. As vaccination campaigns often target a specific age group (e.g., infants, elderly people), frequency of certain reports after immunization (e.g., SIDS, cardiovascular events) may be falsely associated with age-specific vaccines (e.g., rotavirus, influenza). It is therefore recommended to consider age-specific subgroup analyses or age stratification. Background rates should thus be established on a comparable population having *a priori* a similar background risk for the disease. The choice of comparators is also tricky. Comparing the frequency of reports associated to vaccines *versus* all other medicinal products could lead to false vaccine-specific signals (e.g., local reactions). On the other hand, using only case reports after administration of vaccines could lead to age-related signals if inappropriate comparator groups were used (e.g., cardiovascular diseases after influenza immunization in the elderly). Given that infectious diseases may be dependent of both the area and season considered, seasonality as well as geographical scope should be considered in DPA. When the DPA is stratified on potential confounding factors, it is important to provide both crude and adjusted estimates and also to examine the DPA measures in each stratum before pooling data which could mask a putative signal. (European Medicine Agency, 2013)

2.1.2 Observed-to-expected analyses

Among the pharmacoepidemiologic arsenal, observed-to-expected (OE) analyses aim at refining previously detected signals.(Mahaux, Bauchau, & Van Holle, 2016) These methods cannot assess the degree of causality between an event and a medicinal product but they help interpreting the strength of a signal by putting suspected adverse reaction reports into context. OE analyses are particularly valuable during mass vaccination programmes where there is little time to review individual cases and prompt decision-making about a safety concern is required. They can also be useful in signal validation and, in the absence of robust epidemiological data, in preliminary signal evaluation. (European Medicine Agency, 2013)

For vaccines, large populations are usually exposed and potential rare events, related or not, may then be reported in the immunized population. The basic principle of OE analyses is to estimate the number of coincidental associations that would have been expected in any case

under the null hypothesis of no association between the vaccine and the disease, and to compare it with the number of cases actually observed or reported. The latter is easily obtained from pharmacovigilance data, i.e. spontaneous reporting, while the expected number can be derived from background incidence rates standardized according to the characteristics of the immunized population. (Bégaud, 2000; Mahaux et al., 2016). The formula below expresses this concept.

$$\frac{\text{Observed cases}}{\text{Expected cases}} = \frac{\text{Number of case reports in a given area}}{\text{Background incidence rate in this area * person-time at risk}}$$

Like DPA, OE analyses are dependent on reliable background incidence rates for a given adverse event. These rates can be provided by the literature or national statistics/data sources. Nevertheless, case definition for a particular event should be aligned with the diagnostic criteria used for the background incidence rate. This will ensure that the comparison between the number of observed and expected cases is valid. Moreover, the background rates should be established from populations that have not been exposed to the vaccine of interest but that have similar demographic characteristics to the vaccinated population. Additionally, geographical variation should be considered when choosing appropriate background incidence rates.

The person-time at risk is estimated by the number of people exposed to a particular vaccine within a given period of time. However, determining this parameter is often tricky for vaccines, as it depends on both the immunization schedule (i.e., number of doses administered) and the initial hypothesis (i.e., whether there is a dose effect and whether the risk periods overlap). In case of a multiple-dose schedule having an at-risk period shorter that the interval between two doses, each dose contributes independently for an identical at-risk period. As no overlap occurs, the total person-time at risk can easily be determined by multiplying the risk period after a dose by the number of doses administered. In case of an overlap of the at-risk periods between two consecutive doses, the number of persons vaccinated and the average proportion of individuals who received dose 1 and dose 2 should be known in order to be able to calculate the total person-time at risk. This latter would

correspond to the average at-risk time for all vaccinees having received dose 1 and/or dose 2 (cf. Figure 5). (Mahaux et al., 2016)

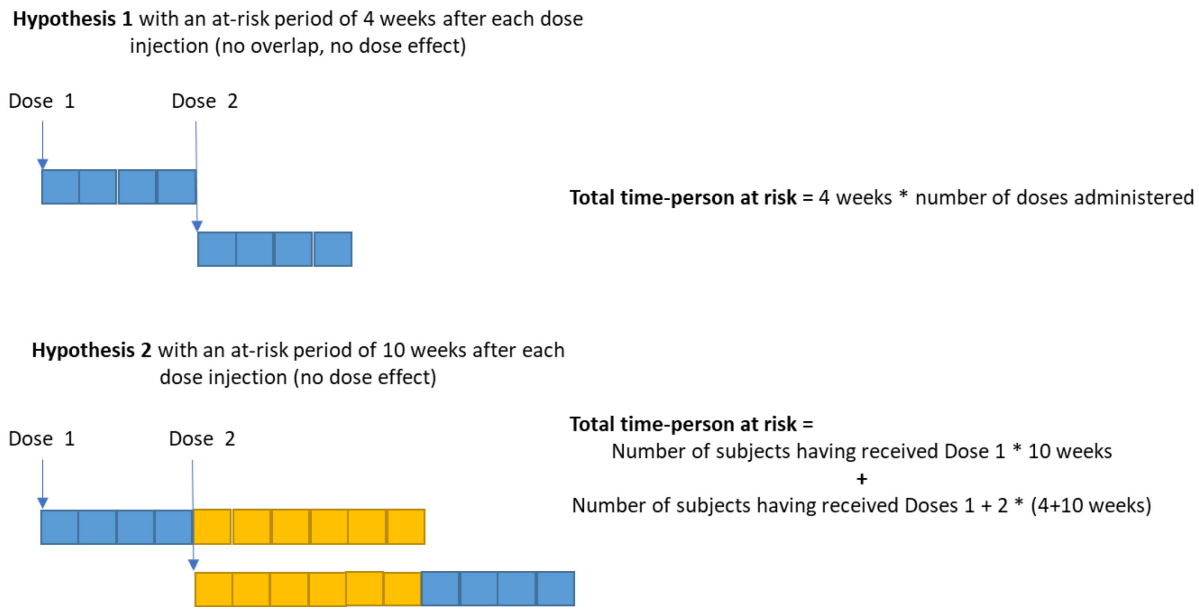


Figure 5: Estimation of the person-time at risk

Defining an appropriate at-risk time window (i.e. the period during which the risk of presenting the disease would be increased by exposure) is essential but not easy to achieve. Under the hypothesis of a causal relationship between an event and a vaccine, selecting a time-window that exceeds the at-risk period would lead to dilute the excess of cases over time by including amounts of time which are not at risk. Conversely, underestimating the time-window would impact the statistical power of the OE analyses by excluding relevant cases potentially attributable to the vaccine.(Mahaux et al., 2016)

When a dose-effect relationship is suspected, a dose-dependent model can be used for OE analyses.

2.1.3 Need for sensitivity analyses

Both DPA and OE analyses depend on the data of spontaneous reporting, which could be influenced by numerous factors including media and public attention, event severity/seriousness, willingness to report, etc. For example, it was found that events occurring a long time after immunization are less likely to be spontaneously reported than events occurring shortly after vaccination, especially if they are expected, common, or mild.(Hazell & Shakir, 2006) As underreporting is a well-known limitation of the SRSs,

sensitivity analyses should thus be recommended when conducting DPA and/or OE analyses, for example by repeating computations for various assumptions about the extent of underreporting. They could also handle various other uncertainties such as the number of confirmed cases (depending on the diagnostic criteria used), estimations of the number of exposed subjects or the uncertainty related to the background incidence rates.(European Medicine Agency, 2013)

2.2 Cohort designs

Cohort studies are often considered as the gold standard for evaluating both effectiveness and safety in the post-marketing phase. Among other strengths, they offer the opportunity of evaluating multiple outcomes and could be both prospective or historical (often improperly named *retrospective cohorts*). For vaccines, they can be valuable when assessing the effectiveness but could be limited for some safety outcomes, especially rare events or events with a long latency period, which would require a considerable sample size or an unreasonably long follow-up.

2.2.1 Classical cohort studies

For practical reasons, observational cohorts are usually conducted within large pre-recorded data sources. While they allow to obtain measures of risk incidence and attributable risk, missing data (i.e., non-recorded information for all or a part of followed persons) are rather common in these settings which could lead to confounding. (N. Andrews, 2012)

The main goal and advantage of this design is to compare the frequency of one or multiple events within an *a priori* defined time period between two or several groups: exposed subjects and controls, which could be subjects exposed to another vaccine or unvaccinated people. In that latter case, attention should be brought to the selection bias inherent to the non-random allocation of vaccines.(IMI ADVANCE Group, 2014) In case of a comparison to an alternate vaccination, both vaccines should target the same population (e.g., children) with a similar indication (e.g., mass campaign). If possible, the comparator group should receive the alternate immunization at the same calendar time period, instead of historical cohort. Matching and adjustment on main known (or suspected) confounding factors or

propensity scores (intended to mimic the randomization process) can be used to restore balance of subjects' characteristics across the groups. (Saddier, 2016)

Figure 6 illustrates the design of parallel-group cohorts.

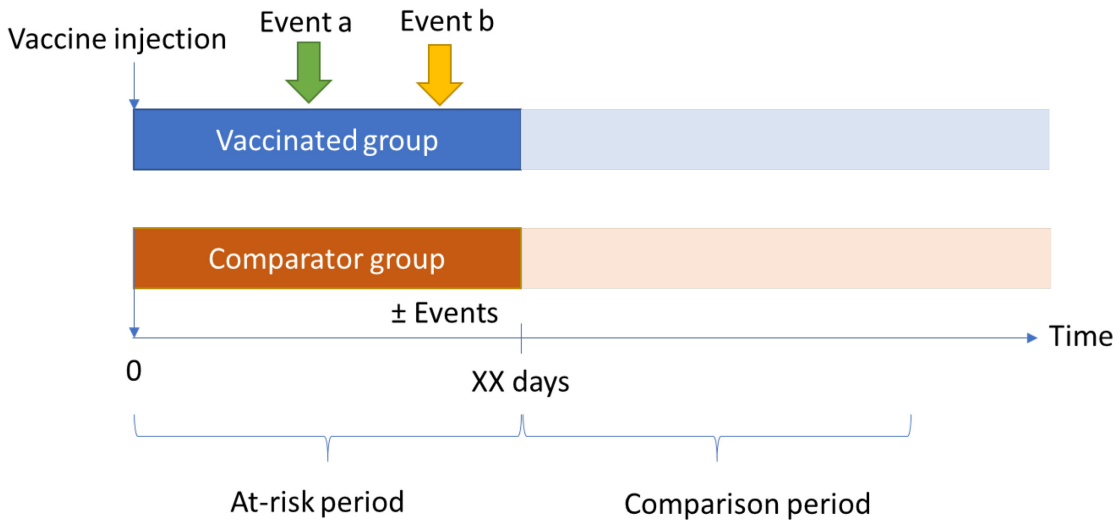


Figure 6: Parallel-group cohort design

The start (index date) and length of the assumed at-risk period should be selected cautiously depending on the nature of the event, the vaccination schedule, the research question and testing hypothesis, etc. Generally, the index date is the day of the vaccine administration but for some events the risk period may be moved forward. The occurrence rate of events of interest could also be compared between the at-risk and comparison periods. The causal inference is from exposure to outcome.

2.2.2 Risk interval cohort studies

The risk interval cohort is an observational study design using vaccinated people only (cases and non-cases). Information should be as complete as possible, especially regarding the date of vaccine administration. The main objective is to compare the occurrence of the event of interest between distinct periods: the *at-risk* period (defined in the same way as the one used in the classical cohort design) and the pre- or post-vaccine control periods (cf. Figure 7). Both the at-risk and control periods should be of the same duration and potential effect of age or seasonality should be taken into consideration. At the opposite of the self-controlled case series (SCCS) design, the comparison is made at the cohort level, not at the individual

level. Consequently, this type of study allows controlling for some time-fixed confounders, but not for time-varying or unmeasured confounders.

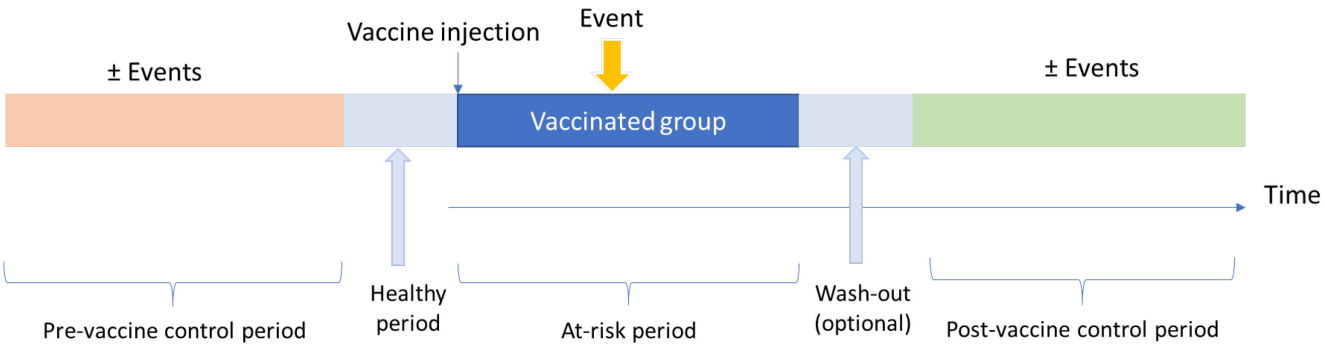


Figure 7: Risk interval cohort design

Both the classical and the risk interval cohorts are relevant to evaluate vaccine effectiveness and the acute safety events.

2.3 Case-control designs

2.3.1 Classical case-control studies

Classical case-control studies offer the opportunity to evaluate a disproportionality of exposure between cases, prevalent or (preferably) incident, and controls. Matching or adjustment on potential confounders are often used (and possibly combined) to ensure comparability of cases and controls. Date of event onset is generally chosen as the index date, even if, for the same reasons as cohort studies, the relevant time-window used for comparing exposures could be put backwards. In any case, assessment of exposure is always assessed retrospectively within a pre-defined look back period at risk (cf. Figure 8). At the opposite of cohort studies, the causal inference is from outcome (disease) to exposure. This design type is particularly well suited for the evaluation of rare events, those with a long latency period or when one intends to explore the relationship between the onset of a disease and different exposures; moreover, it generally requires far smaller sample sizes than cohort studies. If properly conducted (i.e. appropriate sampling), case-control studies provide information that mirrors what could be learned from a cohort study, usually at considerably less cost and time.(UNC Gillings School of Global Public Health, 2015)

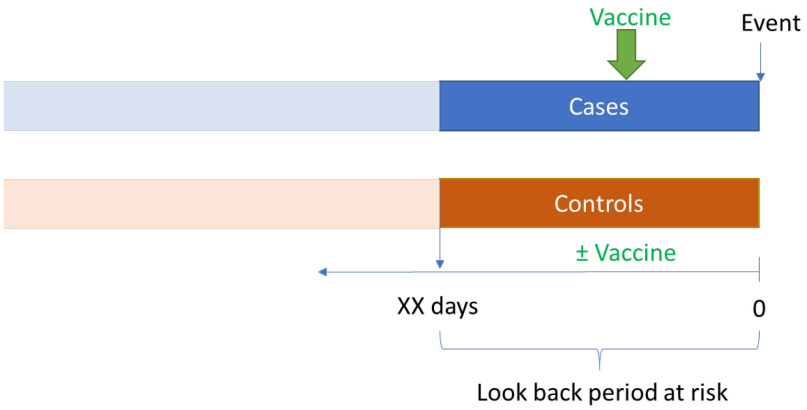


Figure 8: Classical case-control design

The selection of controls is the most complex and challenging step of the design. Moreover, this choice may convey various selection biases will play a major role on the validity of results. Therefore, several methods have been proposed:

- **Base or case-base sampling:** cases and controls are selected from the same source population (i.e., a previously defined cohort: e.g., one single hospital, a registry) such that every person has the same chance of being included as a control.
- **Cumulative density sampling or survivor sampling:** controls are sampled from those people who remained free of the event at the end of follow-up. Controls could never become a case in this setting as their status (case or control) is defined at the end of study.
- **Incidence density sampling or risk set sampling:** in this sampling, cases should be incident and controls should be selected from the at-risk population at the same time as cases occur. Controls must be eligible to become a case if the health outcome develops in the control at a later time during the period of observation. A control selected at a later time point could therefore become a case during the remaining time that the study is running. In this design, the odds ratio approaches the rate ratio of cohort studies, without assuming that the disease is rare in the source population. It gives also the advantage not to be influenced by a differential loss to follow-up among exposed *versus* unexposed subjects. For example, if a large number of smokers left the source population after a certain time point, they would not be available for selection at the end of the study – when using a cumulative density sampling or survivor sampling.

Case-control studies may be subject to recall bias if exposure was measured by interviews and if recall of exposure was likely to differ between cases and controls. Case-control studies should be employed with caution when studying low-level exposures (e.g. less than 10% of

controls are expected to be exposed). (UNC Gillings School of Global Public Health, 2015). Even if mostly used for studying the risk factors of diseases and adverse events (in pharmacovigilance), the case control also is a very valuable design for assessing the effectiveness of drugs and particularly vaccines. In this case, the testing hypothesis is that the exposure will be found less prevalent in subjects presenting the disease compared to controls.

2.3.2 Variant case-control studies

The nested case-control design is a variant method of a case-control study undertaken within a cohort or a data source. Today, this approach is far most used than the classical field case control studies described above. Owing to the access to large sample size (e.g. several millions of individuals in the most used databases), incident cases can be selected and matched, e.g. by a random process, to a large number of controls from the same risk set.. (IMI ADVANCE Group, 2014)

Among other alternate designs, one can cite the following methods (European Network for Centres of Pharmacoepidemiology and Pharmacovigilance (ENCePP), 2018; IMI ADVANCE Group, 2014):

- **Indirect cohort (Broome) method** is a case-control type design which uses cases caused by non-vaccine serotypes as controls. This method should be employed shortly after vaccine introduction as it would be less useful in a setting of very high vaccine coverage and fewer vaccine-type cases.
- **Case coverage method** uses exposure information on cases and population data on vaccination coverage to serve as control. It requires reliable and detailed data on vaccine coverage corresponding to the population from which cases are drawn. This will allow controlling for confounding by stratified analyses. During vaccine introduction, it is also particularly important to address selection bias introduced by awareness of possible occurrence of a specific outcome.
- **Density case-control design** uses incident cases matched to all event-free controls within a pre-specified area (e.g., village), who are at risk of developing disease, at the time that the case occurred (density sampling). This study type produces incidence density rate ratios.
- **Test negative design** uses, as controls, people seeking medical consultation for a similar disease to the one studied (e.g., flu-like symptoms *versus* influenza), but who are tested

negative for the pathological organism. Conversely, cases are those being test-positive. This design helps to reduce misclassification of infection and selection bias.

2.4 Self-controlled designs

Self-controlled methods refer to a study design using the cases as their own controls, automatically adjusting for time-fixed, even unmeasured, confounders (e.g., sex, birth date, etc.). Non-cases are therefore not informative for these methods. The same dichotomy between cohort and case-control is applicable to self-controlled approaches. While self-controlled case series (SCCS) adopts the logic of cohorts, i.e., occurrence of the event is random with vaccine exposure being fixed, the case crossover method adopts the logic of case-control studies with the occurrence of event being known while the exposure is random. (IMI ADVANCE Group, 2014)

2.4.1 Self-controlled case series

This design, developed initially for vaccine safety, is particularly relevant for evaluating rare safety events or when access to an *ad-hoc* vaccinated comparator group is difficult or impossible. (Farrington, 1995; Farrington, Whitaker, & Hocine, 2009; Whitaker, Farrington, Spiessens, & Musonda, 2006; Whitaker, Ghebremichael-Weldeselassie, Douglas, Smeeth, & Farrington, 2018; Whitaker, Hocine, & Farrington, 2009)

This design uses all vaccinated cases within a pre-specified period of observation to compare the occurrence of an event of interest between distinct periods: the at-risk and pre- or post-vaccination control intervals (cf. Figure 9).

The pre-specified periods of time could be based on calendar time (e.g., one year) or on age (e.g., cohort of children aged 6 years old). The main limitation of this design is that the administration of vaccine (i.e., exposure) or follow-up should not depend on previous events occurring in the pre-vaccine control period (e.g. this could occur if the event has high mortality or is a contraindication to vaccination). Otherwise, this would severely bias the findings of the study. Several alternate SCCS variants have been proposed to counteract, in whole or in part, these limitations. For example, to cancel the effect of a postponed immunization due to the occurrence of an event of interest, it is possible to exclude a certain

interval of time before vaccine administration. This period would correspond to a 'low-risk' interval. (N. Andrews, 2012)

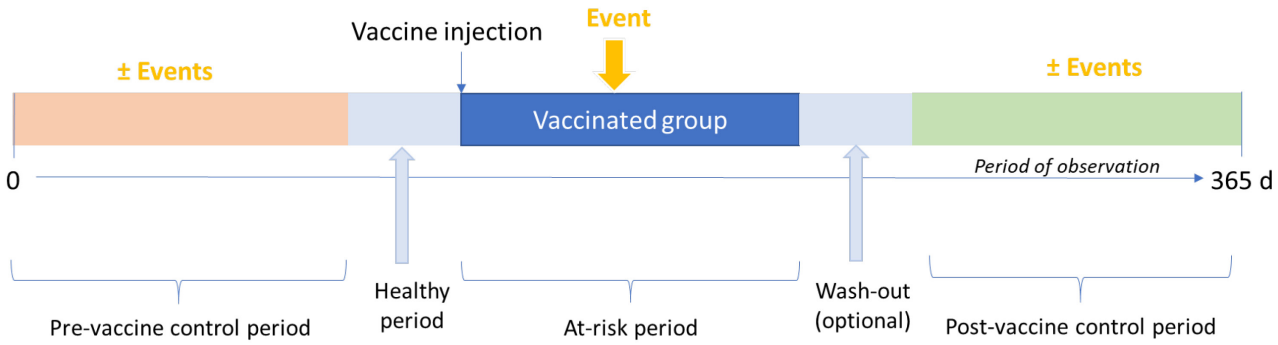


Figure 9: Self-controlled case series design

Among the SCCS-like designs, the self-controlled risk interval uses a shorter period of observation with well-defined intervals in relation to the exposure (i.e., the risk period and the control period).(Greene et al., 2012) In this setting, adjustment on age is therefore not mandatory. (IMI ADVANCE Group, 2014)

2.4.2 Case-crossover design

As mentioned above, the case-crossover studies look like classical case-control studies but using only the vaccinated cases within a pre-specified study period, based on either calendar time or age. This setting compares the disproportionality of exposure according to specific periods (i.e., risk and control intervals) preceding the event onset (cf. Figure 10). This assumes that the exposure is not time-dependent, which is usually not applicable for pediatric or seasonality vaccines.(IMI ADVANCE Group, 2014) Not respecting this limitation could introduce the exposure time bias (i.e., change in exposure probability over time).

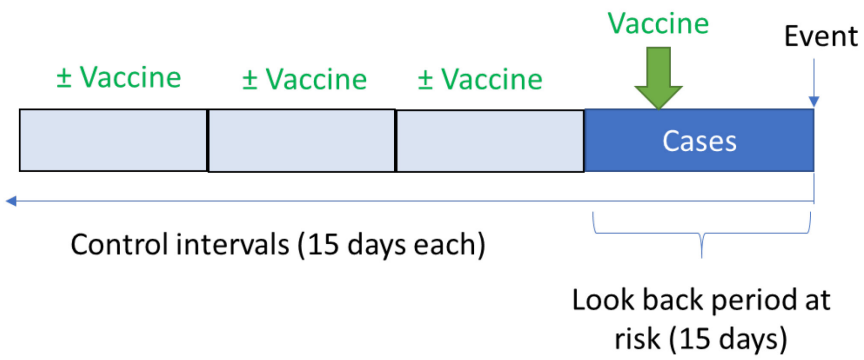


Figure 10: Case-crossover design

2.4.3 Case-time-control design

To address the exposure time bias, alternate designs have been developed such as the case-time control or the case-case time control settings.

The first method supplements the original design with a second time-matched case-crossover in controls using the same exposure (cf. Figure 11).(Suissa, 1995) It assumes that the exposure time trend is similar between controls and cases. It provides odds of vaccination in the pre-specified "risk" *versus* control periods in the case group and in the non-case group. The OR in the control group is an estimate of exposure time trend effect, while the ratio of the two ORs is an estimate of the vaccine exposure effect.(Saddier, 2016) The case-case time control design addresses the issue related to the choice of inappropriate external controls in the case-time control design, by using the future cases as controls. (IMI ADVANCE Group, 2014)

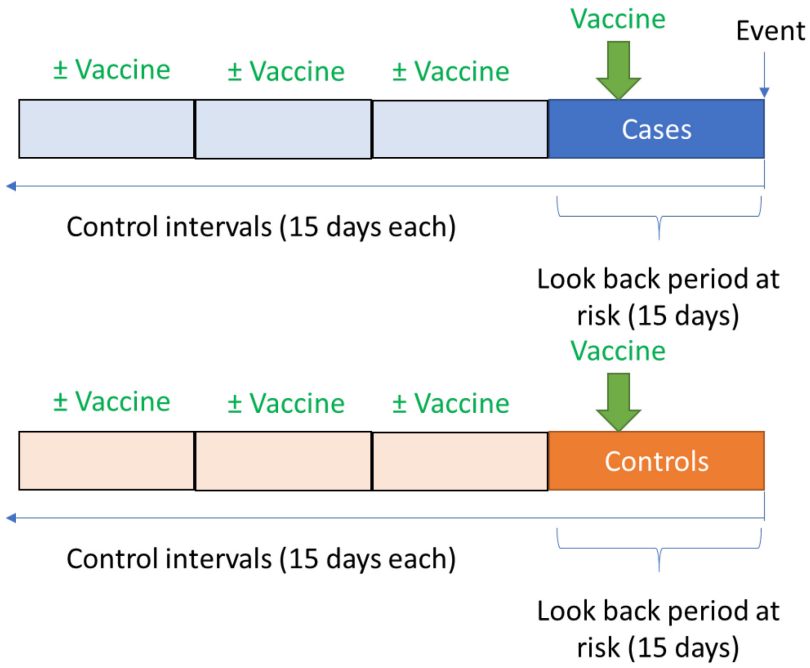


Figure 11 : Case-time control design

It should be acknowledged that most designs presented above (i.e., cohort, case-control or self-controlled studies) are well suited to assess vaccine effectiveness and/or acute and transient safety outcomes. However, most of them perform rather poorly for events with non-acute onset and long duration, even being worse for life-long outcomes.

2.5 Real-world data sources for monitoring vaccine effectiveness and safety

In the framework of vaccination, if excluding *ad-hoc* field studies, various data sources can provide vaccination-related information. Two different types could be considered:

- Generalistic databases
- Immunization registries

The following sections present an overview of potential data sources, but do not intend to constitute an exhaustive review of all existing databases.

2.5.1 Europe

Table 4 presents some of the large generalistic databases existing in Europe and having been used for numerous pharmacoepidemiological research projects including those focusing on vaccines.

No pan-European immunization registry is yet available. However, several European countries have implemented their own immunization information systems which are population-based tools providing vaccine-related data in a specific country or region (cf. Table 5). These systems are particularly useful for monitoring vaccine coverage at local geographical levels, linking individual immunization history with health outcome data for safety investigations, or monitoring vaccine effectiveness and failures. In France, multiple subnational registries capture immunization data. (Derrough et al., 2017)

Table 4: European generalistic data sources used for pharmacoepidemiology research purposes

Data source Name	Country	Creation Date	Origin of Data	Population Size	Average follow-up	Data Collected & Linkage	Vaccine-related Information	Web Link
Echantillon généraliste des bénéficiaires (EGB)	France	2003	Administrative claims	660 000	~11 years	Demographics, date and nature of reimbursed prescriptions/medical procedures (including labs), inpatient data (date, diagnosis), practice	*No details*	https://www.snds.gouv.fr/SNDS/Composantes-du-SNDS
Système national des données de santé (SNDS)	France	2017	Administrative claims	67 million	Unknown	Demographics, medico-administrative data, out and inpatient data including prescriptions, labs, visits, etc., death records (date and cause), sick leaves	*No details*	https://www.snds.gouv.fr/SNDS/Accueil
Clinical Practice Research Datalink (CPRD)	UK: England, Wales, Scotland and Northern Ireland	1987	Electronic medical records	>10 million (7% of total population) 674 GP practices	5.1 years	Demographics, coded diagnosis, therapies, vaccines, health-related behaviours, and referrals to secondary care. It also offers broad linkage capabilities (e.g., hospital episodes, death registration, etc.).	The main drawback of this database for vaccine research is that it does not cover other practices, except GP. As some vaccines are given during school-based programs (e.g., HPV vaccination) or through sexual health or genitourinary medicine clinics (e.g., HBV vaccination), information related to vaccination may be scarce or not representative of the overall exposed population.	https://cprd.com/home
The Health Improvement Network (THIN) database	UK	2002	Electronic medical records	~17 million from over 500 practices	Unknown	Patient demography, clinical data, prescribing, consultations (diagnoses and symptoms), staff and practices, lab tests and results ordered by GP, vaccinations It may be possible to obtain further patient information via the Additional Information Service including: - anonymised questionnaires completed by the patient or GP - copies of patient-based correspondence - a specified intervention (e.g. a laboratory test to confirm diagnosis) - death certificates	Pre-school vaccinations are routinely given in primary care in the UK. The date, type (tetanus, polio, etc.) and dose (first, booster, etc.) of routine vaccinations are recorded in specific structured immunisation fields when they are administered. It should be noted that for many practices, the electronic record is the primary record and there is no paper version for comparison.	https://www.iqvia.com/locations/uk-and-ireland/thin

German Pharmacoepidemiological Research Database (GePaRD)	Germany	2004	Insurance claims	~20 million (17% of total pop)	Unknown	Demographic data, drug dispensations, outpatient and inpatient services and diagnoses	Vaccinations could be identified by outpatient codes used for reimbursement of administration of vaccines. Vaccine dispensations in the pharmacy could not be considered, as physicians generally use vaccines kept in their own medical practices.	https://www.bips-institut.de/fileadmin/bips/images/gepard/GePaRD_description_V1.9.pdf
BIFAP database	Spain	2003	Electronic medical records	> 8 million (20% of total pop)	Unknown	Demographics, prescription details, clinical events, specialist referrals, laboratory test results. Prescription data includes product name, quantity dispensed, dosage regimens, strength and indication.	Vaccinations recorded	http://www.bifap.org/
SIDIAP database	Spain	1998	Electronic medical records	> 5.5 million (74% of the Catalan pop)	Unknown	Demographics, dispensings and prescriptions, diagnoses and dates, clinical parameters, diagnostic procedures (lab, imaging, scales), medical procedures, referral, sick leaves, visits in primary care and others	Immunizations recorded: DT, DTP, Influenza, Haemophilus, Hepatitis B, HPV, Meningitis C, Pneumococcal vaccine, Polio vaccine, Tetanus, Measles, mumps, rubella, Chickenpox, For each vaccine per patient, SIDIAP collects: - Code of vaccine - Description of vaccine - Dose number - Date of immunization - Practice where the immunization is administered	http://www.sidiap.org/
Health Search	Italy	1998	Electronic medical records	2 million	Unknown	Demographics, clinical data, prescriptions, prescriber profile	Vaccinations recorded	https://www.healthsearch.it/?lang=en
PHARMO database	Netherlands	1993	Electronic medical records	~4 million (25% of total pop)	~10 years	Population-based network of healthcare databases combining data from different healthcare settings: general practitioner, in- and out-patient pharmacy, clinical laboratory, hospitals, cancer registry, pathology registry and perinatal registry. All are linked on a patient level through validated algorithm.	*No details*	https://www.pharmo.nl/

Danish Registries	Danemark	1968	Electronic medical records	9 million	Unknown	All data are linkable on individual level. For example, the pharmacy database can be linked to other Danish registries via the patient's civil registration number. Other databases include the Danish Medical Birth Registry, the National Registry of Patients (including inpatient discharge diagnoses coded according to ICD8 from 1977 to 1993 and inpatient, outpatient specialty clinic, and emergency department discharge diagnoses according to ICD10 from 1994 to present), the Danish Psychiatric Central Registry, the Danish Cancer Registry, the Civil Registration System (vital status and residence status), and the Danish Registry of Causes of Death.	Vaccinations recorded	https://www.datatilsynet.dk/

Table 5: Immunization information systems in Europe (extracted from (Derrough et al., 2017))

Country	Name of the Immunization Information Systems	Year	National (N) / Subnational (S)
Belgium (Flanders)	Vaccinnet	2005	S
Denmark	The Danish Vaccination Register (DDV)	2013	N
Finland	The National Vaccination Registry	2011	N
Germany	'KV-Impfsurveillance' ['Associations of Statutory Health Insurance Physicians (ASHIP) vaccination monitoring']	2011	N
Hungary	Országos Szakmai Információs Rendszer (OSZIR) Védőoltási és oltóanyag logisztikai alrendszer	2014 piloting	N
Iceland	Central Immunisation Register	2007	N
Ireland	School Immunisation System (SIS)	2011	N
Latvia	National e-Health System	2016 piloting	N
Malta	National Immunisation Electronic Database	2009	N
Netherlands	Praeventis	2005	N
Norway	SYSVAK – Norwegian Immunisation Registry	1995	N
Portugal (mainland)	Vacinas	2003	S
Romania	National Electronic Registry of Immunization	2011	N
Slovakia	National Health Information System	Unknown, piloting	N
Spain (Andalucía)	Módulo de vacunas DIRAYA	2016	S
Sweden	National Vaccination Registry	2013	N
United Kingdom (England)	Child Health Information System	Late 1980s	S

2.5.2 North America

2.5.2.1 United States

Among the large commercial health databases collecting patient data in the US, one can cite:

- **Optum Research Database**: which collects insurance claims of more than 34 million individuals each year, containing both commercially insured individuals and Medicare managed care enrollees. The database consists of proprietary, deidentified health claims data from a geographically diverse US population (16% West, 20% Midwest, 36% South, and 27% Northeast). This database has already been used for observational research concerning vaccines. (Jain et al., 2015)
- **Truven Health MarketScan® database**: which includes patient demographics, health plan information, medical diagnoses codes, procedure codes, prescriptions, and cost data. It represents about 100 employer-sponsored private health plans covering around 45 million members. Each member in the database has a unique identifier that can be used to track

patients across sites of service and providers over time. This database has already been used for observational research concerning vaccines. (Petigara & Zhang, 2018)

- **Medicare database**: which compiles health insurance claims of about 39 million patients aged 65 years and over and enrolled in Medicare health insurance programme Health insurance claims include pharmacy dispensings, hospital and outpatient claims, and procedure claims. This data source has been used for evaluating the effectiveness and duration of protection provided by the live-attenuated Herpes Zoster vaccine. (Izurieta et al., 2017)
- **Kaiser Permanente databases: :** this historical and particularly fruitful programme contains electronic health records on almost 30 million current and past members in 8 regions of the country. Several articles have been published on the use of these databases (especially Kaiser Permanente Southern California) for vaccine observational research and clinical trials. (Baxter & Klein, 2013)

Aside these large data sources, each US state has its own immunization registry which contains immunizations given to inhabitants throughout life. Table 6 lists examples of these sources.

Table 6: Examples of US immunization registries

State	Name of the Immunization Registry	Web Link
Alaska	VacTrAK	http://dhss.alaska.gov/dph/Epi/iz/Pages/vactrak/default.aspx
Arkansas	Immunization Registry	https://www.healthy.arkansas.gov/programs-services/topics/immunization-registry
California	California immunization registry	https://www.sfcdcp.org/immunizations/immunization-programs/immunization-registry/
Connecticut	Connecticut Immunization Registry and Tracking System (CIRTS)	https://portal.ct.gov/DPH/Immunizations/Connecticut-Immunization-Registry-and-Tracking-System-CIRTS
Delaware	Delaware's Immunization Registry	https://www.dhss.delaware.gov/dph/dpc/immunize-providers.html
Georgia	Georgia Immunization Registry (GRITS)	https://dph.georgia.gov/georgia-immunization-registry-grits
Hawaii	Hawaii Immunization Registry (HIR)	http://health.hawaii.gov/docd/about-us/programs/hawaii-immunization-registry-hir/
Iowa	Iowa's Immunization Registry Information System (IRIS)	https://iris.iowa.gov/IRISPRDJ/clientSearch.do?language=en
Kentucky	Kentucky Immunization Registry	https://chfs.ky.gov/agencies/dph/dehp/idb/Pages/kyir.aspx
Maine	ImmPact Immunization Registry	https://www.maine.gov/dhhs/mecdc/infectious-disease/immunization/providers/immunization-registry.shtml
Maryland	ImmuNet (Maryland's Immunization Information System)	https://phpa.health.maryland.gov/OIDEOR/IMMUN/Pages/immunet.aspx

Massachusetts	Massachusetts Immunization Information System (MIIS)	https://www.mass.gov/service-details/massachusetts-immunization-information-system-miis
Michigan	Michigan Care Improvement Registry (MCIR)	https://www.mcir.org/public/
Minnesota	Minnesota Immunization Information Connection (MIIC)	http://www.health.state.mn.us/miic
Mississippi	Mississippi Immunization Registry	https://msdh.ms.gov/msdhsite/_static/31,0,136.html
New York	Citywide Immunization Registry	https://immunize.nyc/provider-client/servlet/PC
North Carolina	The North Carolina Immunization Registry (NCIR)	https://www.immunize.nc.gov/providers/ncir.htm
San Diego	The San Diego Regional Immunization Registry (SDIR)	https://www.sandiegoimmunizationregistry.org/sdir_home.htm
South Carolina	The Immunization Registry for the State of South Carolina	https://scdhec.gov/health-professionals/electronic-health-records-meaningful-use/immunization-registry
Tennessee	Tennessee Immunization Information System (TennIIS)	https://www.tennesseeiis.gov/tnsiis/
Texas	ImmTrac2 Registry	https://www.dshs.texas.gov/immunize/immtrac/
Vermont	Vermont Immunization Registry	http://www.healthvermont.gov/health-statistics-vital-records/registries/immunization
Washington DC	District of Columbia Immunization Information System (DOCIIS)	https://dchealth.dc.gov/dociis
Washington State	Washington State Immunization Information System (IIS)	https://fortress.wa.gov/doh/cpir/iweb/
West Virginia	West Virginia Statewide Immunization Information System	https://dhhr.wv.gov/oeps/immunization/shotrecords/Pages/default.aspx
Wisconsin	Wisconsin Immunization Registry (WIR)	https://www.dhs.wisconsin.gov/immunization/wir.htm
Wyoming	Wyoming Immunization Registry	https://health.wyo.gov/publichealth/immunization/wyir-wyoming-immunization-registry/

Additionally, the **Vaccine Adverse Events Reporting System (VAERS)** is a is a national passive surveillance system which aims at detecting possible safety issues in US-licensed vaccines. VAERS is co-managed by the Centers for Disease Control and Prevention (CDC) and the U.S. Food and Drug Administration (FDA). VAERS records reports of adverse events after vaccine administration. Anyone can report an adverse event to VAERS. Healthcare professionals are required to report certain adverse events and vaccine manufacturers are required to report all adverse events that come to their attention.

2.5.2.2 Canada

Canada is known for its large population-based data sources allowing real-world research projects in various therapeutic areas. Here are examples of these potential sources:

- **The Manitoba Population Research Data Repository:** based on the comprehensive collection of administrative, registry, survey, and other data about residents of Manitoba. It comprises the Manitoba Immunization Monitoring System which stores over 200,000 immunization records and about 170 data elements since 1986. This registry is devoted to child immunizations only.
- **The "Régie de l'Assurance Maladie du Québec" (RAMQ) database:** which compiles health insurance claims of Canadians living in the Quebec province. With a total of 8 million persons covered, it represents more than 305 million claims submitted annually by health professionals.
- **The Canadian Primary Care Sentinel Surveillance Network (CPCSSN) database**: which contains health records of approximately one million subjects with chronic disease. It covers 11 practice-based research networks of British Columbia, Alberta, Manitoba, Ontario, Quebec, Nova Scotia, Newfoundland and Labrador, and the Northwest Territories. These data are extracted from multiple EMR systems. (Queenan et al., 2016)

Currently under creation, the Canadian Immunization Registry Network will collect vaccination-related data from the ten provincial immunization registries already implemented across Canada. In addition to records details about vaccine administration, these systems will also track reports of adverse events associated with vaccines. (Wilson et al., 2017)

2.5.3 Other countries

Japan does not benefit from a national immunization registry but population-based data sources allowing vaccine observational research are available such as the Japan Medical Data Center database. This large-scale database covers more than 3 million enrollees of employee health care insurance plans and their dependents, and contains claims records for ambulatory care, hospitalization and pharmacy benefits. It has been used recently to assess the effectiveness of influenza vaccination in children. (Shibata, Kimura, Hoshino, Takeuchi, & Urushihara, 2018)

In Taiwan, the National Health Insurance Research Database includes 99% of the Taiwanese population and records, amongst others, clinical diagnoses and prescriptions. Recently, it provided valuable information to understand the effectiveness of influenza vaccination within this country. (Chen et al., 2018)

The Australian Immunisation Register (AIR) compiles any immunization-related information for both children and adults across the country. In 2018, this source was used to document the immunization status of subjects enrolled in an observational study evaluating the recurrence of a hypotonic hyporesponsive episode after immunization. (Crawford et al., 2018)

In South America, immunization registries, local or nationwide, are currently under implementation for several countries. In Brazil, a national database (i.e., Sistema de Informação de Eventos Adversos Pós-Vacinação , SI-EAPV) collects the AEFIs. (Danavaro C, 2014)

2.6 Specific initiatives

2.6.1 Vaccine Safety Datalink (US)

Initiated in 1990, the Vaccine Safety Datalink (VSD) is a collaborative project between CDC's Immunization Safety Office and eight health care organizations:

- Group Health Cooperative
- Kaiser Permanente Northwest
- Kaiser Permanente Northern California
- Kaiser Permanente Southern California
- Kaiser Permanente Colorado
- Health Partners
- Marshfield Clinic
- Harvard Pilgrim

The main goal of VSD is to monitor safety of vaccines, especially those newly approved in the US. Research projects are based on questions or concerns raised from the medical literature and reports to VAERS. Electronic medical records are collected from each participating site. Data of about 5.5 million people are collected each year, representing 1.9% of the US population. Data recorded includes information on vaccines: vaccine type, brand name and

date of vaccination. The VSD also uses information on medical diagnoses, urgent care visits, emergency department visits, and hospital stays. Database linkage between vaccination records, health outcomes (in- and outpatient settings, emergency care) and patient demographics (e.g., birth certificate, census, etc.) is ensured by each participating site.

VSD does not perform data mining but is well-suited for hypothesis testing. Each week, the system evaluates the number of outcomes in vaccinated persons and compares it to the expected number of outcomes based on a comparison group. The system uses sequential statistical analyses and maximized sequential probability ratio test (Max-SPRT).

The outcomes of interest are selected based on:

- Pre-licensure data;
- Known biologic properties of the vaccine.
- Adverse events for similar vaccines.

These outcomes should be serious, relatively uncommon and clearly defined (e.g., Guillain-Barré syndrome rather than “neurologic problems”). Outcomes with an acute onset are preferred. (Centers for Disease Control and prevention, 2018b)

2.6.2 Canadian Vaccine Safety Network - CANVAS (Canada)

CANVAS assesses vaccine safety immediately after implementation of vaccine campaigns. The network is comprised of sites in Vancouver, Calgary, Toronto, Ottawa, Quebec City, Sherbrooke, and Halifax.

For example, each year, CANVAS assesses the safety of seasonal influenza vaccines used in Canada. (Canadian Immunization Research Network, 2018)

2.6.3 AusVaxSafety (Australia)

AusVaxSafety is a national, collaborative active vaccine safety surveillance initiative led by the National Center for Immunization Research & Surveillance (NCIRS) and funded by the Australian Government Department of Health. (National Centre for immunisation research and surveillance (NCIRS), 2018)

Three main activities are routinely conducted to monitor the vaccine safety:

- **Sentinel Active Participant-based Surveillance**:

SmartVax and **Vaxtracker** are software programmes run by general practitioners and immunization clinics that send an SMS or email to patients or parents following a vaccination. De-identified information from SmartVax and Vaxtracker are combined and monitored by AusVaxSafety in order to detect possible safety signals for vaccines.

SmartVax extracts immunization data from practice softwares and sends a series of SMS messages inquiring if patients have experienced an adverse event:

- The SMS asks patients if there were any adverse reactions to the vaccination and requests a "Yes" or "No" reply by SMS.
- YES responses trigger a second SMS. The second SMS inquires if the reaction was medically attended.
- YES responders also receive via SMS a link to an online survey to complete. The survey is simplified and takes less than two minutes to be completed. The survey ascertains the nature, duration and severity of reactions reported.

Vaxtracker is an innovative online active surveillance system that allows people to report, by completing a web-based survey, how their child, or themselves, have responded to a recently administered vaccine.

SmartVax and Vaxtracker are used by more than 200 sentinel surveillance sites including general practices, immunization clinics, hospital- and community-based clinics, and Aboriginal Medical Services spread across all Australian states and territories.

- **Adverse Events Following Immunisation – Clinical Assessment Network (AEFI-CAN)**:

This network investigates severe and/or serious AEFI by creating standardised clinical protocols and facilitating uniform AEFI clinical follow up through a national AEFI-CAN clinical database.

- **National Prescribing Service (NPS) Medicine Insight Data**:

This database collects de-identified patient information from over 600 general practices, this system is currently involved in evaluating the safety of Herpes zoster vaccine in older Australians.

2.6.4 Vaccine Sentimeter

Vaccine Sentimeter is a global surveillance tool of online media discussion about vaccines. It is monitored by the Program for Monitoring Emerging Diseases (ProMED) and the London School of Hygiene and Tropical Medicine. The Vaccine Sentimeter describes the feeling of the person (positive, neutral, or negative), and the topics discussed (according to a typology) in each identified report a specific vaccine. Along with a few other categories, reports about serious AEFI are flagged as high priority reports and forwarded to health ministries and officials at institutions such as the WHO and United Nations International Children's Emergency Fund (UNICEF). Comprised of 100,000 mainstream media sources and Twitter, natural-language processing for automated filtering, and manual curation to ensure accuracy, the Vaccine Sentimeter offers a global real-time view of vaccination conversations online. (Bahk et al., 2016)

3 Epidemiology of central demyelination and multiple sclerosis

3.1 Definitions

Demyelination is a pathologic process leading to the destruction of myelin-supporting cells which are oligodendrocytes and Schwann cells in the central and peripheral nervous system, respectively and/or the myelin lamellae with relative preservation of axons. (Mehndiratta & Gulati, 2014).

Demyelinating diseases of the central nervous system (CNS) can be classified according to their pathogenesis into several categories: demyelination due to inflammatory processes, viral demyelination, demyelination caused by acquired metabolic derangements, hypoxic–ischaemic forms of demyelination and demyelination caused by focal compression. (Love, 2006) Demyelinating diseases include:

- Multiple sclerosis (MS), the most predominant central demyelinating disease,
- Optic neuritis (ON),
- Neuromyelitis optica (NMO),
- Transverse myelitis (TM).
- Acute-disseminated encephalomyelitis (ADEM).

Demyelinating disorders of the peripheral nervous system include, amongst others, Guillain-Barré Syndrome (GBS) and chronic inflammatory demyelinating polyradiculoneuropathy.

3.2 Diagnostic criteria

3.2.1 Multiple sclerosis

Diagnosis of MS is based on the dissemination of CNS lesions in time and space.

MS was initially defined according to four types (Brochet, de Sèze, Lebrun-Frenay, Zéphir, & Defer, 2017) (cf. Figure 12 and Figure 13):

- **Relapsing remitting MS (RRMS):** acute episodes or relapses with partial or complete recovery, clinical manifestations are stable between episodes. RRMS represents about 85% of MS patients.
- **Primary progressive MS (PPMS):** clinical manifestations progress over time from onset without relapses. About 10% of MS patients present this form of the disease.
- **Secondary progressive MS (SPMS):** The initial phase with acute episodes are then followed by a progression of clinical manifestations with or without superimposed relapses. This form usually follows the initial RRMS course for 50% of RRMS patients after 10 years from disease onset and up to 90% of RRMS patients 25 years after the MS diagnosis.
- **Progressive relapsing MS (PRMS):** is an uncommon form of MS (about 5%). It is similar to the PPMS but, in addition to the clinical progression over time, relapses happen every so often.

This classification was recently revised to include additional descriptors such as disease activity (based on clinical relapse rate and imaging findings) and disease progression. (Lublin et al., 2014)

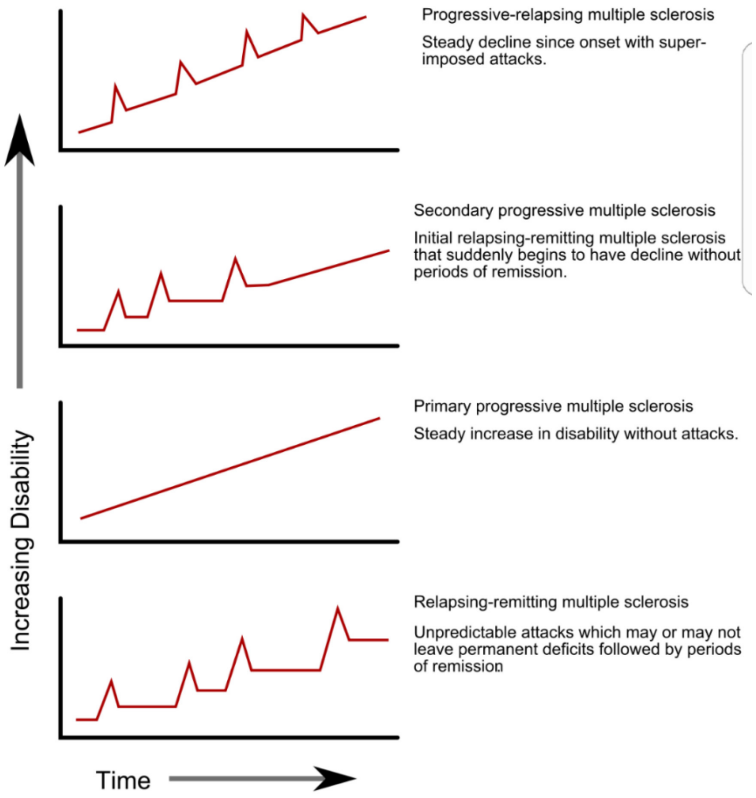


Figure 12 : Definitions of MS forms

Figure 13 : Distribution of MS forms (Multiple Sclerosis International Federation (MSIF), 2013)

Clinical isolated syndrome (CIS) corresponds to an isolated acute episode of demyelination; it is considered as a sub-form of MS by several learning societies such as the United States National Multiple Sclerosis Society and the Multiple Sclerosis International Federation. Although it does not meet the diagnostic criteria for MS, it is often predicting a later MS diagnosis in 30 to 70% of subjects experiencing CIS. In 85% of cases, it involves the optic nerves, brainstem, or spinal cord. (Karussis, 2014; Miller, Barkhof, Montalban, Thompson, & Filippi, 2005)

Several classifications and diagnosis criteria have been proposed since the first observation and description by Charcot and Vulpian in 1862. The disease was initially diagnosed by two time- and space-distant episodes of clinical symptoms of demyelination in the absence of any apparent alternative explanation occurring in patients aged between 1 and 50 years. (Schumacker et al.)

The Schumacher and then Poser criteria were widely used in the last century. In addition to the signs and symptoms evoking MS, Poser used a combination of both clinical and paraclinical criteria. Laboratory exams and electrophysiology required the presence of oligoclonal bands in the cerebrospinal fluid (CSF) and of abnormal/delayed responses of the visual and auditory evoked potentials to confirm the MS diagnosis. (Karussis, 2014)

The concept of clinical evidence of dissemination in time and space was then replaced by the radiological evidence of such dissemination, thanks to the advent of sophisticated neuroimaging (e.g. magnetic resonance imaging (MRI)). New diagnostic criteria were therefore proposed by McDonald in 2001 (McDonald et al., 2001) and subsequent revisions were made in 2005, 2010 and 2017. (Polman et al., 2011; Polman et al., 2005; Thompson et al., 2018)

Table 7 presents the latest version of the McDonald criteria for MS diagnosis.

Table 7: McDonald criteria for MS diagnosis, revised version 2017 (extracted from (Thompson et al., 2018)

Clinical presentation	Additional Data Needed for MS Diagnosis
≥ 2 attacks; objective clinical evidence of ≥ 2 lesions or objective clinical evidence of 1 lesion with reasonable historical evidence of a prior attack	None
≥ 2 attacks; objective clinical evidence of 1 lesion	Dissemination in space demonstrated by an additional clinical attack implicating a different CNS site or by MRI
1 attack; objective clinical evidence of ≥2 lesions	Dissemination in time demonstrated by an additional clinical attack or by MRI OR demonstration of CSF-specific oligoclonal bands
1 attack; objective clinical evidence of 1 lesion (clinically isolated syndrome, CIS)	Dissemination in space demonstrated by an additional clinical attack implicating a different CNS site or by MRI **AND** Dissemination in time demonstrated by an additional clinical attack or by MRI OR demonstration of CSF-specific oligoclonal bands
Addition/modification to the 2010 McDonald criteria	In a patient with a typical clinically isolated syndrome and fulfilment of clinical or MRI criteria for dissemination in space and no better explanation for the clinical presentation, demonstration of CSF-specific oligoclonal bands in the absence of other CSF findings atypical of multiple sclerosis allows a diagnosis of this disease to be made. Symptomatic and asymptomatic MRI lesions can be considered in the determination of dissemination in space or time. MRI lesions in the optic nerve in a patient presenting with optic neuritis remain an exception and, owing to insufficient evidence, cannot be used in fulfilling the McDonald criteria. In the 2010 McDonald criteria, the symptomatic lesion in a patient presenting with brainstem or spinal cord syndrome could not be included as MRI evidence of dissemination in space or time. Cortical and juxtacortical lesions can be used in fulfilling MRI criteria for dissemination in space. Cortical lesions could not be used in fulfilling MRI criteria for dissemination in space in the 2010 McDonald criteria. The diagnostic criteria for primary progressive multiple sclerosis in the 2017 McDonald criteria remain the same as

	those outlined in the 2010 McDonald criteria, aside from removal of the distinction between symptomatic and asymptomatic MRI lesions and that cortical lesions can be used. At the time of diagnosis, a provisional disease course should be specified (relapsing-remitting, primary progressive, or secondary progressive) and whether the course is active or not, and progressive or not based on the previous year's history. The phenotype should be periodically re-evaluated based on accumulated information. This recommendation is an addition to the 2010 McDonald criteria.

Notes:

If the Criteria are fulfilled and there is no better explanation for the clinical presentation, the diagnosis is ''MS''; if suspicious, but the Criteria are not completely met, the diagnosis is ''possible MS''; if another diagnosis arises during the evaluation that better explains the clinical presentation, then the diagnosis is ''not MS.''

An attack, relapse, exacerbation and clinically isolated syndrome (when it is the first episode), are synonyms. CIS is defined a monophasic clinical episode with patient-reported symptoms and objective findings reflecting a focal or multifocal inflammatory demyelinating event in the CNS, developing acutely or subacutely, with a duration of at least 24 h, with or without recovery, and in the absence of fever or infection; similar to a typical multiple sclerosis relapse (attack and exacerbation) but in a patient not known to have multiple sclerosis. Thus, if the patient is subsequently diagnosed with multiple sclerosis (by fulfilling dissemination in space and time, and ruling out other diagnoses), the clinically isolated syndrome was that patient's first attack. A clinically isolated syndrome can be monofocal (reflecting pathology in a single location) or multifocal; the specific manifestations of a clinically isolated syndrome depend on the anatomical location (or locations) of the pathology. Typical presentations include unilateral optic neuritis, focal supratentorial syndrome, focal brainstem or cerebellar syndrome, or partial myelopathy; examples of atypical presentations include bilateral optic neuritis, complete ophthalmoplegia, complete myelopathy, encephalopathy, headache, alteration of consciousness, meningismus, or isolated fatigue. Relapse is defined as a monophasic clinical episode with patient-reported symptoms and objective findings typical of multiple sclerosis, reflecting a focal or multifocal inflammatory demyelinating event in the CNS, developing acutely or subacutely, with a duration of at least 24 h, with or without recovery, and in the absence of fever or infection. Attack, relapse, exacerbation, and (when it is the first episode) clinically isolated syndrome are synonyms.

Dissemination in space can be demonstrated by one or more T2-hyperintense lesions that are characteristic of multiple sclerosis in two or more of four areas of the CNS: periventricular, cortical or juxtacortical, and infratentorial brain regions, and the spinal cord
Dissemination in time can be demonstrated by the simultaneous presence of gadolinium-enhancing and non-enhancing lesions at any time or by a new T2-hyperintense or gadolinium-enhancing lesion on follow-up MRI, with reference to a baseline scan, irrespective of the timing of the baseline MR

MS: multiple sclerosis; CIS: Clinically isolated syndrome; CNS: central nervous system; MRI: magnetic resonance imaging; DIS: dissemination in space; DIT: dissemination in time; PPMS: primary progressive multiple sclerosis; CSF: cerebrospinal fluid; IgG: immunoglobulin G.

The first clinical manifestations of MS are diverse but 21% start with a clinically isolated syndrome of optic neuritis, 46% with motor or sensory deficits, 10% with a brainstem syndrome and 23% with multifocal abnormalities. (Tsang & Macdonell, 2011)

3.2.2 Other central demyelinating diseases

Optic neuritis (ON) is an acute, severe visual inflammation and demyelination of the optic nerve. Clinically, it produces disturbance without any clear diagnostic findings on ocular examination. It generally affects young, otherwise healthy individuals. (Wilhelm & Schabet, 2015)

Neuromyelitis Optica (NMO) involves episodes of optic neuritis (often severe and bilateral leading to fixed visual loss) and acute myelitis which are the major criteria for diagnosis. A contiguous spinal MRI lesion extending over three vertebral segments or NMO-IgG seropositive are used as secondary diagnostic criteria. Brain lesions may also be present in NMO.(Karussis, 2014)

Transverse myelitis (TM) is an inflammation and demyelination across both sides of one level, or segment, of the spinal cord resulting in symptoms of neurological disconnection and dysfunction below the level of the demyelinating area. It is mostly caused by infectious agents such as syphilis, measles, Lyme disease, varicella zoster, herpes simplex, cytomegalovirus, Epstein Barr, influenza, echovirus, human immunodeficiency virus (HIV), hepatitis A, rubella and mycoplasma, either directly or as a postinfectious autoimmune process. It may be also induced by various vaccinations or be idiopathic (i.e. without any apparent cause). The latter may occasionally represent one (or the initial) attack of MS or NMO. (Karussis, 2014)

Acute-disseminated encephalomyelitis (ADEM) is a clinical manifestation of presumed inflammatory or demyelinating cause, occurring mostly in children. It usually starts with an acute or subacute onset affecting multifocal areas of the CNS. It is usually polysymptomatic (presence of fever, confusion, headache) and includes encephalopathy. (Karussis, 2014)

3.3 Incidence and prevalence

3.3.1 Central and peripheral demyelinating diseases

As central or peripheral demyelinating diseases represent a group of disorders having their own epidemiologic parameters, almost no prevalence or incidence estimates for these disorders as a whole is available for the adult population, in the medical literature.

In children, two studies provided interesting estimates. One population-based active surveillance study, using questionnaires sent to pediatricians and ophthalmologists to collect acute demyelinating syndromes - ADS (including ADEM, CIS and NMO) in children aged 1-15 years between September 2009 and September 2010 in the UK, reported an overall annual incidence of 0.983 per 100,000 children per year (95% confidence interval [CI] 8.18-11.71). (Absoud et al., 2013) Despite a slightly different definition of ADS (which covered ON, ADEM, TM), a very similar rate (i.e., 0.9 per 100,000) was found in a Canadian study using the data obtained through the Canadian Pediatric Surveillance Program from April 1, 2004, to March 31, 2007. (Banwell et al., 2009)

3.3.2 Multiple sclerosis

Prevalence

All epidemiologic rates provided below (incidence or prevalence) were presented in the Atlas of MS and were collected during a large questionnaire survey from October 2012 to June 2013. An online questionnaire was drafted (in English only) and send to each country coordinator for completion. (Browne et al., 2014)

The worldwide prevalence of MS was estimated to be 33 per 100,000 in 2013, corresponding to 2.3 million people affected, according to a report by the MS International Federation. (Multiple Sclerosis International Federation (MSIF), 2013) According MSIF, the global prevalence increased over time from 30/100,000 in 2008 to 33/100,000 in 2013. No clear explanation for this trend which could be related to a longer survival of MS patients, a better diagnosis/reporting or other causes, has been drawn so far.

Wide variations were observed with highest rates in North America (140/100,000 population) and Europe (108/100,000), and lowest in East Asia (2.2/100,000 population) and Sub-Saharian Africa (2.1/100,000) (cf. Figure 14).

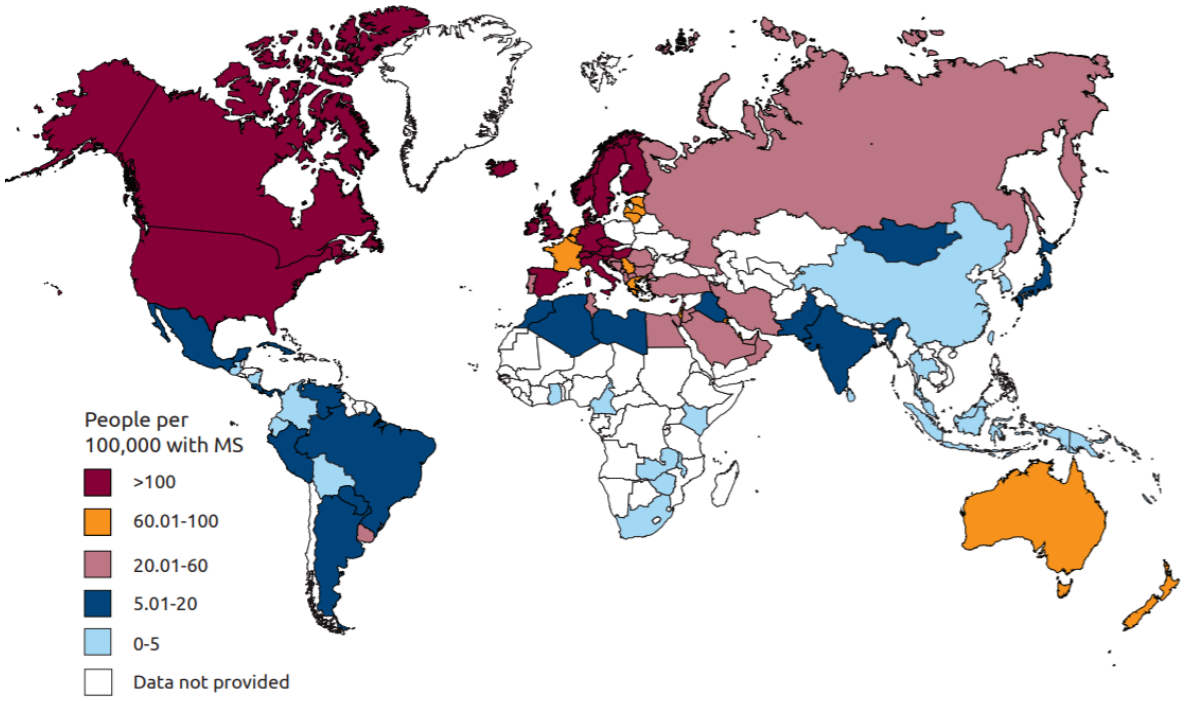


Figure 14 : Prevalence rates of MS across the World (Map extracted from (Browne et al., 2014; Multiple Sclerosis International Federation (MSIF), 2013)

Based on data collected in 2013, Europe also showed large variations depending on the countries considered, from 22 per 100,000 in Albania to 227 per 100,000 in Denmark. These figures confirmed the existence of a North-South prevalence gradient.

Prevalence of MS in France was estimated at 95 per 100,000 in 2013, corresponding to 60,000 people with this disease. (Multiple Sclerosis International Federation (MSIF), 2013). For the same year, a recent cross-sectional study using data from the French National Health Insurance database (SNIIRAM) linked with the National hospital discharge database (PMSI) reported a higher overall MS prevalence of 155.6 per 100,000 inhabitants (95% CI 154.7–156.6) after standardization on the 2013-European population. The difference in the prevalence rates between these two studies could be attributed to the different methods and data sources investigated. The prevalence rates provided by the Multiple Sclerosis International Federation relies on the completion of online surveys while the second reference (Foulon et al., 2017) has investigated nationwide insurance data sources. This latter seems to provide more robust data. Besides, authors of this cross-sectional study reported geographical variations within France. Indeed, the highest standardized prevalence rates were reported in North-Eastern regions of France (e.g., Lorraine, Picardie, or Alsace,

with close to 200 MS cases per 100,000 inhabitants) while prevalence rates were about 130 per 100,000 inhabitants than in South-Western regions (Languedoc-Roussillon, Corse, and Poitou–Charentes). (Foulon et al., 2017)

Incidence

The worldwide annual incidence rate of MS has been estimated at 2.5 (range: 1.1–4) per 100,000 in 2008. Regionally, the median estimated incidence of MS was greatest in Europe (3.8 per 100 000), followed by the Eastern Mediterranean (2), the Americas (1.5), the Western Pacific (0.9) and Africa (0.1). (World Health Organization, 2008)

In 2013, France, with an annual incidence rate of 7.6 per 100,000, ranked among European countries having a high incidence (cf. Figure 15). (Browne et al., 2014)

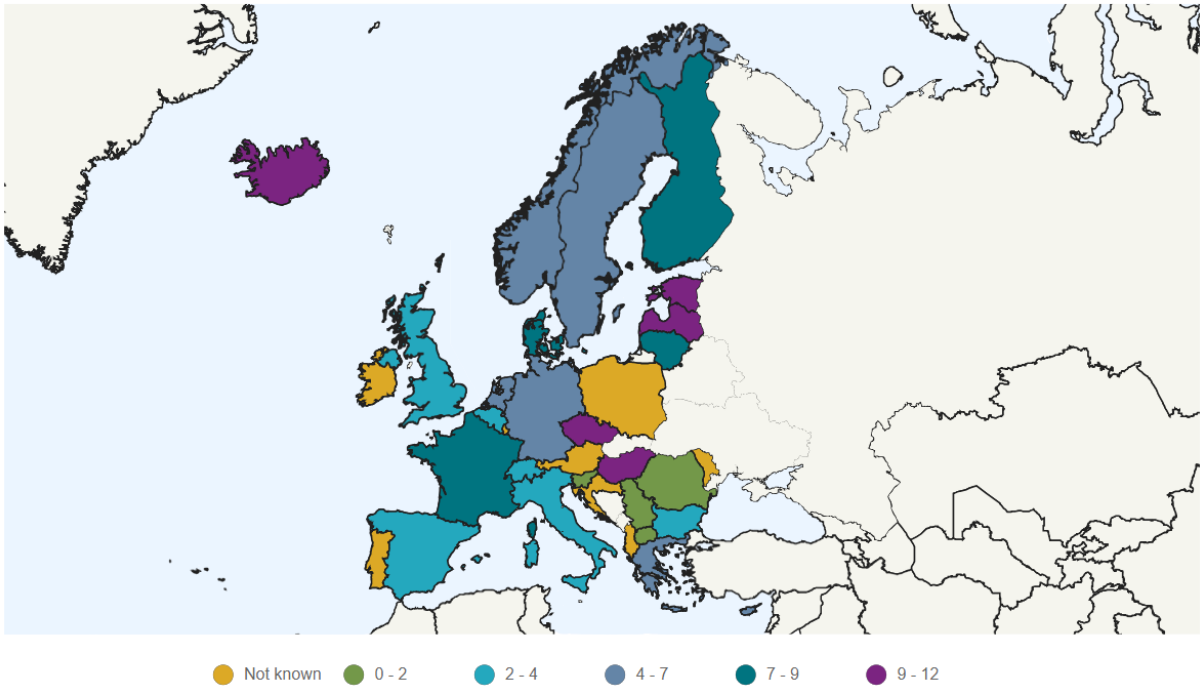


Figure 15 : Incidence rates (per 100,000) of MS across Europe in 2013 (Map extracted from (Multiple Sclerosis International Federation (MSIF), 2013)

Historical trends for France

Among other research activities, the present thesis reviewed the cases of MS that led to the initial signal identified in France during the immunization against hepatitis B in the 1990's. It

was therefore important to illustrate epidemiologic trends of multiple sclerosis within this country.

As historical trends for MS are not available in the Atlas of MS beyond 2008, literature searches identified several epidemiologic studies providing French incidence estimates for the oldest periods covered by the present research work.

A local epidemiologic study aimed at assessing the yearly incidence of MS in Burgundy (French region) between 1993 and 1997. All incident cases diagnosed according to the Poser criteria were reviewed and confirmed by a neurologist working either at the Dijon University Hospital (four neurologists) or in private practices (seven neurologists). With 21 MS cases confirmed over the study period for a catchment area of 94,000 inhabitants (aged under 60 years old), annual incidence rates were 6.1, 3.3 and 4.3 per 100,000 in women, men and both genders, respectively. (Moreau et al., 2000) Another study which used the prevalence of the disease, the average duration of the disease and the population growth, estimated the annual incidence rate of MS for subjects aged 20-44 years between 1994 and 1996 in France. Authors reported a very similar estimate of 4.29 per 100,000. (Fourrier et al., 2001)

The largest real-world study having provided incidence estimates for France was conducted by using the nationwide health insurance system (called Caisse Nationale d'Assurance Maladie des Travilleurs Salariés, CNAMTS) between November 2000 and October 2007. In France, MS is one of the 30 long-term illnesses (Affections de Longue Durée, ALD) for which patients are covered for 100% of their health care costs. Once the diagnosis of MS has been established by a neurologist according to current validated diagnostic criteria, a request was sent to the health insurance system and validated by a CNAMTS doctor. All the data relative to the request for ALD status were systematically collected by the CNAMTS, which covers salaried employees in the private sector, civil and noncivil servants and their families, accounting for 87% of the French population. (Fromont et al., 2012)

The CNAMTS provided the number of requests for ALD status claimed by MS patients over the 1995 – 2008 period from which were derived annual incidence rates overall and per gender (cf. Figure 16). (Fromont et al., 2012)

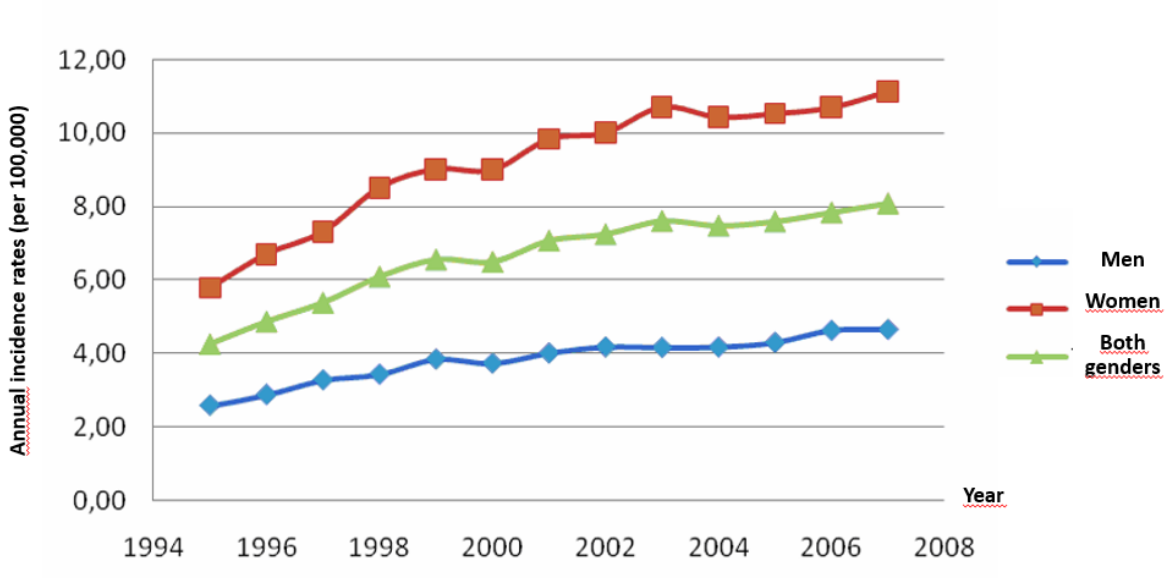


Figure 16: Standardized incidence rates of incident multiple sclerosis in France between 2001–2007; based on cases declared to the CNAMTS and adjusted for age and gender.

Fromont et al, chose to restrict their study period (from November 2000 to October 2007) in order to avoid any potential impact of the commercial launch of (expensive) novel drugs used to treat MS in France. This latter could have possibly prompted the declaration of MS cases by physicians in order to allow their patients to access to the ALD status which grants the full reimbursement of health care expenses. Over this study period, 28,682 new cases of MS were reported to the CNAMTS and obtained an ALD status for MS. A previous study using the capture-recapture approach across multiple French data sources, estimated that the annual incidence of MS cases in France was generally underestimated by 11.5 to 29%. (Sagnes-Raffy et al., 2010) Taking into account this underreporting, the annual incidence rates were then corrected and the following ranges (per 100,000) were found: 7.6 – 8.8 for both genders, 4.2 – 4.8 for men and 11.0 -12.7 for women (cf. Figure 17).

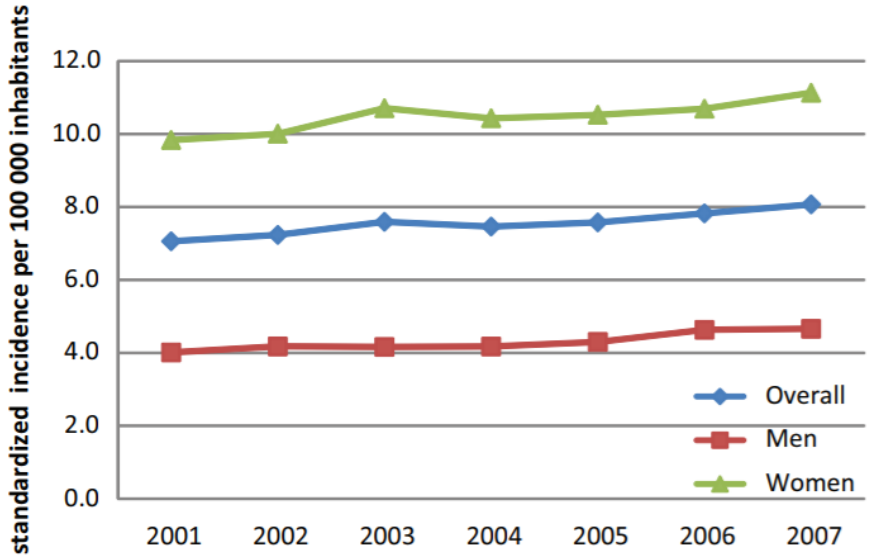


Figure 17: Standardized incidence rates of new multiple sclerosis in France between 2001–2007; based on cases reported to the CNAMTS and adjusted for age and gender.

In this study, authors reported a Northeast to South gradient with MS standardized yearly incidence ranging from 6.4 per 100,000 inhabitants in Provence Alpes Côte d'Azur and Rhône-Alpes (South East), to 10.6 in Alsace (North East). Besides, it was found that the change of diagnostic criteria did not lead to an increase of MS incidence in France. (Fromont et al., 2012)

Other local research initiatives produced more or less similar rates:

- Crude annual incidence in Britanny (2000 – 2004): 5.8 per 100,000 (Hammas, Yaouanq, Lannes, Edan, & Viel, 2017)
- Standardized annual incidence in Britanny (2000 – 2001): 4.41, 6.68 and 2.21 per 100,000, overall, for women and men, respectively. (Yaouanq et al., 2015)
- Annual incidence in Lorraine (North) in 2009, estimated from the capture-recapture approach using the Regional Health Insurance System, medical records and the Loraine registry of MS: 8.5 per 100,000. In this latter study, authors estimated a prevalence of 188.2 (95% CI: 182.7; 193.8) cases per 100,000 inhabitants (El Adssi, Debouverie, & Guillemin, 2012)

3.3.3 CIS

CIS is as an acute demyelinating disorder, which could be considered as a predictive event for MS. CIS is the first occurrence of symptomatic demyelination which could include ON, TM, other forms of monoregional CIS (e.g., isolated brainstem syndrome) or polyregional CIS.

In the USA, a historical cohort study of over 9 million person-years of observation from the multi-ethnic, community-dwelling members of Kaiser Permanente Southern California Health Plan from January 1, 2008 to December 31, 2010, provided reliable incidence estimates for CIS and compared these rates per ethnic group. Of the 468 newly diagnosed CIS cases, the annual incidence was estimated at 4.72 per 100,000. It was also showed that the incidence of CIS varied by race/ethnicity and gender in a similar pattern to MS. (Langer-Gould, Brara, Beaber, & Zhang, 2014)

No European or French estimates of CIS prevalence or incidence were identified by the literature searches.

3.3.4 Optic neuritis

In the US epidemiologic study using the Kaiser Permanente Southern California Health Plan between January 1, 2008 to December 31, 2010 and including more than 9 million person-years of observation, the annual incidence rates of ON among whites was 2.64 per 100,000. (Langer-Gould, Brara, et al., 2014)

Similar rates were produced in two regions of Finland despite the epidemiologic study being conducted between 1970 and 1978. Authors found yearly incidence estimates of 2.2 and 2.5 per 100,000, in Uusimaa and Vaasa areas, respectively. (Kinnunen, 1983)

A literature review focusing on the diagnosis and treatment of optic neuritis mentioned an incidence of 5 cases per 100 000 persons per year in central Europe (Wilhelm & Schabet, 2015), while incidence rates of 0.94 – 2.18 per 100,000 people per year were reported by Brochet et al, in their recent book on MS. (Brochet et al., 2017)

No reliable incidence data was found for France.

3.3.5 Neuromyelitis optica

Incidence and prevalence data for NMO remain scarce.

A recent review and meta-analysis identified nine studies providing incidence or prevalence estimates worldwide. Authors reported a pooled prevalence of 1.82 [85%CI: 1.26 2.36], while individual estimates ranged from 0.51 per 100,000 in Cuba to 4.4 in Southern Denmark. Incidence data were found in four studies and ranged from 0.053 per 100,000 per year in Cuba to 0.4 in Southern Denmark. (Etemadifar, Nasr, Khalili, Taherioun, & Vosoughi, 2015)

As reported in The Atlas of MS, France was found to have an overall NMO annual incidence of 0.2 per 100,000. (Browne et al., 2014)

3.3.6 Transverse myelitis

In the US epidemiologic study using the Kaiser Permanente Southern California Health Plan between January 1, 2008 to December 31, 2010 and including more than 9 million person-years of observation, the annual incidence rate of TM among adult whites was 2.26 per 100,000. (Langer-Gould, Brara, et al., 2014)

Another study including all adult patients with acute transverse myelitis as a first neurological presentation diagnosed from January 2001 to December 2005 at a single institution providing all neurological care for North Canterbury, New Zealand produced a similar annual incidence estimate (i.e., 2.46 per 100,000, respectively). (Young, Quinn, Hurrell, & Taylor, 2009).

No reliable incidence data was found for France.

3.3.7 ADEM

ADEM is an uncommon disorder, predominantly occurring in children.

An US epidemiologic study reviewing the medical records of all persons aged < 20 years diagnosed with ADEM from the three main pediatric hospitals in San Diego County, CA, during 1991-2000, found an overall incidence of ADEM of 0.4 per 100,000 per year. (Leake et al., 2004)

A Canadian study using the data obtained through the Canadian Pediatric Surveillance Program from April 1, 2004, to March 31, 2007 reported an annual incidence rate of 0.2 per 100,000 children. (Banwell et al., 2009)

3.4 Risk factors for MS

Etiology of MS remains unclear while several risk factors, both environmental and genetic, have been assessed.

An in-depth review and meta-analysis of potential environmental risk factors, which considered 44 meta-analyses including 416 primary studies, reviewed the level of evidence for each putative risk factor for MS. While covering a wide range of etiological factors (i.e., vaccinations, comorbid diseases, surgery, traumatic events and accidents, exposure to environmental agents, and biochemical, infectious, and musculoskeletal biomarkers) authors classified the associations according to the level of supportive evidence: convincing evidence, suggestive evidence, weak evidence, absence of association. (Belbasis, Bellou, Evangelou, Ioannidis, & Tzoulaki, 2015)

Three associations were supported by convincing evidence:

- **Epstein Barr virus (EBV) serology**: OR= 4.46 (95%CI: 3.26–6.09), evidence supported by 3,511 cases
- **Infectious mononucleosis** (caused by EBV): OR= 2.17 (1.97–2.39) with 19,519
- **Smoking**: OR= 1.52 (1.39–1.66) with 3,052 cases

As epidemiologic data for MS showed different patterns according to the latitude, the low levels of vitamin D and low exposure to the sun are also considered as potential risk factors. Evidence remains unclear about the strength of these associations with MS. (Leray, Moreau, Fromont, & Edan, 2016)

Familial clustering of MS cases, with multiple siblings affected by the disease within a particular family across several generations, led to the identification of genetic factors, such as the HLA-DRB1*15:01 and single nucleotide polymorphism (SNP) with IL7R and IL2RA genes. Some of these genetic factors are also associated with several autoimmune diseases. (Leray et al., 2016)

3.5 Demographic profile of MS patients

First episodes of MS usually occur between 15 and 60 years, with a mean age of 30 years at disease onset. About 2 to 5% of MS start during in subjects aged less than 18 years. (Browne et al., 2014)

A gender ratio is observed among MS patients with women being about twice (up to thrice) as likely as men to develop MS. (Browne et al., 2014)

White people, particularly those of Northern European descent, are at higher risk of developing MS. Asian and Hispanic people have the lowest risk of developing MS. Contradictory findings were found in the medical literature about the risk for black people. (Langer-Gould, Brara, Beaber, & Zhang, 2013)

4 Vaccination against hepatitis B and risk of central demyelination

4.1 Hepatitis B infection

Hepatitis is an inflammation of the liver, possibly leading to short-term (fulminant hepatitis) and long-term damages (e.g. fibrosis, cirrhosis, hepatocellular carcinoma) and/or affecting the liver function. Among possible causes, excessive (chronic or acute) alcohol consumption, some toxic substances and medications (e.g. paracetamol), and certain medical conditions such as in the nonalcoholic steatohepatitis (NASH), are well-known risk factors for the disease. However, viruses are, by far, the most common cause, notably hepatitis A virus, hepatitis B virus, and hepatitis C virus. (Centers for Disease Control and prevention, 2018a)

Epidemiology of hepatitis B virus (HBV) infection is usually based on the prevalence of hepatitis B surface antigen (HBsAg, formerly Australia Antigen, a serological marker of active HBV infection) in a population. This prevalence of carriers in a given area can be broadly classified into high- (>8% HBsAg prevalence), intermediate- (2%–7%) and low-prevalence (<2%). (Previsani & Lavanchy, 2002)

4.1.1 Prevalence and incidence

4.1.1.1 Worldwide

A reliable meta-analysis conducted by the WHO in 2015, compiled all prevalence data published between 1965 and 2013 for each country of the World. (Schweitzer, Horn, Mikolajczyk, Krause, & Ott, 2015) All studies reporting the HBsAg prevalence in the general population (i.e., not targeted on high-risk groups but including blood donors, pregnant women, and health-care workers) were reviewed and relevant epidemiologic data were extracted to compute the pooled HBsAg prevalence estimates and 95% confidence intervals (CI) weighted by study size.

Of the 17,029 records screened, a total of 1,800 references covering 161 countries, provided data on the prevalence of HBsAg and were considered for inclusion in the meta-analysis.

HBsAg seroprevalence was estimated to be 3.61% (95% CI 3.61 – 3.61) worldwide, i.e. *intermediate* in 2013, the highest endemicities being found in African (pooled estimate: 8.83%, 8.82–8.83) and Western Pacific countries (pooled estimate: 5.26%, 5.26–5.26). It was estimated that, globally, about 248 million individuals were HBsAg carriers in 2010.(Schweitzer et al., 2015)

Given that HBV vaccines were launched in the early 1990's, the meta-analysis was then stratified according to two time periods: 1957 – 1989 *versus* 1990 – 2013; a decrease in prevalence in South East Asian, Western Pacific, and the Eastern Mediterranean regions were observed between the two periods.

4.1.1.2 France

The meta-analysis presented above also produced epidemiologic data for France. (Schweitzer et al., 2015) A total of 20 studies (n= 493,856) provided data for the first period, while 14 (n= 918,198) concerned the second time interval. The corresponding pooled prevalence estimates were 0.29% (95%CI: 0.28 – 0.31) and 0.25% (95%CI: 0.24 – 0.26), respectively. (Schweitzer et al., 2015)

In 2004, the prevalence of HBsAg in France, estimated by the nationwide survey, was 0.65% (i.e. *low* according to the WHO definition), which corresponded to 280,000 HBsAg+ carriers in the country; only one half of them being aware of their positive status for HBV.(Nicand, 2016)

In 2016, the reporting rate for incident cases of acute hepatitis B in France was 0.14 per 100,000. (European Centre for Disease Prevention and Control, 2018) Nevertheless, this figure should be interpreted with caution owing to the passive nature of this disease surveillance, underreporting possibly reaching 85% in France, i.e. only 15% of cases being reported. (Brouard et al., 2013)

Historical data on incidence of hepatitis B or HBV infection remain scarce for France. Indeed, owing to the low level of prevalence, the mandatory reporting of incident cases was suspended between 1985 and 2003. Only two disease-surveillance systems remained active during this period: the laboratories network of Lyon area ("réseau des laboratoires de la communauté urbaine de Lyon (COURLY)") and the sentinel network of general practitioners ("réseau sentinelles des médecins généralistes (Inserm U707)"). (Antona, 2008) The latter

yielded incidence data for the general population between 1991 and 1996 (cf. Figure 18). In 1996, the annual incidence of HBV cases was estimated to be 6 [95%CI: 2 – 12] per 100,000 while it was at 20 [95%CI unknown] per 100,000 in 1991, showing a more than three-fold decrease over the period. In 1996, the median age at disease onset was 31 years. (Flahault et al., 1997)

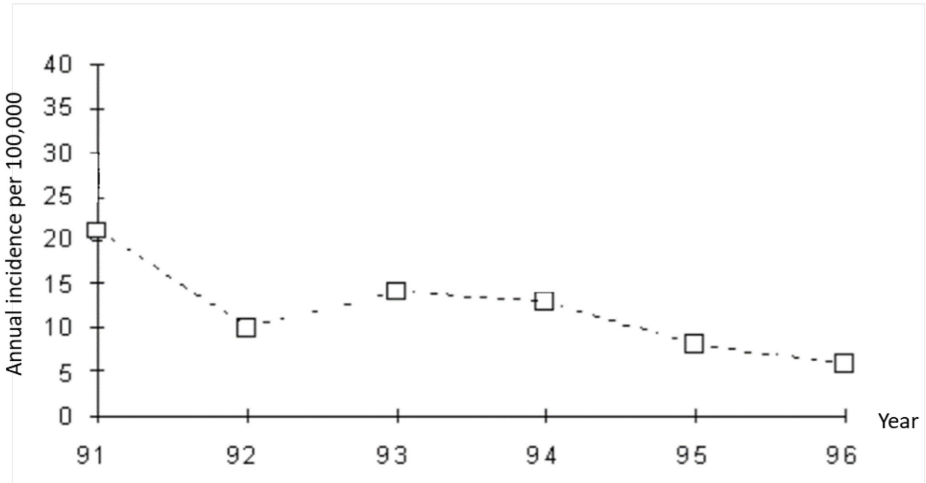


Figure 18 : Annual incidence of HBV cases per 100,000 in the French general population from 1991 to 1996 (extracted from (Flahault et al., 1997))

4.1.2 Burden of the disease

The viral hepatitis pandemic represents a significant burden on lives, communities and health care systems. In 2013, viral hepatitis was the seventh cause of mortality worldwide, accounting for (estimated) 1.4 million deaths per year from acute infection and hepatitis-related liver cancer and cirrhosis. This mortality was comparable to the one due to HIV infection or tuberculosis (cf. Figure 19). Of those deaths, approximately 47% were attributable to hepatitis B virus, 48 % to hepatitis C virus and the remainder to hepatitis A virus and hepatitis E virus. (World Health Organization, 2016)

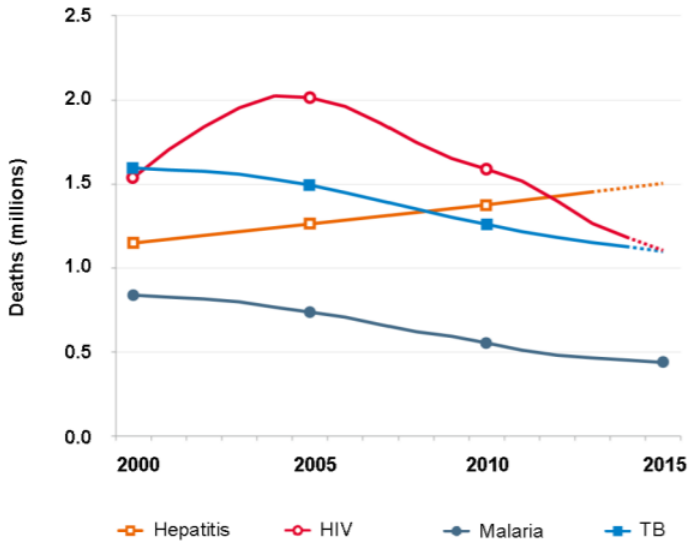


Figure 19 : Estimated global number of deaths attributable to viral hepatitis, HIV, malaria and tuberculosis (TB), from 2000 to 2015 (extracted from (World Health Organization, 2016))

Viral hepatitis is also a growing cause of mortality among HIV carriers; about 2.9 million of them being co-infected by hepatitis C virus and 2.6 million by hepatitis B virus. (World Health Organization, 2016)

4.1.3 Mode of transmission

Blood and bodily fluids (i.e., semen and vaginal secretions) are the main vectors for HBV transmission. HBV can survive outside of the human body for at least 7 days. (Centers for Disease Control and prevention, 2018a)

Although HBV has been detected in saliva, tears, breast milk, sweat, and urine, HBV is not spread through food or water, sharing eating utensils, breastfeeding, hugging, kissing, hand holding, coughing, or sneezing. (Centers for Disease Control and prevention, 2018a; Zheng et al., 2011)

People can become infected in the following situations:

- Birth (spread from an infected mother to her baby during birth)
- Sexual relations with an infected partner
- Sharing needles, syringes, or drug preparation equipment
- Sharing items such as toothbrushes, razors or medical equipment such as a glucose monitor with an infected person
- Direct contact with the blood or open sores of an infected person

- Exposure to blood from needlesticks or other sharp instruments of an infected person.

Most infections worldwide are acquired vertically through perinatal transmission at birth between an infected mother and her child.(Gentile & Borgia, 2014) Other modes of transmission include horizontal transmission to/between young children or institutionalized people, sexual contact, and through injecting drug use. (MacLachlan & Cowie, 2015; Van Damme, Cramm, Van der Auwera, Vranckx, & Meheus, 1995) Additionally, migration from higher prevalence to lower-prevalence countries is an important factor of the burden of HBV infection. (MacLachlan & Cowie, 2015)

In a low-prevalence country, the incidence of vertical and horizontal transmission in childhood is low, most incident infections occurring in adolescence and adulthood through sexual contacts, injecting drug use, and other blood-related exposures. In France, mandatory declarations of incident HBV cases between 2010 and 2014 revealed that the three main modes of transmission were sexual intercourses (38.5%), travel in high endemicity countries (21.5%), and invasive procedures (dialysis, surgery, transplant, etc.) (5.4%). (Nicand, 2016)

4.1.4 Disease progression

Disease progression following the infection with HBV is extremely variable depending on the age at disease onset, the immune status of the patient and the stage at which the disease is diagnosed.

Symptoms of the incubation phase (i.e., 6 to 24 weeks) include nausea, fatigue, diarrhea, vomiting, anorexia, jaundice and/or headache. There are also asymptomatic cases which constitute a reservoir for the disease with subjects becoming silent carriers.

Most adults will recover from the disease and will clear the infection without sequelae but a few (<5-10%) will become chronic carriers, either asymptomatic or progressing to chronic hepatitis possibly resulting to cirrhosis and/or hepatocellular carcinoma. In rare instances, the infection can progress rapidly to a fulminant hepatitis which is a very serious condition with coma and death as possible outcomes. A small proportion of chronic carriers apparently terminate their active infection and become HBsAg-negative (about 2% per year).

HBV infection is transient in approximately 90% of adults and 10% in newborns. (World Health Organization, 2002). The likelihood of progression to chronic infection is inversely

related to age at the time of infection. Around 90% of infants infected perinatally become chronic carriers, unless vaccinated at birth. The risk for chronic HBV infection decreases to 30% of children infected between ages 1 and 4 years and to less than 5% of persons infected as adults (cf. Figure 20). (Ott, Stevens, Groeger, & Wiersma, 2012)

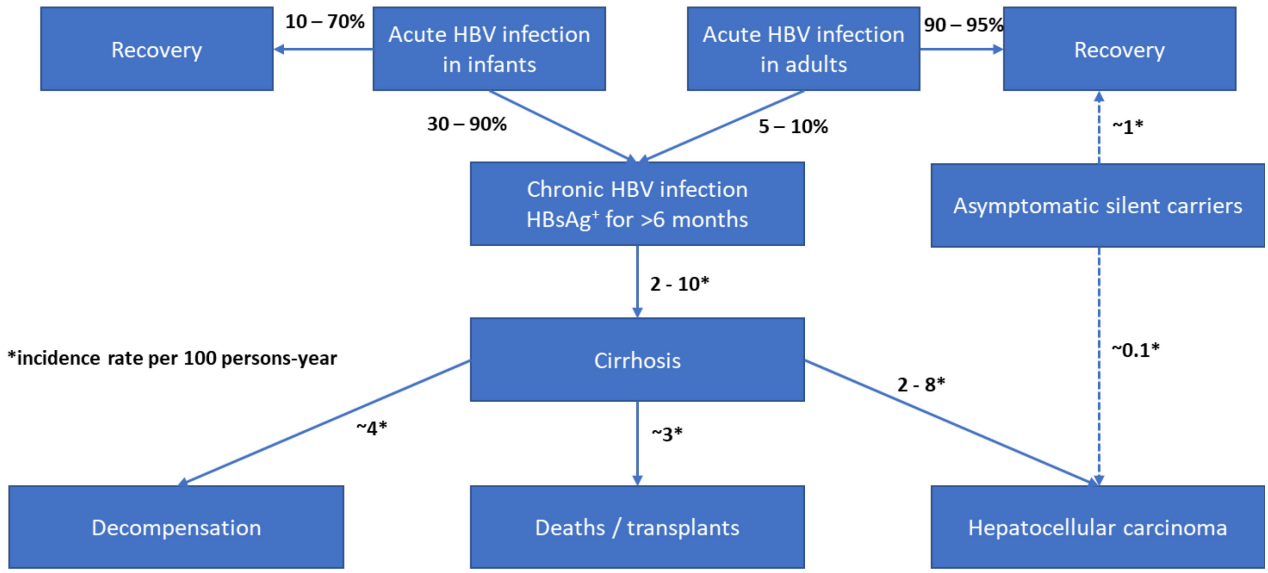


Figure 20 : Progression of Hepatitis B virus infections (reproduced from (Antona, 2008))

Worldwide, it was estimated that 600,000 deaths per year would be related to HBV while 73% of fatal liver cancers would be attributable to hepatitis viruses (i.e., A, B, C, D, E), low and middle income countries having the highest proportions. (Ott, Ullrich, Mascarenhas, & Stevens, 2010)

4.2 Vaccination against Hepatitis B infection in France

4.2.1 History of the French HBV immunization programmes

The first hepatitis B vaccine was obtained and formulated by Philippe Maupas, a French virologist, in 1976. The first commercial launch occurred soon after with Hevac® manufactured by Sanofi Pasteur in 1981. The same year, France became the first country to set up a vaccination campaign against HBV, which initially targeted healthcare professionals. (Aron, 2002). For this population, vaccination became mandatory in January 1991. French vaccination programmes were expanded in 1993 to additional populations including

students and teachers at risk of exposition to hepatitis B, as well as travelers visiting high-endemicity regions. Concomitantly, the WHO recommended to extend the vaccination programmes to all children, including those living in the non-endemic countries. Although the WHO recognized that these children, except those born from an HBV-infected mother, have few chances to be exposed to HBV, they founded their rationale on the fact that vaccinating only at-risk subjects would not eradicate the virus. (DIRECTION GENERALE DE LA SANTE, 2004) Following these recommendations, a massive immunization campaign which targeted newborns, was launched in France on 29 September 1994. Two months later, vaccination was freely offered at schools for adolescents aged 10-11 years. On 10 January 1995, anti-hepatitis B vaccination was part of the recommended immunization schedule for adolescents and newborns. (Denis, Goudeau, & Aufrere, 1998)However, it should be acknowledged that none of the neighbouring countries of France, including those having a higher epidemiologic burden of hepatitis B infections (such as Italy), applied so intense immunization practices.

Because of the identification in 1996 of a pharmacovigilance signal concerning cases of demyelination after immunization, school-based programmes were stopped in October 1998. A nationwide investigation investigating the potential link between the anti-hepatitis B vaccination and the onset of autoimmune diseases, including central demyelination, was launched in 1998. The same year, booster doses were suppressed, leaving a three-injection schedule at 0, 1 and 6 months. In March 2002, national recommendations focused on the immunization of infants, a possible catch-up of adolescents and vaccination for at-risk unvaccinated adults. The final report of the nationwide investigation was made public in 2006. During the 1996-2000 period, the media coverage and the pressure from public opinion was extreme, leading to a major crisis making regulatory decisions and public health communication particularly difficult. In the follow of this crisis, two *ad-hoc* groups of experts successively recommended re-launching a campaign targeting newborns since they cannot express a central demyelination. These advices were not followed by health authorities, none decision being made during almost 20 years. However, in January 2018, the vaccination against HBV, as well as ten other infectious agents, became mandatory for infants in France (cf. Figure 21).

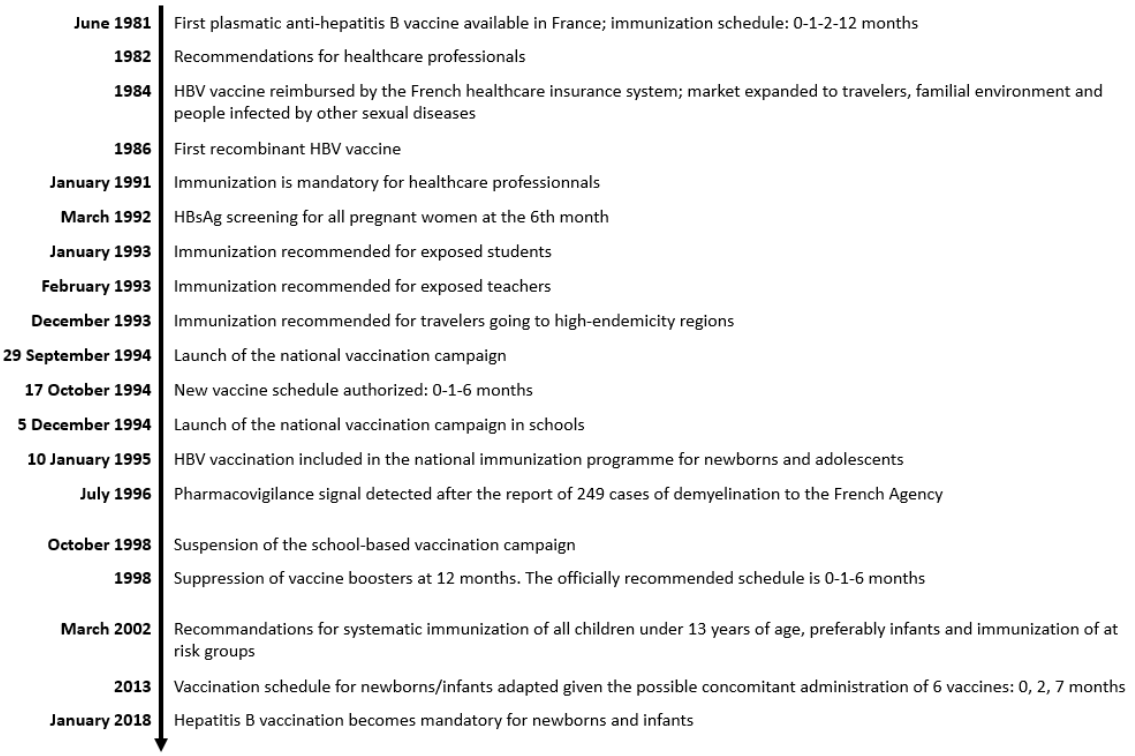


Figure 21 : Important dates for HBV immunization in France

4.2.2 Marketed vaccines

The first available anti-hepatitis B vaccines were plasma-derived, produced by harvesting HBsAg from the plasma of patients carrying chronic HBV infection. The particles were highly purified, and any residual infectious particles were inactivated by various combinations of urea, pepsin, formaldehyde and heat. Although concerns about transmission of bloodborne pathogens, including HIV, from plasma-derived vaccines have proven to be unfounded, public concerns over the safety of the plasma-derived vaccine hampered its acceptance in many populations. Therefore, increased research efforts were made to develop a recombinant vaccine.

In 1986, a HBV vaccine produced by recombinant technology was licensed, a second followed in 1989. Recombinant technology for HBV vaccines involves the insertion of segments of the HBV genome which encodes for HBsAg into a plasmid in yeast or mammalian (Chinese hamster ovary, CHO) cells, thus allowing for the expression HBsAg. This technology offered the potential for producing almost unlimited supplies of vaccine. (World Health Organization, 2013)

A total of eight vaccines were launched on the French market: Hevac B®, Engerix®, Genhevac®, HB VAX DNA®, HBVAXPRO®, Twinrix®, Infanrix Hexa®, HEXAVAC®. They are presented in Table 8. The total of doses sold from 1981 and 2005 are presented in Table 9 and Figure 22. This latter illustrates the peak of sales for years 1993 - 1997, whose top was about 10 times higher than the average level of sales between 1999 and 2005.

Table 8 : Hepatitis B vaccine brand names in France

Year	Vaccine valence	Type of HBV vaccine	Target population	Year of commercialization in France
Hevac B®	Monovalent: HBV	Plasmatic	Adults	1981 - 1993
Engerix®	Monovalent: HBV	Recombinant	All	1989 - onwards
Genhevac®	Monovalent: HBV	Recombinant	All	1989 - 2016
HB VAX DNA®	Monovalent: HBV	Recombinant	Adults	1995 - 2005
HBVAXPRO®	Monovalent: HBV	Recombinant	Adults	2002 - onwards
Twinrix®	Combined: HBV and HAV	Recombinant	All	1998 - onwards
Infanrix Hexa®	Combined: D, T, P, acPertussis, HBV, Hib	Recombinant	Children	2002 - onwards
HEXAVAC®	Combined: D, T, P, acPertussis, HBV, Hib	Recombinant	Children	2003 - onwards

Acronyms: *HBV: hepatitis B virus, D: diphtheria, T: tetanus, P: poliomyelitis, acPertussis : acellular Pertussis, Haemophilus Influenzae b*

Table 9 : Whole sales of anti-hepatitis B vaccines in France per annum (extracted from (Welsch, Decker, & Imbs, 2006))

Year	1981 - 1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005
Hevac B®	1 585 094	601 537	413 703	191 812	230 030	432 622	17 316												
Engerix®			15 699	108 446	659 006	1 571 038	1 725 935	7 963 845	13 447 057	6 540 778	2 860 816	1 677 196	954 912	1 257 353	978 287	1 134 514	1 532 192	1 192 973	1 277 681
Genhevac®			288 548	504 048	1 397 982	1 731 002	3 275 167	6 953 262	9 505 497	7 437 132	4 027 570	1 681 811	642 742	736 655	571 543	601 608	455 698	408 714	374 032
HB VAX DNA®									372 584	1 156 935	1 592 052	1 124 985	920 962	1 019 233	883 220				
HBVAXPRO®																502 656	433 052	432 723	454 556
Twinrix®												91 253	112 883	96 287	94 000	98 433	53 648	61 653	73 516
Infanrix Hexa®																2 314	10 369	14 172	30 244
HEXAVAC®																	51 935	60 525	31 698
Total sales (doses)	1 585 094	601 537	717 950	804 306	2 287 018	3 734 662	5 018 418	14 917 107	23 325 138	15 134 845	8 480 438	4 575 245	2 631 499	3 109 528	2 527 050	2 339 525	2 536 894	2 170 760	2 241 727

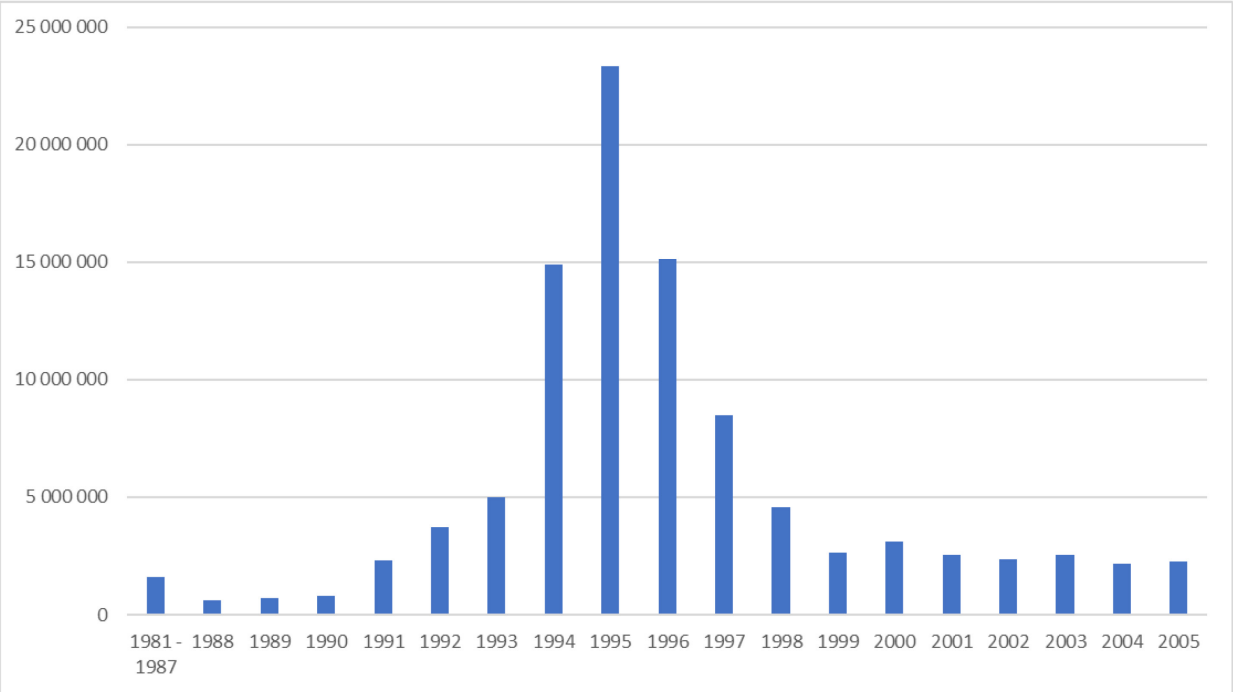


Figure 22: Evolution of the sales of anti-hepatitis B vaccine between 1981 and 2005

4.2.3 Immunization schedules

Unlike the majority of other countries, France chose an initial vaccination schedule including a primovaccination with three doses and one booster dose:

- P1,
- P2 at 1 month after P1,
- P3 at 2 months after P1,
- One booster dose at 12 months after P1

Moreover, booster doses every 5 years were recommended for healthcare professionals.

On 17 October 1994, a new immunization schedule was proposed with only three doses at 0-1-6 months. In 1998, the 0-1-6 schedule was definitively adopted and the booster doses were officially suppressed, except for certain at-risk categories such as healthcare professionals who had received immunization after the age of 25 years and patients with severe renal impairment treated with regular dialysis. (Antona, 2008)

Since 2016, the HBV immunization schedule relies on the systematic immunization of newborns at 2, 4 and 11 months of age with the concomitant injection of five other valences (diphtheria, tetanus, poliomyelitis, acellular Pertussis and haemophilus Influenzae b). This recommendation became mandatory on January 2018. For unvaccinated adults, the

immunization schedule depends on the age of the subject and the risk of contamination, as presented in Table 10. (Ministère des Solidarités et de la Santé, 2018a)

The current recommendations do not support the use of any booster doses, except for emergency cases (only one booster at 12 months after the initial injection), and patients with severe renal impairment or immunocompromised subjects who need constant re-immunization (i.e., annual dosages of anti-HBV antibodies, boosters being administered when their level is below 10 UI/L). (Ministère des Solidarités et de la Santé, 2018a)

Table 10: Current French recommendations for immunization against HBV

SPECIAL POPULATION: Newborns of HBV infected mothers	**MAIN TARGET:** Newborns	**CATCH-UP:** Adolescents between 11 and 15 years	**SPECIAL POPULATION:** Adolescents and adults aged 16 years and over	**SPECIAL POPULATION:** Healthcare professionals
Recommended 3-dose schedule with 0-1-6-month interval ***For premature infants*** (<32 weeks or <2 kg at birth): 4-dose schedule with 0-1-2-6-month interval	**Mandatory** 3-dose schedule with concomitant valences at 2, 4 and 11 months of age	**Recommended** **2 options:** 3-dose schedule with 0-1-6-month interval **OR** 2-dose schedule with 0-6-month interval (ENGERIX® B 20 µg)	**Recommended** 3-dose schedule with 0-1-6-month interval Unvaccinated people at risk for hepatitis B virus: - People coming from endemic countries (e.g., Asia, Africa) - Travellers in endemic countries - Subjects living with HBV infected people - People having multiple sexual partners - Drug users - Prison inmates - HIV or HCV patients - People with chronic liver diseases - People with transplant or requiring blood transfusion ***In emergency cases*** (i.e., urgent need to protect against HBV): accelerated schedule with 3 doses administered within 21 days (D0, D7, D21), followed by a booster dose 12 months after the third dose	**Mandatory** 3-dose schedule with 0-1-6-month interval **Control of anti-HBs antibodies levels:** - If <10 IU/L, up to a maximal of 6 doses is allowed - If ≥10 IU/L, protective immunization

4.2.4 Immunization coverage against HBV in France

4.2.4.1 General population

Vaccination coverage against HBV varied substantially over time and per population targeted. Besides, the methodology used to determine the immunization coverage should also be carefully considered when interpreting these proportions. Both the study design (e.g., survey with vaccination status reported by the subject or medical review of vaccination records) and the definition of vaccinated subjects (e.g., subjects initiating the vaccination schedule (i.e., first dose received) or subjects having completed the full vaccination schedule (i.e., 3 doses administered)) could reveal discrepancies between sources.

In the general population (all ages and genders included), the vaccination coverage fluctuated from 3.1% in 1993 to 21.7% in 2002 (cf. Table 11). (Antona, 2008; Denis et al., 1998) No recent figure was available from the literature.

Table 11 : Vaccination coverage for the general population in France

Year	Panel of 20,000 families, vaccination status self-reported by participants (Denis et al., 1998)	Panel of 20,000 families, vaccination status based on a 3-dose schedule (Antona, 2008)
1993	na	**3.1%**
1994	**13%**	na
1995	**24%**	**10.2%**
1996	**29.1%**	na
1999	na	**20.0%**
2002	na	**21.7%**

na: not available

4.2.4.2 Infants aged less than 24 months

In the main target population (i.e., infants aged less than 24 months), the immunization coverage defined by the percentage of children aged less than 24 months having completed

a full 3-dose schedule fluctuated between 17% in 1996 and 90% in 2017, as reported in Figure 23. (Denis et al., 1998; Institut National de Veille Sanitaire (INVS), 2018)

While infants represented the initial target of the French vaccination campaign launched in 1994, the vaccination coverage stagnated below 30% until 2003. In 2003, the use of 6-valence combined vaccines including HBV was recommended by the French health authorities. This measure markedly improved the HBV immunization coverage in this target population; interestingly the reimbursement of those combined vaccines in March 2008 provoked the sharp increase observed after 2008.

The level of 85% of effective immunization in the infant population, the initial target of the 1994 campaign, was achieved twenty years later, soon after 2014. Surprisingly, the same year, a shortage of the pediatric 5-valence combined vaccines (i.e., those without the HBV valence) occurred while the 6-valence combined vaccines (i.e., vaccines including HBV: Infanrix Hexa®, HEXAVAC®) remained available on the French market. (Denis, 2016)

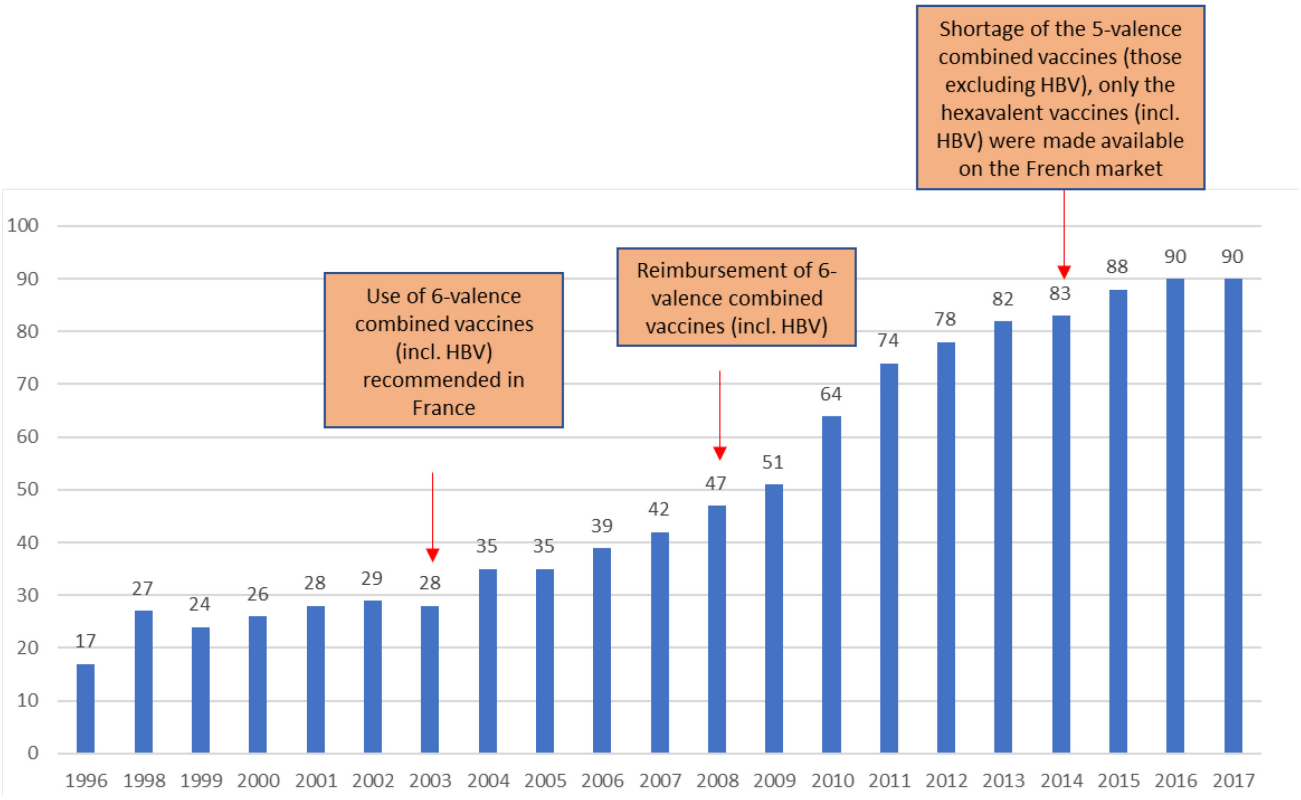


Figure 23 : Vaccination coverage for children aged less than 24 months having completed a full 3-dose schedule (Denis et al., 1998; Institut National de Veille Sanitaire (INVS), 2018)

Even if the immunization rate dramatically progressed over the last decades in France, the threshold which would produce a herd effect in the population, has not been reached yet, although no less than 98 million of vaccine doses were sold between 1981 and 2005. The effectiveness of a complete anti-hepatitis B vaccination series (i.e. 3 doses of hepatitis B vaccine) in preventing perinatal HBV infection (post-exposure immunization) and early childhood and late infection (pre-exposure immunization) was estimated to be 95%. ("Hepatitis B virus: a comprehensive strategy for eliminating transmission in the United States through universal childhood vaccination. Recommendations of the Immunization Practices Advisory Committee (ACIP)," 1991)

4.2.4.1 Adolescents (aged ≤ 15 years)

On 30 June 1996, almost two years after the launch of the national vaccination campaign in schools, immunization of adolescents aged between 11 and 15 years was, at the opposite of newborns, very successful with a coverage ranging from 39% in those aged 11 years up to 87% in those aged 13 years. (Denis et al., 1998).

However, this coverage dramatically decreased after October 1998, date of the suspension of the school-based campaign (cf. Figure 24). Undoubtedly, this political decision has deeply impacted the perception of the general public and has played a major role in this spectacular drop. (Denis, 2016) While the objective of the campaign was to achieve a percentage of 75% in 2015, about one third of this population has been vaccinated at this date, revealing the complete failure of the catch-up strategy.

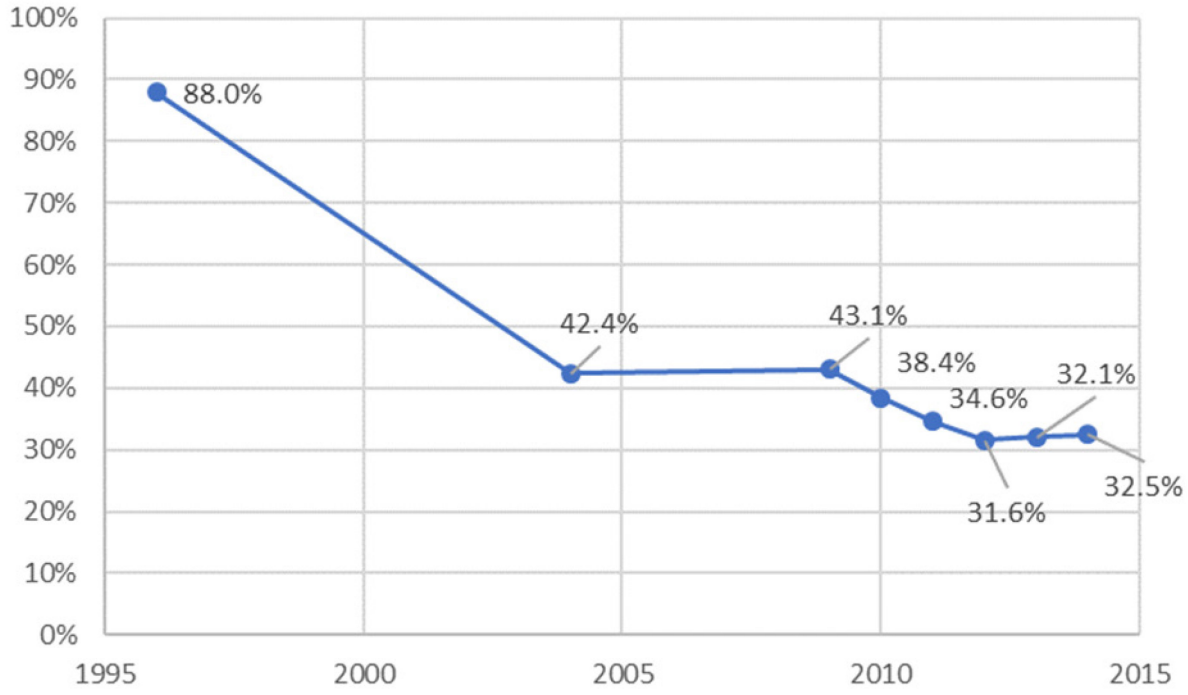


Figure 24: Evolution of the immunization coverage in adolescents aged 14-15 years (Denis, 2016)

4.2.4.2 Young adults (aged 16 – 44 years)

On 30 June 1996, the nationwide survey investigating 20,000 families reported high immunization coverages among young adults, although they were not targeted by the national vaccination campaign implemented two years before (cf. Figure 25). (Denis et al., 1998)

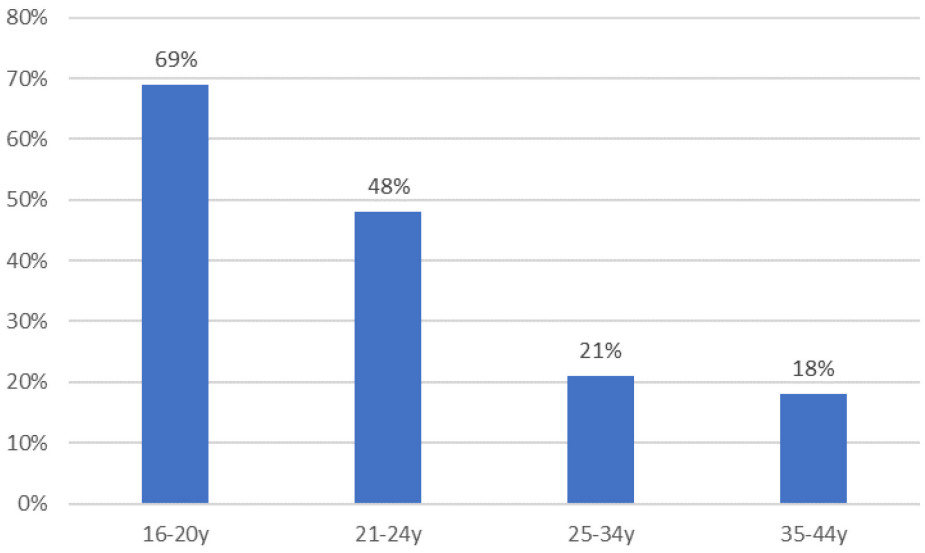


Figure 25: Immunization rates in young adults aged 16-44 years in 1996 (Denis et al., 1998)

By the end of 1998, it was estimated that about 21 million adults were exposed to at least one dose of HBV vaccine while no recommendation claimed to target this specific population (the adult population at risk for HBV infections was estimated at 1 to 1.2 million during the same time period). This corresponded to 59.5% of people aged 15-59 years, belonging to the age class prone to develop central demyelination.

No clear explanation can be drawn to explain such a disjunction between the target and joint populations. In contrast, there is no question about the fact that no other country has ever experienced a similar situation so far, making France a quite particular country regarding the population exposed to the HBV vaccine in the early 1990's. This complete disagreement with the immunization recommendations is key to interpret the pharmacovigilance signal that occurred in France around 1996.

For the most recent years, data on immunization rates among adults remain scarce for France:

- In 2010, a local survey in Ile de France (Paris and suburbs) having included 798 participants aged between 15 and 79 years, found that 53.1% self-declared to be immunized against HBV. (Sauvage, Féron, & Vincelet, 2010)
- In 2015, approximately 60% of at-risk adults (e.g., drug users, homosexuals with multiple partners), as well as 92% of healthcare professionals and 88% of general practitioners were found to be vaccinated against HBV in France. (Launay & Floret, 2015)

Nevertheless, it should be acknowledged that a significant but unquantifiable proportion of these participants may have been vaccinated during their childhood. Consequently, except for the years following the implementation of the national vaccination campaigns in early 1990's, it is almost impossible to determine the number of people being exposed to the vaccine during their adult age.

4.2.4.3 Summary

Key achievements for immunization coverage against HBV across the different French subpopulations are summarized in Figure 26. It appears that the catch-up strategy for adolescents remains problematic while the immunization of newborns can now be considered as effective. As a consequence, an important part of the French population

remains at risk for HBV infection at an adult age despite the administration of a tremendous number of doses (at least 98 million between 1981 and 2005), mostly off-target and off-recommendations.

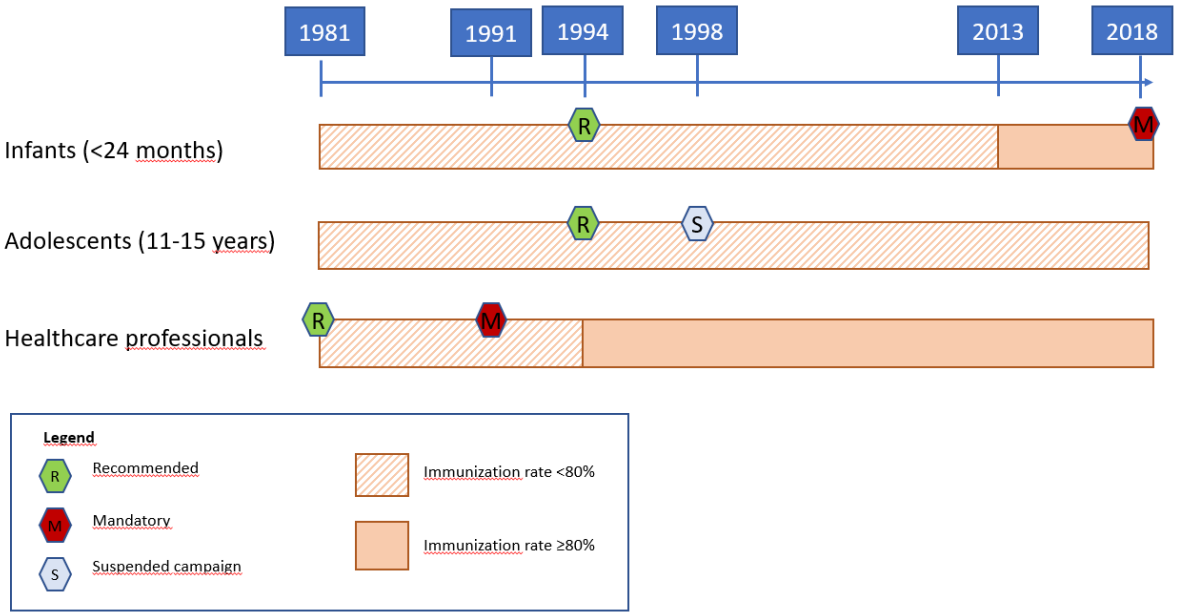


Figure 26 : Key achievements for vaccination against HBV in France

4.2.5 Impact of immunization campaigns

The duration of immunization against HBV lasts at least 30 years and is even thought to be lifelong. (Launay & Floret, 2015)

Nonetheless, the direct impacts of vaccination strategies implemented in France in the 1990's are difficult to measure for two major reasons:

- There is generally a long delay between HBV primo-infection and the possible evolution towards a chronic hepatitis, cirrhosis, and, of course, hepatocellular carcinoma. Therefore, measuring the direct impact of immunization campaigns on these delayed outcomes would require at least 30 years of follow-up and cannot be assessed with confidence.
- France was and is a low endemicity country for HBV, this precluding the observation of a dramatic change in the incidence of hepatocellular carcinomas attributable to vaccination. Moreover, this low endemicity prevented the detection of a massive herd effect due to the vaccination campaign, as reported in Taiwan, for example.

The only indicators of the vaccination benefits consist in the diminution of acute HBV infections in France. Indeed, it was estimated that an increase of 1% in the HBV vaccination coverage would contribute to a 9% decrease in the incidence of HBV acute infections (IRR= 0.91 (95%CI: 0.90 to 0.96), p<0.01). (Miglietta, Quinten, Lopalco, & Duffell, 2018)

When comparing the cases of HBV infection in the pre- and post-vaccination era, a shift in the age distribution is observed. While about 40% of cases were in the 20-29 age range in 1991-1994, most of cases declared between 2003 and 2004 were slightly older, falling in the age category of 30-39 years. This could be attributable to the effectiveness of the vaccination campaign organized in schools between 1994 and 1998. (Antona, 2008) In addition, thanks to this school-based initiative, it was also estimated that 3,000 HBV infections would have been prevented in adolescents between 1994 and 1998. (Denis, 2016)

4.2.6 Comparison with HBV vaccination programmes and recommendations in the USA and Europe

In 2016, the WHO implemented a global strategy aiming at eliminating viral hepatitis as a major public health threat by 2030. To reach this objective, the agency insisted on the importance of several complementary measures: (World Health Organization, 2016)

- Inclusion of HBV vaccination in national childhood immunization schedules
- Implementation of catch-up HBV vaccination strategies
- Achievement of 90% vaccine coverage for the third dose (i.e., complete schedule)

While most developed countries have implemented immunization programmes targeting HBV a while ago, the type of strategy varied across countries making the vaccination coverage heterogeneous. (Lernout et al., 2014)

In the USA, the Advisory Committee on Immunization Practices (ACIP) recommends administration of HBV vaccine and hepatitis B immunoglobulins for infants born of HBV-infected women within the 12 post-birth hours, followed by completion of the vaccine series and a post-vaccination serologic testing. The objective of this early immunization is to provide the infants, as soon as possible after birth, with a first dose of HBV vaccine, in order to minimize, as far as possible, the risk of vertical transmission. For other cases, universal hepatitis B vaccination within the 24 post-birth hours, followed by completion of the vaccine

series is recommended, as well as catch-up vaccination of children and adolescents under 19 years who have not been vaccinated previously, and vaccination of at-risk adults. (Centers for Disease Control and prevention, 2015)

Similarly, all European countries, except Norway, recommend birth immunization of newborns from HBV-infected mothers (cf. Table 12). For infants not born of an infected mother, the HBV vaccination schedule usually starts around the second month of life, for the first dose; the second one being given between 4 and 6 months, and the third injected at 11-12 months.

Catch-up immunizations of unvaccinated children or adolescents are in place in eight European countries: Austria, Belgium, Croatia, France, Germany, Greece, Latvia and Luxembourg.

Table 12 : Comparison of anti-hepatitis B vaccination schedules and recommendations across Europe (ECDC Vaccine Scheduler: https://vaccine-schedule.ecdc.europa.eu/)

General recommendation
Recommendation for specific groups only
Catch-up (e.g. if previous doses missed)
In red, countries where HBV is compulsory

	Birth	Months																Years												
		1	2	3	4	5	6	7	8	10	11	12	13	14	15	16	18	19	2	5-6	7	10	11	12	13	14	15	16	17	18	>= 19
Austria	(1)										(2)										(3)										
Belgium	(1)																													(4)	
Bulgaria	(5)	(6)	(7)	(7)	(7)		(6)																								
Croatia	(8)																							(9)							
Cyprus	(10)																														
Czech Republic	(11)																													(12)	
Denmark	(13)	(14)	(14)	(14)																											
Estonia	(15)																														
Finland	(16)																														
France	(17)																														
Germany	(1)																														
Greece	(20)																														
Hungary	(22)																														
Iceland																															
Ireland	(24)																														
Italy	(25)																														
Latvia	(26)																														
Liechtenstein	(28)																														
Lithuania																															
Luxembourg	(30)																														
Malta	(30)																														
Netherlands	(32)		(33)																												
Norway																															
Poland	(34)																														
Portugal																															
Romania	(35)																														
Slovakia	(36)																														
Slovenia	(37)																														
Spain	(39)		(40)								(40)																				
Sweden	(1)																														
United Kingdom	(41)																														

Footnotes:

1: Babies born to a mother infected with hepatitis B will be offered a dose at birth simultaneously with HB immunoglobulin

2: Minimum interval of 6 month after second dose

3: Primary immunization (0/1/6 months) or catch-up depending on previous vaccination history

4: Vaccination of specific risk groups (see detailed information http://www.health.belgium.be/eportal/Aboutus/relatedinstitutions/SuperiorHealthCouncil/domains/vaccination/index.htm?fodnlang=fr#.VOr0BvnF-QA)

5: Administration within 24 hours after birth.

6: When using a monovalent vaccine, doses are administered at 1 and 6 months

7: When using a combination vaccine (e.g. hexavalent vaccine), doses are administered at 2, 3 and 4 months

8: Babies born to a mother infected with hepatitis B will be offered a dose of immunoglobulins at birth.

9: Catch-up at 6th grade for those not vaccinated in infancy (3-doses scheme). Catch-up expected to end after 2018

10: Babies born to a mother infected with hepatitis B will be vaccinated and receive HB immunoglobulins within 24 hours after birth

11: Babies born to HBsAg-positive mothers will be given a first dose within 24 hours after birth by law

12: 3 doses. If susceptible and no history of vaccination. Mandatory for specific at-risk groups

13: Babies born to a mother infected with hepatitis B will be offered a first vaccine dose at birth simultaneously with HB immunoglobulin. Following vaccine doses are given at one month, 2 month and 12 months of age.

14: For specific at risk-groups only

15: Within 12 hours after birth. Only for at-risk newborns.

16: Risk-groups only (to be given at the earliest age)

17: Babies born to a mother infected with hepatitis B will be offered a first dose at birth simultaneously with HB immunoglobulin, one month of age and 6 months of age. Four doses scheme (0-1-2-6 months) for premature <32 weeks or less than 2 kg. This intervention shall be evaluated at 9 months of age through HBsAg and anti-HBs antibodies testing, preferably one to four months after the last vaccine dose.

18: Three doses in a 0, 1, 6-month schedule. From 11 to 15 years, 2 doses in a 0, 6 schedule

19: Optional dose if monovalent and other combination vaccines are used

20: Babies born to a mother infected with hepatitis B and those whose immune status is unknown will be offered a first vaccine dose at birth simultaneously with HB immunoglobulin in the case of HbsAg mother.

21: Three doses catch-up for unvaccinated adults

22: Babies born to a mother infected with hepatitis B or unknown immune status will be offered a first vaccine dose within 12 hours after birth and simultaneously with HB immunoglobulin in case of HbsAg positive mother. Following vaccine doses are given 1 month later and the third dose, 6 months after first dose.

23: School-based vaccination in 7th grade

24: All babies born to these mothers should receive hepatitis B vaccine at 0, 2, 4 and 6 months and also HB immoglobulin as soon as possible ideally within 24 hours of birth, but no later than 7 days

25: Babies born to a mother infected with hepatitis B will be offered a first vaccine dose within 12-24 hours after birth and simultaneously with HB immunoglobulin. The following and second vaccine dose is given 4 weeks apart from the first. Starting from the third dose, which is given from 61 days of life onwards, the vaccination calendar schedule including the combined hexavalent vaccine should be used.
26: Babies born to a mother infected with hepatitis B or unknown immune status will be offered a first dose within 12 hours after birth. Vaccine administered according to indications.

27: If no previous vaccination. Three doses recommended.

28: Babies born to a mother infected with hepatitis B
29: Hepatitis B vaccination is primarily targeting adolescents aged 11 to 15 years, but can be given at any age (3 doses at 0, 1, 6 months). An accelerated vaccination scheme of adolescents 11-15 years adults in 2 doses (0 and 4-6 months) is possible, but only with vaccines licensed for this regimen, this scheme is valid
when the first dose is administered before the 16th birthday. Vaccination of infants is also possible (hexavalent combined vaccine (DTPa-HBV-IPV-Hib): 4 doses at 2, 4,6, and 15-18 months)

30: Babies born to a mother infected with hepatitis B will be offered a first dose at birth

31: If no history of vaccination
32: Babies born to a mother infected with hepatitis B will be offered a first dose at birth, according to:
http://www.rivm.nl/Documenten_en_publicaties/Algemeen_Actueel/Uitgaven/Infectieziekten/Rijksvaccinatieprogramma/HepB_0_vaccinatie_HepB_dragersmoeders

33: Should be given at 6-9 weeks

34: Administration within 24 hours after birth

35: Within 24 hours after birth. For babies of HBsAG positive mothers, a different schedule applies.
36: Babies born to a mother infected with hepatitis B will be offered a first dose at birth simultaneously with HB immunoglobulin, and two additional doses: one at 1 month and one at 6 months
37: Babies born to a mother infected with hepatitis B will be offered a first dose within 12 hours after birth, one month of age, two months of age and one year of age. Mandatory

38: 3 doses course of vaccination
39: Babies born to a mother infected with hepatitis B will be offered a first dose at birth, 2, 4 and 11 months of age and HB immunoglobulin at birth (first 24 hours of life). Schedule 2,4,11 months will be offered only when high coverage of pregnancy screening is assured

40: Babies born to a mother infected with hepatitis B will be offered a first dose at birth, one month and 6 months of age

41: Babies born to hepatitis B infected mothers. At birth, four weeks and 12 months old

Denmark, Finland, and Iceland are the only European countries still targeting only at-risk populations without promoting universal vaccination programmes against HBV. Conversely, vaccination against HBV is compulsory for ten, mainly Eastern, European countries: Bulgaria, Croatia, Czech Republic, France, Hungary, Italy, Latvia, Poland, Slovakia and Slovenia, while the level of endemicity across all these countries is rather heterogenous as documented in Table 13.

Table 13: Annual notification rates for HBV infections in European countries where the HBV vaccination is mandatory (European Centre for Disease Prevention and Control, 2018)

	Annual notification rate (per 100,000)		
	Acute cases	**Chronic cases**	**Unknown status**
Bulgaria	-	-	-
Croatia	-	-	-
Czech Republic	-	1.66	-
France	0.14	-	-
Hungary	0.56	-	-
Italy	-	-	0.51
Latvia	3.71	14.98	-
Poland	0.13	3.86	6.04
Slovakia	0.92	2.05	-
Slovenia	0.87	1.07	-

4.2.7 Communication and political environment at vaccine launch

The advent of the HBV vaccine was undoubtedly a breakthrough for public health. France acted as a pioneer for both the vaccine discovery and the implementation of vaccination campaigns. Moreover, this vaccine was the first one arguing on a protection against long-term complications including liver cancer. This has probably generated a kind of excessive passion around this particular vaccination.

When the French Minister of health announced the upcoming launch of national immunization school-based campaign in July 1994, the objective was to achieve an immunization coverage of 80% in infants and children aged 11 years. (Benkimoun, 2011) Intensive media communication around the immunization campaigns including impactful promotion messages towards general population and physicians, as well as a massive dissemination of potential threats linked to HBV were implemented to achieve this

ambitious goal; sadly, it also led to the release of unjustified concerns among the French general population.

It should be acknowledged that information around the harmfulness of HBV infection in France was clearly and deliberately exaggerated (Benkimoun, 2011), both by manufacturers and health authorities:

- The predominant mode of transmission presented at that time was saliva as advocated in the communication materials disseminated to the general public in France (cf. Figure 27). However, while the virus can be detected in saliva, it is clearly established that HBV cannot be transmitted through food or water, sharing eating utensils, breastfeeding, hugging, kissing, hand holding, coughing, or sneezing (cf. section 4.1.3 Mode of transmission).
- Moreover, epidemiologic data communicated at the time of vaccination campaign launch were misleading. For instance, it was argued that HBV killed more people in one day than HIV in one year (cf. Figure 28). While the minister announced that 15,000 incident HBV infections occurred each year, the annual incidence of HBV was estimated at 6 [95%CI: 2 – 12] per 100,000 in 1996, corresponding to 3,600 incident cases per year (i.e. about one quarter of the figure announced by health authorities).
- Additionally, the rate of conversion to HBV chronicity was also overestimated, as French public health instances stated that about 30% of infected people will later develop cirrhosis or hepatocellular carcinoma. In fact, as mentioned in section 4.1.3 Mode of transmission, about 5-10% of infected adults become chronic carriers, a risk 3 to 6 times lower.
- Taking into account both overestimations (i.e., number of incident cases per year and rate of conversion to HBV chronicity), it can be estimated that the number of expected liver carcinoma attributable to HBV was exaggerated by a factor of 12 to 24.

Figure 27 : Communication material 1 used in France at the time of national vaccination campaigns (extracted from (Giacometti, 2001))

Figure 28 : Communication material 2 used in France at the time of national vaccination campaigns (extracted from (Giacometti, 2001))

The intensive dissemination of alarming, but distorted if not fallacious, epidemiologic data could explain the success of the vaccination campaign targeting adolescents in the period 1994-1998. Sadly, it also has surely played an important role for the observed disjunction between the target and joint populations, which led to the massive exposure of adults to the HBV vaccine.

4.2.8 Societal acceptability and mistrust

The acceptability of the anti-HB vaccination was rather good at the time of the vaccine launch, as shown by the immunization rate for healthcare professionals which progressed over time since the first recommendation in 1982. (Denis et al., 1998) In 1994, when the national vaccination strategies were implemented, the perception by the French general population was still quite positive.

However, a turning point occurred in 1996, when a pharmacovigilance signal was detected concerning demyelinating events observed in the follow of an administration of HBV vaccine. While the different studies aiming at exploring the nature of this association were not completed, the decision to suspend the school-based vaccination campaign in 1998 was interpreted as a clue supporting a causal link. In addition, the violent debate orchestrated around the polemic produced devastating effects on the immunization coverage of vulnerable populations, especially for infants whose coverage never exceeded 30% until 2000 and even more for adolescents whose immunization rate has never reached the expected target (cf. section 4.2.4 Immunization coverage against HBV in France).

Three successive surveys aiming at evaluating the acceptability of the French population toward vaccinations, were conducted by the National Institute of Prevention and Health Education in 2003-2005. (Balinska & Leon, 2006) They produced interesting findings. Among all vaccines, the anti-HBV vaccine was the one having the biggest defiance/reticence. A proportion of 67.3% of the surveyed general practitioners as well as 46.4% of interviewed pediatricians were still wondering about the utility of such vaccination. Respectively 89.4 and 82.4% had concerns about the safety of the HBV vaccines while 26.6% of all surveyed physicians estimated that these vaccines could be responsible for delayed adverse events occurring at an adult age. Approximately 13% thought that the vaccine manufacturers voluntary biased the safety data regarding HBV vaccines and a similar percentage did not trust the public health instances in France, fearing potential conflicts of interest. It was also estimated than one third of these physicians did not follow the recommendation for newborns for the following reasons:

- This subpopulation is not at risk for HBV infection,
- The position of national health authorities was not clear,
- Parents were afraid/concerned by administering this vaccine to their children,

- The vaccine is unsafe or does not actually confer a long-term immunity.

One should note that, considering the somewhat erratic and contradictory communication of French health authorities, this mistrust was not totally unfounded. For instance, one of the arguments used to promote immunization of newborns was that it would confer a life-long protection. In the same time, and until 1999, periodic boosters (e.g. every five years) were recommended for adults in order to maintain a sufficient level of protection.

Until now (in 2018), these defiance and mistrust are still present in the French society. A large survey on confidence in immunization across 67 countries showed that Europe has the lowest confidence in vaccine safety, France being the worst. (Larson et al., 2016)

4.3 Research question n°1: Is there a link between central demyelination and anti-HBV vaccination?

The first mention of this putative link occurred as early as 1975, in an article entitled "Hepatitis Vaccine: A note of caution". In this article, the author recommended a careful assessment of all vaccine effects on the immune system and he warned that autoimmunity could follow the administration of the HBV vaccine because the infection by the HBV, itself, involves autoimmunity. (Zuckerman, 1975)

In 1996, the hypothesis regarding a potential association between the HBV vaccines and central demyelinating events re-emerged in France following the detection of a pharmacovigilance signal founded on 249 incident demyelinating cases having occurred soon after the administration of a dose of anti-HBV vaccine. (Fourrier et al., 2001) This signal has been detected less than two years after the implementation of the nationwide vaccination campaign.

This polemic gave birth to a violent domestic and also worldwide debate, still active nowadays.

The following sections will review the scientific evidence available so far on our research question and will examine the arguments in favor or playing against the existence of a causal link.

4.3.1 Hypothesis generation

4.3.1.1 Biological plausibility

Two distinct mechanisms have been evoked to support a potential biological plausibility between HBV vaccines and central demyelination (especially MS): (Agence Française de Sécurité Sanitaire des Produits de Santé (AFSSAPS), 2007)

- The injected antigen contains a protein sequence similar to this of myelin, and could induce a specific response with the release of antibodies targeting both the antigen and myelin.
- A bystander effect due to the vaccine adjuvant stimulates the autoimmunity producing heterologous reactions against antigens that differ from the one initially presented. (van Aalst, Ludwig, van der Zee, van Eden, & Broere, 2017)

A structural study was conducted in 2006 to compare the sequence of the HbAg used in commercialized vaccines (i.e., HBS 175-400) with sequences of human proteins. No significant analogy was found between the primary structures of the HBS 175-400 and human proteins. Nevertheless, the three-dimensional structure of the HBS 175-400 has never been compared to those of human proteins. In addition, no study has evaluated so far, the effect of the adjuvant on this structure. For the two aforementioned reasons, the hypothesis of a potential analogy in the protein sequences cannot be completely ruled out.

Owing to the complexity of the etiology of MS (still unclear in many respects), which involves both genetic and environmental factors, it has been acknowledged by a French expert committee that the use of animal models to evaluate a potential link between the HBV vaccines and the occurrence of MS suffers many limitations. (Agence Française de Sécurité Sanitaire des Produits de Santé (AFSSAPS), 2007)

Another theory argued that trace amount of HBV polymerase protein could be co-purified with HBsAg during the manufacturing processes and that this protein could trigger the immunologic reaction chain that lead to MS by a molecular mimicry between HBV-pol and myelin. Indeed, the HBV polymerase shares similar amino acid sequences with the human myelin basic protein, supporting the molecular mimicry theory. Thus, the HBV polymerase, which could be a contaminant in the recombinant or plasma-derived vaccines, could act as a autoantigen and therefore induce autoimmune a demyelinating diseases such as multiple

sclerosis. (Faure, 2005) Besides, this hypothesis was considered by both French health authorities and the US Immunization Safety Review Committee in 2002. This latter concluded that there was a theoretical basis for a HBV-induced immune response that could possibly lead to demyelination, although the evidence supporting this theory remains scant and indirect. (Institute of Medicine Immunization Safety Review, 2002)

4.3.1.2 Published case reports

In the framework of this thesis, a high-level literature review was conducted in Medline via Pubmed to identify the published case reports of demyelination, peripheral and central, having occurred after the administration of HBV vaccines. A total of 12 distinct publications were found accounting for 17 individual cases of demyelination occurring in a close temporal relationship after HBV vaccine administration. (Cabrera-Gomez et al., 2002; Creange, Temam, & Lefaucheur, 1999; Herroelen, de Keyser, & Ebinger, 1991; Hostetler, 2001; Iniguez et al., 2000; Kaplanski, Retornaz, Durand, & Soubeyrand, 1995; Karaali-Savrun, Altintas, Saip, & Siva, 2001; Renard et al., 1999; Santos-Garcia, Arias-Rivas, Dapena, & Arias, 2007; Sinsawaiwong & Thampanitchawong, 2000; Terney et al., 2006; Tuohy, 1989). Reported events were diverse, including ADEM, transverse myelitis, Guillain-Barré Syndrome, first episode of MS, and MS relapse. Gender was balanced with 46.2% of cases occurring in men. Three articles, presenting a total of 4 cases, were published before 1996, year of the detection of the French pharmacovigilance signal. Among these sources, only one publication, relating a single case, originated from France. (Kaplanski et al., 1995)

It is obvious that these 17 case reports represent only a small and non-representative sample of all cases of demyelination having occurred, worldwide, after a HBV vaccination, even if focusing on those for which a causal relationship did not appear by far too unlikely. It is, by definition, impossible to estimate such a number, but it is noteworthy that authors of these publications found their cases convincing enough to hypothesize that the HBV vaccines may have played a role in the occurrence of these AEFIs.

One can also mention the consistency and the specificity of these case reports: investigators originated from different countries around the world but reported similar events (all these cases relied on a demyelinating process, either peripheral or central) having occurred shortly after an immunization against HBV.

4.3.1.3 Description of the case-reports having led to the detection of a pharmacovigilance signal in France

As mentioned previously, a pharmacovigilance signal was generated in July 1996, after 249 cases of demyelination were reported to the French pharmacovigilance system. (Fourrier et al., 2001)

As a consequence, a nationwide investigation was opened by the French Medicine Agency to evaluate the robustness of this signal. The final report was issued in 2001, while the French agency has still pursued its surveillance and the monitoring of the safety profile of HBV vaccines marketed on the territory.

A study which assessed the robustness of this signal by both analysing all validated cases reported during the 1980-2000 period and conducting observed-to-expected (OE) comparisons, was recently submitted to Vaccine. (Mouchet J. & Bégaud B., 2018) This study which formed one of the research axes of the present thesis, is presented in details in the sections below.

4.3.1.3.1 Objectives

The main objectives of the study were to review and describe all cases of central demyelination, including multiple sclerosis (MS), reported in France after HB vaccination between 1980 and 2000, and to conduct several OE analyses in order to assess the robustness of the signal detected from which the polemic arose.

The methods and results pertaining of the OE analyses are reported in section 4.3.1.4 Observed/expected analyses

4.3.1.3.2 Methods

Study design: This descriptive study reviewed all the cases of incident central demyelination occurring after HB vaccination reported to the French pharmacovigilance since the launch of the first HB vaccine in France (i.e., Hevac B® [Sanofi-Pasteur] in 1981) and 31 December 2000 (i.e., cut-off date of the pharmacovigilance report on the putative link between MS and HB vaccination, issued in France in 2001).

Data sources: The complete reports issued by the French pharmacovigilance on the number and summaries of case-reports of demyelination following HB vaccination were used to

identify the cases of interest. Relevant data were abstracted into an Excel standardized matrix including the identification number, the vaccine brand name or type, the vaccination date, the age and gender of the case, the rank of vaccination, the date of event, the event of interest, the vaccination and medical history of the case, the co-administration of other vaccines and additional comments in free text (if any). The level of data completion was dependent on the case report. At least two fields were required for case selection and data extraction: the event of interest and the date of either event occurrence or vaccination.

A high-level scientific and grey literature review was conducted in March 2018 to identify the background incidence rates of MS (overall and by gender) during the years covered by our study, as well as the number of HB vaccine doses sold during that period. Literature searches using a combination of keywords such as incidence, prevalence, multiple sclerosis, demyelination, vaccine, dose, France or French were performed in Medline via PubMed and were then complemented by pragmatic searches in Google and Google Scholar using similar keywords.

Events of interest: For the descriptive analyses, only incident events of central demyelination including MS were considered. At the time of the investigation, i.e. in the early 2000s, these events were all reviewed and confirmed by a senior neurologist. Both adult and paediatric populations were investigated.

Relapses of MS, Guillain-Barré syndrome and peripheral demyelination (including Parsonage-Turner syndrome, chronic polyneuropathy and neuropathy) were excluded.

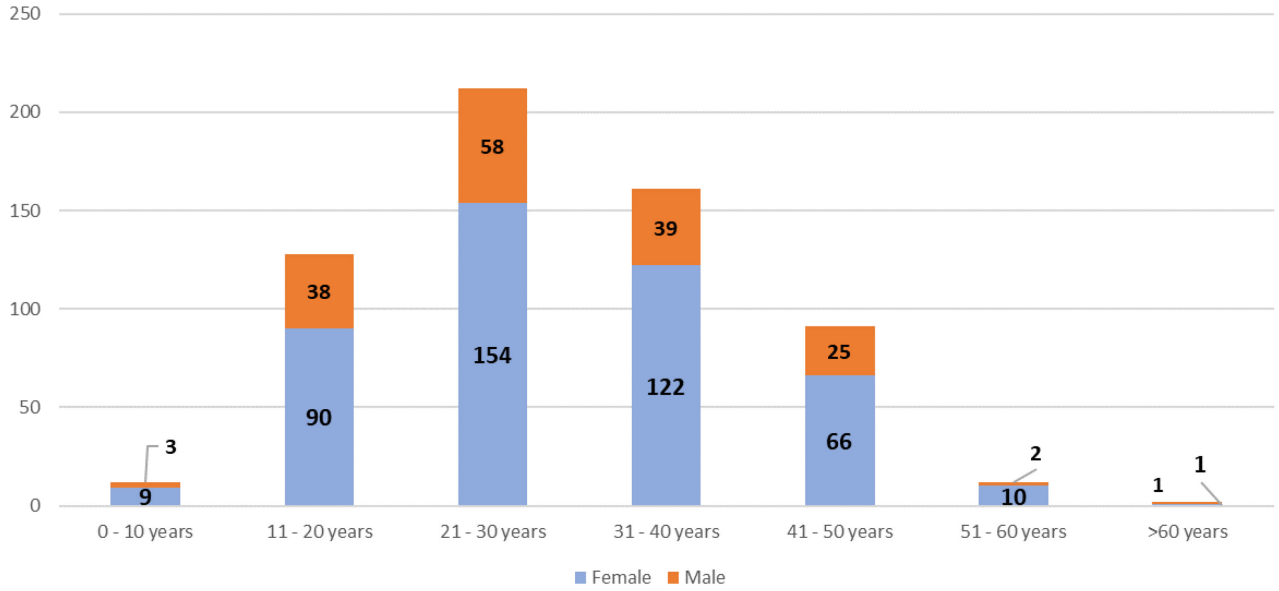
Statistical analysis: All case characteristics were summarized using descriptive techniques: summary statistics (mean, standard deviation (SD), median, minimum and maximum values) were computed for continuous variables while counts and percentages were computed for categorical and binary variables.

Reporting rates (i.e., number of cases reported to the French pharmacovigilance system per 1,000,000 doses of HB vaccines sold) were computed for the whole study period and per year.

Time-to-onset was defined as the time interval between the last vaccine dose injection (regardless of the vaccine rank) and the occurrence of the event, while time-to-report refers to the interval between the event occurrence and the case reporting.

4.3.1.3.3 Results

A total of 624 incident cases of central demyelination were reported to the French Pharmacovigilance from 1981 (date of the first HB vaccine, Hevac B®, launched on the French market by Sanofi Pasteur until 31 December 2000. The first case of interest, not reported at this date, occurred in 1984 but the first case report was recorded in 1992 by the French pharmacovigilance. A total of 422 (67.6%) cases were confirmed as first episodes of MS by an independent senior neurologist. The ratio between events coded as a first episode of MS and those coded as incident central demyelination decreased over the study period. Indeed, all events which occurred between 1984 and 1990 were coded as a first episode of MS, *versus* less than a half (46.9%) for the most recent years (1998 – 2000). Women accounted for most cases (n=457, 73.2%), corresponding to a female/male ratio of 2.7. This trend remained stable over the whole study period. Age of central demyelination cases ranged from 2 to 63.8 years (Q1-Q3: 21.6-38.5). Both mean and median age of cases converged with values of 29.8 years (standard deviation (SD)=11.1 years) and 29.0 years, respectively. Figure 29 presents the age and gender distribution across the 618 case reports for which age and/or gender were documented.



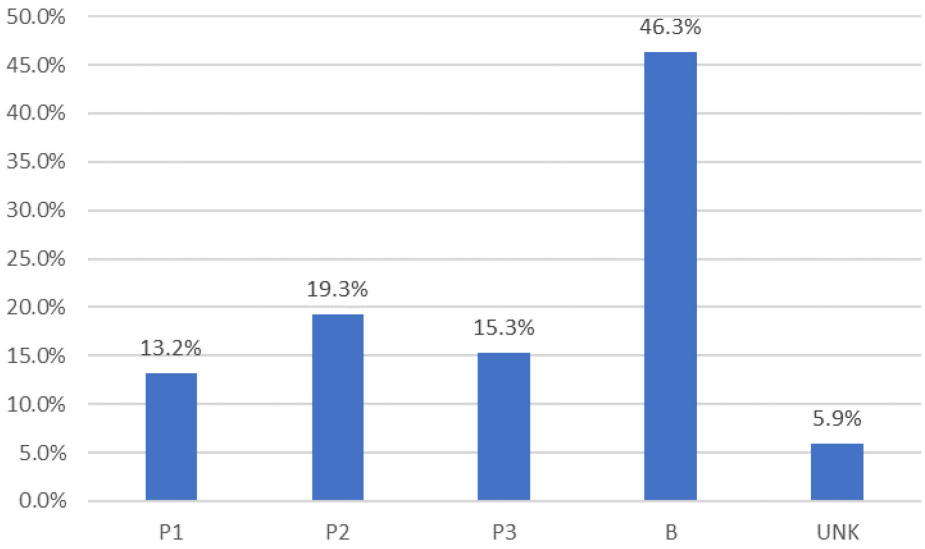
Note: Information regarding age and/or gender was missing for 6 cases.

Figure 29: Age and gender distributions of cases of interest

Among confirmed MS cases, mean age was 30.1 years (SD=11.1 years) while median age was 29.0 years (Q1-Q3: 21.6-38.5).

A total of 86,622,362 doses of HB vaccines were sold over the study period for a total population increasing from 54 to 59 million over this period, i.e. an average of 1.53 doses per inhabitant.

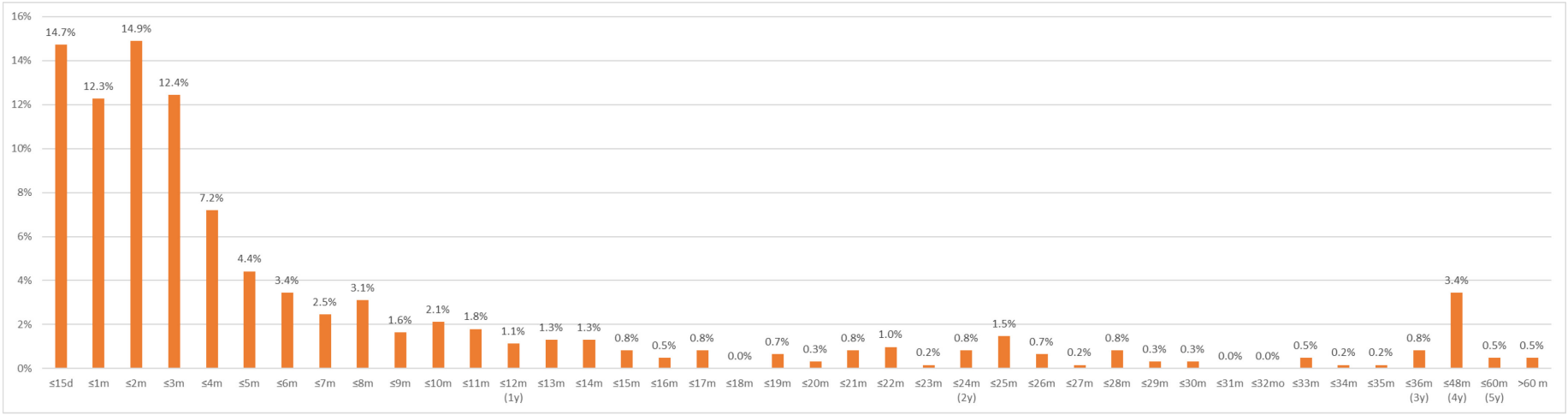
Events of interest were mainly reported after the booster doses (46.3%, n=289) (cf. Figure 30).



Notes: *P1: 1st vaccine dose of primovaccination; P2: 2nd vaccine dose of primovaccination; P3: 3rd vaccine dose of primovaccination; B: booster dose; UNK: unknown, information about vaccine dose after which an event occurred was missing (n=37)*

Figure 30 : Distribution of vaccination ranks for cases of interest (n=624)

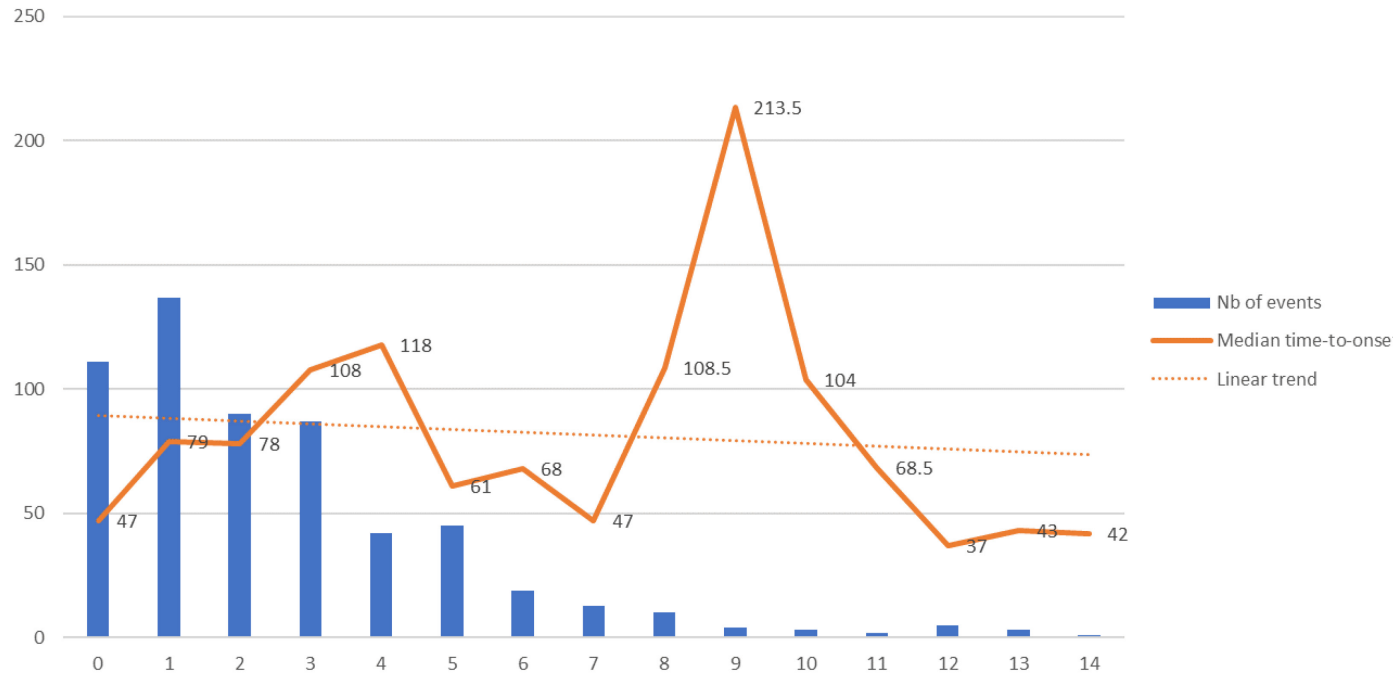
The time-to-onset (i.e., time interval between the HB vaccination and the occurrence of the event of interest) for the whole series of cases ranged from 0 to 2,982 days (i.e., 8 years and 2 months); however, for 69.3% of reported cases, the symptoms appeared within the 6 months following vaccination (cf. Figure 31).



Notes: *'Time-to-onset' refers to time interval between vaccination and occurrence of the event of interest. Information regarding time-to-onset was missing for 13 cases.* ***Abbreviations:*** *d: days; m: months; y: years*

Figure 31: Distribution of time-to-onset for cases reported to the French pharmacovigilance between 1980 and 2000.

The median time-to-onset was 74 days corresponding to 2 months and 14 days, while the mean (221.1 days; SD=345.5) was distorted by outlier values. Overall, the median time-to-onset remained somewhat constant even for cases reported *a posteriori* after a long delay (cf. Figure 32).



Note: *'Time-to-onset' refers to time interval between vaccination and occurrence of event. 'Time-to-report' refers to time interval between occurrence of event and case reporting. Information regarding time-to-onset or time-to-report was missing for 52 cases.*

Figure 32: Relationship between time-to-onset and time-to-report

In absolute values, incidence of events peaked in 1995, 1996, and 1997, these years accounting for 59.8% (n=373) of all cases of interest. However, when looking at the reporting rates, the highest values were for years 1987, 1997 and 1998 with rates of 10.5, 12.5 and 14.7 per 1,000,000 doses sold, respectively. The overall mean reporting rate over the study period was 6.51 per 1,000,000. The time-to-report ranged from 0 to 14 years with mean and median values of 2 and 2.5 years, respectively. This time increased during the study period. For example, the median time to report which was of 6 months in 1992, increased up to 3 years in 2000. Case reporting flared up after 1995 (cf. Figure 33).

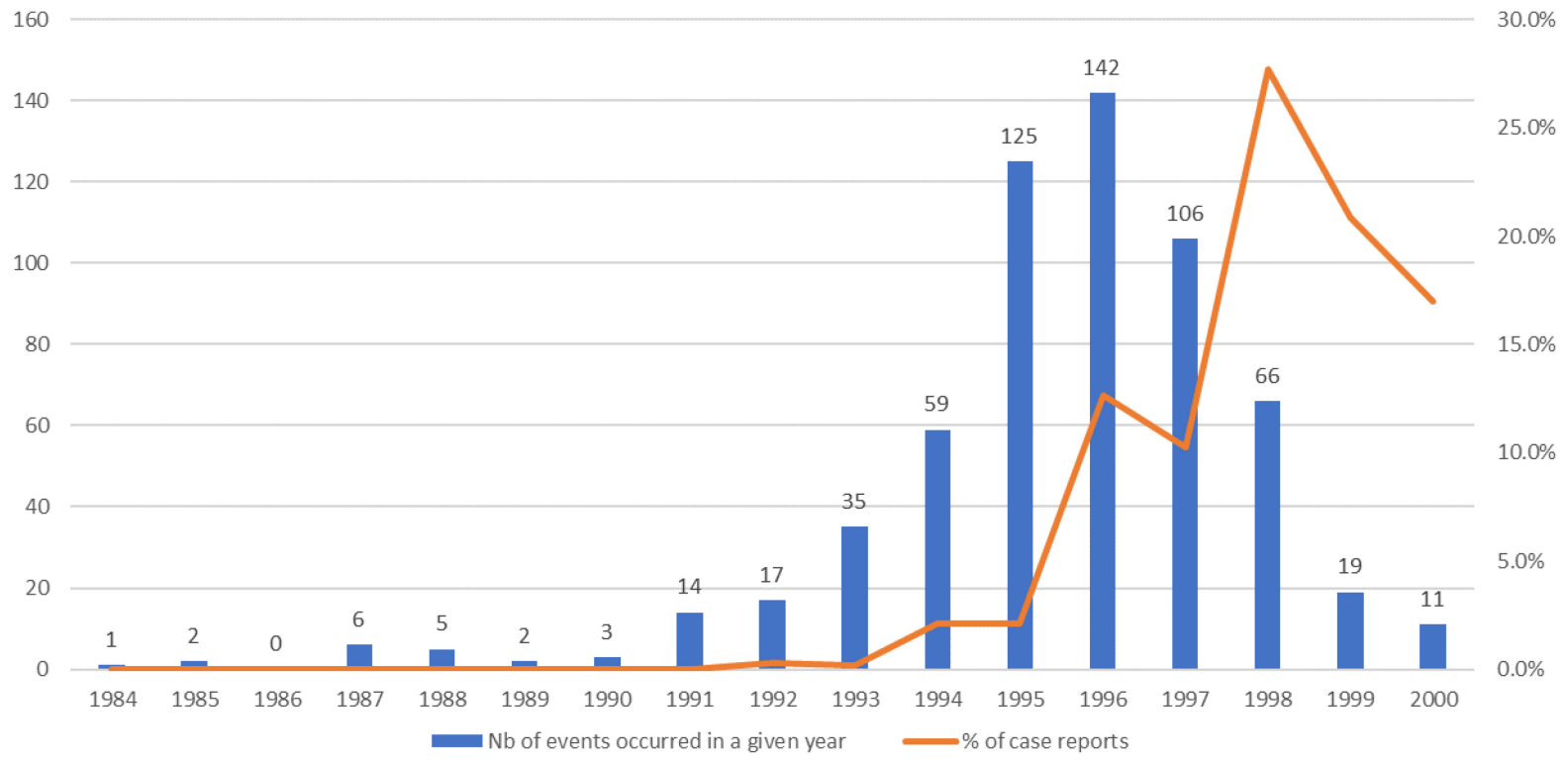
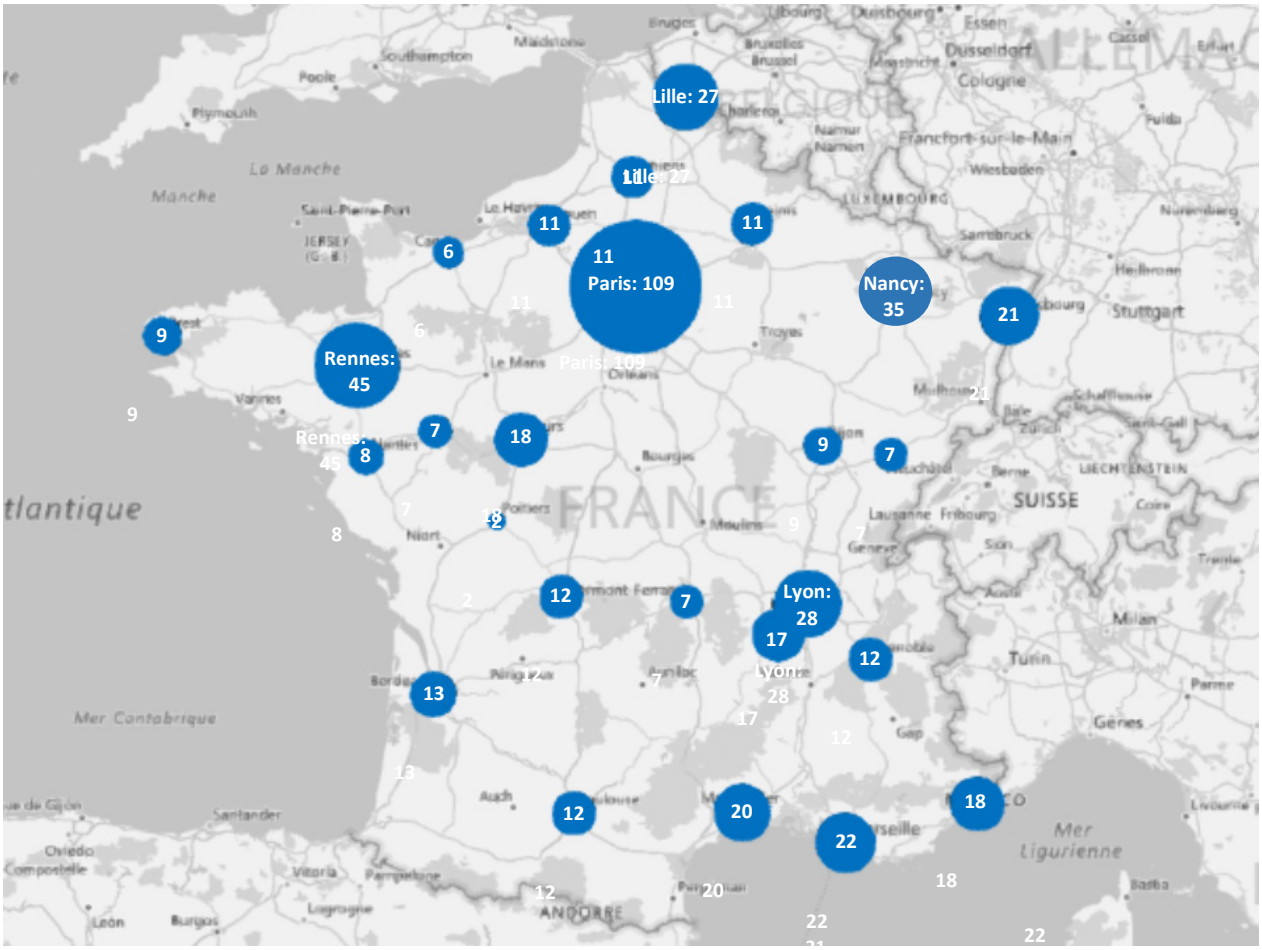


Figure 33 : Case reporting (%) and number of cases occurring in each year of study period

Geographical distribution of case reports is presented in Figure 34.



***Notes**: Information about geographical origin was missing for 127 case reports.*

Figure 34 : Geographical distribution of cases reported to the French pharmacovigilance

4.3.1.3.4 Discussion

Key findings: Of the 624 incident cases of central demyelination after HB immunization reported to the French Pharmacovigilance, our analysis identified 422 incident MS confirmed by a senior neurologist. The female/male ratio of 2.7 is fully aligned with the data of the nationwide "Observatoire Français de la Sclérose en Plaques (OFSEP)" registry in France (i.e., 2.5), which represents 61,022 MS adults in France in 2016.(Observatoire Français de la Sclérose en Plaque, 2017) Overall, the mean age of cases with central demyelination and MS was 29.8 and 30.1 years respectively at the event onset, these being consistent but somewhat lower than the age of MS patients at disease onset (31.9 ±10.5 years) as reported in the OFSEP registry.(Observatoire Français de la Sclérose en Plaque, 2017)

What this study adds? Our analysis showed that the onset of the event of interest was not homogeneously distributed across the rank of vaccine doses, most reported events (46.3%) occurring after the booster dose. During the study period, one booster dose was recommended one year after the initial injection of HB vaccines. After 1994, a new vaccination schedule was proposed with only the three doses of the primovaccination even if the practice of the booster remained common until 1999 (cf. section 4.2.3 Immunization schedules). The short time interval between the last two doses of the immunization schedule (i.e., 12 months) ruled out an age effect which would make vaccinated subjects at higher risk of declaring MS after a certain age. By definition, spontaneous reporting is a mode of passive surveillance of adverse events considered as possibly related to drug use by the observer, mostly a physician in the present case. It relies on both the motivation of physicians to report and their personal opinion regarding the nature of the link between an event and a health product. (Moride, Haramburu, Requejo, & Begaud, 1997) One hypothesis for this non-random distribution of cases according to the dose-rank could be a kind of selective reporting. A physician having observed several consecutive dose-event occurrences in a given person could have been more prone to report the case after the last one, since not yet suspecting a relationship after the initial event.

Analysis of times-to-onset showed a wide dispersion with a median value of 2.5 months that remained somewhat constant over the study period. Conversely, the time-to-report had clearly become longer by the end of the period. Again, this could partly be explained by an effect of the intense media coverage resulting in an extensive retrospective search for and *a posteriori* identification of potential cases of interest. We also observed that reporting rates were doubled in 1987, 1997 and 1998. No clear explanation can be offered for this, even if the mass media coverage could also have played a role at least for years 1997 and 1998, but certainly not for 1987.

4.3.1.3.5 Strengths and limitations

The main strength of this descriptive study relies on the use of comprehensive national reports and reliable data obtained from either French pharmacovigilance or nationwide data sources such as the CNAMTS, which covers 87% of the French population. In addition, cases

reviewed were all confirmed by an independent senior neurologist at the time of the French investigation.

Limitations are those inherent to all pharmacovigilance systems. While French physicians were largely encouraged to report any demyelinating events following the administration of the HBV vaccines, a certain degree of underreporting is likely, all cases being not captured by our analysis. Besides, the time lag between the occurrence and the reporting of events, which has been estimated at a median of 2 years, should be taken into account when discussing the exhaustivity of our analysis. This hypothesis is supported by a communication of the French Agency (September 2011) which provided the distribution of cases of central demyelinating events reported to the French pharmacovigilance system until 31 December 2010 (cf. Figure 35).

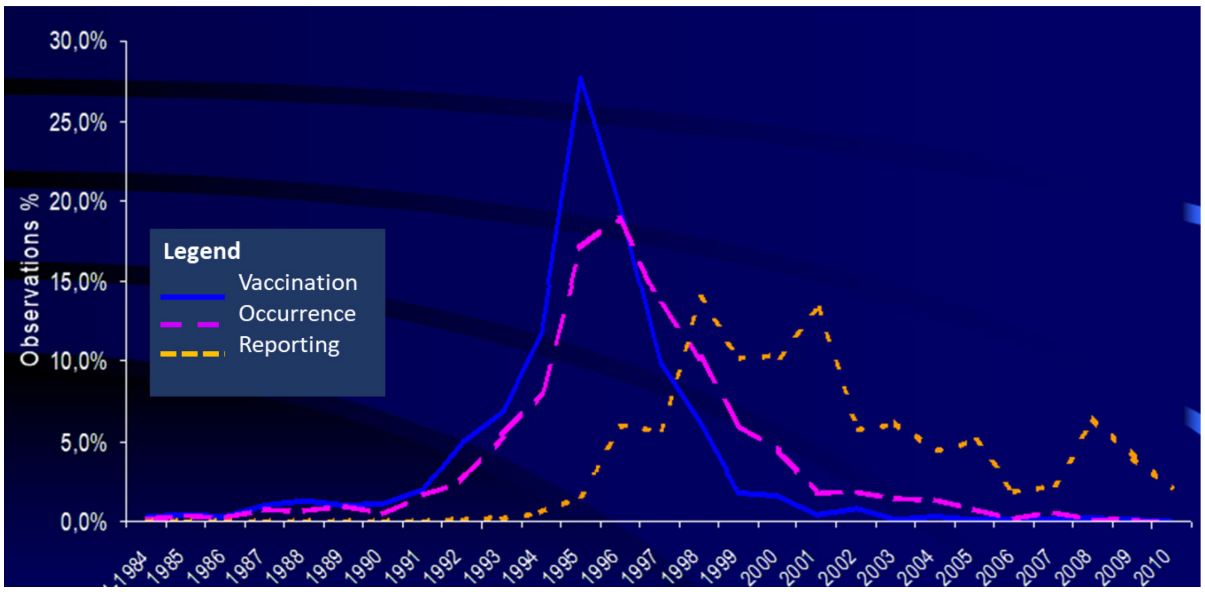


Figure 35 : Distribution of the 1,650 events of central demyelination after anti-HBV vaccination, reported to the French pharmacovigilance system until 31 December 2010

Although this graph appears to be quite similar to our Figure 33 for the period 1984 – 2000, a second peak of reporting was observed in 2002, i.e. outside of our study period. Consequently, these case reports were not included in our analysis. The most streaking element in this graph is the magnitude of the peak observed for the three studied parameters: vaccination, occurrence and reporting. It testifies of the exceptional and abnormal character of the situation in France during the years 1994-1996 with a chain

reaction both for producing and reporting the events of interest. Indeed, this situation reflects the incredible runaway success of the national immunization programme, which was also made possible thanks to an aggressive advertising by manufacturers, leading to an off-target massive exposure of the adult population (cf. section 4.2.4.2 Young adults (aged 16 – 44 years)).

4.3.1.4 Observed/expected analyses based on cases of the French pharmacovigilance

As mentioned in section 4.3.1.3 Description of the case-reports having led to the detection of a pharmacovigilance signal in France, a study aiming at assessing the robustness of this signal by analysing all validated cases reported in 1980-2000 and by conducting observed-to-expected (OE) comparisons, was recently performed. (Mouchet J. & Bégaud B., 2018)

The methods and results of the OE analyses are presented hereafter.

4.3.1.4.1 Methods

The present OE analyses were performed according to methods detailed in section 2.1.2 Observed-to-expected analyses.

OE analyses were restricted to cases of incident MS (i.e., first symptoms of MS, excluding relapses).

Two analytic approaches were used for OE analyses. The first (here referred to as "individual-based") was based on the number of subjects exposed to the HB vaccination, which was derived from the number of vaccine doses sold in France during the study period. The total number of vaccine doses sold each year was divided by the number of injections recommended for a complete immunization schedule: four for years 1980-1994 and three thereafter, given that the vaccination schedule was revised in 1994 to reduce the immunization scheme to three doses at 0, 1 and 6 months. The booster dose at 12 months was therefore no longer recommended after 1994. The number of expected MS cases were derived from the background incidence rates of MS for the French adult population per year of interest (from 1984 to 2000) and two-sided confidence intervals at 95% were computed by using the binomial distribution. The number of expected cases was then compared to the

number of observed cases, i.e. case reports of the disease considered, and OE analyses were stratified by gender.

The second method was based on the total number of person-years “at risk” in the vaccinated population. Considering that (i) the HB vaccine induces specific humoral antibodies against HB surface antigens protective against the HB infection (i.e., anti-HBs titer >10 IU/l) within 1 month after injection and (ii) HB vaccine-induced antibody levels wane over time,(European Medicine Agency, 2000) we chose the month following the injection of one dose as the “at-risk” period. The risk of central neurological event was considered to be identical during the month following each injection, i.e. that one dose generated the same time at risk and could be considered independently. Therefore, the total person-time at risk was computed by multiplying this one-month risk period by the number of doses administered and was then converted into exposed years. Under the null hypothesis of no association, the incidence of MS during “at-risk” time and non-exposed time were expected not to differ. As for the first approach, the numbers of expected MS cases were derived from the MS background incidence rates for the French adult population per year of interest (from 1984 to 2000), and confidence intervals at 95% were computed by using the binomial distribution. OE analyses were then stratified by gender.

Cases for which a time-to-onset was not completed were imputed according to the distribution of observed times between vaccination and the occurrence of events of interest.

In 1999, the French Institute for Public Health Surveillance (InVS) estimated that 5% of HB vaccine doses sold were not actually administered to subjects.(Lévy-Bruhl, Rebière, Desenclos, & Drucker, 1999) The OE analyses were therefore reproduced by using this revised number of vaccine doses.

4.3.1.4.2 Results

Annual incidence rates for MS in France were provided by the National Health Insurance Fund for Employees (i.e., CNAMTS). Whatever the approach used, the number of observed cases never exceeded the expected number. With the individual-based approach, the estimated number of vaccinated people was 26,401,946 over the study period. The expected number of incident MS cases was 1,200 [95%CI: 1,132 – 1,268], while the number of cases

reported to the French pharmacovigilance was 422 during the whole study period, corresponding to an OE ratio of 35.2% (cf. Table 14).

Table 14: Observed-to-expected analysis by using individual-based approach

Year	Annual incidence of MS (per 100,000)	Total number of vaccine doses sold	Estimated number of vaccinated people	Expected number of cases	[95%CI]		Number of 1st episodes of MS reported to French PV	OE ratio
1984	*4*	240,937	60,234	2	1.8	3.1	1	41.5%
1985	*4*	318,605	79,651	3	2.6	3.7	2	62.8%
1986	*4*	453,891	113,473	5	4.1	5.0	0	0.0%
1987	*4*	571,661	142,915	6	5.3	6.1	6	105.0%
1988	*4*	601,537	150,384	6	5.6	6.4	5	83.1%
1989	*4*	717,950	179,488	7	6.8	7.6	2	27.9%
1990	*4*	804,306	201,077	8	7.7	8.4	3	37.3%
1991	*4*	2,287,018	571,755	23	13.5	32.2	10	43.7%
1992	*4*	3,734,662	933,666	37	25.4	49.3	14	37.5%
1993	*4*	5,018,418	1,254,605	50	36.3	64.1	25	49.8%
1994	4	14,917,107	3,729,277	149	125.2	173.1	45	30.2%
1995	4	23,325,138	7,775,046	311	276.4	345.6	90	28.9%
1996	4.75	15,134,845	5,044,948	240	209.3	270.0	92	38.4%
1997	5.25	8,480,438	2,826,812	148	124.5	172.3	72	48.5%
1998	6	4,483,992	1,494,664	90	71.1	108.2	30	33.5%
1999	6.2	2,518,616	839,538	52	37.9	66.2	13	25.0%
2000	6.2	3,013,241	1,004,413	62	46.8	77.7	2	3.2%
	TOTAL	**86,622,362**	**26,401,946**	**1,200**	**1,131.8**	**1,267.6**	**422**	**35.2%**

Abbreviations: *CI: Confidence interval; MS: Multiple sclerosis; Nb: Number; OE: Observed-to-expected*

Notes: *Annual incidence rates of MS in France were provided by the 'Caisse Nationale de l'Assurance Maladie des Travailleurs Salariés' (CNAMTS)*

Surprisingly, the highest OE ratio (105%) was observed for year 1987, the number of reports reaching the number of expected events, this being well before any mass media interest for a potential link between MS and HB vaccination. By using the second approach, the number of person-years "at risk" within the month following immunization was 7,218,530. The expected number of events of interest was 325 [95%CI: 289 – 360] while the total number of reported cases was 100, representing an OE ratio of 30.8%, the latter being of the same order of magnitude as that produced by the first method, i.e. 35.2% (cf. Table 15). However, the number of reported cases reached the number of expected events for years 1984 and 1987 without exceeding it. Nevertheless, these figures were based on too small numbers (1 and 2, respectively) to consider these estimates as reliable.

Table 15 : Observed-to-expected analysis by using 'person-years at risk' approach

Year	Annual incidence of MS (per 100,000)	Total number of vaccine doses sold	Number of months at risk	Number of person-years at risk	Expected number of cases	[95%CI]		Nb of first episodes of MS reported to French PV within 1 month after vaccination*	OE ratio
1984	4	240,937	240,937	20,078	0.80	-0.31	1.92	1.0	124.5%
1985	4	318,605	318,605	26,550	1.06	0.09	2.03	1.0	94.2%
1986	4	453,891	453,891	37,824	1.51	0.70	2.33	0.0	0.0%
1987	4	571,661	571,661	47,638	1.91	1.18	2.63	2.0	105.0%
1988	4	601,537	601,537	50,128	2.01	1.30	2.71	1.0	49.9%
1989	4	717,950	717,950	59,829	2.39	1.75	3.04	0.0	0.0%
1990	4	804,306	804,306	67,025	2.68	2.07	3.29	2.0	74.6%
1991	4	2,287,018	2,287,018	190,584	7.62	7.26	7.99	1.0	13.1%
1992	4	3,734,662	3,734,662	311,221	12.45	12.17	12.73	4.0	32.1%
1993	4	5,018,418	5,018,418	418,201	16.73	16.48	16.97	9.0	53.8%
1994	4	14,917,107	14,917,107	1,243,092	49.72	35.90	63.54	14.0	28.2%
1995	4	23,325,138	23,325,138	1,943,761	77.75	60.47	95.03	24.0	30.9%
1996	4.75	15,134,845	15,134,845	1,261,237	59.91	44.74	75.08	16.0	26.7%
1997	5.25	8,480,438	8,480,438	706,703	37.10	25.16	37.27	14.0	37.7%
1998	6	4,483,992	4,483,992	373,666	22.42	13.14	22.63	8.0	35.7%
1999	6.2	2,518,616	2,518,616	209,884	13.01	5.94	13.29	0.0	0.0%
2000	6.2	3,013,241	3,013,241	251,103	15.57	7.83	15.82	3.0	19.3%
		TOTAL	**86,622,362**	**7,218,530**	**324.65**	**289.3**	**360.0**	**100.0**	**30.8%**

** including 4 imputed cases, estimated from total number of 13 cases without known time to occur and the distribution of time to occur (27.0% within 1 month)*

Abbreviations: *CI: Confidence interval; MS: Multiple sclerosis; Nb: Number; OE: Observed-to-expected.* ***Notes:*** *Annual incidence rates of MS in France were provided by the 'Caisse Nationale de l'Assurance Maladie des Travailleurs Salariés' (CNAMTS)*

Stratifying OE analyses by gender led to similar conclusions, counts of observed cases remaining below the expected figures, except in women for whom the numbers of reported events equalled the number expected for years 1984, 1987, 1990 (person-years at risk approach) and year 1988 (individual-based approach). For men, this was observed for year 1985, and only when using the person-years at risk approach. For both methods used, the OE ratios were consistently higher for women than for men (35.2 *versus* 26.1% and 30.0 *versus* 23.2%, respectively). As expected, the secondary analysis led to slightly higher OE ratios (36.1 and 32.4 %, respectively for individual-based and time-populational approaches) without changing the conclusions.

4.3.1.4.3 Discussion

The two methods produced congruent and inconclusive results, the number of observed cases being lower or equaling the expected number. Stratification by gender led to similar findings. However, these figures are worthy of interest since a certain level of underreporting is an expected and inescapable phenomenon with spontaneous reporting systems.(Alvarez-Requejo et al., 1998) Our overall OE ratios of 35.2 and 30.8% (for the individual-based and at person-years at risk approaches, respectively) would be at 100% if the underreporting factor would have been of 2.8 and 3.2, respectively. In other words, if reporting had been at least three times more intensive than it was, the number of observed events would have reached or exceeded the expected number.

In the present case, the reporting rates of incident central demyelination and first episodes of MS after HB vaccination were 6.5 and 4.8 per 1,000,000, respectively, while the mean population annual incidence rate of MS between 2000 and 2007, based on disease declarations to the French health insurance system, was 6.6 per 100,000, i.e. about 10 times higher.

As already mentioned, it is important to note that the French vaccination campaign launched in the 1990s and initially targeting newborns and adolescents completely missed its target and led to the massive exposure of the adult population. (Fourrier et al., 2001) (Lévy-Bruhl et al., 1999) This resulted in an unprecedented exposure of an adult population at an age prone to developing demyelinating diseases. Consequently, and on the basis of the results of this

study, it is difficult to ascertain whether the reported cases simply corresponded to fortuitous associations or if some of them were caused or anticipated by this massive immunization in predisposed people. In any case, our findings point to two conclusions: (i) if there was a link between the HB vaccine and central demyelination, this link is weak since our results allow to rule out a strong association (e.g. a relative risk higher than 2); and (ii) the current recommendations adopted in most low-endemic countries and targeting newborns with a possible catch-up of at-risk adults should remain the preferred strategy. If those recommendations had been followed, a major crisis would have been avoided and the acceptability of the HB vaccine would have been greater.

4.3.1.4.4 Strengths and limitations

Our OE analyses used two different approaches both based on a conservative hypothesis for estimating the size of the exposed population. Indeed, the total number of subjects receiving HB vaccination, which was estimated from the total number of vaccine doses sold divided by four or three depending upon the period was 26,401,946, while two previous publications provided similar estimates (Fourrier et al., 2001; Lévy-Bruhl et al., 1999). The French National Institute for Public Health communicated a compatible figure for a longer period with about 37 million people exposed to the vaccine between 1981 and 2010.(Haute Autorité de Santé, 2016) In addition, a secondary analysis was performed to test the robustness of the findings, with results converging with the main analyses.

Limitations should also be acknowledged. First, our estimation of the size of the exposed population was derived from the number of doses of vaccine sold, assuming that all people had completed the primo-immunization schedule with the four or three recommended injections. Therefore, we cannot exclude that the actual exposed population was in fact larger than that considered in our computations, making, in any case, our estimations more conservative. Nevertheless, the use of a second approach based on the person-year at risk circumvented the problem, as this method was independent of the size of the exposed population.

As discussed in section 3.3.2 Multiple sclerosis, precise and robust data about the baseline annual incidence rates of MS over our study period are scarce, especially for the first part of

the period (i.e., 1984 – 1993). Thus, for these 10 years, we had to extrapolate the annual incidence rates using both demographic growth and the linear increasing trend for MS observed between 1994 and 2000. Moreover, it has recently been estimated that, for various reasons including changes in diagnostic criteria, annual incidence rates for MS reported in the 1990s were likely to be underestimated by approximately 11 to 29%.(Fromont et al., 2012) However, the impact of the two latter limitations on our conclusions is likely to be minimal, as both tended to reduce the value of the OE ratios. Finally, the time-window chosen as being at risk (i.e., 1 month) is debatable given that in reported cases the median time-to-onset was found to be around 2.5 months. No clear consensus has been established so far on this point but most authors having assessed the putative link between HB vaccination and central demyelination used a window comprised between 0 and 3 months. We used a one-month window both for making our analyses more "specific" (expanding this window to 2 or 3 months would have led to decreasing the OE ratios and for practical reasons. Indeed, given the vaccination schedule (0, 1, 6 and 12 months), choosing a time window of 2 months or larger precluded the use of the "person-years" approach since the periods "at risk" for doses 1 and 2 would have overlapped. As presented in section 2.1.2 Observed-to-expected analyses, the use of an at-risk time-window with an overlap within the immunization schedule imposed to know the number of subjects receiving 1, 2, 3 or more doses, this information being not available from the sources we used.

4.3.1.5 Disproportionality analyses within VAERS

As the access to the French pharmacovigilance database is not public, a disproportionality analysis (DPA) was conducted within the VAERS data source (cf. description of this data source in section 2.5.2.1 United States). Results were published recently (Mouchet & Begaud, 2018b) and discussed by other vaccine experts. (Cohen, Houdeau, & Khromava, 2018; Mouchet & Begaud, 2018a)

4.3.1.5.1 Objectives

By using VAERS, a DPA was conducted to compare the frequency of central demyelination cases reported after anti-HBV vaccination *versus* any other vaccination when administered to a similar population. The primary objective was to estimate the Proportional Reporting

Ratio (PRR) and Reporting Odds Ratio (ROR) of MS having occurred within the 120 days following HB vaccination for adults aged 19 to 49 years when compared with other vaccines.

4.3.1.5.2 Methods

Data source: VAERS receives around 30,000 reports annually, with 13% of them classified as serious (i.e., associated with disability, hospitalization, life-threatening condition or death). Since 1990, VAERS has received over 200,000 reports, most of them consisting of non-serious symptoms such as fever. For the present study, the period from VAERS inception (i.e., cases occurring before 1980) to 26 August 2017 (last date of data extraction) was considered for analysis.

Study population: Cases were defined as reports of MS following immunization with vaccines containing a HB antigen and registered in the VAERS database since the implementation of vaccination programmes against HB. Non-cases were defined as reports of any event other than MS following immunization with vaccines containing a HB antigen and registered in the VAERS database. The reference group included "Other vaccines cases" (i.e., reports of MS following immunization with any vaccine other that HB vaccine) and "Other vaccines non-cases" (i.e. reports of any event different to MS following immunization with any vaccine other that HB vaccine).

Only cases and non-cases aged between 19 and 49 years at the date of the occurrence of the event were considered. This age category was retained as it represents the life period at risk for developing multiple sclerosis according to, among others, the US national MS society (National Multiple Sclerosis Society, 2018).

Vaccine exposure: Six different categories, including five multivalent vaccines, were found in VAERS for vaccines containing a HB antigen (cf. Table 16).

Table 16 : Vaccine categories, brand names and associated codes used for vaccines containing a hepatitis B antigen

Vaccine Category	Brand Names and Associated VAERS* Codes
HEP: Hepatitis B vaccine	ENGERIX-B – code 38, GENHEVAC B – code 1069, RECOMBIVAX HB – code 25, NO BRAND NAME – code 110, FOREIGN – code 24
HEPAB: Hepatitis A and Hepatitis B vaccine	TWINRIX – code 1009, NO BRAND NAME – code 1114
DTPHEP: Diphtheria, Tetanus, Pertussis and Hepatitis B	TRITANRIX – code 914, NO BRAND NAME – code 1112
DTAPHEPBIP: Diphtheria, Tetanus toxoids, acellular Pertussis, Hepatitis B and inactivated Poliovirus	PEDIARIX – code 1082, FOREIGN – code 1146, NO BRAND NAME – code 1110
6VAX-F: Diphtheria, Tetanus toxoids, acellular Pertussis, inactivated Poliovirus, Haemophilus influenza B and Hepatitis B	HEXAVAX – code 1047, FOREIGN – code 1139, NO BRAND NAME – code 1111
HBHEPB: Haemophilus b Conjugate, (Meningococcal Protein Conjugate) and Hepatitis B (Recombinant)	COMVAX – code 287, NO BRAND NAME – code 288

Only events having occurred within 120 days after injection of one dose were considered. HB vaccines induce specific humoral antibodies against HBsAg protective against the HB infection (anti-HBs titer greater than 10 IU/L) within one month after injection and then HB vaccine–induced antibody levels wane over time (European Medicine Agency, 2000). As mentioned earlier, focusing on this short period (0-120 days) allowed us to “maximize” the chances of observing a true pharmacovigilance signal, if any, by restricting analyses to the period potentially at highest risk. In addition, it could be questionable considering events having occurred several years after vaccine administration as potentially attributable. Moreover, the fact that information contained in the VAERS database did not allow an extensive control for potential confounders (other vaccines or drug exposures, medical

history, etc.), was another reason to focus on events having occurred within a short time window after vaccine exposure.

Outcomes of interest: Primary outcomes included the following events: multiple sclerosis, progressive multiple sclerosis, progressive relapsing multiple sclerosis and relapsing-remitting multiple sclerosis. As diagnosis of MS requests at least one attack (often two considered) and one MRI-detectable clinical lesion (Karussis, 2014), it requires a minimal duration of observation to be valid. Consequently, a sensitivity analysis was performed by excluding cases diagnosed within 9 days after the injection. In addition, a sensitivity analysis was performed using a broader category of demyelinating diseases including ADEM, demyelination, CIS, MS, TM, NMO, NMO spectrum disorder, progressive MS, progressive relapsing MS, relapsing-remitting MS, nervous system disorder, neurological examination abnormal and neurological symptom.

Multiple sclerosis relapses were excluded from the events of interest, given that the present analysis focused on the occurrence of a first episode of MS or central demyelination. Corresponding codes used in VAERS are detailed in Table 17.

Table 17: Vaccine Adverse Event Reporting System (VAERS) codes of outcomes of interest

Type of analysis	Events and codes used
Primary analysis	multiple sclerosis – code 10028245, progressive multiple sclerosis – code 10053395, progressive relapsing multiple sclerosis – code 10067063, relapsing-remitting multiple sclerosis – code 10063399.
Sensitivity analysis	acute disseminated encephalomyelitis (ADEM) – code 10000709, demyelination – code 10012305, clinically isolated syndrome (CIS) – code 10071068, multiple sclerosis – code 10028245, myelitis transverse – code 10028527, neuromyelitis optica (NMO) – code 10029322, NMO spectrum disorder – code 10077875, progressive multiple sclerosis – code 10053395, progressive relapsing multiple sclerosis – code 10067063, relapsing-remitting multiple sclerosis – code 10063399, nervous system disorder – code 10029202, neurological examination abnormal – code 10056832 and neurological symptom – code 10060860.

Exclusion: *Multiple sclerosis relapse – code 10048393*

Data analysis: First, descriptive analyses of MS cases per vaccination type (HB *versus* any other vaccines) were carried out prior to calculating any disproportionality ratios (PRR or ROR). The distribution of cases per the following age categories (18-29 years, 30-39 years, 40-49 years) and per gender was documented. The geographical location of cases, either

American, foreign or unknown, was also considered. VAERS also receives reports from US manufacturers which are transmitted by their foreign subsidiaries. Indeed, according to the FDA regulations, if a manufacturer is notified of a foreign case report that related to an event that is both serious and unexpected, it is required to submit it to VAERS. Time to onset between immunization and the event of interest, in addition to the year of vaccination, were also detailed. To conduct such analyses, VAERS data extracts were obtained through the CDC WONDER (Wide-ranging Online Data for Epidemiologic Research) which is an easy-to-use, menu-driven system requiring no computer expertise or special software. 'N-1' chi-squared tests were used to compare proportions for each descriptive variable per group (i.e., MS cases following HB vaccination *versus* those following any other vaccination).

As DPA represents the primary class of analytic methods for analyzing data from SRSs in a drug safety surveillance perspective (Zorych, Madigan, Ryan, & Bate, 2013), we conducted such an analysis by using a two-by-two contingency table. The latter was populated with the "HB cases" (i.e., reports of MS following immunization with any vaccine containing a hepatitis B antigen), the "HB non-cases" (i.e. reports of any event other than MS following immunization with any vaccine containing a HB antigen), the "Other vaccines cases" (i.e., reports of MS following immunization with any vaccines other than HB vaccine), and the "Other vaccines non-cases" (i.e. reports of any event different to MS following immunization any vaccines other than HB vaccine). Results were expressed as PRR and ROR according to the following formulas (cf. section 2.1.1 Disproportionality analyses):

PRR = a/e * c/f and ROR= ad / bc, where
a is the number of MS cases following HB vaccination
b is the number of non-MS cases following HB vaccination
c is the number of MS cases following other vaccination (non-HB)
d is the number of non-MS cases following other vaccination (non-HB)
e is the total of cases (MS and non-MS) following HB vaccination
f is the total of cases (MS and non-MS) following other vaccination (non-HB)
These ratios were provided with their 95% confidence intervals. Both measures (PRR and ROR) have shown to be of importance for assessing potential signals in SRS. (Waller, van Puijenbroek, Egberts, & Evans, 2004) Ratios were estimated globally and then by region (US *versus* foreign). Events associated with an "unknown" vaccine were excluded from the

present analysis. As recommended by Evans et al. (Evans et al., 2001), Chi-squared tests with a Yates' correction were estimated for PRR. In addition, sensitivity analyses using a broader category of demyelinating events (e.g., ADEM, NMO, etc.) were conducted. A sensitivity analysis per vaccine type (multivalent *versus* single hepatitis B vaccine) was planned.

4.3.1.5.3 Results

Descriptive overview of cases: No significant difference was observed between MS cases following HB vaccination and those following another vaccination, except for the geographical origin and the years of vaccination. MS cases following HB vaccination were more likely to be of foreign origin and less likely to be American when compared to MS cases following any other immunization. In addition, MS cases following HB vaccination were more likely to be reported before 2000 whereas MS cases following any other vaccination were more frequently reported after 2000 (cf. Table 18).

Table 18: Descriptive analysis of MS cases reported to VAERS per vaccination type (HB *versus* any other vaccine)

		MS cases following HB vaccination		MS cases following any vaccination (except HB)		p-value
		N	%	N	%	
Symptoms	Multiple sclerosis	180	100.0%	180	99.4%	*0.2986*
	Relapsing-remitting multiple sclerosis	0	0.0%	1	0.6%	
Gender	Female	134	74.4%	125	69.1%	*0.3442*
	Male	45	25.0%	55	30.4%	*0.5515*
	Unknown	1	0.6%	1	0.6%	
Age	18-29	68	37.8%	79	43.6%	*0.4773*
	30-39	68	37.8%	71	39.2%	*0.8658*
	40-49	44	24.4%	31	17.1%	*0.4507*
Onset Interval	0-9 days	66	36.7%	90	49.7%	*0.1074*
	10-14 days	8	4.4%	15	8.3%	*0.7318*
	15-30 days	28	15.6%	23	12.7%	*0.7706*
	31-60 days	30	16.7%	27	14.9%	*0.8539*
	61-120 days	48	26.7%	26	14.4%	*0.2287*
Origin of cases	US	53	29.4%	97	53.6%	***0.0045***
	Unknown	6	3.3%	13	7.2%	*0.7454*
	Foreign	121	67.2%	71	39.2%	***0.0002***
Year of vaccination	Range		1987 - 2015	Range	1968 - 2016	
	1987-2000	128	71.1%	1968-2000	57	31.5%	***<0.0001***
	2001-2017	52	28.9%	2001-2017	124	68.5%	***<0.0001***

Vaccine Type	Hepatitis B	163	90.6%	Influenza vaccine	61	27.5%	***NA***
	Hepatitis A and B vaccine	17	0.4%	Human papillomavirus vaccine	38	17.1%	
				Anthrax vaccine	15	6.8%	
				Hepatitis a	13	5.9%	
				Typhoid vaccine	13	5.9%	
				Poliovirus vaccine	9	4.1%	
				Rabies virus vaccine	9	4.1%	
				Tetanus toxoid	6	2.7%	
				Meningococcal vaccine	5	2.3%	
				Pneumococcal vaccine	5	2.3%	
				Varivax-varicella virus live	5	2.3%	
				Yellow fever vaccine	3	1.4%	
				Lyme vaccine	2	0.9%	
				Bacillus Calmette-Guerin vaccine	1	0.5%	
				Cholera vaccine	1	0.5%	
				Mumps virus vaccine	1	0.5%	
				Plague vaccine	1	0.5%	
				Smallpox vaccine	1	0.5%	
				Tick-borne encephalitis vaccine	1	0.5%	
				Combined vaccines	32	14.4%	

Disproportionality analysis: All computed ratios (both PRR and ROR) were above the classic cut-off value of 2 (routinely used to identify signals (European Medicine Agency, 2006; Evans et al., 2001)) and were found to be statistically significant. ROR ranged from 3.48 to 5.62 with 95%CI not overlapping 1 and PRR gave very similar estimates (ranging from 3.48 – 5.56) with Chi square tests over 4. Both ratios were concordant. It should also be noted that ratios were similar regardless of their geographical origin (US or foreign) (cf. Table 19).

Table 19 : Reporting ratios for multiple sclerosis per region considered

	MS*	Other events	ROR (95%CI)	PRR (Yates' chi-square; p value)
Global (US + non-US + unknown)				
HB vaccine	180	76,740	5.62	5.56
Other vaccines (except HB)	181	429,951	(4.57-6.91)	(335.16; 0)
US only (+ unknown)				
HB vaccine	59	61,203	3.48	3.48
Other vaccines (except HB)	110	397,331	(2.54-4.78)	(66.03; 0)
Non-US only				
HB vaccine	121	15,537	3.58	3.56
Other vaccines (except HB)	71	32,620	(2.67-4.80)	(81.22; 0)

***Symptoms included:** multiple sclerosis, progressive multiple sclerosis, progressive relapsing multiple sclerosis, relapsing-remitting multiple sclerosis*

The sensitivity analysis which excluded the MS cases having occurred within 9 days after injection of one dose led to higher ratios with ROR= 7.02 (95%CI: 5.33-9.25) and PRR= 7.01 ($p<0.05$) for all regions combined (US, foreign and unknown).

Sensitivity analyses using a broader category of demyelinating events led to different patterns (cf. Table 20). When considering all origins (US, Unknown and Foreign), lower but still statistically significant estimates were observed for both PRR and ROR. Moreover, both estimates remained above the threshold of 2 considered for a signal generation. However, when considering each region separately, PRR and ROR for cases of foreign origin were still above this cut-off of 2, while, for American cases, ROR and PRR remained under the threshold. In other words, reporting seemed lower for these less specific events than for MS, at least for cases originating from US.

Table 20: Sensitivity analyses using a broader definition of events

	Cases*	Other events	ROR (95%CI)	PRR (Yates' chi-square; p value)
Global (US + non-US + unknown)				
HB vaccine	342	76,578	2.88	2.88
Other vaccines (except HB)	665	429,467	(2.53-3.29)	(273.79; 0)
US only (+ unknown)				
HB vaccine	102	61,160	1.52	1.52
Other vaccines (except HB)	436	397,005	(1.22-1.88)	(14.14; 0)
Non-US only				
HB vaccine	240	15,418	2.21	2.19
Other vaccines (except HB)	229	32,462	(1.84-2.65)	(75.48; 0)

***Symptoms included:** acute disseminated encephalomyelitis (ADEM), demyelination, clinically isolated syndrome (CIS), multiple sclerosis, myelitis transverse, neuromyelitis optica (NMO), NMO spectrum disorder, progressive multiple sclerosis, progressive relapsing multiple sclerosis, relapsing-remitting multiple sclerosis, nervous system disorder, neurological examination abnormal and neurological symptom.*

No sensitivity analysis per vaccine type (multivalent *versus* single HB vaccine) was carried out as the majority of cases (n=163, 90.6%) were reported after a monovalent HB vaccine.

4.3.1.5.4 Discussion

The main finding of this disproportionality analysis within the VAERS database is that cases of MS were reported significantly more after HB vaccination than after any other vaccination. As explained in section 2.1.1 Disproportionality analyses, a PRR based on more than 3 cases, being equal to or greater than 2 and with a Chi square test equal to or greater than 4 should be considered as a potential signal. For ROR, a cut-off value of 2 with a lower bound of the 95% confidence interval over 1 is routinely used to identify signals (European Medicine Agency, 2006; Evans et al., 2001). Although disproportionality analyses are mainly suited for hypothesis generation and not for causal inference, all our ratios met these requirements and the sensitivity analyses did not alter the global conclusions. Surprisingly, the magnitudes of RORs and PRRs were congruent across US and foreign cases, at least for the primary analysis. This would mean that the disproportionality was still significant regardless of the geographic origin of cases, in conflict with the common belief that a putative link between HB and MS is solely a European, if not French, debate. As the safety profile of a vaccine may differ substantially according to the target or joint populations, our estimates of disproportionality were restricted to reports implying adults (i.e., 18-49 years).

That allowed a comparison across groups *a priori* having a similar background risk, as recommended by the European Medicines Agency in the guideline on good pharmacovigilance practices (European Medicine Agency, 2013).

4.3.1.5.1 Strengths and limitations

To our knowledge, this DPA is the only recent VAERS analysis for MS cases following HB vaccination. A previous paper published in 2005 reported concordant findings (David A Geier & Geier, 2005). In that study, adults having received a HB vaccine had a significant increased odds ratio for MS (OR = 5.2, $p < 0.0003$, 95% CI: 1.9 - 20) contrary to the tetanus-containing vaccine exposed group. In addition, we chose to estimate two different disproportionality ratios (PRR and ROR). The fact that both provided quite similar results reinforces the confidence regarding the robustness of our conclusions.

Nevertheless, several limitations should be acknowledged. First, VAERS is a SRS allowing anyone (e.g. vaccine providers, other healthcare givers, vaccine recipients and their relatives, manufacturers, attorneys and other stakeholders) to report adverse events (Ball et al., 2002). However, as the heated debate about this potential link was mainly publicized in Europe and particularly in France, a notoriety bias seems rather unlikely in the US. This is supported by the fact that reporting ratios found in this study were of the same order regardless of their geographical origin. Furthermore, the lack of standardized diagnosis may have hampered the validity of reported events. In their study, Ball et al, 2002 (Ball et al., 2002) highlighted the limited information contained in many reports. Indeed, after an independent review of VAERS reports by three neurologists, 32% of reviewed cases of MS showed insufficient data to confirm the disease diagnosis. This pleads for the need of supplemental collection for follow-up data and recalls that the results of analyses based on VAERS reports should be interpreted with caution. However, one can assume that this potential misclassification or diagnostic bias was unlikely to differ across the HB-vaccine exposed and the reference groups.

4.3.2 Systematic review of observational comparative studies testing the research question n°1

Since 1996, several observational studies were conducted worldwide to determine whether an association actually existed between central demyelination and anti-hepatitis B vaccination.

A systematic literature review (SLR) and meta-analysis were conducted and published in 2018. (Mouchet et al., 2018a) Methods and results of the SLR are reported hereafter while the methods and results of the meta-analysis are reported in the corresponding sections: 4.3.3 Meta-analysis

4.3.2.1 Objectives

The main objective of the SLR was to identify all observational studies having evaluated the putative link between central demyelination and anti-HBV vaccination.

4.3.2.2 Methods

Data sources and searches: A systematic review was carried out in Medline, Embase, ISI Web of Science, and The Cochrane Library from inception to 10 May 2017. A combination of terms related to vaccination/vaccines and neurological events were used to find pertinent studies (cf. Table 21).

Table 21 : Search strategies used for the systematic literature review

Source	Search string	Terms used
MEDLINE	1	exp Viral Hepatitis Vaccines/
	2	exp Demyelinating Autoimmune Diseases, CNS/ OR exp Guillain-Barre Syndrome/
	3	1 and 2
	4	"vaccin*".ab,ti.
	5	"demyelinat*".ab,ti. OR multiple sclerosis.ab,ti. OR guillain barre.ab,ti. OR acute disseminated encephalomyelitis.ab,ti. OR optic neuritis.ab,ti. OR neuromyelitis optica.ab,ti. OR transverse myelitis.ab,ti. OR acute haemorrhagic leucoencephalomyelitis.ab,ti. OR acute haemorrhagic leuco-encephalomyelitis.ab,ti. OR acute haemorrhagic leuco-encephalitis.ab,ti. OR acute hemorrhagic leuco-encephalitis.ab,ti. OR

		acute hemorrhagic leuco-encephalomyelitis.ab,ti. OR diffuse cerebral encephalomyelitis.ab,ti. OR diffuse cerebral encephalitis.ab,ti. OR acute partial myelitis.ab,ti. OR chronic progressive inflammatory myelopathy.ab,ti.
	6	4 and 5
	7	3 OR 6
EMBASE	**1**	exp hepatitis vaccine/
	2	exp demyelinating disease/ OR exp Guillain Barre syndrome
	3	1 and 2
	4	"vaccin*".ti,ab.
	5	"demyelinat*".ab,ti. OR multiple sclerosis.ab,ti. OR guillain barre.ab,ti. OR acute disseminated encephalomyelitis.ab,ti. OR optic neuritis.ab,ti. OR neuromyelitis optica.ab,ti. OR transverse myelitis.ab,ti. OR acute haemorrhagic leucoencephalomyelitis.ab,ti. OR acute haemorrhagic leuco-encephalomyelitis.ab,ti. OR acute haemorrhagic leuco-encephalitis.ab,ti. OR acute hemorrhagic leuco-encephalitis.ab,ti. OR acute hemorrhagic leuco-encephalomyelitis.ab,ti. OR diffuse cerebral encephalomyelitis.ab,ti. OR diffuse cerebral encephalitis.ab,ti. OR acute partial myelitis.ab,ti. OR chronic progressive inflammatory myelopathy.ab,ti.
	6	4 and 5
	7	3 OR 6
COCHRANE LIBRARY	**1**	MeSH descriptor: [Viral Hepatitis Vaccines] explode all trees
	2	MeSH descriptor: [Demyelinating Autoimmune Diseases, CNS] OR [Guillain-Barre Syndrome] explode all trees
	3	1 AND 2
	4	Vaccin* (ti/ab/kw)
	5	"demyelinat*".ab,ti. OR multiple sclerosis.ab,ti. OR guillain barre.ab,ti. OR acute disseminated encephalomyelitis.ab,ti. OR optic neuritis.ab,ti. OR neuromyelitis optica.ab,ti. OR transverse myelitis.ab,ti. OR acute haemorrhagic leucoencephalomyelitis.ab,ti. OR acute haemorrhagic leuco-encephalomyelitis.ab,ti. OR acute haemorrhagic leuco-encephalitis.ab,ti. OR acute hemorrhagic leuco-encephalitis.ab,ti. OR acute hemorrhagic leuco-encephalomyelitis.ab,ti. OR diffuse cerebral

		encephalomyelitis.ab,ti. OR diffuse cerebral encephalitis.ab,ti. OR acute partial myelitis.ab,ti. OR chronic progressive inflammatory myelopathy.ab,ti.
	6	4 AND 5
	7	3 OR 6
WEB OF SCIENCE	**1**	TS= (Demyelinat* OR multiple sclerosis OR guillain barre acute disseminated encephalomyelitis OR optic neuritis OR neuromyelitis optica OR transverse myelitis OR acute haemorrhagic leucoencephalomyelitis OR acute haemorrhagic leuco-encephalomyelitis OR acute haemorrhagic leuco-encephalitis OR acute hemorrhagic leuco-encephalitis OR acute hemorrhagic leuco-encephalomyelitis OR diffuse cerebral encephalomyelitis OR diffuse cerebral encephalitis OR acute partial myelitis OR chronic progressive inflammatory myelopathy)
	2	TI=vaccin*
	3	1 AND 2 refined to the WOS databases

Pragmatic searches were conducted and bibliographical references of reviews were also screened (i.e. snowballing). No restriction regarding the language or time period was applied. This study followed the recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) statement and the Meta-analysis of Observational Studies in Epidemiology (MOOSE) reporting guideline. (Moher, Liberati, Tetzlaff, Altman, & Group, 2009; Stroup et al., 2000)

Study selection: Eligibility criteria were defined according to the PICOS criteria.(Moher et al., 2009) As a randomized controlled trial would not be *a priori* not ethically acceptable in the present case and would have a good chance to be underpowered for assessing rare outcomes following immunization, only observational studies (i) based upon a comparative design, (ii) having performed matching and/or adjustment on subjects' characteristics at an individual level (i.e., studies considering aggregate data were excluded) and reporting a crude or adjusted relative estimate of risk (i.e. Relative Risk, RR; Odds Ratio, OR; Hazard Ratio, HR; Incidence Rate Ratio, IRR) of developing an acute central demyelinating disorder following vaccination against hepatitis B were selected. Uncontrolled studies (e.g., case

reports case series, expert opinions, ecological studies) as well as “case/non-case” studies (i.e. disproportionality analyses within a pharmacovigilance database) were excluded. Both adults and children were considered. Publication type included peer-reviewed articles and abstracts. The latter were included when sufficient data was presented and no full article was available after contacting the authors.

Outcomes of interest were defined as an incident neurological adverse event including MS and central demyelinating disorders. MS had to be diagnosed or confirmed by a neurologist using established diagnostic criteria, which require the occurrence of at least one central demyelination attack and the demonstration of dissemination of central nervous system lesions in space and time (cf. section 3.2.1 Multiple sclerosis). Relapses of MS, which probably rely on a different pathophysiological mechanism (under the assumption of a causal association), were not considered as an outcome for the present analysis.

Two investigators (Julie Mouchet, University of Bordeaux, France and Emanuel Raschi, University of Bologna, Italy) reviewed the titles and abstracts of all retrieved citations independently. Disagreements were solved through discussion. In the event of doubt, a third person (Bernard Bégaud, University of Bordeaux) was asked to confirm the selection of the study.

Data extraction and quality assessment: For all publications finally retained, data extraction concerned the following items: study design, population characteristics (number of subjects in each group, mean or median age, gender, risk factors for central demyelination or multiple sclerosis), medical event considered, study period, vaccine exposure, crude and adjusted risk estimates and statistical analysis. When necessary, authors of selected publications were contacted to obtain additional information. Quality of each selected study was assessed by using the Newcastle Ottawa Scale for cohort and case-control designs. (Wells et al., 2006) The strength of the evidence generated was evaluated with the GRADE framework. (Guyatt et al., 2011; Meerpohl et al., 2011)

The protocol of the SLR and meta-analysis (n° CRD42015020808) was published on the PROSPERO platform (https://www.crd.york.ac.uk/prospero/) before running the study.

4.3.2.3 Results

Of the 2,804 references identified, thirteen articles describing epidemiological studies including a control group were selected for the SLR (cf. Figure 36). (Ascherio et al., 2001; DeStefano et al., 2003; Eftekharian, Mousavi, Hormoz, Roshanaei, & Mazdeh, 2014; Hernan, Jick, Olek, & Jick, 2004; Hocine et al., 2007; Langer-Gould, Qian, et al., 2014; Mikaeloff, Caridade, Rossier, Suissa, & Tardieu, 2007; Mikaeloff, Caridade, Suissa, & Tardieu, 2009; Ramagopalan et al., 2009; Sturkenboom et al., 1999; Emmanuel Touze et al., 2002; E. Touze, Gout, Verdier-Taillefer, Lyon-Caen, & Alperovitch, 2000; Zipp, Weil, & Einhäupl, 1999)

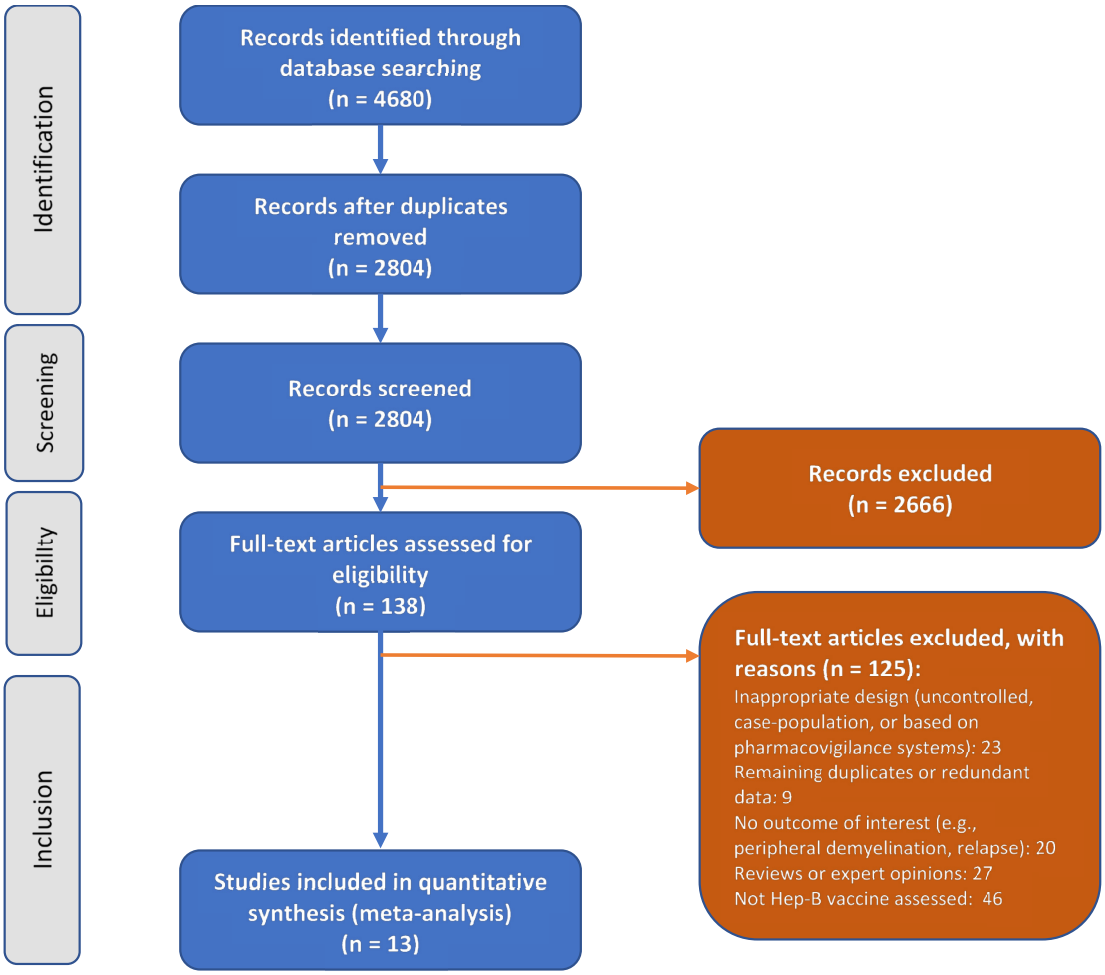


Figure 36 : PRISMA Flow chart

Seven intended to evaluate the link between HB vaccination and the occurrence of MS (Ascherio et al., 2001; DeStefano et al., 2003; Eftekharian et al., 2014; Hernan et al., 2004; Mikaeloff et al., 2007; Ramagopalan et al., 2009; Sturkenboom et al., 1999), two considered central demyelination more broadly (E. Touze et al., 2000; Zipp et al., 1999), and four

investigated both outcomes. (Hocine et al., 2007; Langer-Gould, Qian, et al., 2014; Mikaeloff et al., 2009; Emmanuel Touze et al., 2002)

Table 22 presents the main characteristics of the studies retained for the SLR and meta-analysis, which included a total of 16,799 cases and 15,908 controls for the case-control studies and 134,698 individuals for the historical cohort.

Table 22 : Studies selected for meta-analysis

Reference	Country	Study design	Study period	Sample size	Outcome assessed	Population source	Time window considered at risk	Statistical methods used for bias control	Quality (Newcastle Ottawa Scale – max 9 stars)
Ascherio 2001	USA	Nested case-control	1976 to 1998	Cases: n=192 Breast KC controls: n=111 Healthy controls: n=534	MS	Nurses' Health Study and the Nurses' Health Study II	≤ 2 years Anytime	Matching on year of birth, study cohort, and year of diagnosis (for controls with breast cancer). Adjustment for pack-years of smoking at baseline, latitude of residence at birth (north, middle, or south), history of infectious mononucleosis, history of measles or mumps after the age of 15, and ancestry (Scandinavian, southern European, other white, or non-white)	7 stars
DeStefano 2003	USA	Case-control	January 1, 1995, through December 31, 1999	Cases: n=440 Controls: n=950	MS	3 HMOs that participate in the Centers for Disease Control and Prevention's Vaccine Safety Datalink project	Anytime	Matching on age, sex and HMO Adjustment for race, ethnicity, ancestry (northern European or Scandinavian), family history of demyelinating or other autoimmune diseases, education, marital status, occupation, residency history, cigarette-smoking, pet ownership, and certain groups of high risk for hepatitis B (healthcare workers, dialyzed patients)	7 stars
Hernan 2004	UK	Nested case-control	January 1, 1993, and December 31, 2000.	Cases: n=163 Controls: n=1604	MS	GPRD database	≤3 years	Matching on age, sex, practice, and date of joining the practice Adjustment for age, sex, practice, and date of joining the practice, smoking, clinical course of disease, type of first symptoms	8 stars

Reference	Country	Study design	Study period	Sample size	Outcome assessed	Population source	Time window considered at risk	Statistical methods used for bias control	Quality (Newcastle Ottawa Scale – max 9 stars)
Hocine 2007	France	Self-Controlled Case Series	31 August 1993 - 31 December 1995	Cases: n=287	MS + CNS	18 departments of neurology	≤2 months	No matching as SCCS design (cases act as their own controls) Adjustment for age according to 4 models	8 stars
Langer-Gould 2014	USA	Nested case-control	January 1, 2008 to December 31, 2011	Cases: n=43 Controls: n=249	MS + CNS	Kaiser Permanente Southern California	≤3 months ≤3 years	Matching on date of birth, sex, and zipcode (a surrogate measure for socio-economic status) Adjustment for race/ethnicity, hospitalizations, outpatient visits, emergency department visits, comorbid chronic diseases, and infections within 6 months before symptom onset/index date	7 stars
Mikaeloff 2007	France	Case-control	January 1, 1994 and December 31, 2003	Cases: n=143 Controls: n=1122	MS	French Sclérose en Plaques neuropaediatric MS cohort	≤3 years	Matching on age, sex, and current area of residence Adjustment for age, sex, current area of residence, family history of MS (siblings or parents) and other autoimmune diseases (siblings or parents) and for profession of head of family	7 stars
Touzé 2000	France	Case-control (hospital based)	January 1st, 1994 to December 31th, 1995	Cases: n=121 Controls: n=121	CNS	Patients referred for first time to Fédération de Neurologie	≤2 months	Matching on age, sex and date of medical consultation or hospitalization Adjustment for age, marital status, birth country and urban/rural residence	8 stars

Reference	Country	Study design	Study period	Sample size	Outcome assessed	Population source	Time window considered at risk	Statistical methods used for bias control	Quality (Newcastle Ottawa Scale – max 9 stars)
Touzé 2002	France	Case-control	January 1st, 1994, and December 31st, 1995	Cases: n = 236 Controls: n = 355	MS + CNS	18 departments of neurology	≤2 months	Matching on gender, age and date of referral to neurology department Adjustment for age, exposure outside time window, marital status, number of children, education level, other vaccinations, health occupation, place of residence (urban/rural), country of birth	7 stars
Sturkenboom 1999 (abstract only)	UK	Case-control	Unknown	Cases: n=500 Controls: n=unknown	MS	GPRD database	≤2 months	Matching on age, gender and practice No information about possible adjustment (authors contacted)	Not assessed as only abstract available
Zipp 1999	USA	Historical cohort	1988 to 1995	Exposed: 27,229 Unexposed: 107,469	CNS	Healthcare database consisting of integrated pharmacy and medical claims from six Diversified Pharmaceutical Services affiliated HMO plans	≤2 months ≤3 years	Matching on age and sex No information about possible adjustment	7 stars
Eftekharian 2014	Iran	Case-control	January to May 2014	Cases: n=250 Controls: n=250	MS	Population referring to Hamadan multiple sclerosis society in west of Iran	Anytime	Matching on age and sex No information about possible adjustment	2 stars

Reference	Country	Study design	Study period	Sample size	Outcome assessed	Population source	Time window considered at risk	Statistical methods used for bias control	Quality (Newcastle Ottawa Scale – max 9 stars)
Mikaeloff 2009	France	Case-control	January 1, 1994, and December 31, 2003	Cases: n=349 Controls: n=2941	MS + CNS	French Sclerose en Plaques neuropaediatric MS cohort	≤3 years	Matching on age, sex, and current area of residence Adjustment for age, sex, current area of residence, familial multiple sclerosis history, family history of another autoimmune disease, parental smoking at home before index date, socio-professional status of head of family	6 stars
Ramagopalan 2009	Canada	Case-control	Unknown	Cases: n=14,362 Controls: n=7,671	MS	Canadian Collaborative Project on Genetic Susceptibility to Multiple Sclerosis (CCPGSMS)	Anytime	Adjustment on age and sex	7 stars

***Footnotes**: CNS: Central Nervous System Demyelination, GPRD: General Practice Research Database, HMO: Health Maintenance Organization, KC: Cancer, MS: Multiple Sclerosis, SCCS: Self-Controlled Case Series, USA: United States of America*

Except for the study conducted by Eftekharian et al., the quality of the studies evaluated by the Newcastle Ottawa Scale was good and comparable for all papers included ranging from six to eight stars (cf. Table 23 and Table 24).

Table 23 : Individual quality assessment evaluated with Newcastle Ottawa scale for case-control studies

	SELECTION					EXPOSURE			
	Is the case definition adequate?	Representativeness of the cases	Selection of controls	Definition of controls	Comparability of cases and controls on the basis of the design or analysis	Ascertainment of exposure	same method of ascertainement for cases and controls	Non response rate	**Total score (max 9 stars)**
DeStefano 2003	Yes, with independent validation	consecutive or obviously representative series of cases	community controls	no history of disease (endpoint)	Matching age, sex and HMO	written self report or medical record only	yes	non respondents described	
*********Score	1	1	1	1	2	0	1	0	7
Hernan 2004	Yes, with independent validation	consecutive or obviously representative series of cases	community controls	no history of disease (endpoint)	Matching on age, sex, practice, and date of joining the practice	written self report or medical record only	yes	same rate for both groups	
*********Score	1	1	1	1	2	0	1	1	8
Langer-Gould, 2015	Yes, with independent validation	consecutive or obviously representative series of cases	community controls	no description of source	Matching on date of birth (within1year), sex, and zipcode	written self report or medical record only	yes	same rate for both groups	
*********Score	1	1	1	0	2	0	1	1	7
Touzé 2000	Yes, with independent validation	consecutive or obviously representative series of cases	community controls	no history of disease (endpoint)	Matching on sex, age, date of hospitalization or consultation	written self report or medical record only	yes	same rate for both groups	
*********Score	1	1	1	1	2	0	1	1	8
Ascherio 2001	Yes, with independent validation	consecutive or obviously representative series of cases	community controls	no history of disease (endpoint)	Matching according to year of birth, study cohort, and (for the controls with breast cancer) date of diagnosis.	interview not blinded to case/control status	yes	rate different and no designation	
*********Score	1	1	1	1	2	0	1	0	7
Hocine 2007	Yes, with independent validation	consecutive or obviously representative series of cases	community controls	no history of disease (endpoint)	Self-controlled case series (cases serve as their own controls)	written self report or medical record only	yes	same rate for both groups	

*********Score	1	1	1	1	2	0	1	1	8
Touzé 2002	Yes, with independent validation	consecutive or obviously representative series of cases	hospital controls	no history of disease (endpoint)	MATCHING for gender, age (B 5 years) and date of referral to the neurology department (B 2 months)	written self report or medical record only	yes	same rate for both groups	
*********Score	1	1	0	1	2	0	1	1	7
Eftekharian 2014	No description	potential for selection biases or not stated	hospital controls	no description of source	MATCHING for age and sex	no description	no	non respondents described	
*********Score	0	0	0	0	2	0	0	0	2
Mikaeloff 2009	Yes, with independent validation	consecutive or obviously representative series of cases	community controls	no description of source	Matching on age, sex, and current area of residence	written self report or medical record only	yes	non respondents described	
*********Score	1	1	1	0	2	0	1	0	6
Ramagopalan 2009	Yes, with independent validation	consecutive or obviously representative series of cases	community controls	no history of disease (endpoint)	Adjustment on age and sex	written self report or medical record only	yes	non respondents described	
*********Score	1	1	1	1	2	0	1	0	7
Mikaeloff 2007	Yes, with independent validation	consecutive or obviously representative series of cases	community controls	no history of disease (endpoint)	Matching on age, sex, and current area of residence	written self report or medical record only	yes	non respondents described	
*********Score	1	1	1	1	2	0	1	0	7

Table 24 : Individual quality assessment evaluated with Newcastle Ottawa scale for cohort studies

	SELECTION					Outcome			
	Representativeness of the exposed cohort	Selection of the non exposed cohort	Ascertainement of exposure to implants	Demonstration that outcome of interest was not present at start of study	Comparability of cases and controls on the basis of the design or analysis	Ascertainment of outcome	Was FU long enough for outcomes to occur	Adequacy of FU of cohorts	**Total score (max 9 stars)**
Zipp 1999	somewhat representative of the average individuals in the community	drawn from the same community as the exposed group	secure record (eg surgical records)	no	Matched for sex and age	record linkage	yes (select an adequate FU period for outcome of interest)	No description of those lost or FU rate important	
*********Score	1	1	1	0	2	1	1	0	7

4.3.2.4 Discussion

All the studies included except the one conducted by Hernan et al. in 2004 yielded inconclusive findings, i.e. did not conclude on a significant increase in the risk of demyelinating disorders after vaccination.

In this regard, two studies came out as opposite outliers and deserve discussion. (Ascherio et al., 2001; Hernan et al., 2004) The case-control study by Ascherio et al, 2001 was nested in two cohorts of American women (Nurses' Health Study and Nurses' Health Study II). The authors concluded in the absence of association between hepatitis B vaccination and the subsequent development of MS, the relative risk being 0.7 (95% CI: 0.3 to 1.8) when considering the two years after vaccination. This value, which seems to suggest a protective effect, although not significant, of the vaccine that is *a priori* not supported by any biological plausibility, is surprising. In this respect, one should note that the percentage of women vaccinated against hepatitis B reported in this study was relatively low for a population of nurses (one of the groups considered as at-risk in any country and especially in the USA) and surprisingly lower in MS cases than in controls (51.8% *versus* 66.5%). It is noteworthy that exposure was self-reported by the participants. A proof of vaccination was sought only for women who had reported that they were vaccinated, and confirmation by vaccination records was ascertainable for only 96 out of 301 MS cases (i.e. 32%). Moreover, the very low number of cases (n=9) vaccinated during the two years preceding the disease onset precluded computing a risk estimate for a shorter time-window, e.g. 2 months, which could be more suitable for exploring an association with an acute neurological event. (Collet, MacDonald, Cashman, & Pless, 2000) It is worth noting that this research, on the contrary of other studies retained in our meta-analysis, included only women. However, no evidence of a difference in risk according to gender has been observed so far. (Ramagopalan et al., 2009) A limitation noted by Dr. Ascherio at the committee's March 2002 meeting was the lack of power to detect an increase in the risk of MS within two months after vaccination. He commented, however, that even if demyelination occurred within two months, it might take several months or years for clinical symptoms to become apparent. (Institute of Medicine Immunization Safety Review, 2002)

By contrast, Hernan et al, 2004 remain the only authors who concluded in a significant association between anti-hepatitis B vaccination and MS. This nested case-control study, conducted within the General Practice Research Database (GPRD) in the United Kingdom (UK) from January, 1st 1993 to December 31, 2000, produced a significant odds ratio of 3.1 (95%CI: 1.5, 6.3) after adjustment on age, gender, general physician practice, and date of joining the practice, but not on several putative risk factors such as race or ethnic ancestry. Exposure ascertainment used prospectively recorded data to minimize recall bias. However, records covering the three years preceding the first symptoms were available for only 163 of the 438 MS cases identified. As a consequence of the low adult immunization rate in UK (targeting only at-risk adult populations), only 11 of them were found to be vaccinated against hepatitis B. Interestingly, the authors did not find any association with the risk of MS for influenza and tetanus vaccines, which are *a priori* not suspect in that respect. (Farez & Correale, 2011) Geier et al. came to the same conclusion in 2005 with their study conducted in the VAERS database, the risk of developing MS after anti-hepatitis B vaccination being 5.2-fold higher than for anti-tetanus vaccination. (David A Geier & Geier, 2005)

The most recent study evaluating the risk of central demyelination after hepatitis B vaccination was published in 2014. (Langer-Gould, Qian, et al., 2014) Despite being conducted within a large population-based electronic medical records database (i.e. Kaiser Permanente Southern California), the statistical power required to conclude about such a risk was not achieved. Indeed, hepatitis B vaccination was uncommon in this population, with only 3.3% of controls and 4.0% of cases vaccinated in the 3 years prior index date or symptom onset. Therefore, considering a probably more relevant time-window shorter than one year was not feasible.

4.3.2.5 Strengths and limitations

The first strength of this SLR is that the methods used followed the highest current standards. Various data sources were screened and pragmatic searches were performed to complement findings identified from bibliographical databases. In addition, almost all studies selected for this research project were of good quality, having a NOS score ranging from 7 to 9. Nevertheless, some limitations should be pointed out. First, the variety of study designs used across the selected observational studies made the comparison between

studies difficult. Additionally, most studies did not provide clear-cut results, as most of them were inconclusive. A lack of statistical power was often cited by the authors, as one of potential weaknesses of their research. Finally, it should also be noticed that as for any observational studies, methodological limitations such as selection and information (e.g. recall) biases as well as confounding, should have been more appropriately discussed by the authors and were not always handled appropriately in all identified studies.

4.3.3 Meta-analysis centered on the research question n°1

To address the lack of power often put forward by authors of individual studies and in attempt to counter-balance some specific methodological flaws frequently pointed out in these individual studies (i.e., recall and selection biases), a meta-analysis was performed and published.(Mouchet et al., 2018a) Methods and key findings are presented in the sections below.

4.3.3.1 Objectives

Based on studies identified by the SLR (cf. section 4.3.2 Systematic review of observational comparative studies testing the research question n°1), a meta-analysis was performed to compile the results from the epidemiological studies conducted on both adults and children in order to determine the pooled risk of MS or central demyelination after anti-hepatitis B vaccination.

4.3.3.2 Methods

To conduct this meta-analysis, risk estimates and the corresponding 95% confidence intervals (95%CI) were extracted into Review Manager software [Review Manager (RevMan) [Computer program]. Version 5.3. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014]. In observational settings, authors generally provide several different risk estimates, so choosing the most relevant one for a meta-analysis is not an easy task and could be suspected of subjectivity. Indeed, the strength of the association between exposure and outcome can markedly vary according to the methodological options retained by the authors.

To cope with this difficulty, three different types of results were considered when provided by the authors:

- (i) crude (i.e. non-adjusted) risk estimate. Note: this may concern results obtained from matched sets for case-control studies *but* without further adjustment aiming at controlling for potential confounding,
- (ii) adjusted risk estimate highlighted as the most relevant (when several results were provided) by the authors of the publication,
- (iii) risk estimate computed, when feasible, within the three months following immunization. The latter was chosen for deriving a pooled estimate for a time-window roughly comparable across studies and *a priori* relevant for exploring a risk putatively induced by an acute (I.e. single dose) drug administration.

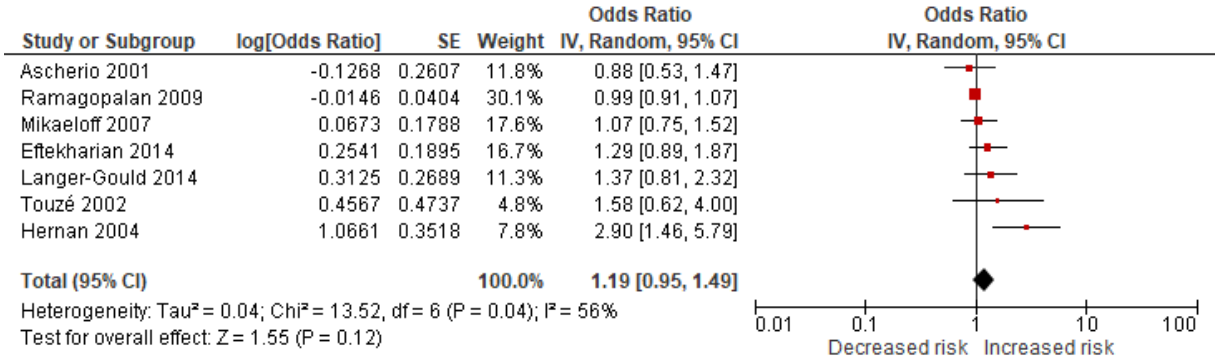
Forest plots were drawn accordingly. Given the non-randomized nature of the included studies and the adjusted odds ratios they provided, a generic inverse variance random-effect model was used to assess the overall risk estimate. (J. P. Higgins & Green, 2011)

Heterogeneity across the included studies was evaluated by the Q Cochran test, and p values <0.10 were considered as statistically significant. (J. Higgins & Thompson, 2002) I^2 statistics were also measured to quantify inconsistencies across estimates. (J. Higgins & Thompson, 2002) When present, source of heterogeneity was investigated. The selected studies were removed one by one from the model, the meta-analysis being repeated without the excluded study in order to obtain less heterogeneity. Subgroup analyses were performed according to the type of population considered for the meta-analysis (i.e., child *versus* adult), study design, and to the studies' methodological quality score. In order to challenge the consistency of findings drawn from non-experimental designs, the analysis was repeated using 99% confidence intervals. Since publication bias is particularly to be feared for non-interventional studies for which preliminary registration in a trial repository is not yet required by health authorities, we planned to test the funnel plot asymmetry provided that the number of studies retained for meta-analysis was larger than 10. Otherwise the power of the test was too low to distinguish chance from real asymmetry.(J. P. Higgins & Green, 2011)

4.3.3.3 Results

From the seven studies having reported crude risk estimates for MS, no statistically significant association was observed (cf. Figure 37), the pooled OR being 1.19 [95%CI 0.95 – 1.46]. The same was true for the association between central demyelination and HB vaccination (evaluated in five studies) with a pooled OR of 1.06 [95%CI 0.88 – 1.28]

A/ Outcome: multiple sclerosis



B/ Outcome: central demyelination

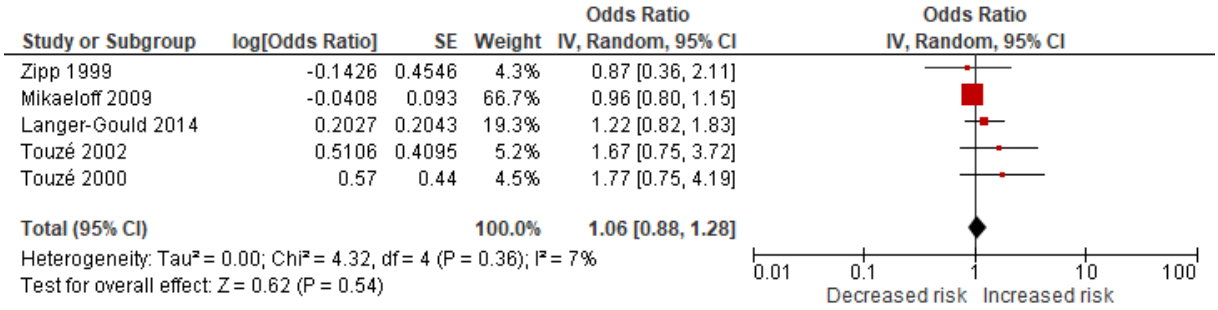
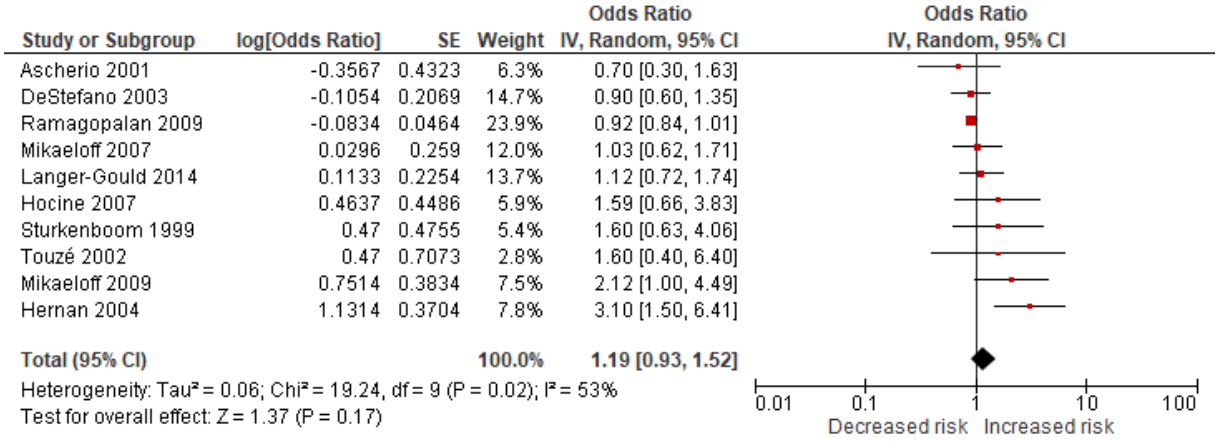


Figure 37: Forest plots of comparison for crude risk estimates following HBV vaccination

For the analysis based on adjusted risk estimates, the values obtained were similar for MS (i.e. 1.19 [95%CI: 0.93 – 1.52]) and slightly higher, without reaching statistical significance, for central demyelination (i.e. 1.25 [95%CI: 0.97 – 1.62]) (cf. Figure 38)

A/ Outcome: multiple sclerosis



B/ Outcome: central demyelination

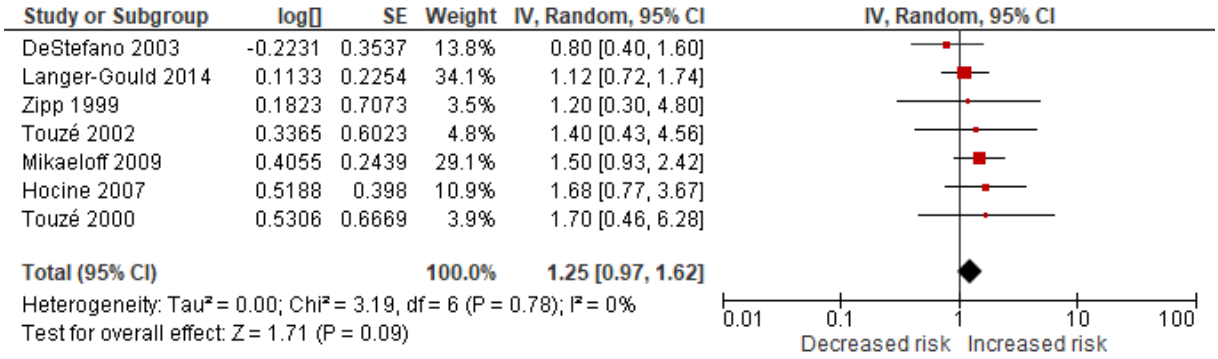
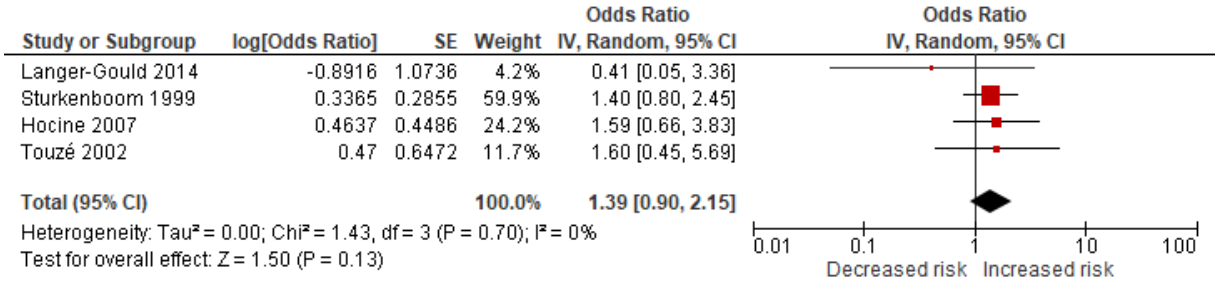


Figure 38: Forest plots of comparison with adjusted risk estimates following HBV vaccination

Finally, restricting the analysis to risk estimates within the 3-month period after vaccine injection led to the highest figures but, again, without reaching statistical significance, either for MS or for central demyelinating events, the pooled odds ratios being 1.39 (95%CI: 0.90 – 2.15) and 1.38 [95%CI: 0.82 – 2.34], respectively (cf. Figure 39).

A/ Outcome: multiple sclerosis



B/ Outcome: central demyelination

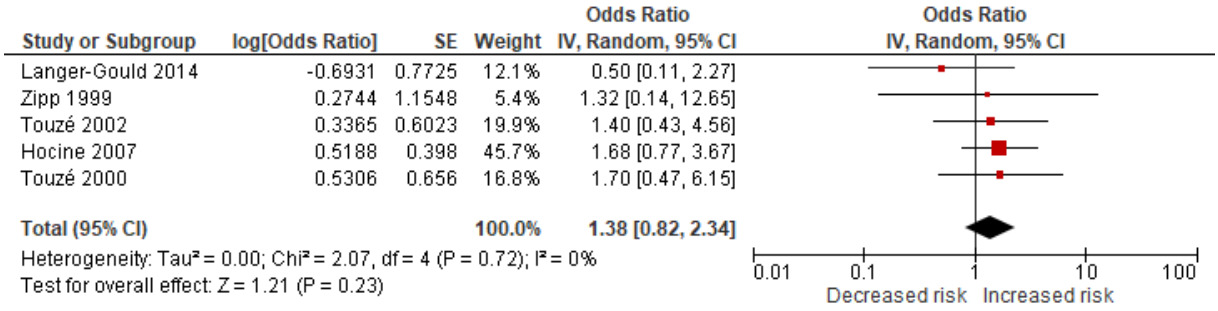


Figure 39 : Forest plots of comparison with risk estimated within 3 months after anti-hepatitis B vaccination

A moderate heterogeneity emerged when computing crude and adjusted pooled risks for multiple sclerosis (I2 =56 and 53%, respectively). Because the limited number of studies precluded the use of a meta-regression, the source of heterogeneity was assessed by removing studies one by one from the meta-analytic model. Only one study (Hernan et al., 2004) was found to introduce heterogeneity. Nevertheless, when it was excluded from the meta-analysis, the results were not markedly affected, even if the crude and adjusted pooled risks for MS decreased to 1.01 [95%CI 0.94 – 1.08] and 1.00 [95%CI 0.86 -1.16], respectively.

When computing crude and adjusted pooled risks for demyelination, heterogeneity was low or even null (I2 = 7 and 0%, respectively).

Results of the subgroup analyses are presented in Table 25. When considering the adult population only, crude risk pooled estimates were 1.25 [95%CI 0.94 -1.66] and 1.29 [0.93 - 1.76] for MS and central demyelination; whereas adjusted estimates were 1.11 [0.88 – 1.41] and 1.29 [0.86 – 1.95], respectively. The main conclusion was therefore not altered as statistical significance was not reached. Similar findings were obtained when restricting the studies to those having the highest quality scores evaluated by the Newcastle Ottawa Scale (i.e. > seven stars) or when restricting the meta-analysis to case-control studies only. When increasing the confidence level at 99%, no change was observed for pooled risk estimates but the intervals became slightly wider, as expected.

Table 25: Subgroup analyses

		Subgroup analyses			Wider Confidence Intervals (CI)	Reference
Scenario considered	**Outcome considered**	Adult pop only[a]	Case controls only[b]	Quality score assessed by Newcastle Ottawa scale >7[c]	99%CI	Pooled risk ratios [95%CI]
1/ Crude risk estimates	Multiple Sclerosis	1.25 [0.94 -1.66]	*No change*	1.19 (0.92 -1.54]	1.19 [0.89 - 1.60]	1.19 [0.95 - 1.46]
	Central demyelination	1.29 [0.93 -1.76]	1.13 [0.88 - 1.45]	1.29 (0.93 -1.76]	1.06 [0.83 – 1.35]	1.06 [0.88 - 1.28]
2/ Adjusted risk estimates	Multiple Sclerosis	1.11 [0.88 – 1.41]	1.17 [0.90 - 1.51]	1.09 [0.86 - 1.39]	1.19 [0.86 – 1.64]	1.19 [0.93 - 1.52]
	Central demyelination	1.29 [0.86 – 1.95]	1.10 [0.85 -1.42]	1.28 [0.90 - 1.82]	1.25 [0.89 - 1.76]	1.25 [0.97 - 1.62]
3/ Risk estimates within 3 months after vaccination	Multiple Sclerosis	*No change*	1.33 [0.81 -2.19]	1.38 [0.70 - 2.73]	1.39 [0.79 - 2.46]	1.39 [0.90 - 2.15]
	Central demyelination	*No change*	1.25 [0.56 -2.80]	*No change*	1.38 [0.69 -2.77]	1.38 [0.82 -2.34]

a. Exclusion of 2 studies [Mikaeloff et al, 2007 and Mikaeloff et al, 2009]
b. Exclusion of 2 studies [Zipp et al, 1999 and Hocine et al, 2007]
c. Exclusion of 3 studies [Sturkenboom et al, 1999 (not evaluated for quality) – Efthekarian et al, 2014 (NOS score = 2) and Mikaeloff et al, 2009 (NOS score = 6)]

As mentioned in section 4.3.3.2 Methods, checking the plausibility of a publication bias by observing the symmetry of a funnel plot was not recommended owing to the limited number of studies, i.e. 10 or fewer, included in the present meta-analysis. (J. P. Higgins & Green, 2011) The strength of the evidence was considered as low owing to the observational nature of studies included and the imprecision of the individual studies according to the GRADE framework (cf. Table 26).

Table 26 : Strength of evidence using GRADE framework

	Strength of evidence						Summary of findings		
Outcome	No of studies (Design)	Limitations	Inconsistency	Indirectness	Imprecision	Publication bias	Odds ratio (95% CI)	**Overall Strength**	**Importance**
Multiple Sclerosis	10 (observational)	Not serious[a]	Not serious[b]	Not serious[d]	Likely[e]	Unlikely	1.19 [0.93 - 1.52]	Low	High
Central demyelination	7 (observational)	Not serious[a]	Not serious[c]	Not serious[d]	Likely[e]	Unlikely	1.15 [0.90 - 1.46]	Low	High

a. A great majority of studies were of good quality as rated by Newcastle Ottawa Scale (score >7)

b. A moderate heterogeneity (I^2=53%) was found.

c. Heterogeneity was found to be null.

d. Population, outcomes and intervention of interest were homogeneous. No indirect comparison was made in selected studies.

e. Confidence of intervals of most risk estimates were large and overlapping the null effect (1).

4.3.3.4 Discussion

The main finding of this meta-analysis is that, for the six situations studied, none of the pooled risk estimates reached statistical significance for the association between anti-hepatitis B vaccination and the occurrence of both multiple sclerosis or central demyelination. Nevertheless, it should be noticed that all these non-significant pooled estimates converged toward a risk ratio around 1.2 - 1.3.

4.3.3.5 Strengths and limitations

This meta-analysis presents several strengths. Firstly, it includes multiple analyses based on three different *scenarii* in order to increase both the robustness and the confidence in the results. Secondly, the great majority of studies were judged as being of good quality, i.e. having individual scores based on the Newcastle Ottawa Scale equal to 7 stars and over. Thirdly, heterogeneity was evaluated as moderate or even null, allowing the selected studies to be pooled. Fourthly, it presents a clear added value to the body of evidence drawn from the five articles having already investigated this issue. (Demicheli, Rivetti, Pietrantonj, Clements, & Jefferson, 2003; Farez & Correale, 2011; Mailand & Frederiksen, 2016; Martínez-Sernández & Figueiras, 2013; Rutschmann, McCrory, Matchar, & Guidelines, 2002) Indeed, two of them were systematic reviews but are clearly outdated as they were published at least thirteen years ago. (Demicheli et al., 2003; Rutschmann et al., 2002) The meta-analysis performed by Farez et al, 2011 included a limited number of studies available on the topic and some methodological options remain unclear such as the surprising selection of an odds ratio equal to 1.0 for the study by Hernan et al. The most recent papers (Mailand & Frederiksen, 2016; Martínez-Sernández & Figueiras, 2013) retained respectively twelve and fifteen studies for a qualitative review but none performed a meta-analysis. The need for an updated systematic review and, overall, a meta-analysis, was thus more crucial than ever, especially as additional observational studies have been published recently. (Eftekharian et al., 2014; Langer-Gould, Qian, et al., 2014)

However, several limitations must be acknowledged. Firstly, the overall pooled estimates obtained in the present meta-analysis failed to reach statistical significance, so no definitive conclusion can be drawn about the possibility of a small (e.g. 10 to 50%) increase in risk.

Secondly, a potential diagnostic bias and more specifically the so-called unmasking phenomenon (i.e., vaccinations lead to diagnosing symptoms that would otherwise have gone unnoticed, resulting in a bias toward an association) (Jacobsen et al., 2012) could be envisaged, even if most of the studies were of the case-control type and the majority of cases were ascertained by a neurologist taking into account the date of demyelinating disorder onset. Thirdly, as already mentioned, several studies, including the most recent one, would have been underpowered if intending to measure a potential increase in risk after hepatitis B vaccination, the main reason being that the prevalence of vaccination was too low in their study samples (unlike the massive off-target immunization of adults during the 1994-2000 campaign in France. The proportion of persons being in age of developing a central demyelination remained low, i.e. < 3 to 5%, in the countries where these studies were conducted). Moreover, it should be noted that the methodological choices made by authors (e.g. factors retained for adjustment or selection of controls) appeared rather heterogeneous across the studies. For this reason, we chose to consider three *scenarii* in order to circumvent this issue.

Another issue might be the statistical model used for this meta-analysis. In the present context, i.e., a meta-analysis based only on observational studies and focusing on a rare dichotomous outcome, an "exact" method would have *a priori* been the best option. (Greenland & Salvan, 1990) However, not only would this have been difficult to implement but it would also have required particular statistical expertise beyond the scope of the study. (Martin & Austin, 2000; Shuster & Walker, 2016) Owing to the low incidence of the events considered, (Sweeting, Sutton, & Lambert, 2004) the Peto one-step odds ratio method was the next best option. (Yusuf, Peto, Lewis, Collins, & Sleight, 1985) However, while it is perfectly suited for clinical trials, a prerequisite for using it is that the groups compared are more or less of the same size, which was definitely not the case for the studies meta-analysed. (Greenland & Salvan, 1990; J. Higgins & Thompson, 2002) Finally, and even if its use has been shown to be questionable for rare events, (Bradburn, Deeks, Berlin, & Russell Localio, 2007) we chose to use a generic inverse variance model (GIVM) as it allowed us to compute adjusted odds ratios from non-randomized studies, for which contingency tables and counts were not appropriate. Otherwise, these studies would have been excluded, leading to a small number of eligible studies and thus hampering any calculation of pooled

estimates. To test the robustness of our model for crude risk estimates, we also used the random-effect Mantel-Haenszel method, which is an option for rare and dichotomous outcomes. (Veroniki et al., 2016) The estimates it provided were fully consistent with those of the main analyses.

4.3.4 Other considerations

In this section, other arguments, interesting to consider in regards to our research question, are listed below.

4.3.4.1 Is there a hypothesis for an excess risk in children to develop multiple sclerosis after anti-HBV vaccination?

Given that first episodes of MS usually occur between 15 and 60 years and the youngest age of onset of MS in the medical literature is 2 years (Chitnis, 2006), children vaccinated below the age of 24 months are not thought to be at risk of developing MS. Besides, an ecological study examined the incidence of MS in adolescents 11–17 years old in the periods immediately before and after the 1992 implementation of a school-based vaccination programme for students aged 11- and 12-year-olds in Canada. (Sadovnick & Scheifele, 2000) The prevaccination study population accounted for 1.14 million person-years of observation while the postvaccination observation period provided 966,000 person-years. Diagnoses of MS were provided from the medical records of the only pediatric hospital in the province, the database of the provincial MS clinic, and pediatric neurologists in the province. A total of nine MS cases with adolescent onset occurred in the prevaccination period, and five cases occurred in the postvaccination period, leading to a non-statistically significant difference. This study provided no evidence associating anti-HBV vaccination with an increased risk for onset of MS during pre-adolescence.

4.3.4.2 Does demyelination need time to occur after immunization?

To date, the neurological process behind demyelination, probably complex, is not entirely known. Although some experts claim that the process leading to the auto-immune response would require several years, several examples of acute (occurring short-term after a triggering stimulus) demyelination can be cited. Among others, the Guillain-Barré syndrome, is a peripheral demyelination in which myelin may be under attack for a few hours before

symptoms appear. In the case of MS, animal studies have shown that if a brain-barrier disruption occurs, central demyelination can develop within some days. (Adler, Martinez, Williams, & Verbalis, 2000)

4.3.4.3 Are MS relapses concerned by an excess risk associated with anti-HBV vaccines?

Although out of the scope of this thesis because it would rely on a different physiopathology, one can wonder whether MS patients who receive HBV immunization could be at a higher risk to develop MS relapses.

A multicenter case-crossover study was conducted in France to examine whether vaccination increased the risk of MS relapses (Confavreux, Suissa, Saddier, Bourdes, & Vukusic, 2001) MS patients were identified from neurology departments associated with the European Database for Multiple Sclerosis network. A total of 643 subjects were included in the study. In the case-crossover design, patients served as their own controls. Cases were defined as subjects having a definite or probable diagnosis of MS according to the Poser criteria and at least one relapse between January 1993 and December 1997. Vaccination histories during the period January 1992 through December 1997 were self-reported by the participants during phone interviews and were confirmed with written documentation, usually a copy of the vaccination record. During the 12 months before the index relapse, 39 had a confirmed anti-HBV vaccination. Vaccination exposure was assessed in terms of a two-month risk period immediately before the index relapse and four two-month control periods during the 10 months preceding the index relapse. The relative risk of relapse was 0.67 (95% CI, 0.20–2.17). The authors concluded that vaccination does not increase the short-term risk of a relapse among patients with MS who had been relapse-free for at least 12 months. However, the authors noted that the findings of their study were inconclusive with regard to putative long-term risks. Limitations of this study included the insufficient statistical power for assessing risks associated with specific vaccines, exclusion of patients with frequent or minor relapses, and an assumption of a constancy of vaccine exposure and risk after each exposure. Study strengths included limited confounding by the nature of the case-crossover study design, high response rates and the validation of vaccine exposures, limited recall bias through collection of exposure data without specific reference to the index relapse, and the fact that results that were unaffected by a change in length of effect periods.

4.3.4.4 Is there a risk difference between the different marketed HBV vaccines?

The study performed by Mikaeloff et al, 2009, which was included in our meta-analysis (Mikaeloff et al., 2009) used a case-control design with children aged <16 years who presented a central demyelinating event between 1994 and 2007. Exposure to HBV vaccine, confirmed by written document, was sought within the 3 years before the event. Although authors did not report an excess risk for the main analysis with an OR of 0.74 (95%CI: 0.54 – 1.02), a subgroup analysis required by the Editor of the journal and restricted to children having received Engerix B® found a statistically significant association with the occurrence of both central demyelination and MS within 3 years following the vaccine administration, with respective risk ratios of 1.74 (95%CI: 1.03 – 2.95) and 2.77 (95%CI: 1.23 – 6.24). However, several limitations were pointed out. It should also be noticed, for central demyelination, an overlap between the confidence intervals of ORs for the two brands, which would render the difference between the two vaccines impossible to prove:

- Engerix B®: OR=1.74 (95%CI: 1.03-2.95)
- Genhevac B® : OR=1.50 (95%CI : 0.71-3.17)

In addition, this study was restricted to subjects who were compliant with vaccinations guidelines, possibly biasing the response rate and therefore under-estimating the risk ratios. Nevertheless, the main inconsistency found by this study was the results obtained for shorter periods:

- Central demyelination : 1-2 years : adjusted OR = 0.45 [95%CI : 0.20-1.01]
- Multiple sclerosis: 1-2 years : adjusted OR = 0.45 [0.12-1.71]

These findings are quite surprising and not consistent with other studies. As a consequence, the risk difference between the different marketed vaccines is rather unlikely.

4.3.4.5 Does the facilitated access to MRI imaging in the 1990' in France lead to the observed signal?

One argument often presented to support the absence of association between HBV and MS was the fact that the number of MRI machines increased substantially in the 1990's in France, which accelerated the MS diagnoses, leading to an artifactual increase of MS incident cases during the 1990's. While the use of MRI machines helped to diagnose MS more rapidly, it represented only one component of the diagnosis process which relies on

both symptoms and imaging (cf. section 3.2 Diagnostic criteria). Besides, even if the number of MS incident cases increased in the 1990's, the relationship with the signal detected in 1996 is not self-evident. Indeed, there was *a priori* no reason for channeling these supplementary cases towards vaccine causation nor for performing MRIs preferentially in persons having received one dose of anti-hepatitis B vaccine. One should note that, during the concerned period, the vast majority of French neurologists rejected any possibility of association between vaccination and multiple sclerosis. Moreover, as mentioned in section 1.5 Pharmacovigilance of vaccines, AEFIs are reported to the SRS when the physician suspects a potential link between the vaccine and the event. Even if the number of MS diagnoses increased during the period of the HBV mass vaccination, the willingness to report possibly related events, was certainly more dependent on the physician's perception than the MS epidemiology.

That being said, it is not debatable that the number of MRI imaging machines dramatically increased over time in France since 1983, date of the first MRI prototype settled in the country. In 1987, 16 MRI machines were available. Twelve years later, in 1999, 182 machines were active within France.(Lavayssiere & Cabee, 2001). Detailed statistics about the number of MRI machines in France are available for the period 2006 – 2015. (Statista, 2018) Figure 40 presents this evolution since 1983. (Lavayssiere & Cabee, 2001; Statista, 2018)

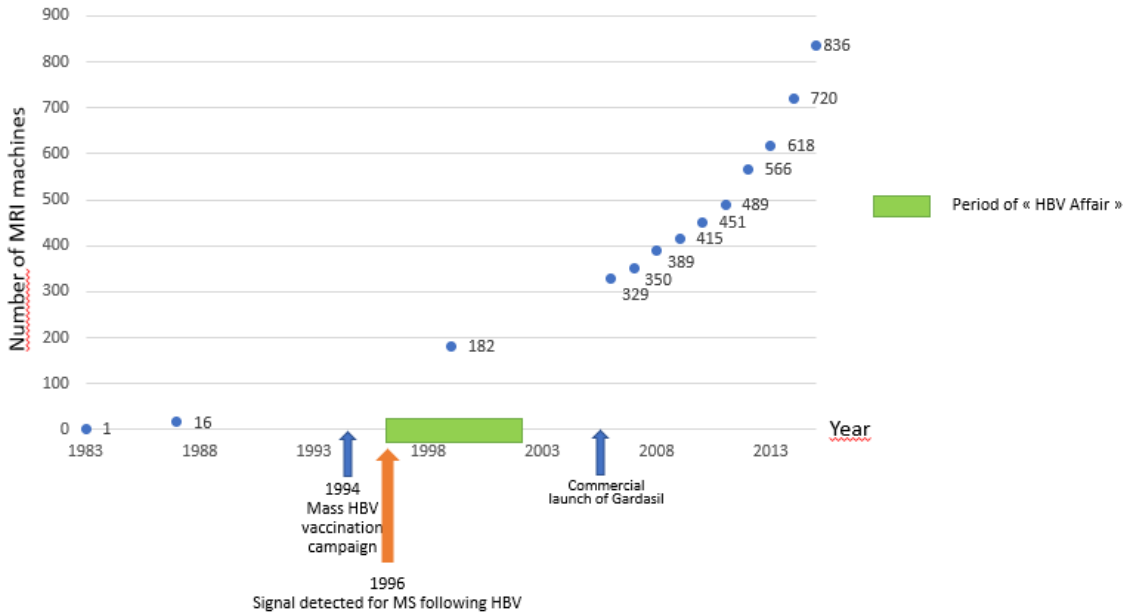


Figure 40: Number of magnetic resonance imaging (MRI) devices in France from 1983 to 2015

Figure 40 confirms that the correlation between the number of MRI machines and the number of MS cases is not evident. Even if the number of MRI machines in France increased during the period of the "HBV Affair" (i.e., 1996 – 2000), the increase was spectacular only for the period 2006 – 2015, i.e. ten years after the generation of the pharmacovigilance signal. Interestingly, the first anti-papillomavirus vaccine (Gardasil®, Sanofi Pasteur MSD) was launched on the French market in 2006. If applying the same reasoning, one would have observed a parallel increase of the number reports for MS cases following HPV immunization. Although several cases of demyelination have been reported and published, no pharmacovigilance signal was detected in the follow of the launch of the HPV vaccination campaign in France. Consequently, it is quite unlikely that the facilitated access to MRI played a major role in the generation of the signal observed for anti-hepatitis B vaccine.

4.3.5 Conclusion

4.3.5.1 Is the benefit-risk balance of anti-HBV vaccination good?

In any case and undoubtedly: yes.

A model was developed to estimate the worldwide population-based HBV-related morbidity (i.e. total and chronic HBV infections) and mortality (i.e. deaths from acute hepatitis B and sequelae like cirrhosis and hepatocellular carcinoma) and the effect of hepatitis B vaccination on these outcomes using the age-specific risk of acquiring HBV infection, development of acute hepatitis B (illness and death), and progression to chronic infection. (Goldstein et al., 2005)

The model estimated that for the year 2000, 620,000 persons died worldwide from HBV-related causes: 580,000 (94%) from chronic infection-related cirrhosis and hepatocellular carcinoma and 40,000 (6%) from fulminant hepatitis B. In the surviving birth cohort for the year 2000, the model estimated that without vaccination, 64.8 million would become HBV-infected and 1.4 million would die from HBV-related diseases. This number of deaths is 127% greater than number of HBV-related deaths observed today, primarily because of population increase and longer life expectancy. Without vaccination, 21% of all HBV-related deaths in the 2000 birth cohort would result from perinatal HBV infection, 48% from infection acquired in the early childhood period, and 31% from infection acquired in the late period.

Routine hepatitis B vaccination of infants would prevent up to 75% of global deaths from HBV-related causes depending on vaccination coverage for the complete schedule. As coverage increased from 50 to 80 to 90%, the proportion of deaths prevented increased from 38% to 60 to 68%. (Goldstein et al., 2005)

4.3.5.2 Answer to the research question n°1: "Is there a link between central demyelination and anti-HBV vaccination?"

All the current evidence which could answer the initial research question of this thesis is summarized in Table 27.

Table 27 : Summary of evidence addressing our research question n°1

Parameters	Hypothesis	Evidence
Biological plausibility	Is there a molecular mimicry between HbAg and human myelin?	Although theorically possible, evidence remains weak and indirect.
Published case reports	Are there numerous case reports of demyelination following HBV vaccination? Are they consistent and specific?	At least 17 case reports published in the scientific literature, and only 4 published before the polemic arose (i.e., 1996). Cases originated from various developed countries. They all relied on a similar demyelinating process.
Description of the French signal	Was the signal consistent and robust?	This signal arose after the reporting of several hundreds of cases of demyelination to the French pharmacovigilance. Its detection revealed a marked disjunction between the target and joint populations of the national immunization programme. French young adults (i.e., 16-44 years, an age prone to develop demyelination) were massively exposed to the HBV vaccines. This unprecedented situation has not been reproduced elsewhere. Moreover, some disturbing facts have to be acknowledged:

		- Most events were reported after booster doses. - Reporting rates doubled in 1987, 1997 and 1998.
Observed/Expected analyses	Did the number of observed cases in France exceed the expected number?	Strictly speaking: no, the number of observed cases always remained below the expected number. However, a certain degree of underreporting is likely and could reverse this conclusion. In our analysis, the interval between observed and expected numbers (i.e., underreporting factor) was about 3, while previous research estimated this factor around 20.
Disproportionality analyses	Does the frequency of reports for demyelination differ between HBV vaccination *versus* other vaccines?	Yes, MS cases were up to five times more likely to be reported after a HB vaccination than after any other vaccination *(findings based on the US VAERS database)*. The origin of cases, either American or foreign, did not alter this conclusion
Observational comparative studies	Was there an association between anti-HBV vaccination and central demyelination found by pharmacoepidemiogical studies?	A total of 13 studies having assessed such as putative link were identified. Only one found a statistically significant association. Several methodological limitations were often put forward: - Recall and selection biases - Lack of power due to low prevalence of vaccine exposure in adults.
Meta-analysis	When pooling all evidence generated so far, is there an association found between anti-HBV vaccination and central demyelination?	The pooled estimates failed to demonstrate a link other than coincidental between vaccine exposure and the outcomes of interest, whatever the type of analysis. Interestingly, all pooled estimates converged to a risk ratio around 1.2 – 1.3 (i.e. an excess of 20 to 30%).

From all the materials reviewed and the research activities conducted, the following conclusions can be drawn:

- There is no doubt regarding the exceptional situation experienced by the French population regarding the massive anti-HBV immunization at the time of its launch. The uncontrolled communication about the risks associated to HBV infection led to an exposure of the adult population at least 10 times greater than it would have been expected, at an age prone to develop central demyelination.
- A pharmacovigilance signal, based on several hundreds of validated cases of central demyelination, was generated two years after the campaign launch.
- An objective analysis of the body of evidence currently available does not permit a clear-cut statement about the existence of an association other than caused by chance. Indeed, some argument could support the hypothesis of a non-fortuitous association, for a proportion of reported cases. Among others, one can cite: a certain degree of biological plausibility, even if weak, the disproportionality observed in VAERS and by other authors, the fact that a moderate degree of underreporting could reverse the conclusions of the observed/expected analyses, the apparent non-random distribution of case-reports according time or the rank of vaccination, etc.
- Even if causal, the strength of this association would be, in any case, weak (RR < 2; in congruence with the average estimate of our meta-analysis, i.e. 1.3) and, overall, would concern only adults and certainly not newborns or child below 12 years.
- This explains the inconclusiveness of almost all observational studies and the meta-analysis: detecting or proving the absence of an excess risk of this order of magnitude would require a sample of considerable, if not unrealistic, size.

4.3.5.3 Can we solve and close the debate?

Behind the question "*can we solve and close the debate?*", there is another questioning about the need of additional studies.

Nowadays, most of international recommendations target infants before the age of 2 years with a possible catch-up of adolescents. The exposure to the HBV vaccine at an adult age is

reserved to specific and limited populations (probably less than 5% of the adult population) and is going to dramatically decrease as most birth cohorts, including in France, have been, are or will be immunized during childhood. Consequently, the reservoir of unvaccinated at-risk adults is going to shrink in the future, notwithstanding massive human migrations from endemic developing countries. Ultimately, for developed countries and except for specific populations such as patients under hemodialysis who require periodic re-immunization, the vaccination at an adult age is thought to disappear. From this point of view, it seems rather useless and unrealistic (considering the level of exposure) to count on additional studies to close the debate.

It is more sensible to admit than a small excess risk cannot and will not be ruled out. Nowadays, it might only concern specific subgroups at high risk for HBV infection (e.g. health professionals, army) in which the benefit and demyelination risk balance of the anti-HBV vaccination could be re-appraised. The incidence of recent immunization, expected to be high in these groups, could overcome the lack of statistical power having jeopardized all studied conducted so far and reproduce the exceptional situation observed in France in the 1990's.

OFSEP is the historical disease-specific registry compiling almost all French patients with MS. It would represent a fantastic research opportunity. Indeed, this source is very likely to contain a large proportion of cases which formed the initial signal in the 1990's, even if, surprisingly, the vaccination status of patients has never been recorded. It could be *a posteriori* determined by a review of medical records and/or by a linkage with the data of the Système National des Données de Santé (SNDS). A self-controlled design (cf. section 2.4 Self-controlled designs) would have the advantage of automatically adjusting for time-fixed, even unmeasured, confounders. To overcome the main limitation of the SCCS (i.e., the administration of vaccine or follow-up should not dependent of previous events occurring in the pre-vaccine control period), a case crossover design would be the first choice. It is clear that reaching the statistical power required for detecting a risk ratio in the order of magnitude of 1.3 would require a considerable sample size, probably problematic to achieve. However, such a design would have the major advantage to be much more robust and convincing than most of pharmacoepidemiologic studies conducted so far.

4.3.5.4 Lessons learned from this research work?

The main interest of this research work was to compile all evidence produced so far on this important public health question: *is there a link between anti-HBV vaccination and central demyelination*? This question caused a violent debate, still active nowadays, which has polarized and divided the French population on the interest of such a vaccination. This research work has also the advantage to have been conducted long after the public health crisis that occurred in the 1990's, therefore in a relatively more serene atmosphere.

As already mentioned, if an excess risk actually existed, it would be weak and would concern adults only. The current recommendations are made in the sense that they minimize the probability of the French population to be exposed at an adult age. Moreover, even for unvaccinated at-risk adults, the benefits of HBV vaccination still outweigh the risks of developing MS. This research work is therefore a true advocacy in favor of the current recommendations.

The main lesson that could be learned from this polemic is about the communication with the general opinion and healthcare professionals. The devastating effects of the unfounded and exaggerated statements about the risks related to HBV infection, the dissemination of erroneous or faked data, as well as the violent debate which followed the media coverage of the French signal, has let profound scars in the confidence towards vaccines in France and elsewhere in the world. These hurdles competed to indirectly support fantasist conspiracy theories against vaccinations, reducing the immunization coverage for susceptible populations and making France the worst example of vaccine defiance in the world. Conversely, the general public and the healthcare professionals should be provided by independent, reliable and fact-based data produced by credible actors promoting public health within our country.

The recent decision of the French government to make eleven vaccinations mandatory for newborns carries a non-negligible risk to drive the public audience and to reinforce the parents' mistrust against vaccination. A softer approach relying on a large information campaign, orchestrated by public actors and not pharmaceutical companies, presenting both the risks of the natural infections and the risks inherent to vaccination, diffused at a peak hour to reach a large audience would have been more satisfactory and probably more

beneficial. Moreover, a sufficient immunization rate (>95%) was already reached for three of the mandatory valences (diphtheria, tetanus and poliomyelitis). (Ministère des Solidarités et de la Santé, 2018b) For the others, it was not yet the case but we were already close to achieve this milestone, at least for hepatitis B for whose immunization coverage was as high as 90% in 2017. Furthermore, it was estimated that up to 70% of children were already compliant with the vaccination against the eleven valences before the implementation of these new recommendations. (Ministère des Solidarités et de la Santé, 2018b) In the lights of this data, one can only wonder whether this political decision, not followed by our neighbours, was justified and relevant. Surely, it will require time to evaluate its impact on the immunization rates in France. More to follow!

5 Vaccination against Human Papillomavirus and risk of demyelination

The polemic related to a potential risk of demyelination following active immunization resurfaced in France at the time of the launch of the first anti-HPV vaccine, Gardasil®, in 2006. The target population, i.e., young adolescent girls aged between 11 and 14 years, was a concern given that this population falls into the age category prone to develop such disorders. Other features were shared between the HBV and the HPV vaccinations, such as the "anti-tumoral" argument claimed for both products. While they should protect against the acute infections, they also confer a protection against their long-term consequences of, which include specific cancers.

For these reasons, it was of great interest to investigate whether a potential risk of demyelination was also (or could be) a concern for HPV vaccines.

5.1 HPV infections

HPV is the cause of the vast majority of cervical cancers and is responsible for a substantial fraction of anogenital and oropharyngeal cancers. There are many HPV serotypes. HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59 were classified as carcinogenic while HPV68 was found as "probably" carcinogenic. All these types are referred to as high risk types. Types 16 and 18 would account for 70% of cervical cancer cases. (de Martel, Plummer, Vignat, & Franceschi, 2017) Conversely, the low-risk types of HPV are known by the numbers 6, 11, 40, 42, 43, 44, 53, 54, 61, 72, 73 and 81. Types 6 and 11 – which are linked to about 90 % of genital warts – are the most common. (Yanofsky, Patel, & Goldenberg, 2012)

In addition to cervical cancer, other cancers can be caused by HPV, especially those of the vulva, vagina, penis, anus and oropharynx which are mainly due to HPV16. Although no effective screening exists for these cancers, they would also be prevented by HPV vaccination. (de Martel et al., 2017)

5.1.1 Prevalence and incidence

By using the GLOBOSCAN 2012 database, it was estimated that 570,000 cases per year in women and 60,000 cases in men would be attributable to HPV worldwide, corresponding to respectively, 8.6% and 0.8% of all cancers occurring globally (cf. Figure 41). (de Martel et al., 2017)

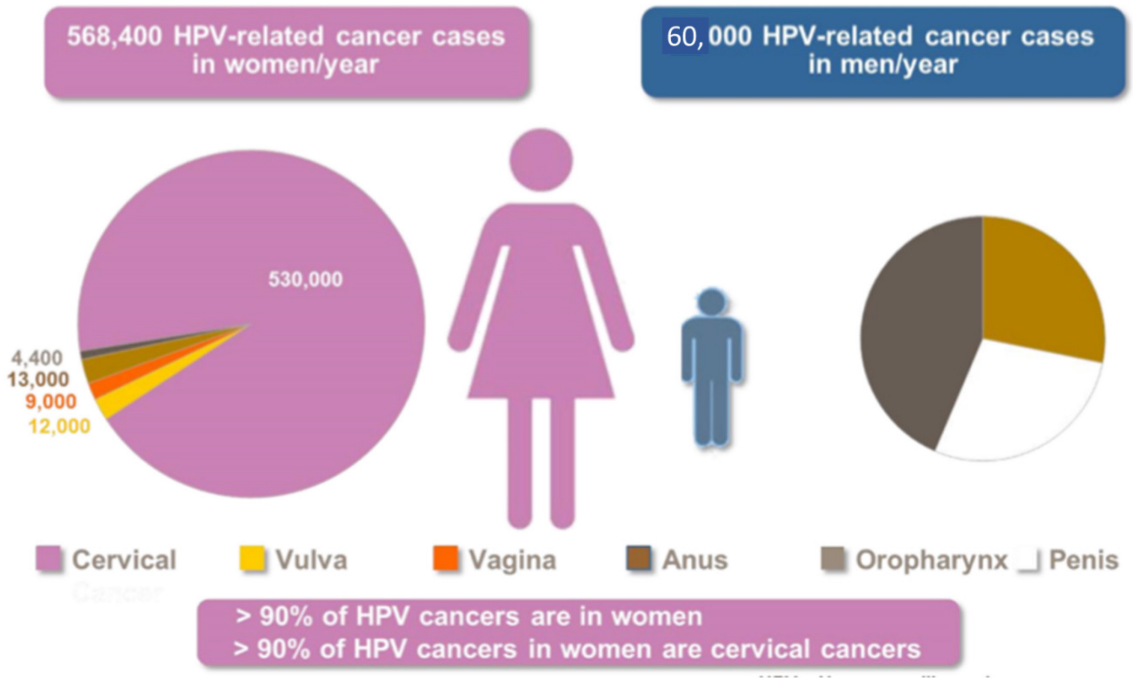


Figure 41 : Distribution of HPV-attributable cancers worldwide

In Europe, HPV would have been responsible of 87,000 cancers in 2012, accounting for 2.5% of all cancers in the European region.

In France, it is estimated that 70% of people (men and women) have been exposed to HPV. The age standardized incidence rate of cervical cancer cases attributable to HPV in 2012 was less than 10 per 100,000 in France, which represents one of the lowest rates in the World. Conversely, France has a high incidence rate of anogenital cancer cases (vulvar, vaginal, anal and penile) and head and neck cancer cases (oropharynx, oral cavity and larynx): above 1.25 per 100,000, each. (de Martel et al., 2017)

Besides, a combination of four large French studies (i.e., EDiTH I–IV studies) (Riethmuller et al., 2009) estimated a proportion due to HPV 6/11/16/18 of:

- 82% (95% CI: 78.5–85.1) in cervical cancer,
- 64% (95% CI: 59.7–68.1) in cervical intraepithelial neoplasia (CIN) 2/3,
- 34% (95% CI: 28.9–38.1) in low-grade squamous intraepithelial lesions (LSIL)
- 83% (95% CI 77.6–87.8) in female external acuminata condylomata cases.

5.1.2 Burden of the disease

The total burden of HPV infections is very difficult, even impossible, to estimate given that it is associated with a range of diseases and cancers at different anatomical sites.

Nevertheless, thanks to an economic study assessing the annual costs associated with management of HPV-related cancers in France (Borget, Abramowitz, & Mathevet, 2011), it was estimated that 21,555 patients were found to be hospitalized for an HPV-related cancer in France in year 2006-2007 (cf. Table 28).

Table 28 : Estimation of the total number of patients hospitalized for an HPV-related cancer in France in 2006-2007

Cancer type	**Cases attributable to HPV (%)**	**Annual number of patients hospitalized in 2006 -2007**	**Number of cases attributable to HPV**
a. Invasive cervical	99.7	7204	7182
b. Vulvar	34.7	1237	429
c. Vaginal	76.8	728	559
d. Anal	84.2	3711	3125
e. Penile	46.7	678	317
f. Head and neck			
Oral cavity	16	10786	1726
Oropharynx/pharynx	28.2	21950	6190
Larynx	21.3	9516	2027
		Total	**21555**

Besides, Figure 42 presents the numbers and proportions for each clinical stage related to cervical HPV for a sample of 6,000,000 cervical smear tests collected in France. (GILBERG S, 2011)

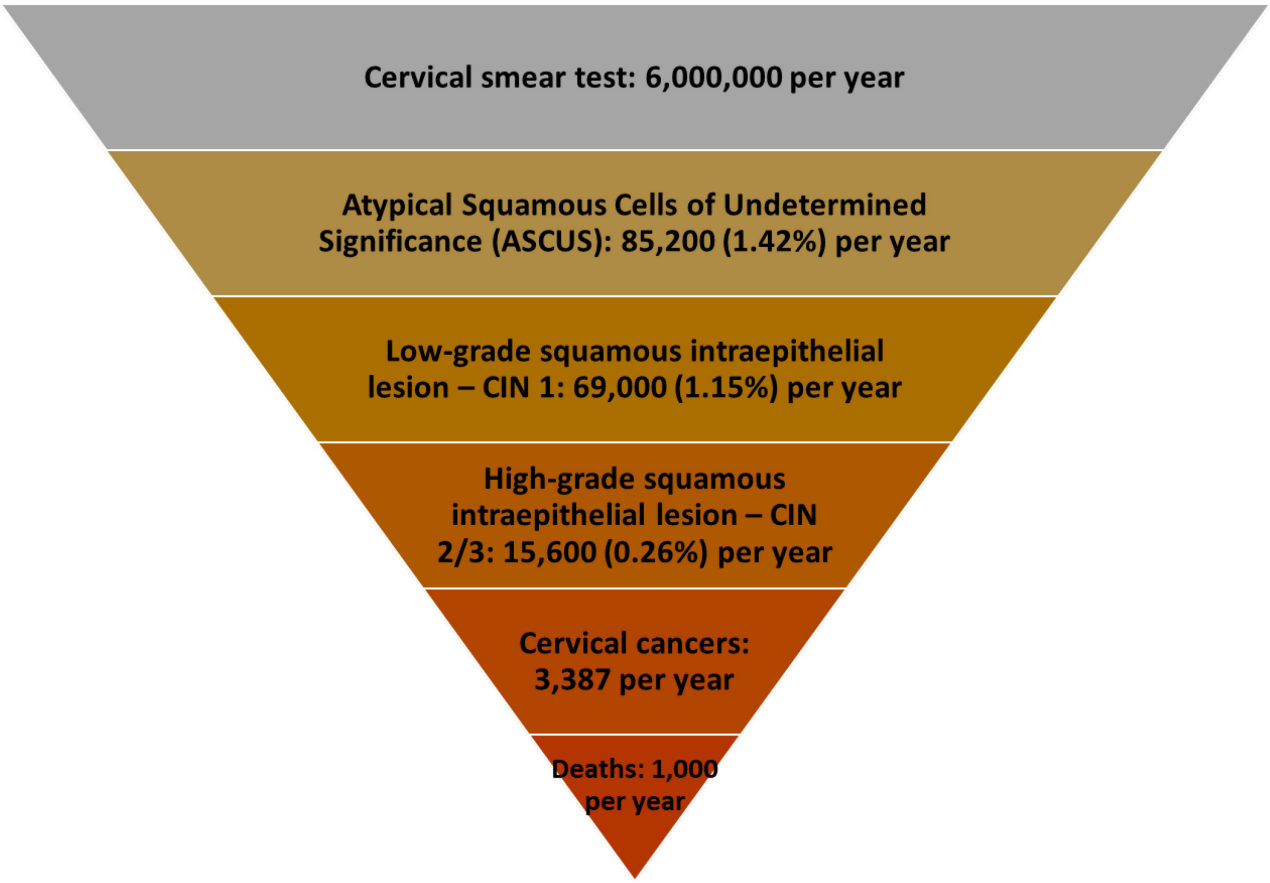


Figure 42 : Proportions of clinical manifestations related to cervical HPV (extracted from (GILBERG S, 2011)

Finally, after a prospective population-based cohort of 61,564 British women recruited between 1987 and 1993, it is noteworthy that, although the youngest age category (15-19y) had the highest risk of being infected by HPV, the annual incidence of CIN3 (i.e., a quite common clinical manifestation of HPV) peaking in the 25-29 years age category (cf. Figure 43). (Peto et al., 2004)

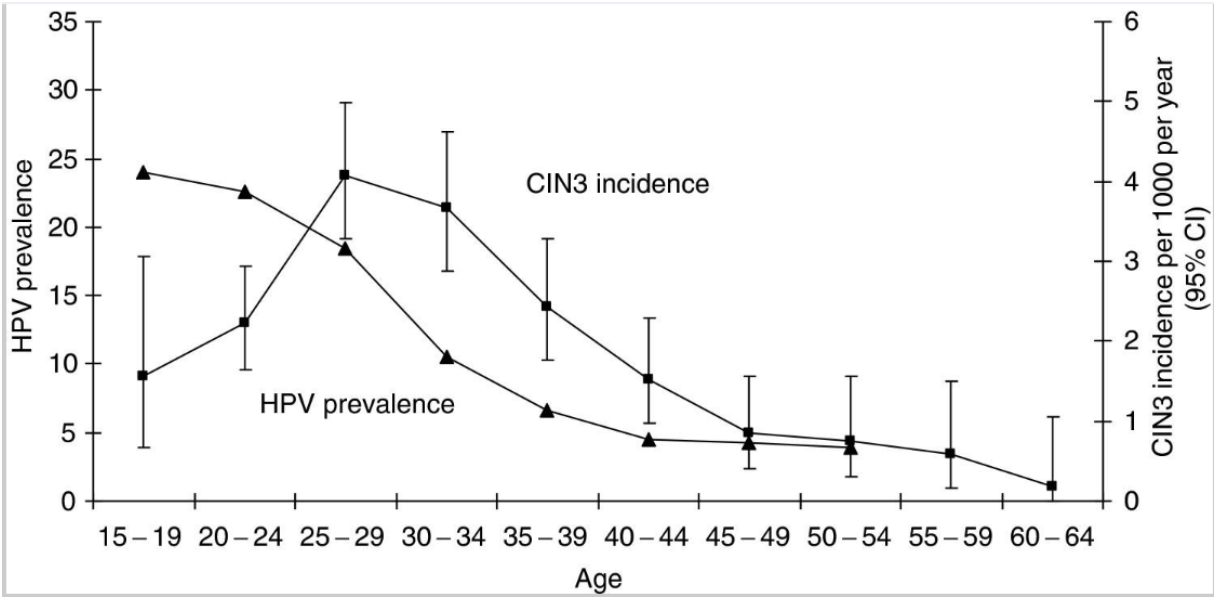


Figure 43 : HPV prevalence and CIN3 annual incidence per age categories (extracted from (Peto et al., 2004))

5.1.3 Mode of transmission

The sexual transmission is the most frequent way of transmission. Only one sexual contact may suffice to transmit HPV; moreover, unlike HBV, condoms do not provide an efficient protection. (Liu, Rashid, & Nyitray, 2016)

5.2 Vaccination against HPV in France

5.2.1 Marketed vaccines in France

Developed in the early 2000s and approved in 2006 in the USA and Europe, the first HPV vaccines were either bi- or quadrivalent.(Galloway, 2003; Lopalco, 2017) Both initial formulations targeted the high-risk serotypes 16 and 18.

Currently, three vaccines are available in France:

- Cervarix® (GlaxoSmithKline), a two-valent vaccine targeting HPV16 and 18, the most carcinogenic types (ii)
- Gardasil® (Merck Inc.), a four-valent vaccine targeting HPV16/18 and also low-risk types HPV6 and 11 that cause genital warts
- Gardasil 9® (Merck Inc.), a nine-valent vaccine targeting HPV6/11/16/18 and the next five most carcinogenic types (HPV31/33/45/52/58).

5.2.2 Current recommendations and immunization schedule in France

In France, the current recommendations target girls aged 11-14 years. Any HPV vaccine can be administered according to a 2-dose schedule with a 6-month interval between the two doses. (Ministère des Solidarités et de la Santé, 2018a)

A catch-up strategy is a possible option for older female adolescents until the age of 19 years. This immunization strategy relies on the fact that immunization against HPV should be administered before being exposed to the virus, i.e., before starting sexual intercourses. (Ministère des Solidarités et de la Santé, 2018a) In that context, three doses will be given: at 0, 2 and 6 months for Gardasil®/Gardasil 9®, or at 0, 1 and 6 months for Cervarix®.

Active immunization against HPV is also recommended for specific subpopulations, as listed below: (Ministère des Solidarités et de la Santé, 2018a)

- For men having sex with men, immunization is recommended up to the age of 26 years with Gardasil® or Gardasil 9®, according to a 3-dose schedule at 0, 2 et 6 months.
- For immunocompromised patients of both genders (as per recommendations in the general population)
- For transplant recipients of both genders, immunization can be administered since 9 years of age.

5.2.3 Comparison with other European countries

Currently included in the immunization programmes of 28 countries (De Vincenzo, Conte, Ricci, Scambia, & Capelli, 2014), HPV vaccination campaigns were initially intended for female adolescents aged 9-14 years, with a possible catch-up of older girls.(Lopalco, 2017) More recently, several countries extended the HPV vaccine recommendations to the male population, in order to prevent some HPV-related cancers but also with the intent to better control, or even reduce the human reservoir of these viruses. (Lopalco, 2017)

In Europe, most countries recommended the initial vaccination before the age of 14 years. A total of 7 countries propose catch-up vaccination strategies for older age categories. Only Latvia made the anti-HPV vaccination mandatory. (cf. Table 29)

Table 29 : Comparative table for HPV vaccination schedules and recommendations across Europe, ECDC Vaccine Scheduler: https://vaccine-schedule.ecdc.europa.eu/)

General recommendation

Recommendation for specific groups only

Catch-up (e.g. if previous doses missed)

In red, compulsory vaccination

	Years														
	9	10	11	12	13	14	15	17	18	19	26	27	29	60	>= 61
Austria	(1)			(2)											
Belgium		(3)													
Bulgaria				(4)											
Croatia						(5)									
Cyprus				(6)											
Czech Republic					(7)										
Denmark				(8)											
Estonia				HPV 9 (9)											
Finland			(10)												
France			(11)				(12)								
Germany	(13)						(14)								
Greece			(15)				(16)			(16)					
Hungary				HPV 2 (17)											
Iceland				HPV 2 (18)											
Ireland				(19)											
Italy				(20)											
Latvia				(21)											
Liechtenstein			(22)				(23)								
Lithuania															
Luxembourg				(24)			(25)								
Malta				(26)											
Netherlands				(27)											
Norway				(28)											
Poland			(29)												
Portugal		HPV 9 (30)													
Romania			(31)												
Slovakia				(32)											
Slovenia			(33)												
Spain				(34)											
Sweden		(35)													
United Kingdom				(36)											

Footnotes:

1: Females and males. 2 doses with at least a 6 month-interval. 9-valent vaccine recommended.

2: For older age groups 3-dose vaccination scheme recommended. Please refer to the original recommendation for the appropriate schedule.

3: Recommended for girls 10-13 years old with 2 doses (schedule 0, 6 months).

4: HPV vaccination is not included in the National Immunization schedule. The vaccination is voluntary, but free of charge for 12-year-old girls.

5: Recommended for boys and girls. Free of charge.

6: Female, two-dose schedule. Vaccination in schools and Governmental immunizations centres from 2016/2017 school year.

7: For all (boys and girls). Recommended only. The vaccination is covered by the health insurance for all children between 13 – 14 years of age.

8: Girls only.

9: Two-dose school-based programme. For more information please refer to http://www.vaktsineeri.ee/HPV-inimese-papilloomiviirus.html.

10: For information related to coverage, please refer to https://www.thl.fi/roko/rokotusrekisteri/HPVraportit2016/.

11: Two doses (0, 6 months): quadrivalent or bivalent vaccine ; or ninevalent (11/14 years).

12: Three doses in a 0, 1 or 2, 6 month schedule (girls aged 15 to 19 years).
13: Females only. At the age of 9-13 years or 9-14 years (depending on the vaccines used) two doses at 6 months interval (Please refer to the product information leaflet). If the interval between doses is <6 months, a 3rd dose is recommended.

14: Females only. In the case of catch-up vaccinations beginning at the age >13 or >14 years (depending on the vaccines used) three doses are necessary (Please refer to the product information leaflets).

15: Females only. 2 doses within a 6-month interval.

16: Females only. 3 doses.

17: School-based vaccination in 7th grade girls. Recommendation only, but free of charge.

18: Females only. 7th grade.2 doses.
19: First year second-level school (females 12 to 13 years of age), 2 dose schedule. for a full description of HPV recommendation for other groups please see:
http://www.hse.ie/portal/eng/health/immunisation/hcpinfo/guidelines/immunisationguidelines.html.

20: 2 or 3 doses, depending on the vaccine and age.

21: The vaccine is offered to girls who are 12 years old.

22: Two doses. Females and males.

23: Females and males. catch-up vaccination preferably before the 20th birthday. On a case-by-case basis from 20 years.

24: Girls only.

25: Catch-up on HPV if not yet done (13-18 years).

26: For females born from the year 2000 onwards. 2 doses in a 0-6 month schedule.

27: For girls only - 2 doses, 6 months apart.

28: Females only. 7th grade.

29: Recommended for girls.

30: Two doses (0-6 month schedule). Females only.

31: 3 doses. Recommended, but not mandatory.

32: Partial reimbursement by the national healthcare system.

33: Girls only. 2-dose vaccination schedule.

34: Two doses. Females only. For more information please refer to http://www.msssi.gob.es/profesionales/saludPublica/prevPromocion/vacunaciones/docs/PapilomaVPH.pdf.

35: Two doses. Females only. Given to 5-6 grade students.

36: Females only. (two doses 6-24 months apart).

5.2.4 Vaccine acceptability by the public opinion

Several cases of demyelination following the immunization against HPV were largely mediatized soon after the marketing launch of HPV vaccines. This having the direct consequence to revive the polemic related to a potential risk of demyelination following active immunization. Thus, the American Center for Disease Control which investigated the top five reasons for not vaccinating daughters among parents with no intention to vaccinate in the next 12 months, found that safety concerns and side effects were ranked third in this study.(Centers for Disease Control and prevention, 2013) Concern about immune-related and neurological diseases triggered by HPV vaccination may also be the result of a "ripple effect" fueled by social and media reports and caused by concomitant findings on the potential associations between adjuvanted influenza A(H1N1) pandemic vaccine with narcolepsy and GBS.(Stowe et al., 2016; Tokars et al., 2012) Notably, despite the absence of scientific evidence on a causal relationship between vaccines and these events, the Japanese government suspended its recommendation for HPV vaccination in 2013.(Saitoh A, 2014) This undermined public confidence in vaccines, with a dramatic decline in vaccination uptake, as observed in Sapporo, a city of 2 million people in Northern Japan, where the three-dose completion rate dropped from 75% to 0.6% after the suspension of the recommendation by the Japanese regulators.(Hanley, Yoshioka, Ito, & Kishi, 2015)

In this context and to avoid any comparable situation, the European Medicines Agency asked the vaccine producers to closely monitor the late-phase benefit and risk balance of their products and to provide detailed analysis of all suspect cases of neurological events, such as leukoencephalomyelitis, acute disseminated encephalomyelitis, and transverse myelitis, with an appropriate causality assessment.(European Medicines Agency, Minutes of the meeting on 6-9 January 2014)

In France, unlike the painful experience of the anti-HBV vaccination campaign in the 1990's, no major confidence crisis was observed for the anti-HPV vaccination so far.

5.3 Research question n°2: Is there a link between demyelination and anti-HPV vaccination?

In the same way we addressed the first research question in section 4.3 Research question n°1: Is there a link between central demyelination and anti-HBV vaccination?, the following sections will examine the arguments in favor or against our research question n°2.

5.3.1 Hypothesis generation

5.3.1.1 Biological plausibility

No data documenting a pathophysiological hypothesis able to explain a relationship between anti-HPV vaccination and demyelination, either central or peripheral, has been found so far.

5.3.1.2 Published case reports

A high-level literature review was conducted in Medline via Pubmed to identify the published case reports of demyelination, peripheral and central, occurring after the administration of HPV vaccines.

A total of 8 distinct publications were found accounting for at least 12 individual cases of demyelination occurring in a close temporal relationship after HPV administration. (Alvarez-Soria et al., 2011; Chang et al., 2016; DiMario, Hajjar, & Ciesielski, 2010; Hu, Tornes, & Lopez-Alberola, 2018; Karussis & Petrou, 2014; Schaffer et al., 2008; Sekiguchi, Yasui, Kowa, Kanda, & Toda, 2016; Sutton, Lahoria, Tan, Clouston, & Barnett, 2009) Reported events were diverse, including ADEM, MS and NMO. Geographical distribution was balanced across the different countries where the HPV vaccines are marketed.

5.3.1.3 Post-licensure studies aiming at detecting signals or evaluating disproportionality

A systematic literature review aiming at identifying post-licensure studies having assessed either the possible existence of a pharmacovigilance signal or a disproportionality within pharmacovigilance databases, for demyelination after HPV immunization, was conducted as a subpart of a bigger systematic review which has been recently published. (Mouchet et al., 2018b)

While corresponding results are presented below, the detailed methods are reported in section 5.3.2 Systematic review of observational comparative studies having addressed the research question n°2.

Table 30 describes the characteristics of the 14 individual post-licensure studies using nationwide pharmacovigilance systems identified by the review.(Angelo, Zima, Tavares Da Silva, Baril, & Arellano, 2014; Baxter, Lewis, Goddard, et al., 2016; Cameron, Ahmed, & Pollock, 2016; Gee et al., 2011; D. A. Geier & Geier, 2015, 2017; Gold, Buttery, & McIntyre, 2010; Labadie, 2011; Ojha et al., 2014; Pellegrino et al., 2013; Slade et al., 2009; Souayah et al., 2011; Vichnin et al., 2015; Willame et al., 2016)

Occurrence of GBS was documented by 10 studies, while incidence of MS was assessed in four studies. Altogether, these studies covered the period between 2004 and 2015. Except two (D. A. Geier & Geier, 2017; Souayah et al., 2011), all articles provided reassurance regarding the safety of HPV vaccines, owing to an absence of safety signal. Indeed, observed incidences of demyelinating disorders were within the expected ranges in the general population.

Regarding the two outlier studies, there were disproportionality analyses conducted within VAERS. Souayah et al, 2011 compared the frequency of acquiring GBS within the 6 weeks following HPV vaccination to the one in the general population, by using the VAERS database between June 2006 and September 2009. They found a 2.5- to 10-fold increased reporting of GBS after HPV vaccination when compared to the general population.(Souayah et al., 2011) Likewise, in the recent case/non-case study conducted within VAERS by Geier et al, 2017 a significant disproportionality was found for central demyelinating disorders and HPV vaccination (ROR= 1.58, 95 % CI 1.13–2.21), with a median onset of initial symptoms ranging from 3 to 37 days after immunization. (D. A. Geier & Geier, 2017)

Table 30 : Post-licensure studies

Citation	Country	Data sources	Study periods	Main findings	Conclusion of the paper
Angelo 2014b	Worldwide	Adverse Drug Reaction reports from spontaneous reporting and clinical trials reported to GSK	18 May 2007 - 17 November 2011	Reporting rate per 100,000 doses: - Guillain-Barré Syndrome (GBS): 0.048 - Optical neuritis: 0.028 - Multiple sclerosis: 0.017	**No signal:** Observed incidences of Guillain–Barré syndrome (confirmed or possible) were within expected range in general population.
Baxter 2016b	US	Vaccine Safety Datalink (VSD)	2007 - 2013	Relative risk of transverse myelitis in the 5- to 28-day Interval following vaccination, compared to remainder of 9 months after vaccination: For transverse myelitis: 0 (95%CI=0.0–15.3) For ADEM: 1.5 (95%CI=0.1–10.7)	**No signal:** Authors found no association between transverse myelitis or ADEM and prior HPV immunization.
Cameron 2016	Scotland	Hospital discharge data from Scottish National Health Service	2004 - 2014	Rates of demyelinating diseases, including multiple sclerosis (MS) exceeded expected levels in 2010 and 2011 and only in 2010, when MS was excluded. Incidence of MS remained within expected levels when analysed alone.	**No signal:** Increases in rates of demyelinating diseases might be due to baseline incidence increase which has been observed worldwide, especially in Northern countries.
Gee 2011	US	Vaccine Safety Datalink (VSD) based on seven surveillance sites	August 2006 - October 2009	Among adults, there was one case of GBS identified following HPV4. Medical record review revealed that this was not an incident GBS case.	**No signal**: Authors confirmed no statistically significant increase in risk of GBS after HPV4.
Geier 2015	US	Case/non-case analysis within VAERS	January 2006 - December 2012	ROR for GBS = 0.75 (95%CI 0.42–1.3)	**No signal for GBS**

Geier 2017	US	Case/non-case analysis within VAERS	2006 - 2014	Cases with Guillain–Barré syndrome as outcome were no more likely than controls to have received HPV4 vaccine (ROR 0.839, 95 % CI 0.601–1.145). Conversely, cases with CNS demyelinating disease were significantly more likely than controls to have received HPV4 vaccine (ROR 1.585, 95 % CI 1.129–2.213); median time to onset of initial symptoms ranged from 3 to 37 days post-HPV4 vaccination.	**Increased reporting risk**: This study provides arguments to support a significant relationship between HPV4 vaccine administration and serious autoimmune adverse event.
Gold 2010	Australia	Therapeutic Goods Administration	June 2006 - August 2009	The observed rate of demyelinating disorders among recipients of quadrivalent HPV vaccine was no higher than expected rate.	**No signal**
Labadie 2011	Worldwide	- VigiBase, the global database of WHO's Programme for International Drug Monitoring - VAERS (Vaccine Adverse Event Reporting System), USA, report on Gardasil® - RIVM (Rijksinstituut voor Volksgezondheid en Milieu), Netherlands, report on Cervarix®	*Not reported*	VAERS: 42 cases of GBS VigiBase: 139 cases of GBS RIVM: 1 case of GBS VigiBase: 336 cases of demyelination	**No signal:** The post licensure safety profile of both vaccines is consistent with the data in the SPC of these vaccines.
Ojha 2014	US	Vaccine Adverse Event Reporting System (VAERS)	January 1, 2010 - December 31, 2012	Nine GBS reports followed HpV4 vaccination.	**No signal:** This analysis of post-marketing surveillance among vaccine-eligible females or males in the United States, does not suggest that Guillain–Barré syndrome was reported more frequently after HpV4 vaccination than after other vaccinations

Pellegrino 2013	Europe + US	Vaccine Adverse Event Reporting System (VAERS) database and the EudraVigilance postauthorisation module (EVPM) database	2006 - 2012	VAERS: 236 cases of ADEM following vaccination implying (but not limited to) HPV vaccines EVPM database: 205 cases of ADEM	**No signal**
Slade 2009	US	Vaccine Adverse Event Reporting System (VAERS)	June 1, 2006 - December 31, 2008	42 cases of Gardasil-related GBS were reported.	**No signal:** The RR of GBS confirmed cases following qHPV was 0.3 per 100,000 person-years. The PRR for GBS after qHPV in 6- to 29-year-olds compared with all other vaccines in 6-to 29-year olds was 0.4. This PRR did not meet criteria required for signal detection.
Souayah 2011	US	Vaccine Adverse Event Reporting System (VAERS)	June 2006 - September 2009	69 reported cases of GBS after vaccination with Gardasil	**Increased trend:** The risk of acquiring GBS within the 6 weeks after Gardasil was 2.5- to 10-fold greater than in the general population.
Vichnin 2015	Worldwide	Post-licensure safety data from active and passive surveillance – Merck	2006 – 2015	Review of 15 clinical and observational studies about autoimmune diseases Among others, Guillain–Barré Syndrome and multiple sclerosis were extensively studied. No increase in the incidence of these events was found compared with background rates.	**No signal:** During the 9 years of post-licensure vaccine safety monitoring and evaluation conducted following the initial licensure of HPV4 in the US, no serious safety concern was identified in the studies conducted worldwide.
Willame 2016	United Kingdom	Historical, observational cohort study conducted within the CPRD GOLD	2005 – 2010	No confirmed case of GBS, MS or Optic Neuritis observed in exposed cohort	**No signal:** This observational study did not show any evidence of an increased risk of autoimmune diseases following vaccination with AS04-HPV--16/18 vaccine.

Footnotes: *ADEM: Acute disseminated encephalomyelitis, CNS: Central nervous system, CPRD GOLD: Clinical Practice Research Datalink General Practice OnLine Database, GBS: Guillain-Barré syndrome, GSK: Glaxo Smith Kline, EVPM: EudraVigilance post-authorisation module, HPV4: Human Papillomavirus quadrivalent vaccine, MS: Multiple sclerosis, OR: Odds ratio, PRR: proportional reporting ratio, qHPV: Human Papillomavirus quadrivalent vaccine, RIVM: Rijksinstituut voor Volksgezondheid en Milieu, RR: Relative risk, SPC: Summary of product characteristics, VAERS: Vaccine Adverse Event Reporting System, VSD: Vaccine Safety Datalink*

5.3.2 Systematic review of observational comparative studies having addressed the research question n°2

A SLR based on the research question n°2 was carried out and published in 2018. (Mouchet et al., 2018b)

Methods and results of the SLR are reported hereafter while the methods and results of the meta-analysis are reported in the corresponding sections: 5.3.3 Meta-analysis centered on the research question n°2

5.3.2.1 Objectives

The present study aimed at identifying all real-world studies having evaluated the link between demyelination, either central or peripheral, and anti-HPV vaccination.

5.3.2.2 Methods

Data sources and searches: A systematic literature review was carried out in Medline, Embase, ISI Web of Science, and The Cochrane Library from inception to 10 May 2017. A combination of terms related to *papilloma vaccination/vaccines* and *demyelinating events* (e.g., MS, ON, or GBS) were used to find pertinent studies (cf. Table 31).

Table 31: Search strategies used

Source	Search string	Terms used
MEDLINE	**1**	exp Papillomavirus Vaccines/
	2	exp Demyelinating Autoimmune Diseases, CNS/ OR exp Guillain-Barre Syndrome/
	3	1 AND 2
	4	"vaccin*".ab,ti.
	5	"demyelinat*".ab,ti. OR multiple sclerosis.ab,ti. OR guillain barre.ab,ti. OR acute disseminated encephalomyelitis.ab,ti. OR optic neuritis.ab,ti. OR neuromyelitis optica.ab,ti. OR transverse myelitis.ab,ti. OR acute haemorrhagic leucoencephalomyelitis.ab,ti. OR acute haemorrhagic leuco-encephalomyelitis.ab,ti. OR acute haemorrhagic leuco-encephalitis.ab,ti. OR acute hemorrhagic leuco-encephalitis.ab,ti. OR

		acute hemorrhagic leuco-encephalomyelitis.ab,ti. OR diffuse cerebral encephalomyelitis.ab,ti. OR diffuse cerebral encephalitis.ab,ti. OR acute partial myelitis.ab,ti. OR chronic progressive inflammatory myelopathy.ab,ti.
	6	4 AND 5
	7	3 OR 6
EMBASE	**1**	exp Wart virus vaccine/
	2	exp demyelinating disease/ OR exp Guillain Barre syndrome
	3	1 and 2
	4	"vaccin*".ti,ab.
	5	"demyelinat*".ab,ti. OR multiple sclerosis.ab,ti. OR guillain barre.ab,ti. OR acute disseminated encephalomyelitis.ab,ti. OR optic neuritis.ab,ti. OR neuromyelitis optica.ab,ti. OR transverse myelitis.ab,ti. OR acute haemorrhagic leucoencephalomyelitis.ab,ti. OR acute haemorrhagic leuco-encephalomyelitis.ab,ti. OR acute haemorrhagic leuco-encephalitis.ab,ti. OR acute hemorrhagic leuco-encephalitis.ab,ti. OR acute hemorrhagic leuco-encephalomyelitis.ab,ti. OR diffuse cerebral encephalomyelitis.ab,ti. OR diffuse cerebral encephalitis.ab,ti. OR acute partial myelitis.ab,ti. OR chronic progressive inflammatory myelopathy.ab,ti.
	6	4 and 5
	7	3 OR 6
COCHRANE LIBRARY	**1**	MeSH descriptor: [Papillomavirus Vaccines] explode all trees
	2	MeSH descriptor: [Demyelinating Autoimmune Diseases, CNS] OR [Guillain-Barre Syndrome] explode all trees
	3	1 AND 3
	4	Vaccin* (ti/ab/kw)
	5	"demyelinat*".ab,ti. OR multiple sclerosis.ab,ti. OR guillain barre.ab,ti. OR acute disseminated encephalomyelitis.ab,ti. OR optic neuritis.ab,ti. OR neuromyelitis optica.ab,ti. OR transverse myelitis.ab,ti. OR acute haemorrhagic leucoencephalomyelitis.ab,ti. OR acute haemorrhagic leuco-encephalomyelitis.ab,ti. OR acute haemorrhagic leuco-

		encephalitis.ab,ti. OR acute hemorrhagic leuco-encephalitis.ab,ti. OR acute hemorrhagic leuco-encephalomyelitis.ab,ti. OR diffuse cerebral encephalomyelitis.ab,ti. OR diffuse cerebral encephalitis.ab,ti. OR acute partial myelitis.ab,ti. OR chronic progressive inflammatory myelopathy.ab,ti.
	6	4 AND 5
	7	3 OR 6
WEB OF SCIENCE	**1**	TS= (Demyelinat* OR multiple sclerosis OR guillain barre acute disseminated encephalomyelitis OR optic neuritis OR neuromyelitis optica OR transverse myelitis OR acute haemorrhagic leucoencephalomyelitis OR acute haemorrhagic leuco-encephalomyelitis OR acute haemorrhagic leuco-encephalitis OR acute hemorrhagic leuco-encephalitis OR acute hemorrhagic leuco-encephalomyelitis OR diffuse cerebral encephalomyelitis OR diffuse cerebral encephalitis OR acute partial myelitis OR chronic progressive inflammatory myelopathy)
	2	TI=vaccin*
	3	1 AND 2 refined to WOS databases

Pragmatic searches were conducted and secondary sources cited in bibliographical references of reviews were also screened (i.e., snowballing). No restrictions regarding the language or time period were applied. The present study followed the requirements of the PRISMA and MOOSE statements. (Moher et al., 2009; Stroup et al., 2000)

Study selection: Eligibility criteria were defined according to the PICOS elements.(Moher et al., 2009) Both adult and child populations were considered for the present study. Publication type included peer-reviewed articles, reports and abstracts.

Outcomes of interest were central demyelination (not limited to MS and ON), MS, ON or GBS, each being considered independently. MS had to be diagnosed by a neurologist using validated diagnostic criteria, which include the occurrence of at least one central demyelination attack and the demonstration of dissemination of central nervous system lesions in space and time.(McDonald et al., 2001; Polman et al., 2011; Poser et al., 1983)

Two investigators (Julie Mouchet, University of Bordeaux, France, and Emanuel Raschi, University of Bologna, Italy) reviewed the titles and abstracts of all retrieved citations independently to select relevant publications. Disagreements were solved through discussion. In the event of doubt, a third person (Bernard Bégaud, Unversity of Bordeaux) was asked to confirm or not the selection of the study.

Data extraction and quality assessment: For all publications finally retained, the following items were abstracted into a standardized Excel data extraction form: study design, population characteristics (number of subjects in each group, mean or median age, gender, risk factors for demyelination), outcome of interest and its definition, study period, vaccine exposure, adjusted risk estimates, statistical analysis and adjustment variables. The results of sensitivity analyses of original articles were also screened and reported when considered relevant. When necessary, authors of selected publications were contacted to obtain additional information or clarification. The quality of individual studies was assessed by using the Newcastle Ottawa Scale for cohort and case-control designs.(Wells et al., 2006)

The protocol of the SLR and meta-analysis (n° CRD42015020809) was published on the PROSPERO platform (https://www.crd.york.ac.uk/prospero/) before running the study.

5.3.2.3 Results

Out of 2,863 references retrieved from the four bibliographic databases and systematically reviewed on the basis of their title and abstract, 139 were initially selected. After full-text reviewing, 19 articles describing epidemiological studies were selected and six additional publications were identified through pragmatic searches, leading to the selection of 11 articles for meta-analysis (Nick Andrews, Stowe, & Miller, 2017; Angelo, David, et al., 2014; Arnheim-Dahlström, Pasternak, Svanström, Sparén, & Hviid, 2013; Baxter, Lewis, Fireman, et al., 2016; Chao et al., 2012; Grimaldi-Bensouda et al., 2017; Langer-Gould, Qian, et al., 2014; Miranda et al., 2017; Scheller et al., 2015; Sridhar et al., 2017; Verstraeten et al., 2008) and 14 pharmacovigilance studies on SRSs for a qualitative review, described in section 5.3.1.3 Post-licensure studies aiming at detecting signals or evaluating disproportionality (Angelo, Zima, et al., 2014; Baxter, Lewis, Goddard, et al., 2016; Cameron et al., 2016; Gee et al., 2011; D. A. Geier & Geier, 2015, 2017; Gold et al., 2010; Labadie, 2011; Ojha et al., 2014;

Pellegrino et al., 2013; Slade et al., 2009; Souayah et al., 2011; Vichnin et al., 2015; Willame et al., 2016) (cf. Figure 44).

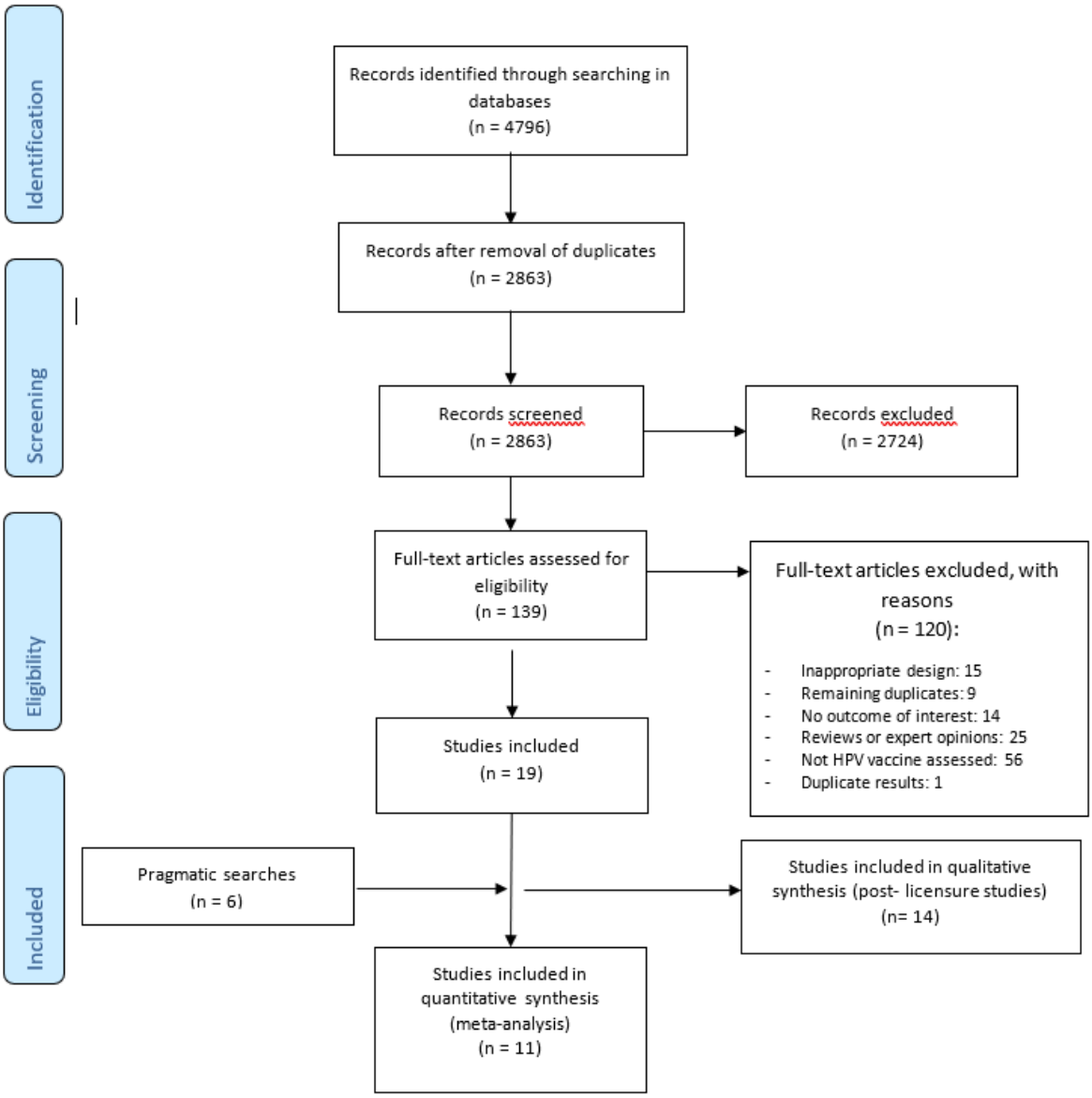


Figure 44 : PRISMA Flow chart

Table 32 presents the main characteristics of the 11 studies (Nick Andrews et al., 2017; Angelo, David, et al., 2014; Arnheim-Dahlström et al., 2013; Baxter, Lewis, Fireman, et al., 2016; Chao et al., 2012; Grimaldi-Bensouda et al., 2017; Langer-Gould, Qian, et al., 2014; Miranda et al., 2017; Scheller et al., 2015; Sridhar et al., 2017; Verstraeten et al., 2008) retained for the meta-analysis: six were cohorts,(Arnheim-Dahlström et al., 2013; Scheller et al., 2015) (Miranda et al., 2017) (Sridhar et al., 2017) (Baxter, Lewis, Fireman, et al., 2016; Chao et al., 2012) two were case-control studies,(Grimaldi-Bensouda et al., 2017; Langer-

Gould, Qian, et al., 2014) one was a self-controlled case series,(Nick Andrews et al., 2017) and two were pooled analyses of randomised clinical trials.(Angelo, David, et al., 2014; Verstraeten et al., 2008)

Table 32 : Studies selected for meta-analysis

Reference	Study design	Case definition	Risk estimate and adjustment factors	Outcome investigated	Experimental group		Comparator group		OR / RR / IRR [95%CI]	Time period
					n (events)	n (total)	n (events)	n (total)		
Angelo 2014	Analysis of 42 studies on HPV 16/18 (Cervarix)	Medical Dictionary for Regulatory Activities (MedDRA) Preferred Terms related to immune-mediated diseases	RRs This exploratory analysis was not corrected for multiple comparisons so it should be interpreted with caution.	MS	1	21358	0	20504	Not assessable (0 cases in controls)	12 months
				ON	8	21358	0	20504	0.58 (0.09-2.96)	12 months
Grimaldi-Bensouda 2017	Case-referent study (PGRx)	Case definitions based on internationally accepted classifications for each disorder. When necessary to confirm diagnosis, cases followed for up to 1 year.	Matched ORs to cases according to (1) age (best match available within maximum range of 2 years as follows: cases ≤17 years-old: age of referent ±1 month, ±3 months, ±6 months, ±1 year from age of case; and cases ≥18 years old: age of referent ±1 year, ±2 years from age of case), (2) place of residence (North vs. South of France) and (3) index date (defined for cases as date of first symptoms evocative of AD and for referents as date of recruitment). Adjustment on age, familial/personal history of autoimmune disease, parents' place of birth, and use of any oral contraceptives or vaccines (other than human papillomavirus vaccine) within the 2 years preceding index date.	Central demyelination	7	113	173	863	0.31 (0.13-0.73)	2 years
				GBS	0	13	2	130	Not assessable (0 cases in vaccinees)	2 months

Arnheim-Dahlström 2013	Nationwide cohorts (Denmark + Sweden)	ICD-10 codes	IRR adjusted for country, age in two-year categories, calendar year, parental educational level (highest attained level of either parent classified as: primary school (nine years) or shorter; secondary school (12 years); short tertiary education; and medium or long tertiary education), parental country of birth (categories: both parents, one parent, or no parent born in Scandinavia), and paternal socioeconomic status (categories: employment with basic, unknown, or no qualification; employment with medium-level or high-level qualifications; self-employed; and not in labour market)	ON	6	230018	61	2374273	0.67 (0.27-1.24)	6 months
Scheller 2015	Nationwide cohorts (Denmark + Sweden)	ICD-10 code G35	RRs adjusted for calendar year, age (2-year intervals), and country Index date = date of diagnosis	MS	73	1193703	4208	19532311	0.9 (0.70-1.15)	2 years
Verstraeten 2008	Analysis of 16 studies on HPV 16/18 (Cervarix)	Medical Dictionary for Regulatory Activities (MedDRA) Preferred Terms related to immune-mediated diseases	RRs adjusted for study effect	MS	1	39160	1	31768	1 (0.01-78.59)	Mean FU 1.8 years
				ON	2	39160	2	31768	1 (0.07-13.80)	Mean FU 1.8 years
Miranda 2017	Nationwide cohort (France, SNIIRAM)	ICD-10 codes	HR adjusted for year of inclusion in the cohort, geographical area, public insurance coverage (i.e. French CMU), previous and current health care consumption and other vaccinations received	Central demyelination	82	842120	219	1410596	1.05 (0.79-1.40)	Anytime (up to 2 years)
				MS	50	842120	134	1410596	0.93 (0.65-1.33)	Anytime (up to 2 years)
				GBS	19	842120	21	1410596	4.00 (1.84-8.69)	Anytime (up to 2 years)
Langer-Gould 2014	Nested case-control study (KPSC)	ICD-9 codes: 340, 341.0, 341.22, 341.8, 341.9, 377.30, 377.32, 377.39, and 336.39	Matched ORs adjusted on race/ethnicity, hospitalizations (0 or ≥1), outpatient visits (0 or≥1), emergency department visits (0 or ≥1), chronic diseases (0 or ≥1), and infections (0 or ≥1) within 6 months before symptom onset (index date)	Central demyelination	36	780	175	3885	1.05 (0.62-1.78)	3 years
				MS	21	780	83	3885	1.6 (0.79-3.25)	3 years

Sridhar 2017	Matched cohort study in HealthCore Integrated Research Database	Algorithm combining incident ON diagnoses with evaluation and management codes using Current Procedural Terminology codes diagnosed by a neurologist or an ophthalmologist along with claims evidence of an MRI	RRs adjusted for demographics such as age, region of residence, Enhanced Charlson comorbidity index in the year prior to vaccination, history of other vaccinations in the 90 days prior to and 30 days after vaccination, and history of other autoimmune diseases in the year prior to vaccination	ON	36	327918	32	327918	1.10 (0.62–1.96)	2 months
Chao 2012	Cohort study (KPSC + KPNC)	ICD-9 diagnostic codes, abnormal laboratory results or pharmacy prescriptions possibly indicative of autoimmune conditions occurring from first HPV4 dose to 180 days	IRR	MS	3	117761	14	412151	1.37 (0.74–3.20)	6 months
				ON	5	117761	22	412151	1.45 (1.00-2.91)	6 months
Baxter 2016	Case-centered analysis in KPNC	ON diagnosis made by either an ophthalmologist or a neurologist within the 3 months following the initial diagnosis. Trained medical records analysts reviewed all identified cases to ascertain the specialist's diagnosis	Relative risk of being in the exposure interval (2-42 days) *versus* the rest of the 9 months	ON	5	NR	NR	NR	4.60 (0.6–40.3)	42 days
Andrews, 2017	Self-controlled case series (Hospital Episodes Database, UK)	ICD-10 code: G610	Self-adjusted for time-fixed confounders	GBS	9	100	NA	NA	1.26 (0.55-2.92)	0-91 days

Footnotes: *CMU: Couverture médicale universelle, GBS: Guillain-Barré syndrome, HPV: Human Papillomavirus, HR: Hazard ratio, KPNC: Kaiser Permanente Northern California, KPSC: Kaiser Permanente Southern California, ICD: International classification of diseases, IRR: Incidence rate ratio, MedDRA: Medical Dictionary for Regulatory Activities, MRS: Multivariate risk score, MRI: Magnetic Resonance Imaging, MS: Multiple sclerosis, NA: Not applicable, NR: Not reported, ON: Optical neuritis, OR: Odds ratio, PGRx: Pharmacoepidemiologic General Research eXtension, RI: Relative incidence, RR: Relative risk, SNIIRAM: Système national d'information inter-régimes de l'Assurance maladie*

The two case-control studies (Grimaldi-Bensouda et al., 2017; Langer-Gould, Qian, et al., 2014) were performed within large real-world data sources (Pharmacoepidemiologic General Research eXtension (PGRx) programme, and the Kaiser Permanente databases) and included a total of 1,290 cases and 5,838 controls. Two cohort studies (Arnheim-Dahlström et al., 2013; Scheller et al., 2015) were conducted in the same nationwide registries for Sweden and Denmark, but at different time periods. A nationwide cohort study was performed within the French "Système national d'information inter-régimes de l'assurance maladie" (SNIIRAM) database.(Miranda et al., 2017) One US matched cohort study used the HealthCore Integrated Research Database (Sridhar et al., 2017), whereas the last two historical cohorts used the Kaiser Permanente Northern and Southern California databases.(Baxter, Lewis, Fireman, et al., 2016; Chao et al., 2012) The cohort studies included a total of 26,852,890 individuals, of which 2,753,578 were exposed to HPV vaccine (200 cases), and 24,099,312 were non-exposed (4,534 cases). The self-controlled case series analysis was performed using Hospital Episode Statistics (HES), and was conducted to investigate the risk of GBS after HPV vaccine in 209 girls aged 12-18 in England.(Nick Andrews et al., 2017)

The quality of the studies evaluated by the Newcastle Ottawa Scale was good and roughly comparable for all papers retained since the scores ranged from six to nine stars (cf. Table 33 and Table 34). By definition, the two studies having pooled safety data from the clinical trials of HPV vaccines (Angelo, David, et al., 2014; Verstraeten et al., 2008) were not eligible for evaluation by the Newcastle Ottawa Scale. Nevertheless, as they compiled results from phase 3 clinical studies, biases usually feared in observational studies such as cohort or case-controls were not a real concern.

Table 33 : Individual quality assessment evaluated with Newcastle Ottawa scale for case-control studies

	SELECTION					EXPOSURE			Total score (max 9)
	Is case definition adequate?	Representativeness of cases	Selection of controls	Definition of controls	Comparability of cases and controls on basis of design or analysis	Ascertainment of exposure	Same method of ascertainment for cases and controls	Non-response rate	
Langer-Gould, 2015 (41)	Yes, with independent validation	Consecutive or obviously representative series of cases	Community controls	No description of source	Matching on date of birth (within 1 year), sex, and zipcode (a surrogate measure for socioeconomic status)	Written self-report or medical record only	Yes	Same rate for both groups	
*********Coding	1	1	1	0	2	0	1	1	**7**
Grimaldi-Bensouda, 2017 (40)	Yes, with independent validation	Consecutive or obviously representative series of cases	Community controls	No history of disease (endpoint)	Matching to cases according to age, region of residence and recruitment consultation date	Written self-report or medical record only	Nes	Non-respondents described	
*********Coding	1	1	1	1	2	0	1	0	**7**
Andrews, 2017 (35)	Yes, with independent validation	Consecutive or obviously representative series of cases	Community controls	No history of disease (endpoint)	Self-controlled case series (cases serve as their own controls)	interview not blinded to case/control status	Yes	Non-respondents described	
*********Coding	1	1	1	1	2	0	1	0	**7**

Table 34 : Individual quality assessment evaluated with Newcastle Ottawa scale for cohort studies

	SELECTION					Outcome			Total score (max 9)
	Representativeness of exposed cohort	Selection of non-exposed cohort	Ascertainment of exposure	Demonstration that outcome of interest was not present at start of study	Comparability of cohorts on basis of design or analysis	Ascertainment of outcome	Was FU long enough for outcomes to occur?	Adequacy of FU of cohorts	
Report ANSM, 2015 (34)	Truly representative of average individuals in community	Drawn from same community as exposed group	No description	Yes	HR adjusted for year of inclusion in cohort, geographical area, public insurance coverage (i.e. CMU), previous and current health care consumption and other vaccinations received	Record linkage	Yes	Complete FU - all subjects accounted for	
*********Coding	1	1	0	1	2	1	1	1	**8**
Arnheim-Dalström, 2013 (37)	Truly representative of average individuals in community	Drawn from same community as exposed group	Secure record (e.g. surgical records)	No	IRR adjusted for country, age in 2-year categories, calendar year, parental educational level, parental country of birth, and paternal socioeconomic status	Record linkage	Yes	Complete FU - all subjects accounted for	
*********Coding	1	1	1	0	2	1	1	1	**8**
Chao, 2012 (39)	Somewhat representative of the average individuals in the community	drawn from same community as exposed group	No description	Yes	*No details*	Independent blind assessment	yes	Complete FU - all subjects accounted for	
*********Coding	1	1	0	1	0	1	1	1	**6**
Scheller, 2015 (42)	Truly representative of average individuals in community	Drawn from same source population as exposed group	Secure record (e.g. surgical records)	Yes	RRs adjusted for calendar year, age (2-year intervals), and country	Record linkage	Yes	Complete FU - all subjects accounted for	
*********Coding	1	1	1	1	2	1	1	1	**9**
Sridhar 2017 (43)	Truly representative of average individuals in	drawn from same source population	Secure record (e.g. surgical records)	Yes	RRs adjusted for demographics such as age, region of residence, Enhanced	Record linkage	Yes	No description of those lost	

	community	as exposed group			Charlson comorbidity index in year prior to vaccination, history of other vaccinations in 90 days prior to and 30 days post vaccination, and history of other autoimmune diseases in year prior to vaccination			or FU rate important	
*********Coding	1	1	1	1	2	1	1	0	**8**
Baxter 2016a (38)	Truly representative of average individuals in community	Drawn from the same source population as the exposed group	Secure record (e.g. surgical records)	Yes	Analyses restricted to vaccines, so need to adjust for differences between vaccinated and unvaccinated individuals.	Record linkage	Yes	No description of those lost or FU rate important	
*********Coding	1	1	1	1	2	1	1	0	**8**

Notes: Angelo, 2014a (336) and Verstraeten, 2008 (44) not eligible for assessment with Newcastle Ottawa Scale as they pooled safety data from clinical studies of HPV vaccines.

5.3.2.4 Discussion

This systematic review did not highlight an increased risk of a demyelinating event attributable to HPV vaccination.

While 10 of the 11 observational comparative studies (Nick Andrews et al., 2017; Angelo, David, et al., 2014; Arnheim-Dahlström et al., 2013; Baxter, Lewis, Fireman, et al., 2016; Chao et al., 2012; Grimaldi-Bensouda et al., 2017; Langer-Gould, Qian, et al., 2014; Miranda et al., 2017; Scheller et al., 2015; Sridhar et al., 2017; Verstraeten et al., 2008) were concordant in finding congruent non-significant risk estimates for demyelination after HPV vaccination, one (Grimaldi-Bensouda et al., 2017) was identified as an outlier with a surprising and significant "protective" odds ratio of 0.31 [95%CI: 0.13 – 0.73]. The fact that this case-control study was designed in the PGRx programme might explain this unexpected finding. Indeed, the controls in that study were sampled from a large population of cases of other diseases recruited by a network of general practitioners.(Grimaldi-Bensouda et al., 2017) In addition, exposure may have been less common in cases for which prodromal illnesses, comorbidities or family history may have prevented vaccination (i.e., depletion of susceptibles). This assumption is strengthened by the fact that a personal or family history of autoimmune diseases was more frequently found in cases (14.7%) than in corresponding matched referents (7.2%). A previous paper with identical objectives, methods and very similar results was published in 2014 (Grimaldi-Bensouda et al., 2014). As cases identified in the latter paper were part of the updated analysis published in 2017, only the most recent paper (Grimaldi-Bensouda et al., 2017) was included in this systematic review in order to avoid the same patients counting twice in the meta-analysis.

Contradictory findings were reported for the potential risk related to GBS, the French nationwide cohort study (Miranda et al., 2017) concluding in a significant, and relatively strong, association between GBS and HPV vaccination with an OR of 4.0 [95%CI: 1.84 – 8.69]. Conversely, this result was not confirmed by the recent self-controlled case series conducted by Andrews et al, 2017 (Nick Andrews et al., 2017) which found a non-significant OR of 1.26 [95%CI 0.55 – 2.92].

5.3.2.5 Strengths and limitations

The main strength of this SLR was complying with methods which followed the highest current standards. Various data sources were screened and pragmatic searches were

performed to complement findings identified from bibliographical databases. In addition, almost all studies selected for this research project were of good quality, having a NOS score ranging from 7 to 9.

The major limitation of this SLR ensues from the complexity of a comparison of individual studies having diverse features, using different sources and designs with their own drawbacks and strengths.

5.3.3 Meta-analysis centered on the research question n°2

To compile the results obtained from individual studies, a meta-analysis was conducted and published in 2018. (Mouchet et al., 2018b) To our best knowledge, this study was the first meta-analysis published on this research question.

5.3.3.1 Objectives

The present study aimed at assessing the risk of developing demyelination after HPV immunization by meta-analysing risk estimates from all comparative pharmacoepidemiologic studies identified by the SLR (cf. section Systematic review of observational comparative studies having addressed the research question n°2).

5.3.3.2 Methods

Study selection: The quantitative review included observational studies with an *ad hoc* reference group reporting an adjusted relative estimate of risk (e.g. Odds Ratio, OR; Hazard Ratio, HR; Incidence Rate Ratio, IRR) of developing an acute demyelination after vaccination against human papillomavirus. Pooled analyses of HPV randomized trials having investigated at least one outcome of interest were also included. Post-licensure studies based on SRSs, case reports, case series, expert opinions, and studies without a reference group were excluded.

Data analysis and synthesis: Risk estimates and the corresponding 95% confidence intervals (95%CI) were extracted with Review Manager software [Review Manager (RevMan) [Computer program]. Version 5.3. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014]. Each neurological event (i.e., broad category of central demyelination, MS, ON, GBS) was considered separately, since the biological mechanisms leading to these

events have little in common. Separate forest plots were drawn accordingly. Adjusted risk estimates assessed on the longest periods of each study were chosen for the primary analysis. The strength of the evidence generated was evaluated with the GRADE framework by considering the clinical outcome that was the most shared by the meta-analysed studies.(Balshem et al., 2011; Guyatt et al., 2011)

A sensitivity analysis using risk estimates assessed at 6 months after HPV vaccination was performed to rule out the impact of a shorter time window on the pooled risk estimate. Given both the non-randomized nature of most of the studies included and the use of adjusted ORs, generic inverse variance random-effect models were used to estimate the overall risks for the selected outcomes.(DerSimonian & Laird, 1986) Heterogeneity across the included studies was evaluated by using the Q Cochran test, and p values <0.10 were considered as statistically significant.(J. Higgins & Thompson, 2002) I^2 statistics were also measured to quantify inconsistencies across estimates.(J. Higgins & Thompson, 2002) Source of heterogeneity was investigated when necessary. No specific procedure was applied to assess publication bias quantitatively because of the absence of specific recommendations concerning observational studies, and because this bias is particularly difficult to appraise in non-interventional studies for which preliminary registration in a trial repository is not yet required by the health authorities.(Food and Drug Administration, 2007) In addition, the usual methods for assessing such a bias, which are partly based on the sample sizes of the included trials (J. P. Higgins & Green, 2011), are irrelevant for observational studies. Rather than using specific statistic tools, the likelihood of a publication bias was assessed qualitatively by considering the number of studies funded by a private company or by a stakeholder interested in the marketing of the vaccine under consideration. Indeed, the more published studies are sponsored in such a way, the higher the risk of publication bias.

5.3.3.3 Results

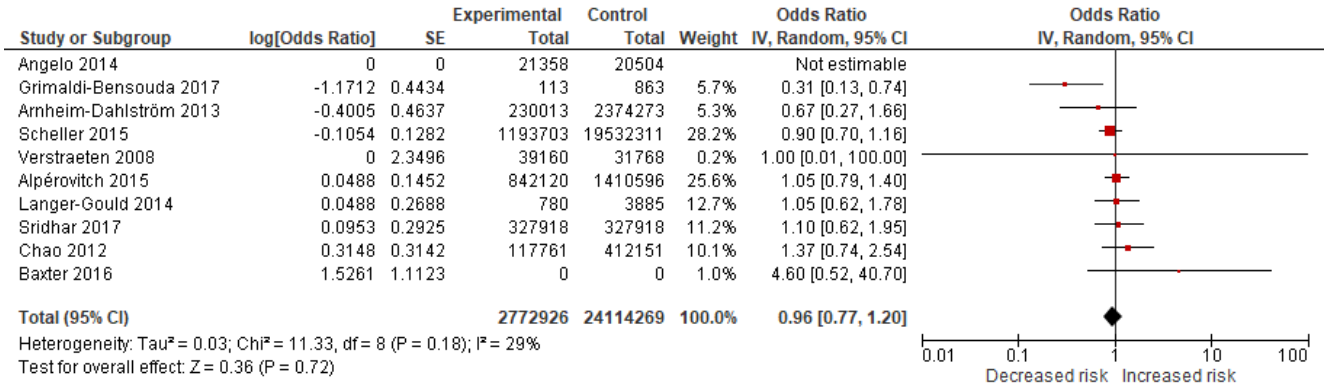
Selection of studies

A total of 11 articles were selected for the meta-analysis (Nick Andrews et al., 2017; Angelo, David, et al., 2014; Arnheim-Dahlström et al., 2013; Baxter, Lewis, Fireman, et al., 2016; Chao et al., 2012; Grimaldi-Bensouda et al., 2017; Langer-Gould, Qian, et al., 2014; Miranda et al., 2017; Scheller et al., 2015; Sridhar et al., 2017; Verstraeten et al., 2008)

Broad category of central demyelination

A total of ten studies (Angelo, David, et al., 2014; Arnheim-Dahlström et al., 2013; Baxter, Lewis, Fireman, et al., 2016; Chao et al., 2012; Grimaldi-Bensouda et al., 2017; Langer-Gould, Qian, et al., 2014; Miranda et al., 2017; Scheller et al., 2015; Sridhar et al., 2017; Verstraeten et al., 2008) reported adjusted risk estimates for central demyelinating diseases occurring after HPV vaccination. Individual risk estimates markedly differed and ranged from 0.31 (95%CI: 0.13-0.74] to 4.60 [95%CI: 0.52-40.70]. The resulting meta-analysis did not support a statistically significant association between HPV vaccination and central demyelinating diseases (cf. Figure 45a), the pooled OR being close to 1 with a value of 0.96 [95%CI 0.77 – 1.20]. A moderate but non-significant heterogeneity emerged when computing adjusted pooled risks (Q= 11.33, p= 0.18; I^2 = 29%). The limited number of studies and the non-significance of the observed heterogeneity did not justify the use of a meta-regression. Nevertheless, the source of heterogeneity was assessed by removing studies one by one from the meta-analytic model. As mentioned earlier, one study (Grimaldi-Bensouda et al., 2017) was identified as an outlier with its "protective" risk estimate of 0.31 [95%CI: 0.95 – 40.70]. Nevertheless, when this study was removed from the meta-analysis, the results were practically not affected, with a pooled odds ratio slightly increasing to 1.00 [95%CI 0.85 – 1.18]. A sensitivity analysis was performed with the five studies (Arnheim-Dahlström et al., 2013; Chao et al., 2012; Langer-Gould, Qian, et al., 2014; Miranda et al., 2017; Scheller et al., 2015) having provided risks of central demyelination for the 6-month period following HPV vaccination. When considering this shorter, and probably more specific, period, the pooled estimate increased slightly to 1.06 [95%CI 0.85-1.32; Q=2.21, p= 0.70; I^2 = 0%], but remained far from statistical significance (cf. Figure 45b).

A/ Forest plot for demyelinating diseases



B/ Forest plot for demyelinating diseases within 6 months after HPV

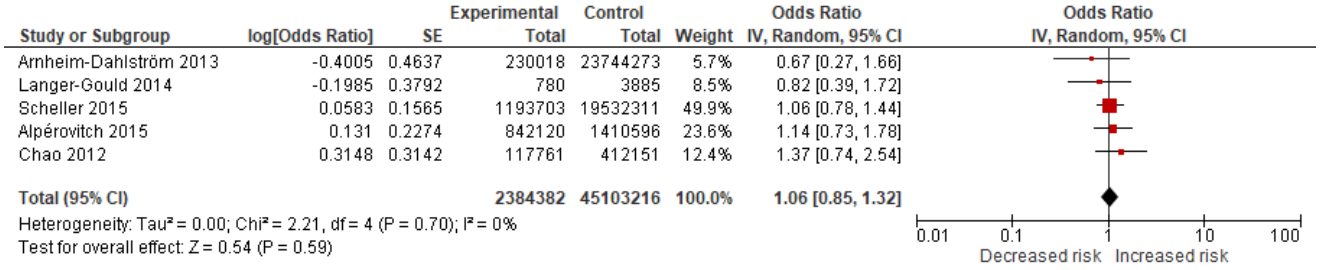


Figure 45 : Forest plots for demyelinating diseases

Central demyelination occurring after HPV vaccination was the most documented outcome with ten individual studies providing risk estimates for this condition. To assess the strength of evidence generated by this meta-analysis, the GRADE framework was applied to this outcome (cf. Table 35). Strength of evidence was judged low owing to the observational nature of studies included in this meta-analysis.

Table 35 : Strength of evidence using GRADE framework

	Strength of evidence						N° of patients		Summary of findings		
Outcome	No of studies (Design)	Limitations	Inconsistency	Indirectness	Imprecision	Publication bias	HPV	Comparator	Odds ratio (95% CI)	**Overall Strength**	**Importance**
Central demyelination	10 (observational)	Not serious[a]	Not serious[b]	Not serious[c]	Unlikely[d]	Unlikely	245 / 2,772,926	4,883 / 24,114,269	0.96 [95%CI 0.77 – 1.20]	⊕⊕◯◯ LOW	CRITICAL

a. Most studies were of good quality as rated by Newcastle Ottawa Scale (score >7)

b. As heterogeneity was found moderate (I^2=29%).

c. Population, outcomes and intervention of interest were very homogeneous. No indirect comparison was made in selected studies.

d. Risk estimates were all within a tight range of values and very large samples (nationwide cohorts) were assessed.

Multiple sclerosis

A total of six individual studies provided risk estimates for MS occurring after HPV vaccination.(Angelo, David, et al., 2014; Chao et al., 2012; Langer-Gould, Qian, et al., 2014; Miranda et al., 2017; Scheller et al., 2015; Verstraeten et al., 2008). Individual relative risks were more congruent than for central demyelination and ranged from 0.90 [95%CI: 0.70-1.16] to 1.60 [95%CI: 0.79-3.24]. The pooled risk ratio obtained from the meta-analysis was 0.98 [95%CI: 0.82-1.19; Q= 3.51, p= 0.48; I^2 = 0%] (cf. Figure 46).

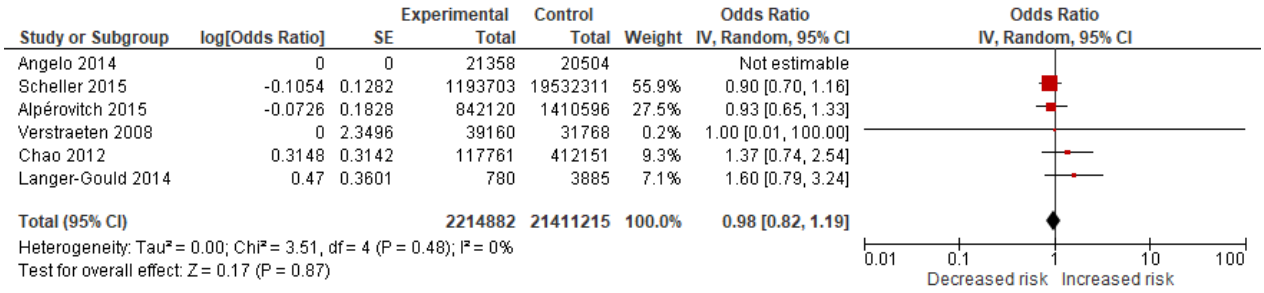


Figure 46 : Forest plot for multiple sclerosis

The cohort using Danish and Swedish nationwide registries of girls weighted almost 56% of the pooled sample; after its exclusion, the pooled estimate did not vary substantially (pooled OR 1.10 [95%CI: 0.83-1.46; Q= 2.42, p= 0.49; I^2 = 0%]).

Optic neuritis

A total of six individual studies provided risk estimates for ON occurring after HPV vaccination.(Angelo, David, et al., 2014; Arnheim-Dahlström et al., 2013; Baxter, Lewis, Fireman, et al., 2016; Chao et al., 2012; Sridhar et al., 2017; Verstraeten et al., 2008) Individual risk estimates ranged from 0.58 [95%CI: 0.09-3.74] to 4.60 [95%CI: 0.52-40.70]. None of the individual estimates reached statistical significance and the meta-analysis led to a pooled risk of 1.25 [95%CI: 0.93-1.66; Q= 4.66, p= 0.46; I^2 = 0%] (cf. Figure 47).

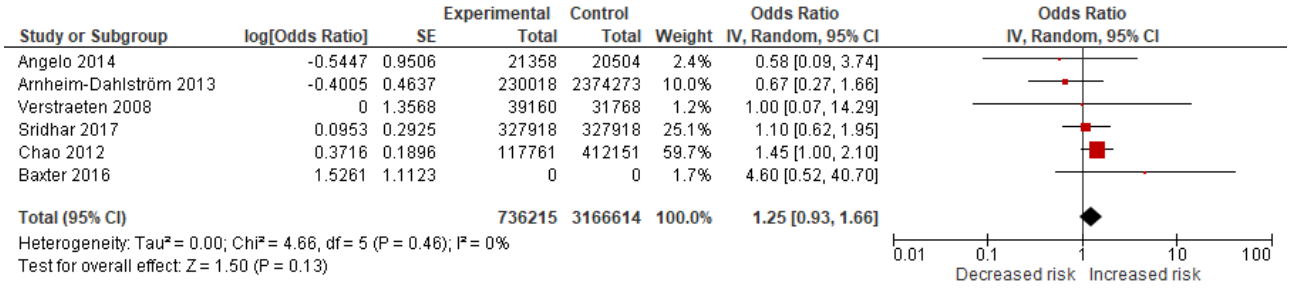


Figure 47 : Forest plot for optic neuritis

The cohort study conducted within the Kaiser Permanente databases (Chao et al., 2012) represented almost 60% of the total weight of the pooled sample; after its exclusion, the pooled estimate decreased by about 25% (pooled OR 0.99 [95%CI: 0.63-1.56]; Q= 3.06, p= 0.55; I^2 = 0%).

Guillain-Barré Syndrome

When considering the age range recommended for vaccination, the Guillain-Barré syndrome is certainly a particularly relevant outcome to be scrutinized when assessing the neurological safety of anti-HPV vaccines. Surprisingly, only three studies (Nick Andrews et al., 2017; Grimaldi-Bensouda et al., 2017; Miranda et al., 2017) assessed the risk of developing a GBS in the follow of a HPV vaccination, and only two allowed to quantify this risk and provided estimates ranging from 1.26 (95%CI: 0.55-2.92] to 4.00 [95%CI: 1.84-8.69]. Owing to the low number of studies, no meta-analysis was performed for this outcome. It is noteworthy that the two estimates were higher than 1 and one reached statistical significance. (Miranda et al., 2017)

Publication bias

Of the 11 studies considered for meta-analysis, almost half (n=5; 45.5%) were sponsored by pharmaceutical companies (Angelo, David, et al., 2014; Chao et al., 2012; Grimaldi-Bensouda et al., 2017; Scheller et al., 2015; Verstraeten et al., 2008). However, results from industry-sponsored studies were similar in magnitude to those funded by apparently non-industry sources.

5.3.3.4 Discussion

The meta-analysis did not highlight any significant association for all outcomes investigated, pooled odds ratios remained close to one and not-significant for the broad category of central demyelination, MS and ON. However, unlike other conditions, the two studies having considered the Guillain-Barré syndrome led to risk ratio greater than 1, the association being found significant in one study. (Miranda et al., 2017)

Restricting the at-risk period to the 6 months following HPV vaccination did not alter the conclusion, the pooled risk estimate remaining non-statistically significant.

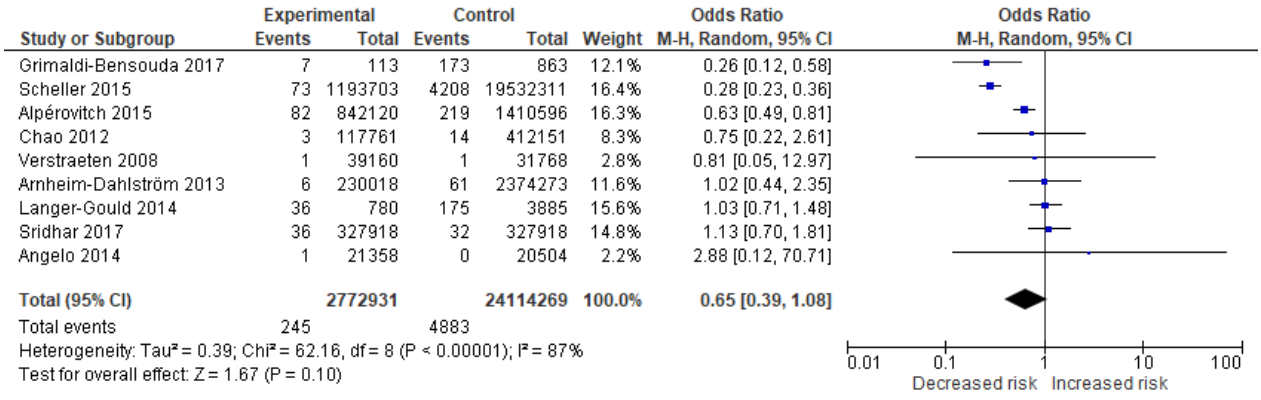
Performing a meta-analysis for GBS was not feasible owing to the small number of observational studies having considered this outcome, with only two studies yielding a risk estimate.(Nick Andrews et al., 2017; Miranda et al., 2017)

5.3.3.5 Strengths and limitations

The present study has several strengths. To our knowledge, it was the first meta-analysis having assessed the potential association between HPV vaccines and demyelinating diseases. Moreover, it complied with the highest current methodological standards (e.g., PRISMA and MOOSE). Second, while two reviews were previously published (Mailand & Frederiksen, 2016; Pellegrino et al., 2014), our meta-analysis (i) retrieved six additional studies, (ii) considered different demyelinating diseases, and (iii) tested, in a sensitivity analysis, the impact of a shorter at-risk period (i.e., 6 months) in order to increase both the robustness of and the confidence in the results. Third, almost all studies meta-analysed were judged as being of good quality, i.e. having individual scores based on the Newcastle Ottawa Scale of at least seven stars. Fourth, heterogeneity was evaluated as moderate and non-significant (or absent for some analyses), allowing the selected studies to be pooled. Furthermore, to increase the amount of evidence, this meta-analysis was supplemented by a review of post-licensure studies based on pharmacovigilance reports.

Some limitations should also be acknowledged. First, only a limited number of observational studies was eligible for the meta-analysis, which precluded some outcomes being studied (e.g., GBS). Secondly, the rarity of the outcomes of interest, with an estimated incidence in general population of 1 to 2 per 100,000 and per year for GBS,(Burns, 2008) would have

required unusually large samples, at least for prospective (i.e. cohort) analyses, which explains why several studies included were clearly under-powered to assess such a risk, if any, with a sufficient reliably. Thirdly, the existence of a potential publication bias was not assessed quantitatively, given the absence of a suitable method for observational studies. However, this bias was qualitatively appraised by comparing results obtained by industry-funded studies with those of publicly sponsored studies. Another limitation might be the meta-analytic model used for this research. Owing to the low incidence of the events considered (Sweeting et al., 2004), the Peto one-step odds ratio method could have been the preferred option.(Yusuf et al., 1985) However, while it is perfectly suited for clinical trials, a prerequisite for using this method is that the groups compared should have more or less the same size, which was definitely not the case for some of the studies meta-analysed.(Greenland & Salvan, 1990; J. P. Higgins & Green, 2011) For this reason, and even if its use could appear questionable for events of rare occurrence (Bradburn et al., 2007), we chose to use a generic inverse variance model as it allowed us to compute adjusted ORs from non-randomized studies, for which contingency tables and counts were not appropriate. Otherwise, these studies would have been excluded, leading to a number of eligible studies hampering any calculation of pooled estimates. To test the robustness of our model, we also used the Mantel-Haenszel random-effect method, which is an option for rare and dichotomous outcomes and imposes using crude risk estimates for computation.(Veroniki et al., 2016) The results obtained were consistent with those reported above. For central demyelination, the pooled risk ratio was 0.65 (95%CI: 0.39 – 1.08) *versus* 0.96 (95%CI: 0.77 -1.20) for the model chosen (cf. Figure 48).



Note: Baxter 2016a not included as this study did not provide details about numbers of events and number of patients in each group.

Figure 48 : Sensitivity analysis with Mantel-Haenszel statistical model

5.3.4 Conclusion

All evidence concerning the second research question of this thesis is summarized in Table 36.

Table 36 : Summary of evidence addressing our research question n°2

Parameters	Hypothesis	Evidence
Biological plausibility	Is there a physiopathological hypothesis supporting an association between HPV and demyelination?	Unlikely as not documented in the literature so far.
Published case reports	Are there numerous case reports of demyelination following HPV vaccination? Are they consistent and specific?	At least 12 case reports published in the scientific literature Cases originated from various developed countries and were either peripheral or central demyelinating events.
Existence of a safety signal	Has a signal been detected so far? Is the frequency of occurrence/reports for demyelination higher after the HPV vaccination?	A close monitoring of the vaccine safety has been implemented since the vaccine launch. No safety signal has been detected so far. Two studies conducted within VAERS found higher reporting rates/ occurrence frequency for: - GBS (frequency was 2.5-10-fold higher when compared to general population) - Central demyelination (ROR about 1.6 after HPV vaccination when compared to other vaccines.
Observational comparative studies	Is there an association between anti-HPV vaccination and demyelination?	A total of 11 studies having assessed such as putative link were identified. All were congruent in finding non-significant risk estimates for demyelination after HPV vaccination
Meta-analysis	When pooling all evidence generated so far, is there an association between anti-HPV vaccination and demyelination?	The meta-analysis did not highlight any significant association for all outcomes investigated, pooled odds ratios remained close to one and not-significant for the broad category of central demyelination, MS and ON.

		Owing to the limited number of studies, it was sensible to conduct a meta-analysis to evaluate the potential risk of GBS after anti-HPV vaccination.

After reviewing all the material available and the studies conducted to answer our research question n°2, the risk of central demyelination after anti-HPV vaccination seems, at this date, unlikely. Nevertheless, a doubt remains regarding a possible excess risk of GBS in the follow of an anti-HPV immunization. More specific studies would be needed. Moreover, the rarity of this event renders its evaluation difficult. Nevertheless, GBS was selected as an outcome of interest for routine surveillance by the near-real time monitoring system of the Vaccine Safety Datalink in the USA. On the basis of more than 2.7 million doses administered until end of 2015, no association was found between GBS and anti-HPV vaccination. Besides, from the studies already conducted, it was estimated that this excess risk, if any, would be lower than 1 per 1,000,000 doses sold. (World Health Organization, 2017a)

6 General conclusion

Vaccines are exceptional health products carrying a major mission of public health. They have the power to alleviate, or suppress, within a short term, the burden of an infectious disease. Probably more than any other medicine, they could save lives and healthcare costs by avoiding the risks brought by infections and their potential immediate or long-term complications, for which cures are not always available.

The two examples reviewed in this thesis have a specific dimension which is an additional source of hope: they can prevent some forms of cancer. Nevertheless, and as any other drug, they could not be 100% safe, especially concerning rare events almost impossible to detect during the clinical development. In the two examples reviewed, a doubt is still remaining regarding a possible excess risk of central demyelination in the follow of an anti-HBV vaccination or for GBS after HPV immunization. Nonetheless, and beyond doubt, a strong association with these risks can be ruled out for both vaccines, making the benefit and risk balances still largely positive for both products if used in their current target populations. In that context, an independent, clear and scientifically-based communication is the key element to promote vaccination programmes and to generate the adherence of the general population. The regrettable affair of the French campaign of anti-HBV vaccination clearly demonstrated how difficult it is to restore a lost confidence in a vaccine. It took approximately 20 years to place France at the level of immunization coverages of comparable European countries, at least for infants. Political decisions also carry a heavy responsibility in ensuring trust towards vaccination programmes. In both examples presented here, the decision to suspend immunization programmes (against HBV for France and HPV for Japan) was interpreted, by media and public opinion, as an acknowledgment of a vaccine-attributable risk with long-lasting deleterious consequences on the vaccines' image.

On a more positive note, lessons were also learned from previous vaccine scandals. For example, at the time of the HPV vaccines' launch, a stringent monitoring of their safety profile was anticipated and sophisticated methods such as the near-real time surveillance were implemented in order to detect any potential safety signal in the shortest delay possible.

In the current era of technology devices and as already implemented in Australia (i.e., SmartVax and Vaxtracker), the future of vaccine pharmacovigilance could rely on the implementation of a collaborative GP-patient network-based solution using SMS and smartphones. While collecting potential adverse effects of vaccines, it would also be a unique opportunity to place the patients at the heart of the surveillance system, giving them a voice and potentially contributing to restore their confidence in vaccines and even, in the actors and decision-makers in the field of public health.

7 French Summary

Même si les vaccins représentent une avancée majeure pour la santé publique, le risque d'effets secondaires constitue une menace réelle pour son acceptation par le grand public et les professionnels de santé. La France se classe parmi les pays ayant la plus grande défiance envers les vaccins. L'origine de cette perte de confiance est, entre autres, liée à la polémique intense dans les années 1990 autour du vaccin anti-hépatite B (HB) et d'un risque de sclérose en plaques.

Le but de ce travail thèse était d'évaluer la vraisemblance du lien pouvant exister entre vaccination et réaction de démyélinisation, en considérant deux exemples : les vaccins contre le virus de l'hépatite B (VHB) et ceux contre le papillomavirus (HPV).

7.1 Introduction à la vaccinologie

Un vaccin est constitué d'une préparation d'antigènes, ayant pour but d'induire, chez le sujet qu'il le reçoit, une immunité à long terme et spécifique des agents infectieux ciblés ou des toxines qu'ils produisent.

En comparaison des molécules chimiques constituant les principes actifs de la majorité des médicaments, les vaccins présentent des caractéristiques spécifiques :

- Ils sont utilisés en prévention ;
- Ils sont, donc, dans la grande majorité des cas, administrés à des sujets sains, incluant les enfants ;
- Ils ciblent des cohortes de naissance ou des groupes à risque, et peuvent être administrés à des âges spécifiques ou dans des circonstances particulières (ex. épidémie ou voyage à l'étranger)
- Ils peuvent être délivrés par l'intermédiaire de campagnes publiques de vaccination ;
- Ils peuvent constituer un prérequis pour l'inscription à l'école ou dans d'autres structures publiques.

De plus, ils peuvent avoir un impact épidémiologique rapide, réduisant la mortalité et épargnant des coûts en santé. Cela a, d'ailleurs, été reconnu par un collège d'économistes

de la santé qui ont placé la vaccination chez l'enfant à la quatrième place sur une liste de 30 mesures coût-efficaces pour améliorer la santé publique. (World Health Organization, 2018b)

Si la vaccination protège les individus directement en induisant une immunité active, elle peut également offrir des bénéfices indirects pour les populations non vaccinées. Ce phénomène, appelé l'immunité collective (*herd immunity* en anglais), fait référence à la protection des personnes non vaccinées du fait qu'au-delà d'un certain seuil, la couverture vaccinale empêche la circulation de l'agent infectieux au sein des populations à risque naïves. (Kim et al., 2011) L'existence et l'ampleur de cette immunité collective est dépendante de l'agent infectieux, de son degré de contagiosité, du mode de transmission de la maladie, de la zone géographique considérée, de l'efficacité du vaccin et du niveau de couverture vaccinale. (John & Samuel, 2000)

Les seuils de couverture vaccinale aboutissant à une immunité collective sont donc définis pour chaque vaccin et maladie infectieuse.

Malgré les bénéfices attendus, l'acceptation d'un risque potentiel lié à la vaccination est très faible pour le grand public. Les vaccins nécessitent ainsi une surveillance renforcée afin d'identifier au plus vite tout événement secondaire grave pouvant être lié à la vaccination. Le suivi des événements mineurs et une communication adaptée et transparente sont également nécessaires pour minimiser le risque de crise sanitaire et de rejet par le grand public.

Ainsi, la pharmacovigilance des vaccins comporte, comme pour les autres produits de santé, trois grands piliers ("Global safety of vaccines: strengthening systems for monitoring, management and the role of GACVS," 2009):

- La détection de signal ;
- La génération d'une hypothèse causale ;
- L'évaluation de cette hypothèse.

En effet, la survenue d'un événement indésirable après une vaccination n'implique pas nécessairement une relation causale. Etant donné la nature complexe d'une préparation vaccinale (mélange d'antigènes, antibiotiques, stabilisants, conservateurs et dispositif médical), les événements indésirables peuvent être liés à chacun de ces composants, mais

aussi au procédé de production, de stockage ou mode d'administration. (World Health Organization, 2013)

7.2 Schémas d'études visant à évaluer l'efficacité et la sécurité des vaccins en vie réelle

Les études observationnelles sur les vaccins sont généralement conduites sur un grand nombre de personnes vaccinées ou exposées, dans le but d'évaluer leur efficacité en vie réelle ou d'identifier des événements rares n'ayant pas été capturés, de ce fait, durant les études précédant la commercialisation. La mise en place d'un programme de vaccination doit être considérée comme un moment clé pour mieux appréhender l'efficacité et la sécurité en vie réelle de ces produits. En effet, cette période peut permettre d'estimer les taux de survenue d'un événement donné dans une population encore non exposée au vaccin, juste avant l'expansion du programme de vaccination qui aboutira à une diminution rapide des sujets naïfs.

L'exposition au vaccin est relativement facile à déterminer du fait que celui-ci s'administre en une ou plusieurs doses selon un calendrier bien spécifique. Par ailleurs, les informations relatives à l'acte de vaccination (ex. date d'injection, numéro de lot, nom commercial, etc.) sont, le plus souvent, disponibles dans diverses sources de données (ex : registre d'immunisation, dossiers médicaux, bases de données des assurances).

Pour la détection de signaux de sécurité, les analyses de disproportionnalité et les analyses attendu/observé sont fréquemment employées. Ces deux méthodes utilisent généralement les données de la notification spontanée ou celles de bases de remboursement de soins. Si elles ne permettent, en aucun cas, de quantifier l'importance d'un risque ni d'évaluer la vraisemblance d'une relation de causalité entre l'administration d'un vaccin et la survenue d'un événement, elles sont cependant très utiles pour détecter rapidement un signal de pharmacovigilance et constituent souvent l'une des premières analyses servant à générer une hypothèse et à prendre des mesures rapides lorsqu'un problème de sécurité lié à un vaccin semble survenir.

Il est à noter que les schémas d'études visant à évaluer le profil de sécurité des vaccins, utilisent souvent les sujets comme leurs propres témoins, en considérant uniquement :

- Les sujets vaccinés, ou
- Les cas d'un événement d'intérêt, ou
- Les cas vaccinés.

Les études de cohorte sont souvent considérées comme la référence pour l'évaluation de l'efficacité et de la sécurité des produits de santé dans la phase post-AMM. Dans le cadre des vaccins, elles sont d'intérêt pour l'évaluation de l'efficacité mais peuvent toucher leurs limites pour l'étude de la sécurité, en particulier concernant des événements rares ou avec une longue période de latence ; dans ce cas, une taille de population considérable ou une durée de suivi extrêmement longue serait requise. Les études de type cas/témoins sont mieux adaptées à ce type de recherche ; l'inférence causale se faisant alors de la survenue de l'événement vers l'exposition. Cette dernière est toujours déterminée de manière rétrospective selon une fenêtre de temps définie *a priori*. Du fait que de nos jours, il est relativement aisé d'accéder à des bases de données et cohortes de grandes tailles, les études de type cas/témoins nichées (*nested case-control studies*) sont de loin le *design* le plus utilisé.

Les études auto-contrôlées ont la particularité de considérer les sujets comme leurs propres témoins, ajustant de ce fait de manière automatique pour les facteurs de confusion indépendants du temps, y compris ceux non mesurés ou non mesurables. Adoptant la même logique que celle qui distingue les études de cohortes des cas/témoins, il existe deux grands types d'études auto-contrôlées : les séries de cas ou « *self-controlled case series* » qui miment les études de cohortes, et les études de cas croisés ou « *case-crossover studies* » qui se rapprochent des études cas/témoins.(IMI ADVANCE Group, 2014) Plusieurs variantes ont été développées à partir de ces méthodologies et sont couramment employées pour l'évaluation des bénéfices et risques des vaccins.

7.3 Epidémiologie des démyélinisations centrales et de la sclérose en plaques

La démyélinisation est un processus pathologique qui conduit à la destruction des cellules constituées de myéline que sont les oligodendrocytes et les cellules de Schwann pour le système nerveux central et périphérique, respectivement ; ainsi que la lame de myéline qui préserve les axones. (Mehndiratta & Gulati, 2014).

Les maladies démyélinisantes du système nerveux central peuvent être classées selon plusieurs catégories : les démyélinisations liées à un processus inflammatoire, les démyélinisations virales, les démyélinisations consécutives à un dysfonctionnement métabolique acquis, les démyélinisations consécutives à une ischémie/hypoxie, etc. (Love, 2006) Elles comportent:

- La sclérose en plaques (SEP), qui est la forme prédominante,
- La névrite optique,
- La neuromyélite optique,
- La myélite transverse,
- L'encéphalomyélite aiguë disséminée.

Les maladies démyélinisantes du système nerveux périphérique incluent, entre autres, le syndrome de Guillain et Barré et la polyradiculoneuropathie chronique inflammatoire.

Le diagnostic de la SEP repose une dissémination dans le temps et l'espace des lésions du système nerveux central. Les critères de McDonald demeurent les critères diagnostiques les plus utilisés et ont été révisés pour la dernière fois en 2017.

La prévalence mondiale de la SEP a été estimée à 33 cas pour 100,000 habitants en 2013, ce qui correspond à 2,3 millions de personnes affectées par la maladie, selon le rapport de la Fondation Internationale de la SEP. (Multiple Sclerosis International Federation (MSIF), 2013) Selon cet organisme, la prévalence mondiale a augmenté de 30/100 000 en 2008 à 33/100 000 en 2013. En France, la prévalence a été estimée à 155.6 pour 100 000 (standardisée sur la population Européenne) en 2013, correspondant à environ 99 000 patients. (Foulon et al., 2017) L'incidence annuelle mondiale de la SEP a été estimée à 2,5 (étendue : 1,1 – 4) pour 100 000 en 2008. En 2013, la France, avec un taux de 7,6 pour 100 000, se classe parmi les pays européens avec la plus forte incidence annuelle. (World Health Organization, 2008)

L'étiologie de la SEP est complexe et reste mal comprise. Cependant, plusieurs facteurs de risque, environnementaux et génétiques, ont été mis en évidence. Une méta-analyse ayant considéré 416 études individuelles et 44 autres méta-analyses, a identifié trois facteurs de risque non génétiques, à savoir (Belbasis et al., 2015):

- Sérologie positive pour le virus Epstein Barr: OR= 4,46 (95%IC: 3,26–6,09) ;
- Mononucléose infectieuse (qui est causée par ce même virus): OR= 2,17 (1,97–2,39) ;
- Tabagisme : OR= 1,52 (1,39–1,66).

Le profil démographique des patients atteints de SEP fait apparaitre un âge compris entre 15 et 60 ans pour la première poussée avec une moyenne à 30 ans. (Browne et al., 2014) Les femmes sont les plus touchées avec un risque en moyenne deux fois supérieur à celui des hommes. (Browne et al., 2014) Des différences ethniques ont également été mises en évidence, les populations blanches, en particulier les descendants des pays européens nordiques, étant les plus à risque. (Langer-Gould et al., 2013)

7.4 Vaccination contre l'hépatite B et risque de démyélinisation centrale

7.4.1 Epidémiologie des infections aigues et chroniques du virus de l'hépatite B

Une hépatite consiste en une inflammation du parenchyme hépatique, pouvant conduire à des complications à court terme (comme l'hépatite fulminante) ou à plus long terme (comme l'hépatite chronique active, la fibrose évolutive, la cirrhose qui peut en résulter ou le carcinome hépatique). Parmi les causes possibles de ces atteintes du foie, on peut citer une consommation excessive d'alcool, certaines substances toxiques ou médicamenteuses (ex. paracétamol), certaines maladies comme la stéatose hépatite non-alcoolique. Cependant, les virus (A, B et C) sont, de loin, la cause majeure des hépatites. (Centers for Disease Control and prevention, 2018a)

En France, la prévalence des hépatites B a été estimée par une méta-analyse menée par l'Organisation Mondiale de la Santé, qui a considéré 20 études (n= 493 856) entre 1957 – 1989 et 14 autres études (n= 918 198) pour la période 1990 – 2013. Les deux périodes ont été sélectionnées pour évaluer l'impact de la mise en place de la vaccination anti-hépatite B au début des années 1990. Les estimations agrégées de la prévalence sont de 0,29% (95% IC: 0,28 – 0.31) et 0,25% (95%CI: 0,24 – 0,26), pour les deux périodes respectivement.

(Schweitzer et al., 2015). Le critère de faible endémicité défini par l'OMS, est une prévalence inférieure à 2%. La France se classe donc comme un pays de très faible endémicité avec une prévalence dix fois inférieure au seuil retenu. Concernant l'incidence des hépatites B, les données restent très rares en France, du fait du faible niveau d'endémicité et de la suspension de la déclaration obligatoire des cas incidents entre 1985 et 2003. (Antona, 2008) Cependant, il a été estimé que l'incidence annuelle des infections par le virus de l'hépatite B était de 6 [95% IC: 2 – 12] pour 100 000 en 1996, alors qu'elle était de 20 [95%IC non communiqué] pour 100 000 en 1991. (Flahault et al., 1997).

Le mode principal de transmission du virus de l'hépatite B est le sang et les fluides corporels tels que le sperme et les sécrétions vaginales. (Centers for Disease Control and prevention, 2018a) Bien que le virus ait pu être retrouvé dans la salive, les larmes, le lait maternel, la sueur et l'urine, aucune contamination ne semble possible via la nourriture ou l'eau, le partage des couverts, l'allaitement, les baisers, les poignées de main, la toux ou l'éternuement. (Centers for Disease Control and prevention, 2018a; Zheng et al., 2011). En France, les déclarations obligatoires des cas incidents d'infection par le virus de l'hépatite B entre 2010 et 2014 ont montré que les trois modes de transmission les plus fréquents étaient les relation sexuelles (38,5%), les voyages dans les pays de haute endémicité (21,5%), et les procédures médicales invasives comme la dialyse, la chirurgie ou la transplantation (5,4%). (Nicand, 2016)

La progression de l'infection par le virus de l'hépatite B est très variable selon l'âge, le statut immunitaire du sujet et le stade auquel l'infection est diagnostiquée. Ainsi, il est estimé que 95% des adultes et 10% des nouveau-nés contaminés présenteront une infection aigue transitoire. (World Health Organization, 2002). Le risque de devenir porteur chronique de l'infection est inversement proportionnelle à l'âge. Alors que 90% des enfants infectés à la naissance deviendront des porteurs chroniques, ce risque diminue à 30% pour les enfants infectés à l'âge de 1 à 4 ans, et devient inférieur à 5% pour les personnes infectées à l'âge adulte. (Ott et al., 2012)

7.4.2 La vaccination contre le virus de l'hépatite B en France

Le premier vaccin contre l'hépatite B (Hévac®) a été développé et commercialisé par Sanofi Pasteur dès 1981. La même année, la France est le premier pays à organiser une campagne de vaccination qui cible les professionnels de santé. (Aron, 2002) Il faudra attendre 10 ans plus tard pour que cette mesure devienne obligatoire. En 1993, les programmes de vaccination s'étendent à d'autres populations incluant les étudiants et les professeurs à risque d'exposition au virus de l'hépatite B, ainsi qu'aux voyageurs des pays avec une forte endémicité. Dans le même temps, l'OMS recommande d'étendre les programmes de vaccination à l'ensemble des enfants, y compris dans les pays de faible endémicité. Bien qu'elle reconnaisse que ces enfants, en dehors de ceux nés d'une mère infectée, aient très peu de risque d'être exposés au virus de l'hépatite B, l'OMS a basé son raisonnement sur le fait que vacciner uniquement les personnes à risque ne serait pas suffisant pour éradiquer le virus. (DIRECTION GENERALE DE LA SANTE, 2004) En suivant ces recommandations, une campagne nationale de vaccination visant les nouveau-nés est lancée en France. Deux mois plus tard, la vaccination anti-hépatite B est proposée à chaque enfant scolarisé en classe de sixième. Le 10 Janvier 1995, la vaccination anti-hépatite B est recommandée et est inscrite dans le calendrier vaccinal des nouveau-nés et adolescents.(Denis et al., 1998) Il faut, cependant, reconnaitre qu'aucun des pays voisins de la France, incluant ceux ayant une prévalence plus importante d'infections par le virus hépatite B (comme l'Italie), n'a adopté de mesures aussi strictes.

En raison de la détection d'un signal de pharmacovigilance en 1996 concernant des cas de démyélinisation survenus après l'administration du vaccin anti-hépatite B, et des résultats, pourtant non concluants, de deux études pharmaco-épidémiologiques, la campagne de vaccination scolaire est suspendue le 1er Octobre 1998. Une enquête nationale investiguant le lien potentiel entre la vaccination anti-hépatite B et la survenue de maladies auto-immunes, incluant les maladies démyélinisantes centrales a été lancée en 1998. La même année, les rappels ont été supprimés et le schéma de vaccination s'est allégé passant de 4 à 3 doses (à 0, 1 et 6 mois).(Antona, 2008) La couverture médiatique et la pression de l'opinion publique, intenses durant la période 1996 – 2000, provoquent une crise majeure. Toute décision règlementaire et/ou communication pour la santé publique devient particulièrement difficile. En Mars 2002, de nouvelles recommandations nationales sont

émises et visent l'immunisation des jeunes enfants, avec un rattrapage possible des adolescents, ainsi que la vaccination des adultes à risque non vaccinés dans l'enfance. Le rapport final de l'enquête nationale de pharmacovigilance est publié en 2006. En Janvier 2018, la vaccination contre le virus de l'hépatite B est rendue obligatoire chez l'enfant.(Ministère des Solidarités et de la Santé, 2018a)

L'enquête nationale a révélé une disjonction complète entre les populations visées par les recommandations et celles réellement vaccinées. Alors que la couverture vaccinale chez les plus jeunes, c'est-à-dire les nouveau-nés qui constituaient la cible initiale de la campagne, n'a jamais dépassé 30% avant 2003, la population adulte, pourtant en dehors des recommandations à l'exception des adultes à risque, a été massivement exposée. En effet, malgré des ventes records de doses de vaccins (plus de 98 millions entre 1981 et 2005), la cible d'une couverture vaccinale à 95%, nécessaire pour atteindre l'immunité collective contre le virus de l'hépatite B, n'a jamais été atteinte en France, que ce soit chez les nouveau-nés ou les adolescents. Pour les nouveau-nés, la couverture vaccinale a stagné jusqu'en 2003 où elle avoisinait les 30%. Elle s'est ensuite accélérée grâce à la mise sur le marché puis au remboursement d'un vaccin hexavalent, comprenant le vaccin anti-hépatite B. De nos jours, il est estimé que 90% des enfants de moins de 24 mois sont vaccinés contre le virus de l'hépatite B. (Denis et al., 1998; Institut National de Veille Sanitaire (INVS), 2018) Pour les adolescents, une tendance opposée a été observée. Alors que la couverture vaccinale des jeunes de 14-15 ans était de 88% en 1995, elle s'est effondrée après l'arrêt de la campagne de vaccination scolaire. En 2015, seulement 32,5% des enfants de cette tranche d'âge étaient vaccinés. (Denis, 2016) Pour la population adulte, il a été estimé en 1998, que 59,5% des personnes âgés de 15 à 59 ans avaient reçu au moins une dose de vaccin contre le virus B. Cette tranche d'âge qui correspond à l'âge à risque pour le développement d'une SEP, est en dehors de toute recommandation vaccinale, à l'exception des adultes à risque qui ne représentaient que 1 à 1,2 million de personnes en 1998. Cette situation exceptionnelle de dérapage de la campagne vaccinale, qui n'a jamais été observée en dehors de la France, est un élément clé pour comprendre l'apparition du signal de pharmacovigilance dans les années 1990.

Si les raisons de cette disjonction restent obscures, nul ne peut ignorer l'intense communication par les médias et les instances publiques, des risques de l'infection par le

virus de l'hépatite B. Certaines informations, notamment concernant le mode de transmission via la salive, le poids épidémiologique des infections HBV en France ou encore le taux de passage à la chronicité, étaient totalement erronées. (Benkimoun, 2011)

7.4.3 Association entre vaccination contre l'hépatite B et risque de démyélinisation centrale ?

Plausibilité biologique

Deux mécanismes distincts ont été évoqués pour tenter d'accréditer une plausibilité biologique entre la vaccination entre le virus de l'hépatite B et la survenue d'une démyélinisation centrale (en particulier la SEP) :(Agence Française de Sécurité Sanitaire des Produits de Santé (AFSSAPS), 2007)

L'antigène du vaccin contient une séquence protéique similaire à celle de la myéline et pourrait induire une réponse spécifique produisant des auto-anticorps qui cibleraient l'antigène et la myéline.

Un effet du type « *bystander effect* » dû aux adjuvants du vaccin stimulerait l'autoimmunité produisant des réactions hétérologues contre des antigènes différents de ceux présentés initialement. (van Aalst et al., 2017)

Une autre explication concerne la présence, à l'état de trace, de la polymérase virale qui pourrait être co-purifiée avec l'antigène HBsAg durant le procédé de fabrication. Cette protéine pourrait entrainer une réaction immunitaire à l'origine d'un épisode de démyélinisation du fait du mimétisme moléculaire entre la myéline et la polymérase HBV. En effet, l'enzyme comporte des séquences d'acides aminés proches de celle de la myéline humaine. (Faure, 2005)

Néanmoins, les arguments concernant la plausibilité biologique restent indirects et fragiles.

Reports de cas dans la littérature

Dans le cadre de ce travail de thèse, une revue de la littérature pragmatique (non-systématique) a été conduite pour identifier les cas publiés de démyélinisation, centrale ou périphérique, survenus après injection de vaccin anti-hépatite B. Douze publications présentant 17 cas individuels de démyélinisation survenus après administration d'un dose de vaccin, ont été identifiés (Cabrera-Gomez et al., 2002; Creange et al., 1999; Herroelen et al.,

1991; Hostetler, 2001; Iniguez et al., 2000; Kaplanski et al., 1995; Karaali-Savrun et al., 2001; Renard et al., 1999; Santos-Garcia et al., 2007; Sinsawaiwong & Thampanitchawong, 2000; Terney et al., 2006; Tuohy, 1989) Il est évident que ces 17 cas ne représentent qu'une part infime et non-représentative de l'ensemble des cas, impossibles à dénombrer, de démyélinisation survenus, dans le Monde, après une vaccination anti-HBV. Il est intéressant de noter que les auteurs de ces publications ont considéré que ces cas étaient suffisamment convaincants pour envisager une relation causale entre le vaccin et la survenue de ces événements.

Analyse du signal de pharmacovigilance observé en France dans les années 1990

Dans le cadre du présent travail de thèse, tous les cas confirmés de démyélinisation centrale survenus après la vaccination anti-HBV et déclarés à la pharmacovigilance française entre 1981 et 2000 ont été re-saisis. Après une analyse descriptive, une comparaison entre les nombres attendus et observés des événements d'intérêt pour la période considérée, a été menée selon deux approches méthodologiques. Les résultats de ces travaux ont fait l'objet d'une publication, soumise au journal « Vaccine ». Les principaux résultats sont présentés ci-dessous.

Au total, 624 cas incidents de démyélinisation centrale ont été notifiés à la pharmacovigilance française entre 1981 (date de lancement du premier vaccin commercialisé en France : Hévac B® [Sanofi Pasteur]) et le 31 décembre 2000. Le premier cas est survenu en 1984 mais n'a été notifié qu'en 1992. Les femmes représentent la majorité des cas (n=457, 73.2%), correspondant à un ratio femme/homme de 2,7. L'âge de survenue s'étale de 2 à 63,8 ans (Q1-Q3: 21,6-38,5). Les âges moyen et médian convergent avec des valeurs de 29,8 ans (écart type=11,1 ans) et 29,0 ans, respectivement. Parmi ces notifications, 422 (67.6%) cas de première poussée de SEP ont été confirmés par un neurologue sénior ; l'âge moyen à la survenue de l'événement étant de 30,1 ans (écart type=11,1 ans).

Plus de 86 622 360 doses de vaccin anti-HBV ont été vendues en France durant la période considérée. La population générale française étant passée de 54 et 59 millions durant cette même période, cela correspond à une moyenne 1,53 doses par habitant (tous âges confondus), témoignant d'une exposition particulièrement importante, jamais égalée dans

un autre pays. Les événements d'intérêt ont été majoritairement observés après les doses de rappels (46,3%, n=289). Le temps médian de survenue a été estimé à 74 jours, ce qui correspond à 2 mois et 14 jours. En valeurs absolues, l'incidence des événements a atteint des pics en 1995, 1996 et 1997, représentant 59,8% de l'ensemble des cas. Cependant, en considérant les taux de notification (nombre de cas notifiés pour 100 000 doses vendues), les valeurs maximales ont été observées pour les années 1987, 1997 et 1998 avec des taux de 10,5, 12,5 et 14,7 pour 1 000 000, respectivement. Le taux moyen de notification est de 6,51 pour 1 000 000.

Les analyses comparant le nombre de cas notifiés de démyélinisation à celui qui aurait été attendu dans la population vaccinée sur la même période en l'absence de vaccination (comparaison attendu/observé) ont été conduites selon deux approches méthodologiques distinctes : une approche « populationnelle » qui a estimé la population exposée au vaccin à partir des doses vendues et une approche « personne-temps à risque » qui a considéré une fenêtre à risque de 1 mois après l'injection d'une dose. Les deux méthodes ont produit des résultats similaires et non conclusifs, le nombre de cas notifiés restant, dans tous les cas, inférieur au nombre attendu. Ces résultats sont cependant à interpréter avec prudence car ils ne prennent pas en compte la sous-notification, phénomène inhérent à tout système de surveillance passive. Les ratios obtenus étaient de 35,2 et 30,8% pour les méthodes populationnelle et personne-temps à risque, respectivement. Cela signifie qu'il suffirait que la notification soit 3 fois plus forte (ou de considérer qu'à peine un tiers des cas aient été notifiés) pour que les nombres observés et attendus se rejoignent.

Analyse de disproportionnalité dans la base de données américaine VAERS

Une analyse de disproportionnalité entre les cas de démyélinisation centrale survenus après la vaccination anti-HBV et toute autre vaccination a été conduite dans la base de données de pharmacovigilance des vaccins aux Etats-Unis : VAERS. Ce travail a été publié récemment. (Mouchet & Begaud, 2018a, 2018b)

Les conclusions principales de ce travail ont montré que la fréquence de notification de cas de démyélinisation centrale après la vaccination anti-hépatite B était de trois à cinq fois supérieure à celle associée à toute autre vaccination. Ces ratios étaient supérieurs aux seuils

généralement admis pour la génération d'un signal. Ces résultats étaient, par ailleurs, indépendants de l'origine des cas (Etats Unis ou autres pays).

Revue systématique des études observationnelles comparatives évaluant le lien entre vaccination anti-HBV et démyélinisation centrale

Une revue systématique de l'ensemble des études observationnelles comparatives ayant évalué le lien entre vaccination anti-HBV et démyélinisation centrale a été conduite en utilisant à la fois plusieurs sources de données bibliographiques (Medline, Embase, Cochrane, ISI Web of Science) et des recherches dans la littérature grise et dans les références secondaires des articles identifiés comme pertinents. Ce travail a fait l'objet d'un protocole rédigé avant la conduite de l'étude et publié dans le registre en ligne PROSPERO. Suivant les recommandations PRISMA, le processus de revue des articles et l'extraction des données ont été menée en parallèle et de manière indépendante par, au moins, deux chercheurs.

Parmi les 2 804 références identifiées, treize articles ont été retenus.(Ascherio et al., 2001; DeStefano et al., 2003; Eftekharian et al., 2014; Hernan et al., 2004; Hocine et al., 2007; Langer-Gould, Qian, et al., 2014; Mikaeloff et al., 2007; Mikaeloff et al., 2009; Ramagopalan et al., 2009; Sturkenboom et al., 1999; Emmanuel Touze et al., 2002; E. Touze et al., 2000; Zipp et al., 1999) Sept de ces références ont tenté d'évaluer le lien entre la vaccination anti-HBV et la survenue de SEP (Ascherio et al., 2001; DeStefano et al., 2003; Eftekharian et al., 2014; Hernan et al., 2004; Mikaeloff et al., 2007; Ramagopalan et al., 2009; Sturkenboom et al., 1999), deux ont considéré une catégorie plus large d'événements démyélinisants centraux (E. Touze et al., 2000; Zipp et al., 1999), et quatre ont évalué les deux types d'événements (démyélinisation centrale et SEP). (Hocine et al., 2007; Langer-Gould, Qian, et al., 2014; Mikaeloff et al., 2009; Emmanuel Touze et al., 2002). Les études cas-témoins incluaient un total de 16 799 cas et 15 908 témoins. La seule cohorte historique ne comptait pas moins de 134 698 sujets.

A l'exception d'une étude conduite par Eftekharian et al., la qualité des études évaluée par l'échelle Newcastle Ottawa était bonne et comparable pour tous les articles, avec des scores variant de 6 à 8 étoiles. Toutes les études, excepté celle d'Hernan et al. conduite en 2004,

sont restées non conclusives avec des intervalles de confiance pour l'indicateur d'association (risque relatif ou odds ratio) chevauchant la valeur 1.

Méta-analyse des études identifiées

Dans le but d'augmenter la puissance statistique et de contrebalancer certaines faiblesses méthodologiques pouvant avoir affecté certaines études (comme un biais de mémoire ou de sélection), une méta-analyse a été conduite et publiée dans la revue « Vaccine » (Mouchet et al., 2018a). La méthodologie utilisée était conforme aux recommandations MOOSE.

Etant donnée la nature observationnelle des études identifiées, les auteurs ont souvent fourni plusieurs estimations du risque, rendant difficile le choix d'une seule mesure pour la méta-analyse. Il a donc été décidé de considérer trois estimations : brute, ajustée, et celle estimée pour une fenêtre de trois mois après la vaccination. L'hétérogénéité a été quantifiée et son origine déterminée en enlevant une après une, les études incluses. Des analyses en sous-groupe (enfants *versus* adultes, schéma d'étude, score de qualité) ont été réalisées. Deux types d'événement ont été traités séparément : les premiers épisodes de SEP et les événements démyélinisants centraux.

Dans le cas des estimations brutes, la méta-analyse a produit un odds ratio (OR) poolé de 1,19 [95%CI 0,95 – 1,46] pour la SEP et de 1,06 [95%CI 0,88 – 1,28] pour les événements démyélinisants. Pour les mesures ajustées, les OR poolés étaient de 1,19 [95%CI: 0,93 – 1,52] et 1,25 [95%CI: 0,97 – 1,62], respectivement. Pour le troisième scénario, considérant une fenêtre de temps réduite à 3 mois après la vaccination, les valeurs étaient de 1,39 (95%CI: 0,90 – 2,15) et 1,38 [95%CI: 0,82 – 2,34]. Les analyses en sous-groupe ont également abouti à des résultats non statistiquement significatifs. En conclusion, bien que les OR convergent, en moyenne, vers une valeur de 1,2-1,3, aucune des estimations poolées n'a atteint le seuil de significativité statistique.

7.4.4 Conclusion

De l'ensemble des documents revus et des activités de recherche menées sur la problématique : « *Existe-t-il un lien entre la vaccination anti-hépatite B et la survenue de démyélinisation centrale ?* », les conclusions suivantes peuvent être avancées :

- Il n'existe aucun doute pour ce qui concerne le caractère exceptionnel et unique au Monde, de l'exposition massive des adultes français aux vaccins contre le virus de l'hépatite B, suivant la mise en place des campagnes nationales de vaccination.

- La communication excessive concernant les risques associés à l'infection par le virus de l'hépatite B a conduit à une exposition des adultes au moins dix fois supérieure à celle attendue, ceci à un âge susceptible de déclarer une SEP.

- Un signal de pharmacovigilance, basé sur plusieurs centaines de cas validés de démyélinisation centrale, a été généré deux ans après le lancement de la campagne vaccinale.

- L'analyse objective de l'ensemble des données actuellement disponibles, ne permet pas d'aboutir à une position tranchée sur l'existence d'une association autre que fortuite. Certains arguments pourraient supporter une association entre le vaccin et la survenue de démyélinisation centrale. Parmi ceux-ci, on peut citer : une certaine plausibilité biologique (bien que faible et indirecte), la disproportionnalité observée dans VAERS, le fait qu'un degré somme toute modéré de sous-notification pourrait renverser les conclusions de notre analyse attendu/observé, ou la distribution non aléatoire des rangs de vaccination des cas rapportés à la pharmacovigilance française (majorité des cas survenant après la dose de rappel).

- Cela étant, même dans l'hypothèse d'une relation causale, la force de l'association serait, dans tous les cas, faible, c'est-à-dire correspondant à un risque relatif inférieur à 2, et ne concernerait que les adultes, et non pas les nouveau-nés ou les enfants en dessous de l'âge de 12 ans (du fait qu'ils ne sont pas encore totalement myélinisés à cet âge). Le fait que ces derniers soient les cibles principales des programmes actuels de vaccination en France explique très vraisemblablement la baisse drastique du nombre de notifications d'affections démyélinisantes associées au vaccin. Ceci explique l'absence de conclusion robuste de la plupart des études observationnelles et de la méta-analyse. Détecter ou prouver l'absence d'un risque en excès de cet ordre de grandeur, exigerait une taille d'échantillon considérable, voire irréaliste.

7.5 Vaccination contre le papillomavirus humain et risque de démyélinisation

La polémique concernant un risque potentiel de démyélinisation après une vaccination a refait surface en France au moment du lancement du premier vaccin anti-papillomavirus (HPV), Gardasil®, en 2006.

La population cible, à savoir les adolescentes âgées de 11 à 14 ans, représentait une inquiétude, étant donné que cette tranche d'âge tombe dans la catégorie d'âge à risque de développer une SEP. D'autres caractéristiques étaient communes aux vaccinations anti-HBV et anti-HPV, comme l'argument « vaccination anti-cancer » mis en avant pour les deux produits, notamment par les firmes pharmaceutiques. En effet, en conférant une protection contre les infections par les virus concernés, les vaccins offriraient également une protection contre leurs conséquences à long terme qui incluent certains types de cancer (cancer invasif du col utérin pour le papilloma virus et hépatocarcinome pour le virus de l'hépatite B).

Pour les raisons évoquées ci-dessus, il semblait d'un intérêt majeur d'investiguer si le risque de démyélinisation était ou pourrait être une crainte pour les vaccins anti-HPV.

7.5.1 Epidémiologie des infections à papillomavirus en France

Les papillomavirus représentent la cause majeure des cancers du col de l'utérus et sont responsables d'une part non négligeable, des cancers anogénitaux et des cancers oropharyngés. Il existe plusieurs sérotypes différents. Parmi ceux-ci, certains sont cancérogènes comme les types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59 et, possiblement, le 68. Ils sont classés comme les sérotypes à haut risque. Ainsi, les types 16 et 18 contribueraient pour 70% des cas de cancers cervicaux. (de Martel et al., 2017). HPV16 serait également largement impliqué dans les cancers de la vulve, du vagin, du pénis, de l'anus et de l'oropharynx. (de Martel et al., 2017)

En France, il a été estimé que 70% des hommes et des femmes avaient déjà été exposés au virus HPV. Le taux d'incidence annuel standardisé sur l'âge pour les cancers cervicaux attribuables à HPV en 2012 avoisinerait 10 pour 100 000 femmes en France, ce qui représente l'un des taux les plus faibles dans le monde. A l'inverse, avec un taux supérieur à 1,25 pour 100 000 habitants, la France compte parmi les pays ayant la plus forte incidence annuelle pour les cancers anogénitaux (vulve, vagin, anus et pénis) ainsi que pour les cancers de la tête et du cou (oropharynx, cavité orale et larynx). (de Martel et al., 2017)

Le mode de transmission le plus fréquent est la voie sexuelle. Un seul contact peut suffire à transmettre le virus. De plus, à l'inverse du virus HBV, les préservatifs ne semblent pas représenter un moyen de protection efficace. (Liu et al., 2016)

7.5.2 La vaccination contre le papillomavirus en France

En France, les recommandations actuelles ciblent les adolescentes âgées de 11 à 14 ans. Le schéma de vaccination comprend deux doses à six mois d'intervalle. Aucun rappel n'est préconisé. (Ministère des Solidarités et de la Santé, 2018a)

Une stratégie de rattrapage est possible pour les jeunes femmes jusqu'à l'âge de 19 ans. Cette mesure repose sur le fait que l'immunisation contre le virus HPV doit être menée avant d'être exposé au virus, c'est-à-dire, avant le premier rapport sexuel. (Ministère des Solidarités et de la Santé, 2018a) Dans le cas de ce rattrapage, le schéma d'administration comporte trois doses à 0, 2 et 6 mois pour Gardasil®/Gardasil 9®, ou à 0, 1 et 6 mois pour Cervarix®.

La vaccination est également recommandée pour certaines populations bien spécifiques: (Ministère des Solidarités et de la Santé, 2018a)

- Pour les hommes ayant des rapports sexuels avec d'autres hommes, l'immunisation active est recommandée jusqu'à l'âge de 26 ans, selon un schéma d'administration à trois doses à 0, 2 et 6 mois (pour Gardasil® ou Gardasil 9®).
- Pour les patients immunodéprimés de deux sexes, selon les mêmes recommandations que celles pour la population générale.
- Pour les patients des deux sexes recevant une transplantation, l'immunisation peut être administré dès l'âge de 9 ans.

7.5.3 Association entre vaccination contre HPV et risque de démyélinisation ?

Une démarche similaire à celle ayant été utilisée pour évaluer la plausibilité de l'association entre HBV et démyélinisation centrale, a été employée pour cette deuxième question de recherche.

Plausibilité biologique

A ce jour, aucune donnée fiable ne documente d'hypothèse physiopathologique entre les vaccins anti-HPV et la survenue d'une démyélinisation, centrale ou périphérique.

Reports de cas dans la littérature

Une revue de la littérature pragmatique a été conduite dans Medline (via Pubmed) pour identifier les reports de cas de démyélinisation, centrale ou périphérique, étant survenus après administration d'un vaccin anti-HPV.

Un total de 8 publications présentant 12 cas individuels de démyélinisation apparus après une vaccination contre HPV, ont été identifiées. (Alvarez-Soria et al., 2011; Chang et al., 2016; DiMario et al., 2010; Hu et al., 2018; Karussis & Petrou, 2014; Schaffer et al., 2008; Sekiguchi et al., 2016; Sutton et al., 2009)

Les événements rapportés étaient de nature diverse et incluaient des cas de SEP, de neuromyélite optique ou d'encéphalomyélite aiguë disséminée. La distribution géographique des cas était répartie sur l'ensemble des pays dans lesquels sont commercialisés les vaccins anti-HPV.

Etudes ayant pour objectif de détecter des signaux ou d'évaluer une disproportionnalité dans les systèmes de pharmacovigilance

Une revue systématique, ayant fait l'objet d'une publication dans la revue « Pharmacological Research » (Mouchet et al., 2018b), a été menée dans le but d'identifier toutes les études post-enregistrement ayant évalué soit l'existence d'un signal de pharmacovigilance, soit d'une disproportionnalité observée dans une base de cas de pharmacovigilance, pour ce qui concerne des événements démyélinisants survenus après une vaccination anti-HPV.

Quatorze études ont été retrouvées. (Angelo, Zima, et al., 2014; Baxter, Lewis, Goddard, et al., 2016; Cameron et al., 2016; Gee et al., 2011; D. A. Geier & Geier, 2015, 2017; Gold et al., 2010; Labadie, 2011; Ojha et al., 2014; Pellegrino et al., 2013; Slade et al., 2009; Souayah et al., 2011; Vichnin et al., 2015; Willame et al., 2016). La survenue d'un syndrome de Guillain et Barré (SBG) était documentée dans dix études, alors que quatre articles se concentrent sur des cas incidents de SEP. Les références retenues couvraient une période allant de 2004 à 2015. En dehors de deux études (D. A. Geier & Geier, 2017; Souayah et al., 2011), tous les

articles ont fourni des éléments rassurants quant au profil de sécurité des vaccins anti-HPV, et n'ont pas mis au jour de signal de pharmacovigilance. En effet, les incidences observées dans la population vaccinée pour les événements démyélinisants n'étaient pas supérieures aux estimations attendues pour la population générale.

Revue systématique des études observationnelles comparatives ayant évalué le lien entre vaccination anti-HPV et démyélinisation centrale

Une revue systématique de l'ensemble des études observationnelles comparatives ayant évalué le lien entre vaccination anti-HPV et démyélinisation a été conduite en utilisant plusieurs sources de données bibliographiques (Medline, Embase, Cochrane, ISI Web of Science) ainsi que des recherches dans la littérature grise et dans les références secondaires des articles identifiés comme pertinents. Ce travail a fait l'objet d'un protocole rédigé avant la conduite de l'étude et publié dans le registre en ligne PROSPERO. Suivant les recommandations PRISMA, le processus de revue des articles et l'extraction des données ont été menée en parallèle et de manière indépendante par, au moins, deux chercheurs.

Sur les 2863 références identifiées, onze articles ont été sélectionnés. (Nick Andrews et al., 2017; Angelo, David, et al., 2014; Arnheim-Dahlström et al., 2013; Baxter, Lewis, Fireman, et al., 2016; Chao et al., 2012; Grimaldi-Bensouda et al., 2017; Langer-Gould, Qian, et al., 2014; Miranda et al., 2017; Scheller et al., 2015; Sridhar et al., 2017; Verstraeten et al., 2008) Six concernaient des études de cohortes (Arnheim-Dahlström et al., 2013; Scheller et al., 2015) (Miranda et al., 2017) (Sridhar et al., 2017) (Baxter, Lewis, Fireman, et al., 2016; Chao et al., 2012) deux étaient des études cas-témoins (Grimaldi-Bensouda et al., 2017; Langer-Gould, Qian, et al., 2014), l'une était une série de cas auto-contrôlés,(Nick Andrews et al., 2017) et deux étaient des analyses poolées d'essais cliniques randomisés.(Angelo, David, et al., 2014; Verstraeten et al., 2008)

La qualité méthodologique des études retenues était bonne et comparable, avec des scores allant de six à neuf étoiles pour l'échelle de Newcastle-Ottawa. Dix des 11 articles identifiés reportaient des résultats convergents avec des estimations du risque de démyélinisation après vaccination anti-HBV, non statistiquement significatifs. (Nick Andrews et al., 2017; Angelo, David, et al., 2014; Arnheim-Dahlström et al., 2013; Baxter, Lewis, Fireman, et al., 2016; Chao et al., 2012; Langer-Gould, Qian, et al., 2014; Miranda et al., 2017; Scheller et al.,

2015; Sridhar et al., 2017; Verstraeten et al., 2008) La dernière étude (Grimaldi-Bensouda et al., 2017) se distingue des autres du fait qu'elle rapporte une valeur "protectrice" d'odds ratio (0,31 [95%CI: 0,13 – 0,73]) difficilement explicable en l'état.

Méta-analyse des études identifiées

Dans le but d'augmenter la puissance statistique et de contrebalancer certaines faiblesses méthodologiques pouvant avoir affecté certaines études (comme un biais de mémoire ou de sélection), une méta-analyse a été conduite et publiée dans la revue « Pharmacological Research » (Mouchet et al., 2018b). La méthodologie utilisée était conforme aux recommandations MOOSE.

Les résultats de la méta-analyse ne supporte pas l'hypothèse d'une association statistiquement significative entre la vaccination anti-HPV et la survenue de maladies démyélinisantes centrales, l'OR poolé étant très proche de 1 avec une valeur exacte de 0,96 [95%CI 0,77 – 1,20]. Une analyse de sensibilité n'ayant retenu que les cinq études produisant des estimations du risque pour un délai de 6 mois après la vaccination anti-HPV (Arnheim-Dahlström et al., 2013; Chao et al., 2012; Langer-Gould, Qian, et al., 2014; Miranda et al., 2017; Scheller et al., 2015) n'a pas altéré les conclusions précédentes (OR= 1,06 [95%CI 0,85-1,32).

Six études se sont concentrées sur la problématique de l'association du vaccin anti-HPV avec la survenue de cas incidents de SEP (Angelo, David, et al., 2014; Chao et al., 2012; Langer-Gould, Qian, et al., 2014; Miranda et al., 2017; Scheller et al., 2015; Verstraeten et al., 2008). A nouveau, la méta-analyse de ces études n'a pas mis en évidence de lien entre la survenue de SEP et la vaccination anti-HPV avec un odds ratio de 0,98 [95%CI: 0,82-1,19].

On ne dénombre que deux études ayant produit une estimation du risque de survenue d'un syndrome de Guillain et Barré dans les suites d'une vaccination anti-HBV. Les odds ratios publiés étaient respectivement de 1,26 (95%CI: 0,55-2,92] to 4,00 [95%CI: 1,84-8,69]. (Grimaldi-Bensouda et al., 2014; Miranda et al., 2017) En raison du nombre trop faible d'estimations du risque fournies pour SGB, il n'a pas été possible de réaliser de méta-analyse pour cet événement. Il faut toutefois noter que les deux estimations étaient supérieures à 1, atteignant le seuil de significativité statistique dans une étude. (Miranda et al., 2017)

7.5.4 Conclusion

Après la revue de l'ensemble de preuves et des études conduites pour répondre à notre deuxième question de recherche, il apparait que le risque de démyélinisation centrale après la vaccination anti-HPV semble, à ce jour, improbable. Cependant, un doute subsiste concernant un possible risque en excès de syndrome de Guillain et Barré consécutif à la vaccination anti-HPV. D'autres études seraient nécessaires pour répondre à cette question. Toutefois, d'après les données de l'Organisation mondiale de la santé, il a été estimé, à partir des 2,7 millions de doses vendues jusqu'en 2015, que si le risque existait, il serait inférieur à un cas pour un million de doses vendues. (World Health Organization, 2017a)

7.6 Conclusion générale

Les vaccins représentent des produits de santé dont l'impact en santé publique est considérable. Ils ont le pouvoir de rapidement diminuer le poids épidémiologique des maladies infectieuses. Probablement plus que tout autre médicament, ils peuvent épargner un grand nombre de vies et des coûts de santé considérables en prévenant les risques inhérents à l'infection naturelle ainsi que ses conséquences immédiates ou à long -terme.

Les deux exemples examinés dans cette thèse ont une dimension supplémentaire, source d'un espoir immense : ils pourraient prévenir certaines formes de cancers. Néanmoins, comme tout autre médicament, ils ne sont pas exempts de risques, en particulier concernant des effets indésirables rares, quasiment impossibles à détecter durant le développement clinique. Si une association forte peut être exclue pour chacun des exemples étudiés dans cette thèse, la possibilité d'un risque en excès pour les événements démyélinisants centraux après la vaccination anti-HBV ou la survenue de syndrome de Guillain et Barré suivant une injection de vaccin anti-HPV, n'est pas à exclure. Toutefois, la balance bénéfice/risque de ces deux vaccinations demeure largement positive dans les populations visées par les recommandations actuelles. Dans ce contexte, une communication scientifique, indépendante et claire, à l'opposé de celle ayant prévalu en France pour la campagne contre l'hépatite B, est la clé pour promouvoir les programmes de vaccination et regagner et maintenir la confiance et l'adhésion de la population. Les décisions politiques portent aussi une lourde responsabilité dans la défiance s'étant installée contre la vaccination, notamment en France, en particulier les conditions de lancement de la campagne anti-HBV

de 1994. De plus, les suspensions des campagnes nationales de vaccination peuvent avoir des conséquences délétères à long terme. Le futur de la pharmacovigilance des vaccins pourrait résider dans la mise en place d'un réseau collaboratif entre le patient et son médecin, via l'utilisation de SMS et smartphones, comme cela existe déjà en Australie. En plus de collecter les effets secondaires des vaccins, cela représenterait une opportunité unique de placer le patient au cœur du système de surveillance, lui offrant une voix et contribuant à restaurer sa confiance envers les vaccins, et, par la même occasion, envers les décideurs de santé publique.

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9 Annexes

Published articles

Mouchet, J., et al. (2018). "Hepatitis B vaccination and the putative risk of central demyelinating diseases - A systematic review and meta-analysis." Vaccine **36**(12): 1548-1555.

BACKGROUND: The anti-hepatitis B immunization campaigns launched in the early 1990s were a major public health breakthrough and targeted various populations (at-risk adults, newborns, adolescents). However, debate is still active about a possible link between this vaccine and central demyelination. This study provides a pooled estimate of this risk based on a comprehensive review and meta-analysis of all available epidemiologic studies. METHODS: A systematic review was conducted in Medline, Embase, ISI Web of Science and the Cochrane Library from database inception to 10 May 2017. Grey literature was searched and snowballing was also undertaken. Only observational studies including a control group were retained. Primary outcome was multiple sclerosis diagnosed by recognized criteria. Study selection was performed by two independent reviewers with disagreements solved through discussion. This meta-analysis based on crude, adjusted estimates, or risks limited to the 3months following immunization was performed using a generic inverse variance random-effect model. Heterogeneity was investigated; sensitivity and subgroup analyses were performed when necessary. This study followed the PRISMA statement and the MOOSE reporting guideline (Study protocol registered in PROSPERO: CRD42015020808). FINDINGS: Of the 2804 references reviewed, 13 studies with a control group were analysed. None of the pooled risk estimates for either multiple sclerosis or central demyelination following HB immunization reached statistical significance. When considering adjusted risk ratios, the following non-significant figures were obtained: 1.19 (95%CI: 0.93 - 1.52) and 1.25 (95%CI: 0.97 - 1.62), for multiple sclerosis and central demyelination, respectively. CONCLUSIONS: No evidence of an association between hepatitis B vaccination and central demyelination was found.

Mouchet, J., et al. (2018). "Human papillomavirus vaccine and demyelinating diseases-A systematic review and meta-analysis." Pharmacol Res **132**: 108-118.

Approved in 2006, human papillomavirus (HPV) vaccines were initially targeted for girls aged 9-14years. Although the safety of these vaccines has been monitored through post-licensure surveillance programmes, cases of neurological events have been reported worldwide. The present study aimed to assess the risk of developing demyelination after HPV immunization by meta-analysing risk estimates from pharmacoepidemiologic studies. A systematic review was conducted in Medline, Embase, ISI Web of Science and the Cochrane Library from inception to 10 May 2017, without language restriction. Only observational studies including a control group were retained. Study selection was performed by two independent reviewers with disagreements solved through discussion. This meta-analysis was performed using a generic inverse variance random-effect model. Outcomes of interest included a broad category of central demyelination, multiple sclerosis (MS), optic neuritis (ON), and Guillain-Barre syndrome (GBS), each being considered independently. Heterogeneity was investigated; sensitivity and subgroup analyses were performed when necessary. In parallel, post-licensure safety studies were considered for a qualitative review. This study followed the PRISMA statement and the MOOSE reporting guideline. Of the 2,863 references identified, 11 articles were selected for meta-analysis. No significant association emerged between HPV vaccination and central demyelination, the pooled odds ratio being 0.96 [95% CI 0.77-1.20], with a moderate but non-significant heterogeneity (I(2)=29%). Similar results were found for MS and ON. Sensitivity analyses did not alter our conclusions. Findings from qualitative review of 14 safety studies concluded in an absence of a relevant signal. Owing to limited data on GBS, no meta-analysis was performed for this outcome. This study strongly supports the absence of association between HPV vaccines and central demyelination.

Mouchet, J. and B. Bégaud (2018). "Central Demyelinating Diseases after Vaccination Against Hepatitis B Virus: A Disproportionality Analysis within the VAERS Database." Drug Saf **41**(8): 767-774.

INTRODUCTION: Hepatitis B (HB) vaccination programs were set up worldwide in the early 1990s. Despite their major focus on reducing the burden of HB infection, they have seldom achieved the targeted population coverage in most countries, including the USA, with around 24.5% of adults being vaccinated against HB. Among proposed reasons for this is the persisting doubt about a possible link between HB vaccination and the occurrence of cases of multiple sclerosis (MS). OBJECTIVE: Our objective was to evaluate a potential safety signal between MS and HB vaccination. We conducted a disproportionality analysis (DPA) using the cases reported to the Vaccine Adverse Event Reporting System (VAERS). METHODS: We calculated the proportional reporting rate (PRR) and reporting odds ratio (ROR) of MS having occurred within the 120 days following HB immunization in adults aged 19-49 years when compared with other vaccines using the reports recorded in the VAERS database. Both ratios were estimated globally and then according to the origin of reports (USA vs. non-USA). We then performed a sensitivity analysis using a broader category of demyelinating events. FINDINGS: MS cases following HB vaccination were more likely to originate from outside the USA and to be reported before 2000 than those associated with other immunizations. All computed ratios were found to be statistically significant, with PRRs ranging from 3.48 to 5.56 and RORs ranging from 3.48 to 5.62. When considering the geographical origin, similar RORs were obtained for both US and non-US cases. CONCLUSION: In VAERS, MS cases were up to five times more likely to be reported after an HB vaccination than after any other vaccination. Since DPA is mainly suited for hypothesis generation, further studies evaluating the nature of the link between MS and HB vaccination would be of considerable importance.

Mouchet J, Bégaud B. Authors' Reply to Cohen et al.'s Comment on "Central Demyelinating Diseases after Vaccination Against Hepatitis B Virus: A Disproportionality Analysis within the VAERS Database". Drug Saf. 2018 Dec;41(12):1429-1430. **No abstract**

Mouchet J, Bégaud B. Hepatitis B vaccination and central demyelination - History, description and observed/expected analyses of 624 cases reported to the French pharmacovigilance over a 20-year period. Vaccine. 2019 Mar 6.

BACKGROUND: Confidence in vaccines is essential for achieving targeted immunization coverage. The current skepticism about vaccine safety feeds on controversies such as the suspicion about a link between hepatitis B (HB) vaccination and central demyelination (CD) after the massive HB immunization campaign in France in 1994-2000. This study assesses the robustness of this signal by analysing all validated cases reported in 1980-2000 and by conducting observed-to-expected (OE) comparisons. METHODS: After characterizing case profiles, reporting rates per 1,000,000 vaccine doses sold were computed for the period and per year. OE comparisons were conducted by using individual-based and person-year approaches and were stratified by gender. FINDING: A total of 624CD cases including 422 incident cases of multiple sclerosis (MS) were reported over 20 years. Women accounted for 73.2% (n = 457). Mean age was 29.8 years (SD = 11.1). Incidence of events peaked in 1995-1996 and 1997, these years accounting for 59.8% (n = 373) of cases. Events were mainly reported after booster doses (46.3%, n = 289). The overall reporting rate was 6.5 per 1,000,000 doses sold. The OE analyses produced inconclusive results, the number of observed cases remaining below the expected number. CONCLUSIONS: The complete disjunction between target and joint populations in the 1990s French HB immunization campaign created an unpreceded situation with ~26 million of adults exposed at the age of MS onset. Two findings are noteworthy: the non-random distribution of reports according to the rank of vaccination or years of survey, and the fact that the number of reports sometimes approached the baseline incidence of MS, irrespective of underreporting. While the nature of the link remains unclear, our results are not consistent with a strong association between HB vaccine and MS. Current recommendations targeting newborns with a possible catch-up of at-risk adults should remain the preferred strategy in low-endemic countries.

Articles under development

Mouchet J. and Bégaud B. (2019). "How to measure underreporting factors in spontaneous reporting systems? An example with anti-hepatitis B vaccine and central demyelination."