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Pharmacovigilance and Multiple Sclerosis (MS): drugs as risk factors for MS, and safety profile of drugs used in MS treatment

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SCIENTIFIC ENVIRONMENT

The studies include in this thesis were conducted by the Alma Mater Studiorum-University of Bologna, Unit of pharmacology, Department of Medical and Surgical Sciences.

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ABSTRACT

Background: Multiple sclerosis (MS) is a neurodegenerative disease of central nervous system with strong inflammatory component, which represent the main cause of clinical damage. Several risk factors have been associated with MS onset and progression such as vitamin D level, virus infections, smoke and obesity. However, the role of drugs exposure in MS development is still under-investigated.

On the other hand, an important aspect of MS management is represented by safety of authorized therapies. In the past, these therapies have been associated with occurrence of unexpected adverse drug reaction (ADR) and recently case reports have documented idiosyncratic liver injury (DILI) in MS patients under pharmacological therapy. This observation could be a spurious phenomenon or could represent an actual new ADR. Pharmacovigilance studies, by including millions of data, allow to detect possible signals of new ADR during post marketing drug use in clinical practice.

Objective: The main objectives in this thesis were to provide data on the possible role of drugs in MS development, as well as on safety profile of MS therapies. In details, we intended to 1) conduct a literature review of available evidence on the role of drugs in MS development and its worsening (part 1), 2) conduct a signal detection analysis to assess MS events during any drug treatments (part 2), 3) analyze signals of DILI events during MS therapies (part 3).

Methods: For the first part, we screened scientific literature, by starting from an *ad hoc* search strategy in Medline and EmBase, with the terms “drugs exposure” and “MS onset/worsening”. Each included record was classified according to the phase of the disease (onset Vs worsening), type of study (case report, case series, case-control study, cohort study and

clinical trial) and drug under study. Relevant indicators were extracted as well as number of cases for longitudinal studies, whereas Naranjo algorithm was applied for case reports and case series. Finally, quality of studies was assessed by using proper scales according to the nature of the study (part 1 of this thesis).

Then, FDA Adverse Event Reporting System (FAERS), a freely available database downloaded from the FDA website, was explored. After data management of the raw archive (q1_2004-q2_2016), case and non-cases were extracted. Case were represented by reports indicating "*Multiple Sclerosis*" as adverse event, whereas other ADRs were considered non-cases. In order to decrease spurious signals, we excluded records in case of: 1) presence of MS drugs, 2) presence of vaccines, 3) presence of non-MS drugs with MS in the field "indication". In order to increase the specificity of analysis an *ad hoc* index, named cleaning index, was developed by considering the proportion of records remained after application of exclusion criteria. A case/non-cases strategy of analysis was applied and reporting odds ratio (ROR) with relevant confidence interval (95%CI) was calculated. Signals were claimed when drugs presented at least 10 cases, significant ROR (lower limit > 1) and cleaning index ≥70% (part two of this thesis).

For the third part, we applied the above mentioned case/non-case strategy to analyze update archive (q1_2004-q4_2016). Cases were represented by records with at least one MS therapy and at least one preferred term (PT) suggesting a DILI event. Two definitions of DILI were considered: overall liver injury (OLI, broad definition) and severe liver injury (SLI, narrow definition) focused only on severe events. Then, ROR (95%CI) was calculated and signal was claimed when specific drug showed at least 3 cases with significant ROR. Finally, case-by-case analysis was performed, by detecting concomitant presence of drugs already associated with liver damage or specifically used to treat hepatitis.

Results: From analysis of literature several case reports and case series suggested a possible link between drug exposure and MS onset or its worsening, whereas longitudinal studies were few and sometime with contrasting results. Drugs acting on immune system were the most studied suggesting a link between their use and MS development, hormone balances and infections were also investigated but without homogeneous conclusions among different studies. However, the low quality was find in most studies, and the high frequency of missing information did not allow comparison of different findings.

FAERS analysis showed signals for drugs acting on immune system such as etanercept, adalimumab, infliximab. Disproportionality signals were found also for contraceptives, insulin and bisphosphonates. Our advanced strategy of analysis actually allowed to exclude from the signals drugs generally used as symptomatic agents in MS, for instance baclofen and methylprednisolone.

As for DILI events, interferons as well as mitoxantrone showed significant results. In fact according to previous publications they have been already classified as potentially hepatotoxic drugs. Signals were found also for alemtuzumab, teriflunomide and the symptomatic agent fampridine. Finally, case-by-case analysis showed concomitant presence of drugs potentially involved in liver damage and generally used to treat MS symptoms such as baclofen.

Conclusions: In part 1 and 2 we highlighted that a possible role of drugs in MS pathogenesis cannot be completely excluded, especially for biologics, hormones and antifectives. They can represent cause *per se* of disease onset or proxy of concomitant/previous disease able to trigger MS onset.

As regards MS therapies, in part 3 we found possible links between MS drugs exposure and subsequent DILI events. However, concomitant presence of other medications raises concerns on possible interactions with them that can trigger DILI events (e.g., gabapentin). We suggest

continue liver test during MS therapies in order to detect early enzymes abnormalities potentially involved in subsequent liver damage.

In conclusion, this thesis highlighted the importance of pharmacovigilance studies especially for rare and unpredictable ADRs. We encourage healthcare professionals and consumers to report as detailed as possible every ADRs to the relevant authority and to scientific community. This effort is crucial for better characterizing signals and for allowing to perform sensitivity analyses on specific groups of cases, in order to obtain useful information for future clinical and regulatory decisions.

LIST OF PUBLICATION

1) **Antonazzo IC**, Raschi E, Vignatelli L, Baldin E, Riise T, D'Alessandro R, De Ponti F, Poluzzi E. "Occurrence of Multiple Sclerosis After Drug Exposure: Insights From Evidence Mapping". Drug Saf. 2017;40(9):823-34.

2) **Antonazzo IC**, Raschi E, Forcesi E, Riise T, Bjornevik K, Baldin E, De Ponti F, Poluzzi E. "Multiple sclerosis as an adverse drug reaction: clues from the FDA Adverse Event Reporting System". Expert Opin Drug Saf. 2018.

3) **Antonazzo IC**, Poluzzi E, Forcesi E, Riise T, Bjornevik K, Baldin E, Muratori L, De Ponti F, Raschi E. Liver injury with drugs used for multiple sclerosis: A contemporary analysis of the FDA Adverse Event Reporting System. Mult Scler. 2018:1352458518799598.

ABBREVIATIONS

25(OH)D	25-hydroxyvitamin D
95%Cl	95% confidence interval
ADR	Adverse drug reaction
AML	Acute myelocytic leukemia
BBB	Blood brain barrier
BMI	Body mass index
CAN	Central nervous system
CIS	Clinically isolated syndrome
CNS	Central nervous system
CS-1	Connectin segment-1
DHODH	Dihidro-orate dehydrogenase (dhodh) enzyme
DILI	Idiosyncratic liver injury
DMF	Dymethyl fumarate
DMTs	Disease modifying treatments (drugs)
EAE	Experimental autoimmune encephalomyelitis
EBV	Epstein Barr virus
FAERS	Adverse event reporting system
GA	Glatiramer acetate
HLA	Human leucocyte antigens
HR	Hazard ratio
ISoP	International society of pharmacovigilance
ISPE	International society for pharmacovigilance
JCV	Polyomavirus JC
LLT	Lowest level term
MHC	Major histocompatibility complex
MS	Multiple sclerosis
Nrf2	Nuclear factor (erythroid-derived 2)-like 2
OLI	Overall liver injury
OR	Odds ratio
PhV	Pharmacovigilance

PPMS	Primary progressive multiple sclerosis
PRMS	Progressive relapsing multiple sclerosis
PT	Preferred term
ROR	Reporting odds ratio
RRMS	Relapsing remitting multiple sclerosis
SLI	Severe liver injury
SMQ	Standardized medDRA queries
SNP	Single nucleotide polymorphism
SOC	System organ class
SPMS	Secondary progressive multiple sclerosis
USA	United State of America
VAERS	Vaccine adverse event reporting system
VCAM-1	Vascular cell adhesion molecule 1
VDR	Vitamin D receptor
WHO	World Health Organization

CONTENTS

1. INTRODUCTION

1.1 Epidemiology of Multiple Sclerosis

This year is the 180th anniversary of the first case of "Sclèrose en plaque dissèmonèes" a disease currently known as Multiple Sclerosis (MS) (1). MS Generally affects 30 year or older individuals and only 2-5% of cases are diagnosed before 18 years old (2). MS has a complex pathological mechanism characterized by the contemporary presence of inflammatory and degenerative processes of central nervous system. It is well known that MS has a different incidence and prevalence worldwide (3, 4). A gradient can be identified from equator (less cases) to the northern regions (more cases, Figure 1.1a). In fact, Northern Europe, Northern America Southern Australia and New Zealand have higher prevalence of MS with over 30 cases per 100,000 inhabitants; lower prevalence about 5-30 cases per 100,000 inhabitants is registered in Southern Europe, Southern USA and Northern Australia; whereas less than 5 cases per 100,000 inhabitants can be detected in Asia and South America (5). Of particular interest is the case of Sardinia, an island of Italy, that shows higher prevalence (more than 100 cases per 100,000 inhabitants) as compared to the rest of the Country (3), maybe due to population characteristics. This represents a case of isolated population with peculiar environmental characteristics as well as lower genetic variability due to scarce migration phenomenon. It should be mentioned that according to the last MS report, latitude effect seems to be less incisive than other risk factors if we focus on general MS incidence worldwide (6).

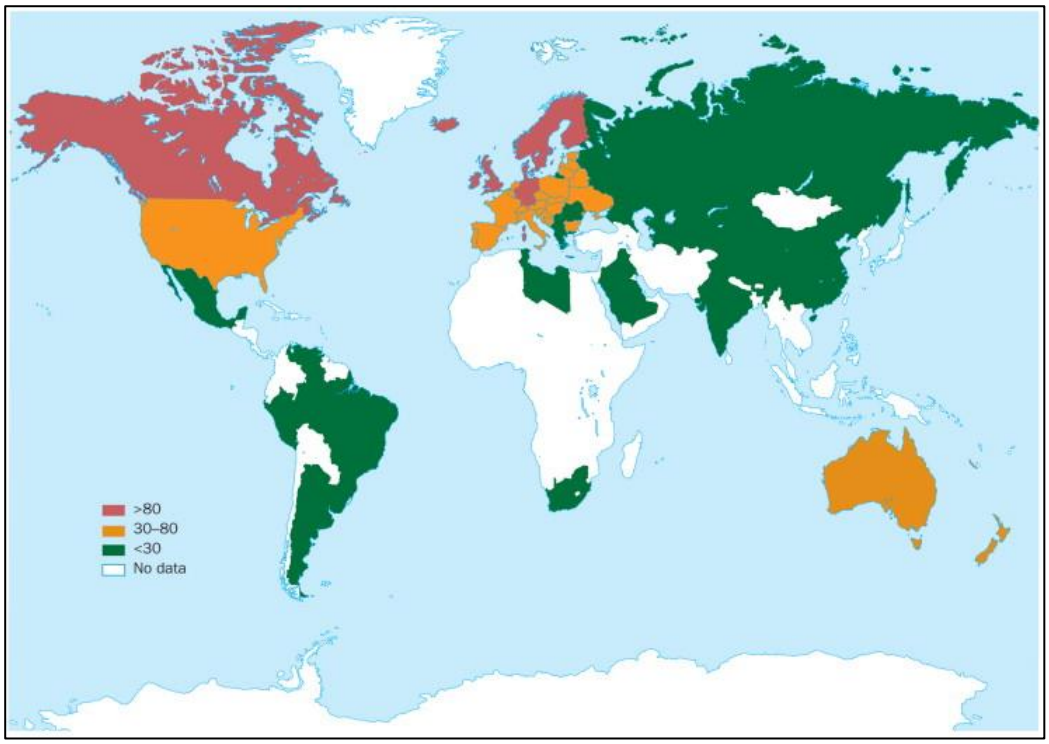


Figure 1.1a. Prevalence of multiple sclerosis worldwide. The figure illustrates prevalence of MS across different part of the Earth with prevalence reported per 100,000 inhabitans population. Reprint by permission from Elsevier: The Lancet Neurology 3: 710,19 © Copyright 2004.

The number of new diagnosed people increased from 2.1 million in 2008 to 2.3 million in 2013 (2). This can be justified by both improvements in diagnostic criteria and increased awareness of clinicians about early symptoms and clinical course of disease (7).

For many decades clinicians have had several misconceptions about MS also because many aspects of disease were unclear. One of those was possible sex difference in MS risk (8). In the early 1900s clinicians though that MS was a typical male disease with ratio male:female distribution of 3:2. Then, National MS society updated this ratio to 1:1. Only in the last decades the incidence rate was updated to 2:3.6 (6). The initial misclassification of disease was due to gender stereotypes. In fact, in the past, women were considered more prone to suffer from hysteria and this fact contributed to underdiagnosis of the disease, whereas men, which were considered more

important in the society, also for their power in workforce, received a proper diagnosis (8). However, sex difference and latitude distribution do not represent hard risk factors of disease because also many other factors may have a role in this disease such as sun exposure, vitamin D level, infection and genetic variants (6).

1.2 General characteristics and pathogenesis of Multiple Sclerosis

MS is an example of multifactorial disease that is influenced by environmental factors and genetic predispositions. While pathophysiological mechanisms are incompletely studied, its clinical manifestations is well documented.

In this intricate scenario, four clinical phenotypes of MS can be individualized: relapsing remitting (RRMS), secondary progressive (SPMS), primary progressive (PPMS) and progressive relapsing (PRMS) (9). By the time, these variants of disease were grouped into two main representative phenotypes: relapsing and progressive disease (10). The RRMS represents the most diagnosed type of MS accounting for almost 85% of total diagnoses of MS. By a clinical point of view, typically the RRMS is characterized by relapses followed by recovery (partial or complete). In this context, the first episode of disease is called Clinically Isolated Syndrome (CIS) which is not enough to have a diagnosis of MS, because it does not fulfill the criteria of dissemination in time even if involved in inflammatory events (11). On the contrary, few evidence are available for progressive form that remains almost completely under-investigated. Progressive MS is generally characterized by gradual accumulation of damage without recovery; clinicians refer to PPMS when it represents the first diagnosis whereas SPMS when represents a worsening of RRMS (10).

Migration of T- and B- lymphocytes in the Central nervous system (CNS) represents the crucial and first step to develop multiple sclerosis (12). However, the complete process has not been elucidated and many steps remain still unknown. Demyelination and neurodegeneration is generally associated with inflammation especially during relapse of disease (13, 14). Dysregulation of immune system due to intrinsic (e.g., cytokines receptors, microRNA) and extrinsic factors (e.g., regulatory T-cell dysfunction) affects T cell with consequent migration of them from peripheral areas into central nervous system (15). Impairment of T cells function leads also an indirect impairment of B cells with abnormal proliferation of these in the blood (16). These act against blood brain barrier (BBB) causing its increased permeability with subsequent infiltration of lymphocytes (17). In this phase of disease several mechanisms act simultaneously: production of immunoglobulin G and interleukin 6, activation of MHC class II-dependent antigen-presenting (18) resulting in demyelination of the CNS meanwhile production of metalloproteinase-9 destroys BBB (19).

All the processes described above are heavy influenced by genetic and environmental risk factors (e.g. virus infection (20)).

Knowledge of pathological mechanisms involved in MS disease allowed to create new therapies, albeit far to be considered as capable to resolve the disease, devoted to act on specific target in order to decrease severity of relapse.

1.3 Multiple Sclerosis and risk factors

Multiple sclerosis is an autoimmune neurodegenerative disease. The pathological pathways involved in its onset and worsening are still far to be completely elucidated. However, some risk factors have been identified. Among those, the most plausible risk factors are: genetic

predispositions, which include mainly genetic variant that increase MS risk, and environmental/biologic factors that can be associated to the disease onset (i.e., vitamin D level and sun exposure, virus infections, smoking and obesity).

1.3.1 Genetic variants

Studies on genetic susceptibility indicate that MS risk increases with decreased of genetic distance. The risk to develop a new episode of MS is about 9.2 for individuals with MS cases among first-degree relatives, 3.4 among second-degree and 2.9 for those with third-degree (21). Additionally, MS risk seems to be 10 folds higher for children of conjugal with MS than single affected parent (22, 23). Differences can be detected also among sibling, in fact monozygotic twin have a risk roughly 24-30% compared to heterozygote with 3-5% (24, 25).

In spite of evidence on possible association between genetic variants and MS risk, this disease is not considered a Mendelian disease because genetic variants represent only one of the several factors able to influence MS onset.

Association between multiple sclerosis and Major histocompatibility complex (MHC) have been supposed since 1970 (26, 27). In particular, genetic modification in genes encoding for human leucocyte antigens (HLA) class II have been associated with increased risk to develop the disease: HLA-DR15, HLA-DR16 and HLA-DQ6 genes in general population and HLA-DR4 for Sardinia and Mediterranean populations (28). In addition, individuals with allele HLA-DRB1*15.01 variants seem to have higher risk to develop the disease than general population (29).

Genome wide association studies have identified new genetic variants which may be associated with MS development (30, 31). These variants interest HLA-I and non-HLA loci (32, 33). Mutations in these loci are involved in alteration of structures and functions of receptors and their ligands. In particular, mutations of gene IL2RA and IL7RA are involved in interleukin 2 and 7 alpha

receptors modification; whereas STAT3, TNFRSF1A and TYK2 modifications encode for signal transducer and activator of transcription 3, tumor necrosis factor receptor 1 and tyrosine kinase 2 enzyme alteration potentially involved in MS pathology.

In spite of several genetic variants have been already associated with MS, influence of other ones cannot be completely excluded. It should be remembered that as happen for non Mendelian diseases, presence of one of these variants cannot represent cause *per se* of disease (34, 35).

Lastly, epigenetic alterations such as histone modifications which are primary involved in myelination and degeneration pathways can influence disease development and its progression (36). More studies need to evaluate the role of genetic and epigenetic factors in MS pathogenesis taking into account also environmental factors able to influence both of them.

1.3.2 Vitamin D level

Vitamin D is a nutrient introduced into human body through two main routes: orally and by UV exposure (37, 38). Pre-vitamin D3 is naturally synthesized in the skin when UBV strikes the skin with consequent conversion of 7-dehydrocholesterol in pre-vitamin D3, which spreads into the body by using cardiovascular system (39). The oral intake of vitamin D is characterized by vitamin D_2 (mainly from vegetables sources) and vitamin D_3 (mainly from animal sources). In liver pre-vitamin D_3 is converted in vitamin D_3 and then in 25-hydroxyvitamin D (25(OH)D) (40). Ergocalciferol (vitamin D_3) and colecalciferol (vitamin D_2) after conversion in 25-hydroxyvitamin D can undergo a second hydroxylation in the kidney producing 1,25-dihydroxyvitamin D (called also calciferol see figure 1.3.2a) (41). Generally, 25-hydroxyvitamin D is used as marker of vitamin D concentration in the blood instead of vitamin D_3 or 1,25-dihydroxyvitamin D because has long half-

life and it is directly depending on vitamin D concentration but it is also less influenced by hormones than vitamin D metabolism (38, 40).

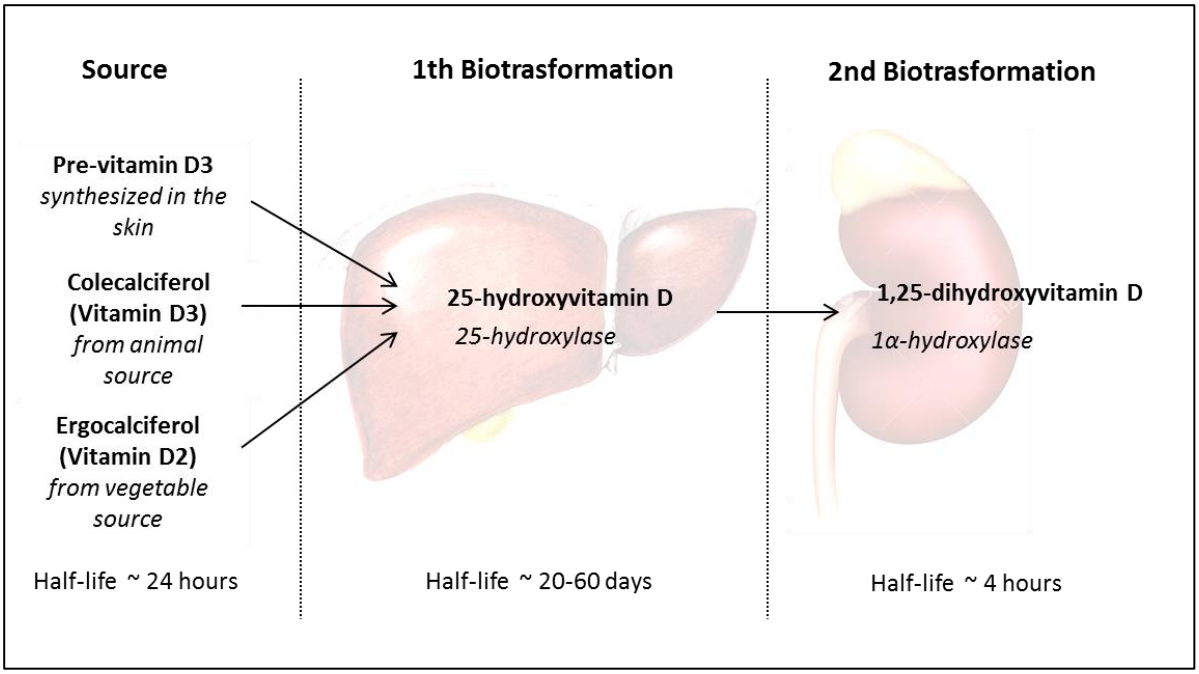


Figure 1.3.2a. Metabolism of vitamin D. Figure illustrates Vitamin D metabolic pathway. Vitamin D from skin, animal and vegetable sources are converted in 25-hydroyvitamin D in the liver and then in its active form 1,25-dihydroxyvitamin D in the kidney less in other tissue.

After the first hypothesis of possible role of vitamin D in MS pathogenesis in 1974, many studies were conducted to assess the possible association between this nutrient, and in particular its deficiency, with multiple sclerosis onset (42). A nested case control study, including 148 cases and 296 controls, found a decreased risk to develop MS in individuals with higher level of 25-hydroxyvitamin D. The study suggested that this inverse association is true only for white individuals because no significant results were found for Blacks and Hispanics (43). In 2012, Salzer and colleagues including roughly the same number of cases from Swedish population, identified an inverse association between 25(OH)D≥75nmol/L and MS risk (44). Another study explored the role of vitamin D instead of 25(OH)D (45). In this case, high concentration of vitamin D was associated

with decreased risk to develop MS during follow-up. In addition, also vitamin D supplements (≥400 UI/Day) was inversely associated with MS risk (45). In a multinational case-control study, self-reported vitamin D supplements was associated with reduced MS risk (46). Also other studies with different methods to detect vitamin D intake were consistent with previous results (47-49). Finally, also a Mendelian randomization studies found an association between specific single nucleotide polymorphism (SNP) and MS. In particular these SNPs are correlated with vitamin D level and can be used in order to avoid generic confounders, almost impossible to control, that can affect study on vitamin D concentration such as sun exposure (50-52).

Animal studies by using an experimental autoimmune encephalomyelitis (EAE), the mice model of MS, found that vitamin D and its metabolites have a protective role in disease onset and progression (53). Two mechanisms can be supposed, 1) 1,25-Dihydroxyvitamin D3 acting on Th1 cells and activating macrophages inhibits their activity and blocks disease progression; 2) stimulation the proliferation of encephalitogenic cells (53). Other studies suggested that several pathways are involved in animal model, and hypothetically also in human, in protective effect of vitamin D including: IL-10 pathway (54), modulating Rag-1 dependent cell such as autoreactive T cell (55), and modulating the vitamin D receptor (VDR) on T cell surface (56). Furthermore, 25(OH)D and $1,25(OH)_2D$ seem to decrease Th17 cell differentiation (57, 58), same results were observed in MS patients (59).

Vitamin D levels seem to influence also disease activity and progression. In fact, two longitudinal cohort studies found that high level of 25(OH)D was associated with decrease disease activity and slower progression, predicting lower disability during follow-up (60, 61). The role of vitamin D supplements during MS therapies is still debated (62). However, a trial found reduced MRI disease activity in patients treated with interferon beta and vitamin D (63).

Finally, considering the disease characteristics and the possible concomitant influence of other risk factors rather than *mere* vitamin D levels, further studies need to assess the role of this supplement in MS therapies.

1.3.3 Epstein Barr virus infection

Microbial infections have been suggested as possible cause implicate in MS development in susceptible individuals. However, these agents may also trigger MS development by accelerate subclinical autoimmune processes (64). Among the possible infections proposed as cause of MS development (bacterial and virus) only Epstein Barr virus (EBV) has been associated with increased MS risk (65).

A prospective study among US military personnel, including 222 MS cases and 444 matched controls, aimed to estimated MS risk among EBV positive individuals. MS risk was higher, 36-fold, among individuals with anti-EBNA complex IgG titers above 320 compared with individuals with anti-EBNA titer lower than 20 (66). Further, another study aimed to detected possible link between several viruses (HBV, human herpes virus, herpes simplex virus, varicella zoster virus and measles) and MS risk. Among the analyzed virus only EBV had significant results suggesting a role in MS disease (67). A study published in 2010, included 305 MS cases and 610 matched controls, by using blood sample collected before MS onset had the purpose to establish possible temporally sequence between EBV infection and MS onset (68). Results from this study suggested that after seroconversion (individuals affected by EBV virus) the risk to develop MS increased if compared to people without seroconversion (individuals without EBV infection).

The mechanisms implicated in MS development after EBV infection is still not completely understood. A possible hypothesis includes several dysregulations in immune system functions

due to virus infection (69). In particular, virus infection inhibits functional suppression at T-cell level, additionally EBV homing in B-cell contributes to the distinct characteristics of EBNA-specific T cell in MS subjects. Finally, after EBV infection neurons of central nervous system are more prone to induce immune response with consequent inflammatory processes due to EBV antigens on their surface (69). So, EBNA1-T cell are more prone to recognize myelin antigens inducing inflammatory response that is involved in CNS damage (70). Finally in these intricate pathways also genetic component can affect EBNA1-T cell selection and proliferation and therefore deserves further evaluations (70).

1.3.4 Smoking

Several studies have been conducted in order to explore association between smoking and multiple sclerosis. The first time that smoke can represent a risk factors for MS development was suggested in 1965, by using a case control study that found higher proportion of MS cases among smokers compared to controls (71). This find was in contrast with two studies conducted in United Kingdom, which were planned for other purposes and that found a non-significant association between smoking and MS development (72, 73).

In 2002 a Canadian case-control study including 200 cases and 202 sex- and age-matched controls found an association between cigarette smoking and MS risk (74). Then, two large prospective cohort studies which included US female nurse confirmed previous results (75). Some years later, the same authors conducted a new nested case-control study with 201 cases and 1,913 controls (76). They found an increased risk to develop MS (Odds ratio: 1.3; 95%CI: 1.0-1.7) in ever-smokers compared to never-smokers. Another study focused on relapse risk found increased risk to have a progression of disease (Hazard ratio: 3.6; 95%CI: 1.3-9.9) in MS smoker individuals.

In addition, cigarette smoking seems to lead a reduction of functional level in MS patients even if had a good EDSS score (77). A recent study has suggested that smoke is able to influence also therapeutic effects of MS treatments decreasing their efficacy and increasing probability to experience relapses during treatments (78).

Association between smoke and increased MS risk have been supported by several studies conducted in Norway (79), England (76), Serbia (80), Sweden (81), Iran (82) and Australia (83).

Finally, some meta-analyses have been conducted and updated in the last years and all of them agree on the role of smoke in MS development. Two of them, including 26 and 29 studies concluded that smoker men are more susceptible than women to develop the disease (OR: 2.14; 95%Cl: 1.80-2.55) (84, 85), and current smokers are more prone to develop MS than past smokers (OR: 1.83; 95%Cl: 1.42-2.37) (84, 86).

The complete mechanisms cause of MS development or its worsening are far to be completely elucidated. Smoking cigarette can affect other inflammatory response, immune system activity as well as epigenetic pathways (87, 88). Free radicals and in particular hydrogen cyanide are produced during cigarette combustion. Basic research showed that this compound is responsible of demyelinating processes in central nervous system (89, 90). Oxidants can cause alteration of B and T cells through alterations of cell membrane functions increasing number of pro-inflammatory agents (91-93). Smoking can affect also gene expression through epigenetic pathways. An increased risk of MS was observed in individuals with HLA genes alterations. The most studied genes are HLA-DRB*1501 and HLA-A*0201 only for Nordic individuals (94-96). In this intricate scenario, further studies need to elucidate the role of environmental and genetic factors interplay in MS development.

1.3.4 Obesity

Several studies investigated the role of body mass index (BMI) in MS pathogenesis (97-103). Two studies setted in Nurse home called Nurse's Health I study (121700) and Nurse's Health II (116671) by using cox regression model found that obesity (BMI≥30 Kg/m^2) at age 18 was associated with higher risk to develop MS (102). A Danish study that included individuals who were born in the early 20^{th} century suggested that those were more prone to develop MS if they had higher BMI at age 7 to 13 (101).

Case control studies carried out in Norway, Italy, Sweden and US including about 3,000 MS cases and much more controls showed an increased risk to develop MS for individuals with higher BMI during childhood and adolescence (98-100).

The biologic mechanisms involved in the observed phenomenon are still far to be completely elucidated, even if also vitamin D can be involved. The main hypothesis supports an interplay between vitamin D level in obese individuals and MS development. In fact, a meta-analysis found an inverse association between vitamin D level and BMI (104), this can be due to: 1) scarce sun exposure due to impaired mobility of obesity people; 2) change in vitamin D metabolism in liver and bones; 3) over storage of vitamin D in the body fat (104-106). Obesity is associated also with inflammatory processes that can increase MS susceptibility (107). Adipocytes produce several pro-inflammatory cytokines such as IL-6, tumor necrosis factors and leptin able to induce alteration in immune system functions with consequent triggering of MS development (108, 109).

1.4 Therapeutic armamentarium for Multiple Sclerosis

Management of multiple sclerosis is challenging for clinicians and patients, which currently deal within an increasing number of therapeutic alternatives (110). Nowadays, therapeutic strategy is focused on the reduction of the risk of relapses and disability progression after MS diagnosis (110). The uncomplete knowledge of pathophysiological mechanisms of MS onset and its worsening increased the needs to develop drugs able to target different pathways. So far, 11 drugs (included in this thesis) plus two new molecules (not included in this thesis because approved only recently) have been authorized for MS treatment. In MS therapies, 3 milestones can be reminded: first authorized therapy in 1995, first monoclonal antibody specifically addressed to MS in 2005 and first oral treatment in 2011 (Figure 1.4a).

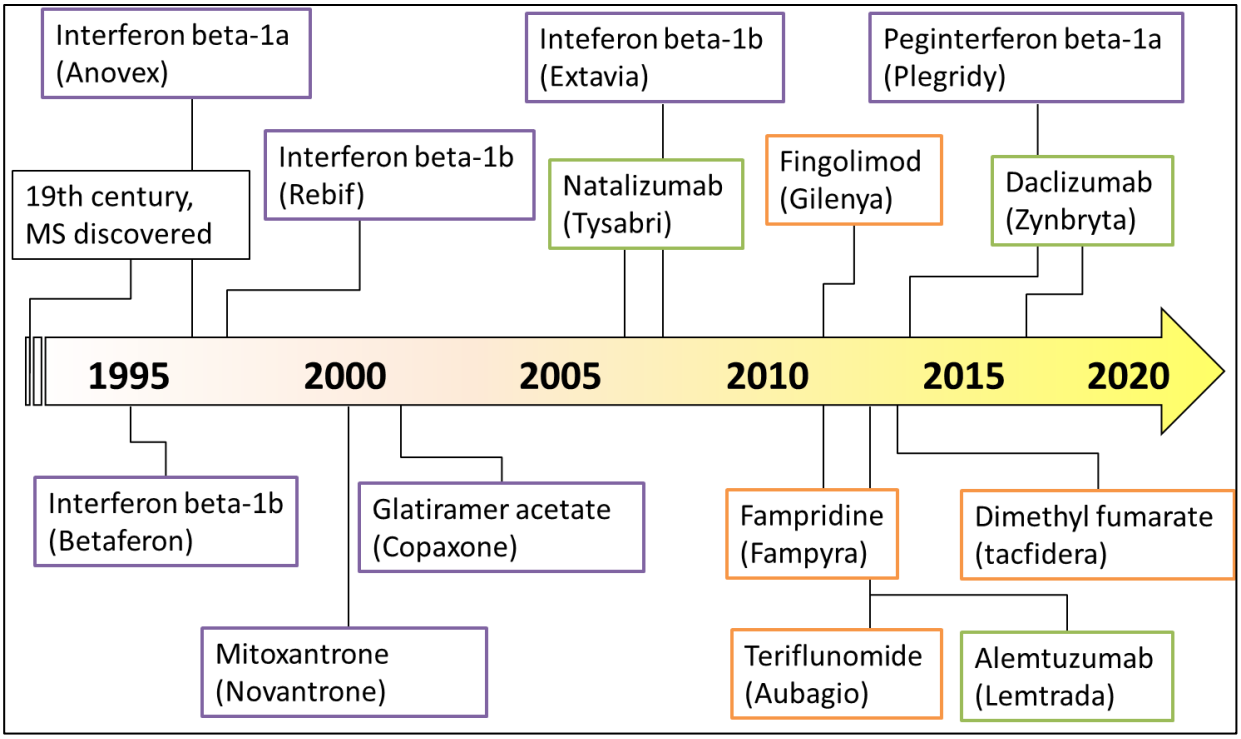


Figure 1.4a. Time sequence of approved MS therapies. The figure illustrates the chronological sequence of available drugs; in violet injectable therapies, in orange oral ones and in green injectable and monoclonal antibodies therapies.

The huge heterogeneity of available therapeutic armamentarium makes almost impossible to prioritize treatment options, so usually interferons were considered the first choice followed by another option in case of scarce risk-benefit profile and according to the patient characteristics (10). However, a sort of categorization based on the clinical effect can be proposed: 1) therapies with moderate efficacy or indicated for initial treatment of disease such as interferon beta 1a, interferon beta 1b, glatiramer acetate, fingolimod, teriflunomide and dimethyl fumarate; 2) high efficacy or indicated in later disease modifying therapies such as mitoxantrone, natalizumab, alemtuzumab and daclizumab (recently withdrawn from the market); and 3) symptomatic therapy such as fampridine.

1.4.1 Moderate efficacy or standard initial treatment

Moderate efficacy or standard treatment category includes the first used option therapy, interferons, and part of the second line treatments: glatiramer acetate, fingolimod, terifluonimide and dimethyl fumarate.

These substances represent a heterogeneous group of drugs with different route of administration (e.g., injectable and oral), scheme of therapy, doses and mechanisms of action.

In the following sections the most important characteristics of each included therapy will be briefly described.

1.4.1.1 Interferons beta

Interferons are the first drugs specifically authorized for MS treatment. Firstly, was Interferon beta 1b called Betaseron® authorized in 1993, subsequently, other three drugs were approved with the same indication: two formulations containing interferon beta 1a (Anovex® and Rebif®) in 1997 and 2000 respectively, and its pegylated form marketed as Plegridy® in 2014 (111-114).

Although the exact mechanism of action is still unknown, different possible mechanisms of action have been proposed for their therapeutic effects. Among those, the mains are: suppression of cells functions and reduction of interferon gamma secretion by activated lymphocytes; activation of macrophage; and downregulation of class II MHC induced by interferon gamma with consequent reduction of antigen-presenting glial cells (115). Additionally, interferons beta suppress T cell proliferation, increases natural killer production and decreases blood brain barrier (BBB) permeability by reducing activity of metalloproteinases (116, 117).

Interferons beta have the longer period on market and many data are available on their safety profile. Usually, treatment with these drugs is associated with flu-like symptoms, local injection-site reactions, lymphopenia, complex headache and pain (118). In addition, adverse events associated with self-injection are cause of drug discontinuation in a range between 14% and 44% of treated patients with consequent relapse of disease (119). However, self-injectable devices improved compliance to this therapy (120) decreasing most of uncomfortable aspects associated with self-administration. However, the most dangerous adverse effect is represented by liver damage associated with their use (121). In fact, liver damage ranged from mild and moderate enzyme elevation, generally resolved with drug discontinuation, to acute hepatic failure.

Liver enzymes monitoring and drug discontinuation are recommended in case of abnormalities during ongoing treatment (121).

In spite of adverse effects associated with interferons use, usually, they are considered and used as first line of treatment for both first symptoms and relapses. However, a recent meta-analysis including all authorized therapies for MS treatment reveal unclear results on real benefit of early treatment with interferons compared to other therapies (122).

1.4.1.2 Glatiramer acetate

Glatiramer acetate (GA) was discovered in 1970 (123), but only in 2001 Copaxone®, the brand name of active substance GA, was approved by FDA (124). Glatiramer acetate is a mixture of polypeptides randomly polymerised from four amino acids: L-glutamic acid, L-alanine, L-tyrosine and L-lysine (124, 125). The mechanism of action of this drug has still been incompletely understood. However, some proprieties are well known. Firstly, it binds major histocompatibility (MHC) class II molecules, preventing the presentation of other antigens and reducing T-cell activation. GA acts on monocytes and dendritic cells with consequent inhibition of first cells and less production of TNF-alpha, IL-12 and increased production of IL-10 by the second ones (126). Animal studies suggested also a neurotrophic effect of GA in murine model. In fact, neurotrophic factors such as production of BDNF and neutrophin 3 and 4 were detected in peripheral and central nervous system of animals after treatment (126).

Usually, a dose of 20 mg/mL per day is well tolerated by patients and according to the clinical trials the most reported adverse events are injection-site reaction as well as vasodilatation, dyspnea, depression, tachycardia, dizziness and tremor (124, 127).

In conclusion, glatiramer acetate acting on different levels on immune system activity results in a general anti-inflammatory and neuroprotective response with consequent benefits of treated individuals (126, 128).

1.4.1.3 Dimethyl fumarate

Dymethyl fumarate (DMF) is an oral therapy included in MS armamentarium by FDA in 2013 and marketed as capsules of both 120mg and 240 mg dose (129).

This agent is a derivate of fumaric acid, a metabolite of Krebs cycle, that claims anti-inflammatory, cytoprotective and immunomodulating functions (130-132). The complete mechanism of action of this drug is far to be completely elucidated, however, some lines of evidence are available from molecular and animal studies. In fact, DMF is responsible of activation of nuclear factor (erythroid-derived 2)-like 2 (Nrf2) transcriptional pathway (129, 132, 133). In vitro studies highlighted that DMF (129) induced anti-oxidative pathways by regulating Nrf2 (132, 133). In particular, monomethyl fumarate (an active metabolite of DMF) was able to stabilize and translocate Nrf2 in the nucleus (133). This step increases production of anti-oxidative enzymes resulting in major concentration of oxygen species scavenger (130-133). Finally, these agents (DMF and its metabolites) seem to decrease also oxidative stress in astrocytes and neurons (132, 133).

Animal studies pointed out beneficial effects of DMF in murine model of MS (133, 134) by decreasing inflammatory infiltrate and increasing anti-inflammatory cytokine (e.g. interleukin-10). In addition to the Nrf2 effects, this agent inhibits also expression of inflammatory cytokines, chemokines and adhesion molecules due to nucleus translocation block of factor-kb (135).

Finally, DMT showed apoptotic effects on human T cell (136, 137) and this may be responsible of depletion of circulating lymphocytes during DMT treatment.

The most reported adverse effect associated with DMT use are: nausea, diarrhea abdominal pain and flushing (129). As regards flushing this may be caused by monomethyl fumarate bindings with nicotinic receptor that cause PGE_2 formation via GPR109A and COX-2 pathways (138).

Even if the exactly mechanisms of action of DMT deserve more evaluations, this drug seems well tolerated by patients (139). In addition, its route of administration can be a factor that may increase also adherence to the treatment.

1.4.1.4 Fingolimod

Fingolimod was approved by FDA in 2010 and marketed with brand name Gylenia ® (140). It has been authorized as single agent in MS disease and it is able to reduce both clinical exacerbation of disease and accumulation of physical disability (141).

Fingolimod is an analogue of sphingosine and acts as antagonist of sphingosine 1-phosphatase receptors (S1PRs) except $S1PR_2$. The S1PRs are largely expressed on surface of many types of cells and are involved in several cell pathways: differentiation, survival, movement, angiogenesis, inflammation and immunity (142). This drug regulates traffic of T- and B- cells across lymphoid tissues, bone marrow and circulatory system. In particular, once fingolimod binds its receptors on the lymphocytes surface inhibits their egression from thymus and lymph nodes with consequent reduction of circulating lymphocytes (143). Further, fingolimod is able also to reduce humoral response and interferes with degenerative process in the brain and spinal cord (141).

Among treated patients hyperpolarization of atrial myocytes resulting in reduction of heart rate and reduction of pulmonary function were observed (144, 145). Both of the above ADRs are consequences of its effect on S1PR receptors of cardiac and pulmonary cells. Finally, this agent has been associated with cystoid macula oedema (140, 146). Considering the pharmacologic effect on cardiac and pulmonary cells co-administration of fingolimod with other drugs with similar effects such as β-adrenergic receptor antagonists (e.g. atenolol), class Ia and IIa of antiarrhythmic, calcium channel blockers (e.g. dialtiazem), digoxin, anticholinesterase inhibitors (e.g., neostigmine) and pilocarpine should be avoided (140, 144, 147). Regulators suggest no vaccination with attenuated virus during treatment and in the following two months after drug discontinuation because of high risk of infection; as this regards continuous monitoring of patients for possible opportunistic infections are suggested (140, 147).

In spite of mentioned risks benefit of this therapy was highlighted also in a recent publication that suggested lower rate of switch from first treatment to another one for those firstly treated with fingolimod (148). However, it should be recognized that several aspects can influence the first treatment choice (i.e., disease activity, time gap between first symptoms and final diagnosis and age) and all of them have a role also in adherence to the treatment.

1.4.1.5 Teriflunomide

Teriflunomide, marketed with brand name Aubagio®, was approved by FDA in 2012 (149). The main mechanism of action involves inhibition of lymphocytes proliferation by blocking these cells in S phase of mitotic division. Teriflunomide blocks production of *de novo* synthesis of pyrimidine by reversible inhibition of dihidro-orate dehydrogenase (DHODH) enzyme in the mitochondrial and highly expressed in proliferating lymphocytes (150). As result of above activity,

a general cytostatic effect on proliferating T- and B-lymphocytes were obtained, with consequent reduction of disease activity (151).

This agent was the second drug, licensed for MS treatment after interferons, and it is able to reduce disease activity during acute phase (152). In clinical trials teriflunomide showed a good risk benefit profile having moderate adverse events (≥2% of treated individuals) such as alopecia, headache, diarrhea and hypertension (152).

By pharmacological point of view, teriflunomide is a metabolite of leflunomide used to treat rheumatoid arthritis (153). This last agent has already been associated with hepatic failure, therefore as precautionary measure FDA recommend periodic hepatic enzymes tests also in new treated patients with teriflunomide (149).

Finally, it should be noted that teriflunomide represents the first oral therapy available for MS patients. This is a crucial aspect that should be taken into account during treatment choice for patients. In fact, is easy to suppose that patients should be more confident with oral therapies rather than injectable ones and this can also contribute to their adherence to the treatment.

1.4.2 High efficacy or later disease modifying therapies

These agents are usually used as second- or third- line of treatment and include mitoxantrone and monoclonal antibodies. A recent network meta-analysis, by analyzing 33 studies among all the authorized MS therapies, showed that alemtuzumab, natalizumab and the recent approved drug ocrelizumab presented high efficacy in MS patients compared with other MS therapies (154). In the next section, we briefly describe pharmacological characteristics of these agents as well as those of mitoxantrone.

1.4.2.1 Mitoxantrone

Mitoxantrone (marketed as Novantrone ®) was approved for multiple sclerosis treatment by the FDA in 2000 (155, 156). It is an anti-neoplastic anthracednedione derivate (157), targets topoisomerase II interfering with DNA repair as well as inhibiting DNA replication and RNA synthesis by producing crosslinking and single- double- strand breaks (157). These mechanisms involve in apoptotic processes resulting in inhibition of T- and B- lymphocytes cell proliferation (157, 158). Another possible mechanism of action could be represented by decreasing secretion of pro-inflammatory cytokines (157).

A recent Cochrane meta-analysis confirmed the clinical efficacy of mitoxantrone on disease progression in MS patients (159). The most frequent adverse effects reported during clinical trials were: nausea, vomiting, alopecia and urinary tract infections (159). However, use of this drug seems to be associated with increased risk to develop acute myelocytic leukemia (AML) suggesting a careful evaluation of leukocytes blood levels before new cicle with mitoxantrone (159). As regards cardiotoxicity induced by long treatment with mitoxantrone, this can be reduced by performing specific tests (i.e, electrocardiogram and echocardiography) in all potential candidate for this therapy (155, 159). As see above for interferons, also during mitoxantrone treatment transient ALT elevation can occur due to toxicity of drug (121). Therefore, periodically monitoring of aminotransferase levels is recommended and therapy with this agents should be avoided in patients experienced hepatic failure (121, 155).

Finally, a post marketing French study, by including a large population, found positive impact of mitoxantrone into long-term period of treatment (160). However, longitudinal base registry study RENEW (Registry to evaluate Novantrone Effects in Worsening Multiple Sclerosis Study) highlighted the importance to made a carefully evaluation of patients before new

treatment in order to reduce the risk of ADRs (i.e., cardiotoxicity) associated with this therapy in peculiar sub-group of patients (i.e., first line therapy non responders) (161).

1.4.2.2 Natalizumab

Natalizumab represents the first monoclonal antibody specifically developed for MS treatment. It was authorized by FDA and marketed worldwide in 2004 with brand name Tysabri® (162).

The mechanism of action of natalizumab is not yet completely elucidated. Natalizumab acts by blocking migration of T lymphocytes through the blood-brain barrier (BBB). In particular, this antibody binds α4 subunit of α4β1 and α4β7 integrins expressed on surface of lymphocytes. This action prevents possible binding with their receptors which include vascular cell adhesion molecule 1 (VCAM-1) expressed on vascular endothelial cells, osteopontin, fibronectin, and connectin segment-1 (CS-1) expressed in parenchymal cells (162, 163). A treatment with 300 mg of natalizumab via infusion is able to inhibit lymphocytes migration in the central nervous system with consequent reduction of disease activity (162, 163).

The most common side effect is acute infusion reaction, but probably the most known ADR associated with natalizumab treatment is the development of progressive multifocal leukoencephalopathy (PML) a severe and sometime fatal complication (164). The PML is a demyelinating disease firstly detected in HIV patients and other immunocompromised patients (165). This disease interests the central nervous system (CNS) due to reactivation of polyomavirus JC (JCV) (164, 165). Clinically, patients who experienced PML have neurological deficit caused by lesions in the white matter of CNS that appear as demyelinated areas with infected oligodendrocytes located at the periphery (165).

Nowadays, several improvements have been made for PML prevention and management. So far, three risk factors have been associated with PML development: 1) positive serostatus for anti-JCV antibodies; 2) prior treatments with immunosuppressant drugs; 3) use of natalizumab for more than 2 years (166-169). Severity of PML was a critical issue in clinical practice that raised concern among regulatory agencies. For this reason natalizumab had been temporarily suspended from the market until 2006 when after carefully evaluation of PML cases it obtained again authorization for MS treatment with some guidelines in order to decrease this risk in treated patients (170-172).

In spite of possible occurrence of severe PML, natalizumab has been representing a great therapeutic option for MS patients. Analysis of JCV antibody for possible natalizumab candidate patients, continuing monitoring during and after treatment discontinuation could lead to detect early PML onset contributing to improve patients' management (169).

Natalizumab could be considered a sort of milestone in MS therapy history. In fact, the positive results observed during clinical practice contributed to increase studies on new antibodies which are currently approved for MS treatment such as alemtuzumab and ocrelizumab (recently approved but not included in this thesis).

1.4.2.3 Alemtuzumab

Alemtuzumab was authorized for the treatment of B-cell chronic lymphocytic leukemia in 2001 (173), and then as MS treatment in 2013 with brand name Lemtrada® (174). It is a humanized monoclonal antibody IgG1 against the CD52 protein, glycosylphosphatidylinositol (GPI)-anchored protein, expressed on surface of T (CD^{3+}, CD^{4+} or CD^{8+}) and B (CD^{19+}) lymphocytes

and less on surface of other circulating cells (i.e., monocytes, macrophages and eosinophil granulocytes) (175, 176). The complete mechanism of action is far to be understood, because many aspects and roles of antibody and its targets are still under-investigate. The bond between antibody and its receptor cause cellular cytolysis and complement-mediated lysis involved in depletion and repopulation of lymphocytes (176). This is the most accredited theory on mechanism of action of alemtuzumab involved in immunity cells remodeling resulting in reduction of inflammatory processes (175, 176).

Treatment scheme consists in 12 mg/day on 5 consecutive days following by infusion of 12 mg/day for 3 consecutive days after 1 year from the first infusion (174). The most common side effect is infusion reaction generally resolved with methylprednisolone or other symptomatic drugs (177). Treatment with alemtuzumab is also associated with severe adverse events (177, 178). In fact, severe and sometime fatal autoimmune disorders (i.e. autoimmune thyroid disease), idiopathic thrombocytopenic purpura, goodpasture syndrome, and in a minority of cases thyroid cancer were reported (174, 177, 178).

Apparently patients treated with alemtuzumab do not experience infections such as PML a typical complication of natalizumab that have been raised concern on its use in previous years. However, monitoring of individuals cured with this agent should be advocated in order to detect early ADRs associated with its use.

1.4.2.4 Daclizumab

Daclizumab is a humanized IgG1 monoclonal antibody binds the alpha subunit (CD25) of the human interleukin-2 receptor expressed on activated lymphocytes. The complete pathway of

action is still under-investigate, but the main effect of this antibody consists in general inhibition of lymphocytes activity in MS patients (179).

This drug represents a paradigmatic example of agent already used as efficient therapy in organ transplanted patients (180), but extremely dangerous if used for different pathological condition (179). It was authorized as MS therapeutic option in summer 2016 with brand name Zimbryta® (179). However, after few months EMA's Pharmacovigilance Risk Assessment Committee (PRAC) restricted its use only for patients with no benefits from other MS therapies because of some reports of elevation of hepatic transaminase enzymes and liver injury (181). Then, the pharmaceutical company owner of daclizumab voluntary withdrawn it from the market after seven cases of encephalitis and meningoencephalitis involved in MS treated patients (181).

Nowadays, daclizumab does not represent anymore a valid therapeutic option for MS patients, but it is still included in this thesis because on the market when data on safety profile of MS therapies were analyzed.

1.4.3 Symptomatic therapy

Multiple sclerosis patients during early phase of disease as well as relapse experience a widely number of symptoms. Neurologists and other specialists manage these symptoms by using a wide range of pharmacological and non-pharmacological options in combination with disease modified treatments (DMTs). Pharmacological treatments include steroids during phase acute of disease (i.e., 0.5 g daily for 5 days of methylprednisolone), amantadine for fatigue, baclofen and gabapentin (or combination of these drugs) for spasticity, gabapentin (as first choice) and memantine (as second one) for oscillopsia and other drugs for minor symptoms (182).

Among all the therapeutic options used for MS symptoms, only fampridine was specifically approved as symptomatic agent for MS disease (183). In the next section we focus on this drug providing some pharmacological details.

1.4.3.1 Fampridine

Fampridine, also called dalphampridine, was approved by the FDA and marketed with brand name Ampyra®, as symptomatic drug for multiple sclerosis patients in 2010 (183).

Fampridine is a broad inhibitor of K^+ channels present on the surface of axons (184). The mechanism of action is far to be completely understood. The most accredited hypothesis suggests its role in prolonging of action potential in damaged neurons (185), and new data indicates also possible stimulation of N- and L- calcium channels (186). All of these mechanisms can reinforce synaptic transmission and increase muscle twitch tension, resulting in improving walking speed in treated individuals (184).

It should be note that this agent does not have any effect on MS pathology, but can be used to improve walk impairments and to decrease fatigue in these patients (184). Fampridine seems to have a relatively safe profile, no drug-drug interactions were detected during clinical trials and the most common side effects were urinary tract infections, insomnia, dizziness, headache and balance disorders. For this reasons moderate use is recommended in patients with past history of convulsions, kidney failure and concomitant use of medication for seizure (184, 187).

Further studies could elucidate the effects of this therapy when administered together with other MS therapies in patients experienced first symptoms of disease and during relapses.

However, it should be remarked that it remain the first therapy specifically devoted to treat MS symptoms, that could be used as model for future therapies.

2. STUDY RATIONALE AND OBJECTIVE

2.1 Rationale

Several environmental and genetic risk factors have been already associated with MS development and its progression. However, the role of other potential risk factors cannot be excluded, as for example drug exposure. Data on drug exposure and new MS events can provide new insights on MS pathological pathways, also in light of concomitant diseases traceable by using drugs.

On the other hand, the safety profile of MS drugs raises concerns among clinicians, patients and regulators. Several new drugs have been approved by EMA and FDA in the last decades, although data on their safety profile were still very scarce. For this reason, pharmacovigilance studies by using large databases are crucial in order to better define the side effects, especially by detecting rare ADRs, that were not properly addressed in clinical trials. This study phase is extremely important because of consequences in regulatory actions (restriction or withdrawn of drug) as well as in clinical management of the disease.

2.2 Objectives

The objectives of the present thesis are the following:

1) Identify and evaluating the strength of evidence on drug exposure and multiple sclerosis onset and worsening, by developing an evidence mapping review of available findings in the literature.
2) Providing new insights on signals of disproportionality for MS development after drug exposure, by analyzing FDA Adverse Event Reporting System database (FAERS).
3) Providing new insights on signals of possible association between exposure to drugs for MS treatment and drug induced liver injury (DILI), by analyzing the FAERS database.

3. METHODS

3.1 Part 1: Evidence mapping of the literature

3.1.1 Study design

Since relationship between drug exposure and MS onset (or its worsening) is still debated we performed a literature review of available data. First, we defined a specific search strategy including terms “Multiple sclerosis” and “drug exposure” that was applied in two different databases: Medline and EmBASE.

After merging records originated from the two sources and removal of duplicates, snowballing of reviews was performed in order to include additional relevant articles. Full texts of eligible articles were downloaded and analyzed, then each article was classified according to: 1) type of drug(s) included in the study, 2) type of study (clinical trials, cohort study, case-control study, case reports and case studies), and 3) stage of disease (onset, worsening).

3.1.2 Causality and quality assessment

For each included study the appropriate index summarizing the results was retrieved. In particular, odds ratio, relative risk, hazard ratio were selected for longitudinal studies, whereas Naranjo algorithm was performed for case reports and case series; all these data were included in the analysis (188). Type of association was classified according to above listed index as follows: positive (↑ increased risk), negative (↓ decreased risk) and no association (↔ if no effect was suggested).

As final steps, an analysis of quality of included studies was performed by applying appropriate scale: we used the Newcastle Ottawa scale for longitudinal studies (cohort and case-control studies), whereas for case reports and case series the assessment was based on International Society for Pharmacoepidemiology (ISPE) and International Society of Pharmacovigilance (ISoP) guidelines (189). RTCs were excluded from this analysis because 1) no clinical trials are usually designed to study onset or worsening of new disease and 2) MS is rarely detected as adverse event in these studies due to its characteristics (i.e., long latency and still scarces awareness on drug-related component).

3.2 Part 2 and 3: Analysis of FAERS database

3.2.1 Data source: Food and Drug Administration Adverse Event Reporting System (FAERS)

For the analyses included in studies 2 and 3, the FDA Adverse Event Reporting System (FAERS) was used. This database is one of the three large freely-available data source created by FDA for pharmacovigilance purpose: FAERS for drugs, VAERS for vaccine and CAERS for both food supplements and herbal remedies.

FAERS is maintained by the FDA and represents the most suitable source of data to conduct pharmacovigilance studies. In fact, it collects reports from United State of America (USA) and severe ADRs from other regions of the World as for example from Eudravigilance maintained by European Medicines Agency-EMA, and Vigibase, by Uppsala Monitoring Centre of WHO, which include European and worldwide ADRs. This organization of data sharing makes impossible to

collapse the three sources of data in a unique database due to a large amount of overlapping reports.

The FAERS is an open-source database that include anonymous data, for this reason ethical committee authorization is not request. Data are available on dedicated page of FDA website (190), and can be downloaded periodically in 4 quarterlies per years (Q1 including data from January to March, Q2 from April to June, Q3 from July to September and Q4 from October to December). As shown in figure 3.2.1a this database consists of several datasets linked each other by specific Keys.

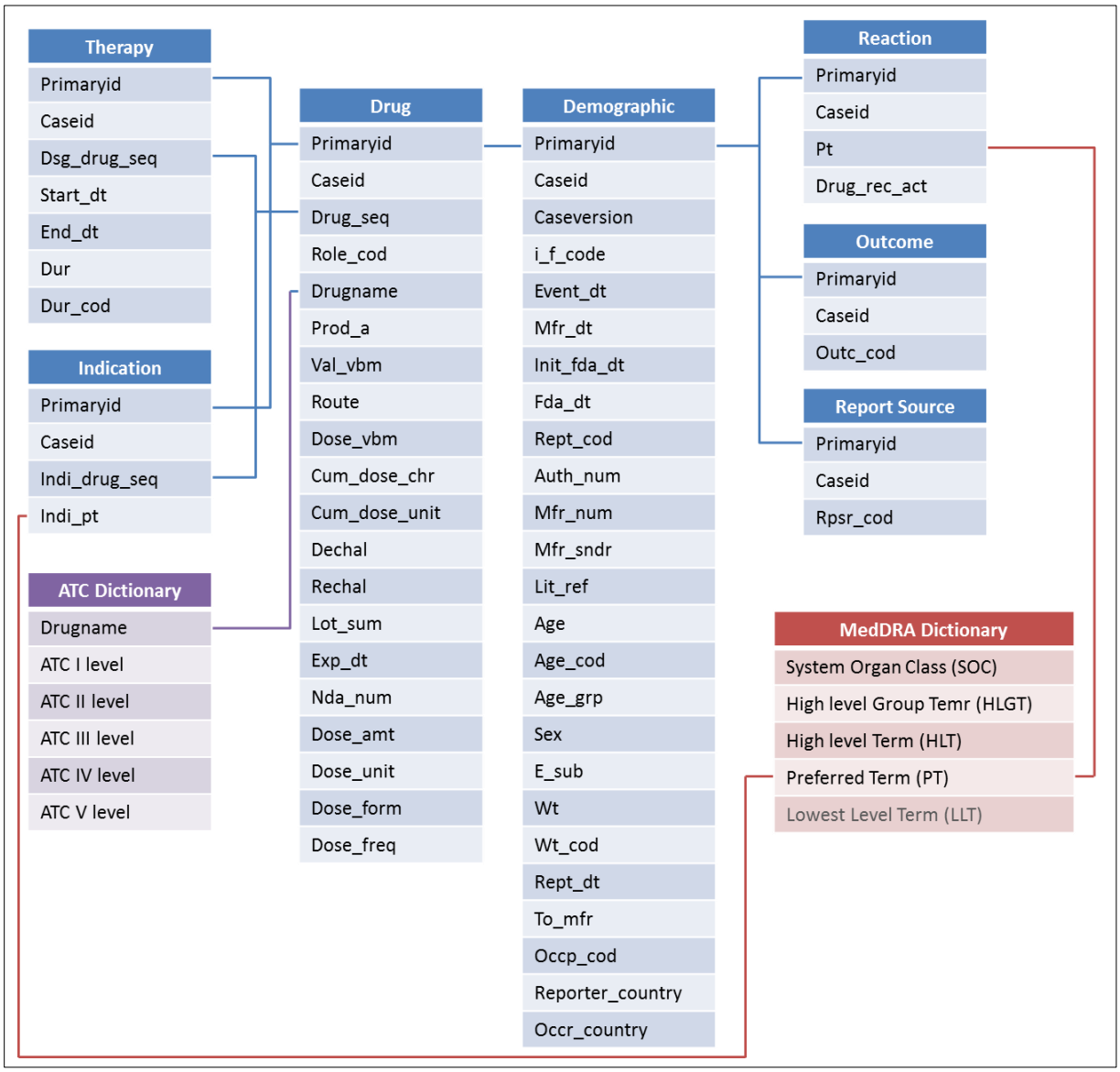


Figure 3.2.1a. Food and Drug administration adverse event reporting system (FAERS) database complete structure for pharmacovigilance analysis. Figure illustrates FAERs database structure with particular focus on link among different dataset.

The entire database provides information on demographic characteristics, drug therapies and adverse events for each case. As mentioned above, each dataset is linked with relevant ones through specific keys (i.e., “primary-id” to identify the cases, “drug_seq” for drug information, “preferred term” for indication and adverse event). The same keys are used by researches to link

database information with external tools, which allow to codify data and to perform aggregated analyses (see next section for details).

Here below, we detailed the main data generally used in pharmacovigilance studies:

- **Demographic data.** This information are available in “Demographic” datasets and included data on sex, age, country and date of reported adverse event.
- **Drug data.** These data are essential for the analysis and as shown in table 3.2.1a, they are linked to “Therapy” and “Indication” datasets (these last datasets are actually used only in some studies because of a higher rate of missing data). Information on drugs (brand name or substance name), dose, dechallenge (it indicates if symptoms were improved after drug discontinuation) and rechallenge (it indicates if symptoms reappear after drugs re-administration) are available with variable completeness. Crucial importance has information on the role of drugs, as defined by the reporter: in pharmacovigilance analyses, “primary” and “secondary suspected” drugs are usually taken into account, whereas “concomitant” and “interacting” drugs are preferably used as covariates for adjusted analyses. A rigorous datamining approach is needed before using these data, since drug name is a free text variable and misspelling errors can occur. From “Therapy” dataset is possible to retrieve data on date of start and end of therapy, whereas “indication” dataset provides information on reason for drug use. This last information is codified by using MedDRA dictionary.
- **Adverse Event data. “**Reaction”, “Outcome” and “Report” source datasets contain the following information: the first two datasets include data on the specific observed ADRs (codified by PTs - preferred terms – of the MedDRA dictionary)

and their consequences (e.g., death, hospitalization, other), whereas the third one indicates the reporter of the case (i.e., physician, lawyer, customer, others).

Use of MedDRA dictionary and ATC dictionary (described in the next sections) allow to codify data available in the database and to conduct aggregate analysis on specific class of drugs or ADRs associated with specific organ/system classes of ADRs.

3.2.1.1 ATC classification

The ATC code is an alphanumeric code developed by the World Health Organization (WHO) (191) to harmonize the identification of medicines in the world. As shown in Figure 3.2.1a this code is included in a separate dictionary that is linked with FAERS through substance name.

The code characterizes the authorized and commercialized drugs or combination of them. It is an hierarchical system based on anatomical, pharmacological, therapeutic and chemical characteristics of drug. It should be take into account that drugs used in different therapeutic areas are characterized by different ATC codes (e.g., acetylsalicylic acid, ATC code: A01AD05, B01AC06, N02BA01, M01BA03, N02BA51, B01AC56 and N02BA71), whereas unique ATC can identify different administration routes.

The ATC code is characterized by 5 levels: 1st level consider the anatomical group, 2nd the therapeutic subgroup, 3rd the pharmacological subgroup, 4th the chemical subgroup and 5th the chemical substance (Figure 3.2.1.1a).

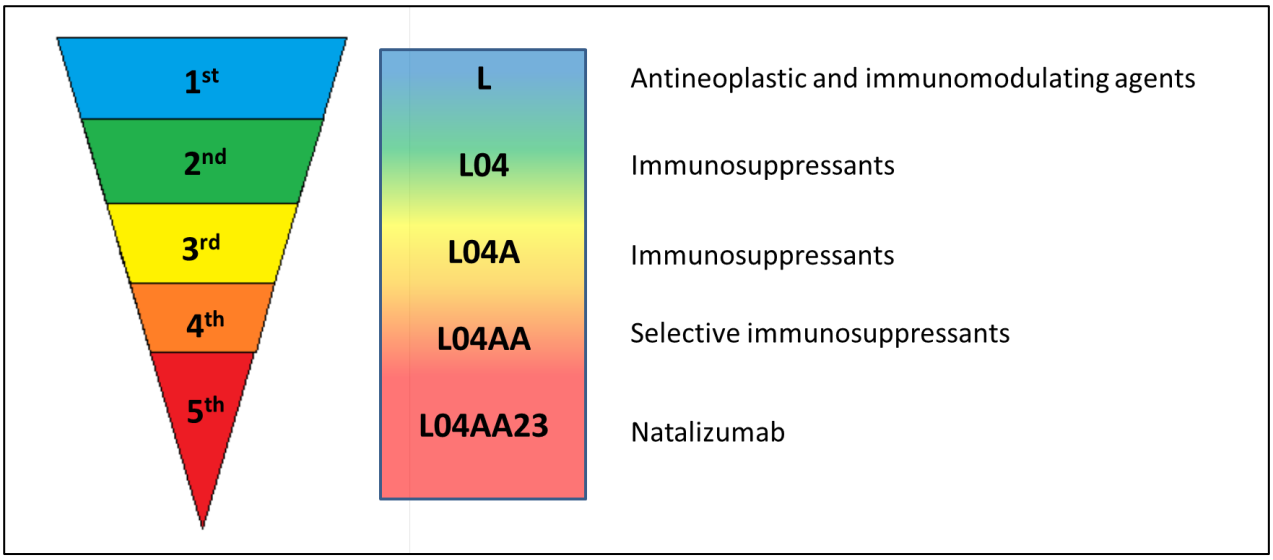


Figure 3.2.1.1a. ATC classification of drugs. In the figure is shown an example of classification from first to last level (Natalizumab; ATC code: L04AA23).

In pharmacovigilance studies, codifying each substance in relevant ATC code is crucial in order to perform aggregate analyses. In selected research questions, substance name can be used when ATC code is not yet available.

3.2.1.2 MedDRA dictionary

Medical dictionary for regulatory activities (MedDRA) is a hierarchical classification of medical terms for pharmacovigilance and regulatory affairs (192). The structure of classification includes 5 levels, from the most general level, System Organ Class (SOC), to the most specific level named Preferred Terms (PTs) and its shadows lowest level term (LLT). This dictionary is linked with FAERS by using PT level, and it is used to codify both indications and adverse reactions. In MedDRA classification, a single PT can belong to more than one SOC (among which one is considered the

Primary SOC). Generally in pharmacovigilance studies Primary SOC is considered, because more specific (Figure 3.2.1.2a). Aggregate analysis can be made by using selection of PTs that can be already available in Standardized MedDRA Queries (SMQ). This approach is more appropriate to study complex ADRs (e.i., Drug induce liver injury).

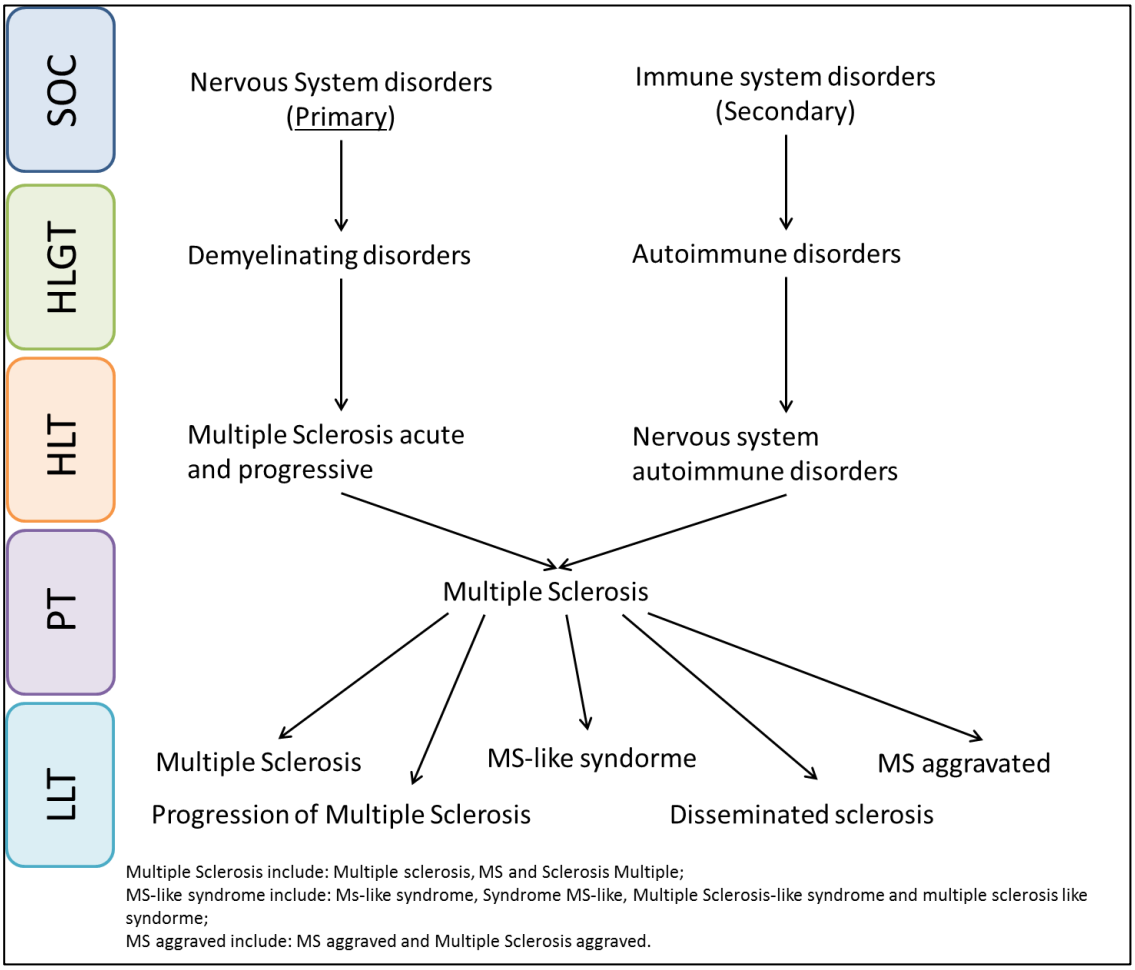


Figure 3.2.1.2a. MedDRA classification of medical terms. Figure illustrates classification of PT Multiple Sclerosis.

3.2.2 Statistical analysis of part 2 and 3

3.2.2.1 Part 2: Multiple sclerosis drug induced

For this study we downloaded FAERS quartiles from 2004 to first semester of 2016. After datamining of raw data (removing duplicated and records presenting 3 out of 4 identical content in sex, age, country, drugs and adverse event fields), drugs and adverse events were codified as previously described.

According to the purpose of this thesis (detecting signal of drug-induced MS), we selected all cases that include as adverse event the terms "*multiple Sclerosis*"; the rest of records in database were considered as non-cases. In order to increase the specificity of analysis, we applied the following exclusion criteria to each record: 1) presence of drugs approved for MS treatment (e.g. interferons beta, mitoxantrone, teriflunomide, glatiramer acetate, fingolimod and natalizumab); 2) presence of drugs reporting "multiple sclerosis" as indication of therapy in the database; 3) presence of vaccines, because data on these entities are more specifically collected in a dedicated database (VAERS). Then, we developed an additional indicator named "Cleaning index" that represents the ratio between number of final records and number of initial records (before application of exclusion criteria; see formula 1):

$$Cleaning\ index = \frac{Final\ cases\ (after\ exclusion\ criteria)}{Initial\ cases\ (before\ exclusion\ criteria)} X\ 100$$

Formula 1. Cleaning index calculus. Formula of Cleaning index calculus according to additional exclusion criteria

Finally, a case/non-case strategy of analysis was performed by calculating the reporting odds ratio (ROR) with respective 95% of confidence interval (95%CI) for each drug (see Table 3.2.2.1a and formula 2 and 3 below) (193).

Table 3.2.2.1a. The 2x2 contingency table for ROR analysis. Table illustrate a general example of ROR calculus.

		Drug of interest		Total
		Yes	NO	
Adverse event of interest	Yes	**A**	**B**	**A+B**
	NO	**C**	**D**	**C+D**
Total		**A+C**	**B+D**	**A+B+C+D**

A=reports containing suspect drugs + suspect adverse event (AD)
B=Reports containing suspect AD + other reported drugs (excluding drug of interest)
C=Reports with drug of interest + other ADs (excluding AD of interest)
D=reports containing other drugs + other ADs

$$Reporting\ Odds\ Ratio = \frac{\mathrm{A/C}}{\mathrm{B/D}}$$

Formula 2. Reporting odds ratio calculus. Reporting odds ratio calculus according to table 3.2.2.1a.

$$95\%CI = e^{\ln(ROR)} \pm 1.96\ x\ \sqrt{\frac{1}{A} + \frac{1}{B} + \frac{1}{C} + \frac{1}{D}}$$

Formula 3. ROR confidence intervals calculus. The 95% confidence interval (upper and lower limits) calculus according to table 3.2.2.1a.

Disproportionally signal was detected when drug had significant ROR (lower limit above 1), at least 10 cases and cleaning index equal or higher than 70% (see article 2 in the end of the thesis for more details).

Additionally, since the incidence of MS is higher in female sex, we performed a sensitivity analysis of drugs by considering sex strata subjects.

3.2.2.2 Part 3: MS therapies and DILI events

For this study, we included 13 years of FAERS data from q1-2004 to q4-2016 by applying the datamining techniques (see the previous section). This study aimed to detect possible idiosyncratic liver injury (DILI) signals in MS treated individuals. In this case, we included reports with at least one PT associated with DILI events according to two strategies of analysis: only Severe liver injury (SLI) by using a dedicated MedDRA query and Overall liver injury (OLI) by using a selection of specific PTs (194, 195). Exposure were defined as presence of disease treatment authorized for MS such as modifying therapies (Interferon beta-1a, interferon beta-1b, glatiramer acetate, fingolimod, teriflunomide, dimethyl fumarate, mitoxantrone, natalizumab, alemtuzumab, daclizumab) and symptomatic drug (fampridine). Since alemtuzumab and daclizumab have also other therapeutic indications we selected only cases presenting substance name + MS indication in INDIPT field or substance + MS authorized brand name (Lemtrada and Zinbryta).

A case/non-case analysis was performed: for each included drug the Reporting Odds Ratio (ROR) with 95% confidence interval (95CI) was calculated. Signal was claimed when at least 3 cases where retrieved and 95CI lower limit exceeded 1. In addition, since analysis focused on drugs rather than patients, and therefore possible alternative cause of DILI events may account in the observed events, we adjusted the RORs for co-reported drugs previously associated with DILI events (194, 196-198).

Finally, presence of concomitant therapies associated with DILI events were also assessed in a case-by-case analysis. Presence of hepatotoxic drugs as well as drugs for hepatitis were evaluated and ranked according to the recent categorization proposed by Bjornsson (199), which includes five categories based on number of published case reports: A (with ≥ 50 published reports), B (12-49), C (4-11), D (1-3), E (none) (199).

The analyses of part 2 and 3 were performed by using PostgreSQL program (200) and R software (201).

3.2 Ethical issues

In this thesis, we used aggregate and anonymous data therefore the ethical authorization is not requested.

4. RESULTS

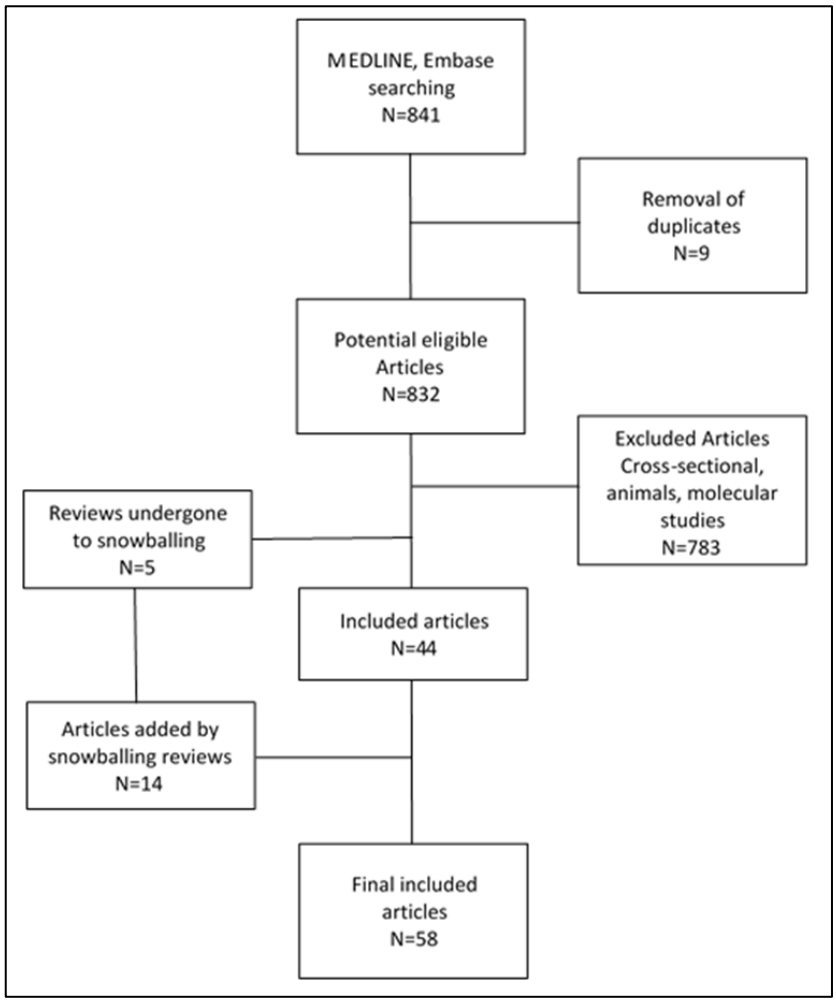
4.1 Part 1: Evidence mapping of the literature

Results showed in this section were published in relevant article (202).

In order to evaluate possible role of drugs in MS development we first conducted a review of available evidence by an evidence mapping approach.

From 832 potential eligible studies retrieved by using specific search strategy in Medline and Embase, 44 articles were included and 14 ones were added after snowballing of 5 reviews (Figure 4.1a).

Figure 4.1a. Flowchart of literature selection and final included articles.



Analysis of included articles showed possible presence of two outcomes in the same paper, therefore 46 out of 58 articles provided data on MS onset and 14 on its worsening (Table 4.1a and 4.1b).

As indicated in table 4.1a and 4.1b drugs acting on immune system were the most studied drugs and the relevant evidence included several case reports and case series as well as longitudinal studies. The major number of case reports and case series occurred in patients treated with infliximab, adalimumab and etanercept. After the application of Naranjo algorithm, a score between possible and probable were ascribed to these reports.

As for drugs acting on nervous system, valproic acid was associated with increased risk to develop MS in the unique available cohort study (Hazard Ratio: 2.41; 95%Cl: 1.32-4.43), whereas contrasting results were obtained for anesthetics (see supplemental material of study 2 for details).

Oral contraceptives were the most investigated drugs, albeit with contrasting results. Three cohort studies and one case-control study showed a protective effect of these therapies in MS onset and worsening, whereas one case-control study found a possible relationship between contraceptive use and increased MS risk; and three cohort studies did not find any association (table 4.1a and 4.1b). As regards assisted reproduction treatments increased number of relapses were observed in 2 cohort studies and 1 case series.

Antibiotics were investigated in two studies: Alonso et al. (203) reported no association between antibiotics and MS onset, whereas Norgaard et al. (204) found an increased number of events in exposed individuals.

Other valuable evidence includes a case report, that suggests possible association between use of cannabinoid receptor-1 antagonist and new MS onset, and a cohort study that found no

association between amiloride exposure and MS onset and worsening (Hazard Ratio: 1.34; 95%CI:0.81-2.20).

Table 4.1a Synopsis of literature results: drugs and onset of Multiple Sclerosis.

	ONSET				
Drug	**Type of study**				
	Randomized controlled trials	Cohort study	Case control	Case series	Case report
Drugs acting on immune system (24)					
Adalimumab					↑ (7)
Etanercept					↑ (8)
Infliximab		↔ (1)			↑ (6)
TNF antagonist		↔ (1)	↓ (1)	↑ (1)	
Anakinra			↑ (1)		
Methotrexate			↔ (1)		
Leflunomide			↔ (1)		
Hydroxychloroquine/chloroquine			↔ (1)		
Other DMARDs			↑ (1)		
Interferon alfa				↑ (1)	↑ (1)
Drugs acting on nervous system (4)					
Valproic acid		↑ (1)			
Anaesthetic		↑ (2)	↔ (2)		
Drugs acting on endocrine system (9)					
Contraceptive		↔ (3)	↑ (1)		
		↓ (2)	↓ (1)		
Diethylstilbestrol		↔ (1)			
Synthetic human type insulin				↑ (1)	
Drugs acting on microbial infections (5)					
Penicillin			↓ (1)		
			↑ (1)		
Cephalosporin			↔ (1)		
Tetracyclines			↔ (1)		
			↑ (1)		
macrolides			↔ (1)		
			↑ (1)		
Pivmecillinam			↑ (1)		
Sulfonamide/Trimethroprim			↑ (1)		
Nitrofurantoin			↑ (1)		
Quinolones			↔ (1)		
HIV treatment		↓ (1)			
Ethambutol				↑ (1)	
Sulfasalazine					↑ (1)
Other antibiotics			↑ (1)		
			↔ (1)		
Others (4)					
Amiloride	↔ (1)				
Histamine 1 receptor blockers			↓ (1)		
Cannabinoid receptor-1b antagonist (rimonabant)					↑ (1)
Drug abuse			↑ (1)		

NOTE: A single article counts as many fold as the number of drug-event pair investigated. A single article may be cited in both tables 1 and 2.

↑ increased risk ↓ decreased risk ↔ no association.

Antonazzo et al; Drug Saf. 2017 Sep;40(9):823-834 **(205)**

Table 4.1b Synopsis of literature results: drugs and worsening of Multiple Sclerosis.

	WORSENING				
Drug	**Type of study**				
	Randomized controlled trials	Cohort study	Case control	Case series	Case report
Drugs acting on immune system (5)					
Interferon gamma	↑ (1)				
Atacicept	↑ (1)				
TNF antagonist	↑ (1)			↑ (1)	
Lenercept	↑ (1)				
Drugs acting on nervous system (3)					
Fluoxetine					↑ (1)
Carbamazepine				↑ (1)	
Anaesthetic		↔ (1)			
Drugs acting on endocrine system (5)					
Contraceptive		↓ (2)			
Assisted reproduction treatment		↑ (2)		↑ (1)	
Others (1)					
Amiloride		↔ (1)			

NOTE: A single article counts as many fold as the number of drug-event pair investigated. A single article may be cited in both tables 1 and 2.
↑ increased risk ↓ decreased risk ↔ no association.
Antonazzo et al; Drug Saf. 2017 Sep;40(9):823-834 (205).

Causality assessment, by using Naranjo algorithm, for the majority of case reports and case series pointed out a possible or probable link between drug exposure and observed adverse event (see supplemental material of article 2 at the end of this thesis).

The assessment of quality of case reports and case series by using ISPE and ISO guidelines revealed a general paucity of important data in the reports such as medical history of patient, presence of concomitant therapies, physical examination results and other parameters useful to assess the plausibility of suspected association between drug and reported adverse event (Figure 4.1a).

Missing or not gradable data were observed also in longitudinal studies by using Newcastle-Ottawa scale. In particular, in case–control studies, authors did not report non-response rate and how exposure was ascertained (Figure 4.1b). As regards cohort studies, all included records showed some missing data among evaluated items (Figure 4.1b); the main limitations were detected in follow-up of the cohort that seems inadequate for the aim of the studies (evaluation of MS onset), the non-comparability of the cohorts and the absence of assessment of disease at the date entry (Figure 4.1b).

Figure 4.1a Quality assessment of case reports and case series. Antonazzo et al; Drug Saf. 2017 Sep;40(9):823-834 (205).

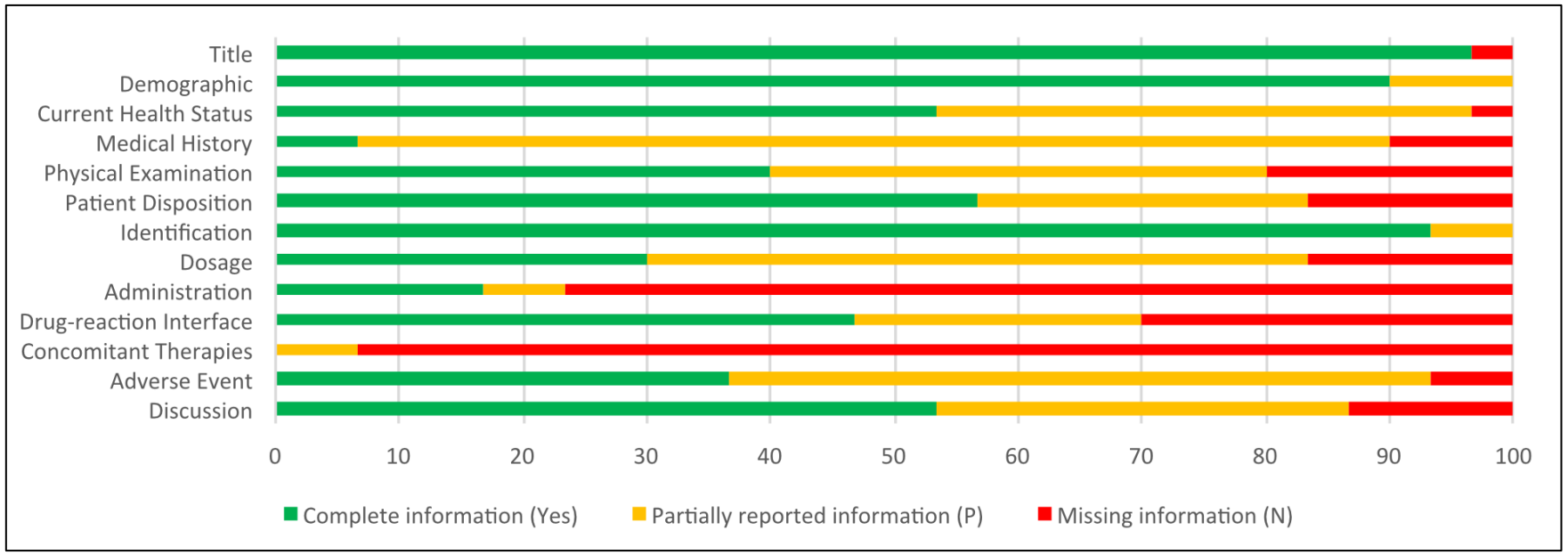
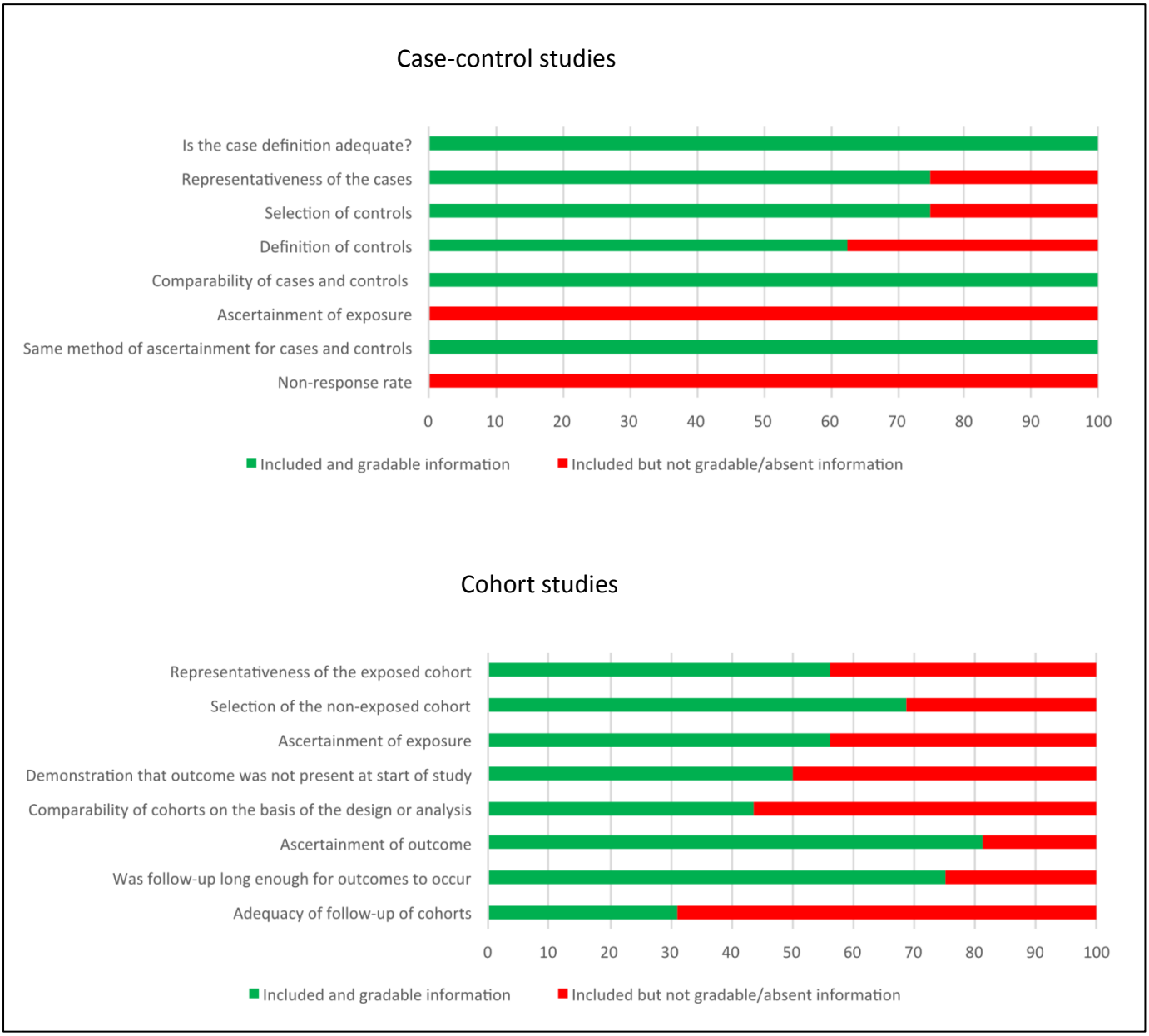


Figure 4.1.b. Quality assessment of case control and cohort studies. Antonazzo et al; Drug Saf. 2017 Sep;40(9):823-834 (205).

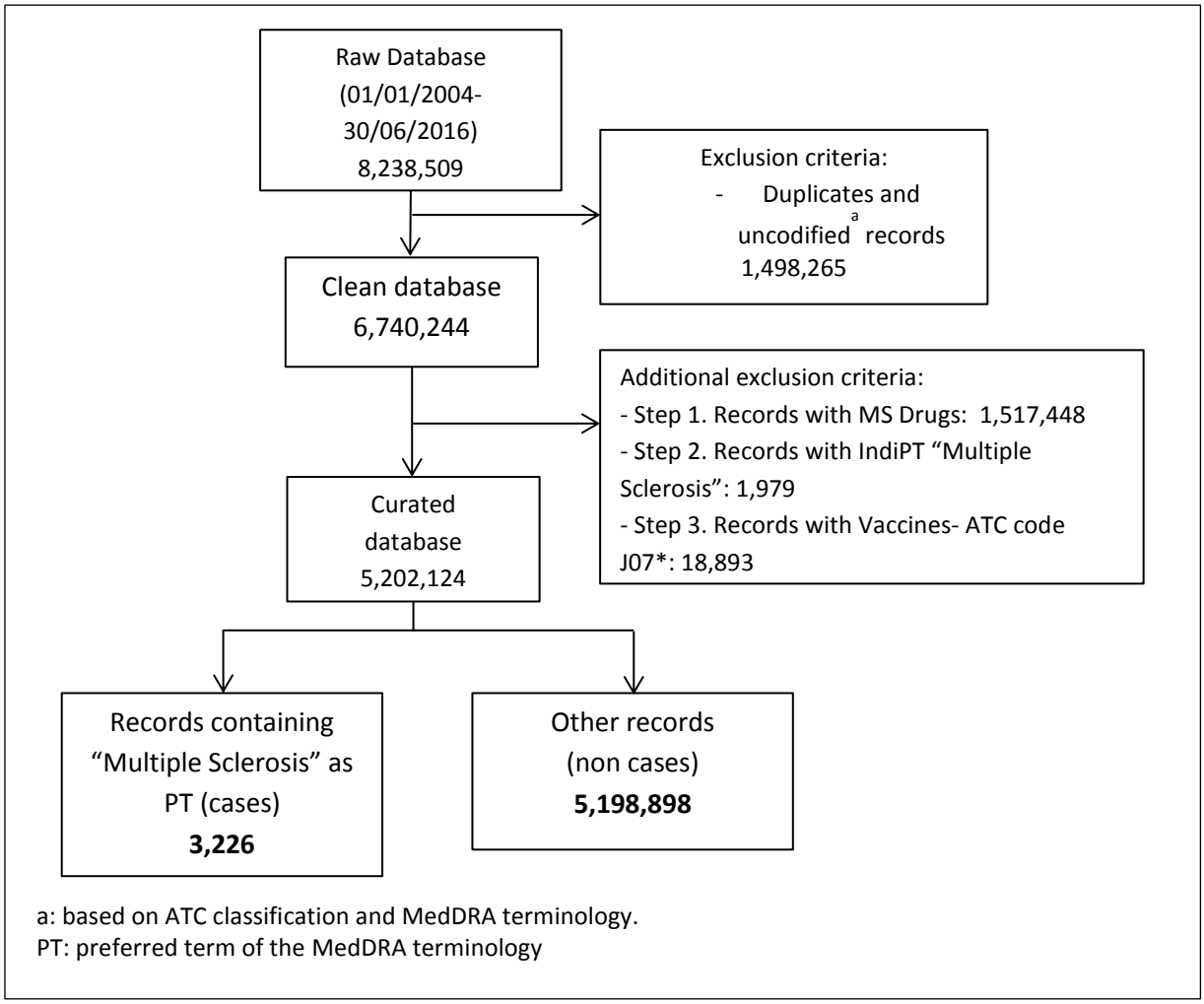


4.2 Part 2: Drug-induced multiple sclerosis

Results showed in this section were published in relevant article (205).

After analyzing the relevant literature, we investigated new signals of possible drug contribution in multiple sclerosis occurrence by using the Food and Drug Administration Event Reporting System database (FAERS). Over the 13 years of data collection (from 01/01/2004 to 30/06/2016), FAERS database collected 8,238,509 reports. Among them after data mining and application of exclusion criteria 5,202,124 reports were included in the analysis. From this database we selected 3,226 cases (indicating "multiple sclerosis" as ADR) and 5,198,898 non-cases (Figure 4.2 a).

Figure 4.2a. Synopsis of case and non-cases selection.



Most of the included cases were female, aged 30-65 years and lived in USA. For 2,666 (83%), only a single drug was reported as suspected agent. The highest percentage of reports were submitted by consumers and physicians (74%).

According to criteria to define a signal (at least 10 cases, cleaning index ≥70% and significant ROR), immunomodulating drugs were the most reported drugs (1,343 cases), of them etanercept (445 cases, ROR 2.48; 95%Ci: 2.24-2.74), adalimumab (329, 2.05; 183-2.30), and infliximab (119, 2.25; 187-2.70) were the most frequently reported with also higher cleaning index (Table 2.1a).

Significant RORs, albeit with less cases, were found also for drugs acting on nervous system and musculo-skeletal system: varenicline (80, 2.05; 1.64-2.56), clozapine (33, 1.43;1.01-2.01), desvenlafaxine (13, 1.95; 1.13-3.37), alendronic acid (50, 2.56; 1.93-3.389) and its salt (14, 2.63; 1.56-4.45).

Sildenafil (27, 1.87; 1.28-2.73) typically used by men and etonorgestrel (46, 2.20; 1.64-2.94) used as contraceptive drugs by women showed significant disproportionality.

As showed in table 4.2a application of cleaning index (see method for details) allowed to exclude from the analysis drugs with possible misleading results because erroneously associated with multiple sclerosis events. Drugs such as baclofen, gabapentin and its analogue pregabalin, and methylprednisolone were excluded from significant results, and in fact they are also widely used as symptomatic drugs during first episode and subsequent relapses of MS.

Table 4.2a Reporting Odds Ratio (ROR) of active substances (ATC V level). In bold, drugs with statistically significant ROR (see methods for details). Expert Opin Drug Saf. 2018 Jul 30 **(202)**.

Substance	ATC code	N (%)[a]	Sex M/F/UNK	ROR (95%Cl)
ANTINEOPLASTIC AND IMMUNOMODULANTING AGENTS (ATC: L)				
Etanercept	**L04AB01**	**445 (93)**	111/291/43	**2.48 (2.24 - 2.74)**
Adalimumab	**L04AB04**	**329 (81)**	94/214/21	**2.05 (1.83 - 2.30)**
Infliximab	**L04AB02**	**119 (88)**	33/79/7	**2.25 (1.87 - 2.70)**
Methotrexate	**L01BA01**	**39 (78)**	4/32/3	**1.87 (1.36 - 2.56)**
Ustekinumab	**L04AC05**	**36 (100)**	16/16/4	**4.97 (3.57 - 6.90)**
Peginterferon alfa-2a	L03AB11	31 (78)	15/14/2	1.13 (0.79 - 1.61)
Abatacept	**L04AA24**	**31 (86)**	7/21/3	**3.78 (2.65 - 5.39)**
Golimumab	**L04AB06**	**28 (93)**	1/26/1	**3.70 (2.55 - 5.37)**
Imatinib	L01XE01	15 (88)	5/10/0	0.96 (0.58 - 1.60)
Tocilizumab	**L04AC07**	**15 (88)**	2/11/2	**1.90 (1.14 - 3.15)**
Rituximab	L01XC02	14 (39)	2/11/1	0.60 (0.36 - 1.02)
Certolizumab pegol	L04AB05	13 (100)	3/9/1	1.20 (0.70 - 2.08)
Ciclosporin	L04AD01	13 (76)	6/7/0	0.89 (0.51 - 1.53)
Trastuzumab	L01XC03	12 (75)	0/12/0	1.34 (0.76 - 2.36)
Mycophenolic acid	**L04AA06**	**11 (79)**	2/9/0	**1.87 (1.03 - 3.38)**
Interferon alfa-2b	**L03AB05**	**10 (100)**	2/8/0	**5.10 (2.74 - 9.50)**
NERVOUS SYSTEM (ATC: N)				
Pregabalin	N03AX16	112 (59)	24/82/6	2.98 (2.47 - 3.60)
Varenicline	**N07BA03**	**80 (81)**	14/59/7	**2.05 (1.64 - 2.56)**
Gabapentin	N03AX12	70 (41)	23/44/3	3.72 (2.93 - 4.71)
Quetiapine	N05AH04	69 (63)	13/55/1	2.15 (1.69 - 2.73)
Duloxetine	N06AX21	39 (47)	4/35/0	1.58 (1.15 - 2.16)
Paracetamol	N02BE01	37 (32)	8/28/1	1.03 (0.75 - 1.43)
Fentanyl	N02AB03	34 (59)	5/29/0	1.29 (0.92 - 1.80)
Sodium oxybate	N07XX04	34 (64)	1/33/0	3.86 (2.75 - 5.42)
Venlafaxine	N06AX16	31 (55)	4/26/1	1.94 (1.36 - 2.76)
Clozapine	**N05AH02**	**33 (70)**	13/20/0	**1.43 (1.01 - 2.01)**
Sertraline	N06AB06	28 (52)	7/18/3	1.66 (1.14 - 2.41)
Olanzapine	N05AH03	23 (64)	3/20/0	1.27 (0.84 - 1.92)
Acetylsalicylic acid	N02BA01	22 (59)	6/16/0	0.85 (0.56 - 1.29)
Risperidone	N05AX08	21 (54)	2/19/0	1.09 (0.71 - 1.68)
Paroxetine	N06AB05	21 (70)	4/16/1	1.02 (0.67 - 1.57)
Carbamazepine	N03AF01	20 (34)	5/14/1	2.02 (1.30 - 3.14)
Oxycodone	N02AA05	17 (40)	4/12/1	0.83 (0.51 - 1.34)
Lamotrigine	N03AX09	17 (71)	0/16/1	0.89 (0.55 - 1.43)
Levetiracetam	N03AX14	16 (55)	3/12/1	1.39 (0.85 - 2.27)
Topiramate	N03AX11	15 (43)	1/13/1	1.61 (0.97 - 2.67)
Buprenorphine	N02AE01	13 (76)	3/10/0	0.95 (0.55 - 1.64)

Desvenlafaxine	**N06AX23**	**13 (72)**	2/11/0	**1.95 (1.13 - 3.37)**
Morphine	N02AA01	12 (35)	4/8/0	0.99 (0.56 - 1.74)
Fluoxetine	N06AB03	12 (55)	3/9/0	1.09 (0.62 - 1.91)
Bupropion	N06AX12	11 (33)	0/11/0	0.73 (0.41 - 1.33)
Modafinil	N06BA07	11 (21)	1/10/0	3.12 (1.72 - 5.64)
Caffeine	**N06BC01**	**11 (73)**	4/7/0	**4.05 (2.24 - 7.33)**
Eletriptan	**N02CC06**	**10 (91)**	4/6/0	**7.6 (4.08 - 14.16)**
Lithium carbonate	N05AN01	10 (50)	7/3/0	2.44 (1.31 - 4.54)
Lorazepam	N05BA06	10 (59)	3/6/1	1.73 (0.93 - 3.22)
ALIMENTARY TRACT AND METABOLISM (ATC: A)				
Esomeprazole	**A02BC05**	**80 (78)**	9/70/1	**3.17 (2.54 - 3.96)**
Insulin lispro	**A10AB04**	**33 (89)**	9/24/0	**1.54 (1.09 - 2.17)**
Insulin (human)	**A10AB01**	**20 (87)**	8/12/0	**1.90 (1.23 - 2.95)**
Insulin glargine	A10AE04	16 (73)	4/12/0	0.89 (0.54 - 1.45)
Omeprazole	A02BC01	11 (37)	0/11/0	0.86 (0.48 - 1.56)
MUSCULO-SKELETAL SYSTEM (ATC: M)				
Baclofen	M03BX01	98 (49)	27/66/5	9.83 (8.04 - 12.03)
Rofecoxib	M01AH02	54 (56)	17/37/0	1.79 (1.37 - 2.35)
Zoledronic acid	M05BA08	53 (54)	8/43/2	1.72 (1.31 - 2.26)
Alendronic acid	**M05BA04**	**50 (83)**	1/43/6	**2.56 (1.93 - 3.38)**
Celecoxib	M01AH01	27 (57)	5/21/1	1.34 (0.91 - 1.95)
Denosumab	M05BX04	26 (87)	0/23/3	1.32 (0.90 - 1.95)
Botulinum toxin	M03AX01	21 (68)	1/20/0	2.28 (1.48 - 3.50)
Pamidronic acid	M05BA03	12 (27)	0/12/0	2.57 (1.46 - 4.53)
Ibandronic acid	M05BA06	12 (86)	0/10/2	1.43 (0.81 - 2.52)
Alendronate sodium	**M05BA05**	**14 (78)**	0/14/0	**2.63 (1.56 - 4.45)**
GENITO-URINARY SYSTEM AND SEX HORMONES (ATC: G)				
Ethinylestradiol	G03CA01	65 (73)	0/60/5	1.26 (0.99 - 1.61)
Etonorgestrel	**G03AC08**	**46 (84)**	0/41/5	**2.20 (1.64 - 2.94)**
Levonorgestrel	G03AC03	38 (78)	0/38/0	0.73 (0.53 - 1.01)
Sildenafil	**G04BE03**	**27 (96)**	26/0/1	**1.87 (1.28 - 2.73)**
Medroxyprogesterone	G03AC06	11 (42)	0/11/0	0.48 (0.26 - 0.86)
Tolterodine	G04BD07	11 (61)	1/10/0	3.61 (1.99 - 6.52)
Drospirenone and estrogen	G03AA12	10 (67)	0/10/0	0.50 (0.27 - 0.92)
Conjugated estrogens	G03CA57	10 (38)	0/10/0	0.42 (0.23 - 0.79)
OTHER DRUGS				
Teriparatide	H05AA02	68 (67)	3/65/0	1.28 (1.01 - 1.63)
Ribavirin	J05AB04	39 (70)	14/24/1	1.03 (0.75 - 1.42)
Atorvastatin	C10AA05	35 (69)	12/19/4	1.09 (0.78 - 1.52)
Isotretinoin	**D10AD04**	**30 (81)**	16/13/1	**1.68 (1.17 - 2.41)**
Rosuvastatin	**C10AA07**	**29 (76)**	6/22/1	**1.46 (1.01 - 2.10)**
Omalizumab	**R03DX05**	**21 (88)**	1/19/1	**2.11 (1.37 - 3.24)**
Levofloxacin	**J01MA12**	**19 (73)**	1/14/4	**1.62 (1.03 - 2.55)**

Valsartan	C09CA03	17 (71)	4/13/0	1.01 (0.63 - 1.63)
Octreotide	**H01CB02**	**16 (100)**	1/15/0	**2.01 (1.23 - 3.29)**
Somatropin	H01AC01	15 (83)	1/13/1	1.49 (0.90 - 2.48)
Budesonide	R01AD05	15 (79)	0/15/0	1.07 (0.65 - 1.78)
Methylprednisolone	H02AB04	14 (5)	3/11/0	1.90 (1.12 - 3.21)
Salmeterol	R03AC12	13 (72)	2/11/0	0.64 (0.37 - 1.10)
Fluticasone	R03BA05	13 (50)	2/11/0	0.53 (0.3 - 0.91)
Prednisone	H02AB07	12 (15)	2/10/0	0.68 (0.39 - 1.20)
Formoterol	R03AC13	12 (86)	1/11/0	1.00 (0.57 - 1.77)
Naloxone	**V03AB15**	**12 (86)**	2/10/0	**1.82 (1.03 - 3.21)**

(a) Cleaning index

The sex stratified analysis showed distribution of female component among retrieved cases (table 4.2b). Data were in line with the general analysis apart for few drugs which lost significance in male group due to low number of cases (table 4.2b).

Table 4.2b. Reporting Odds Ratio (ROR) of active substances (ATC V level) in sex stratification. In bold, drugs with statistically significant ROR (see methods for details)

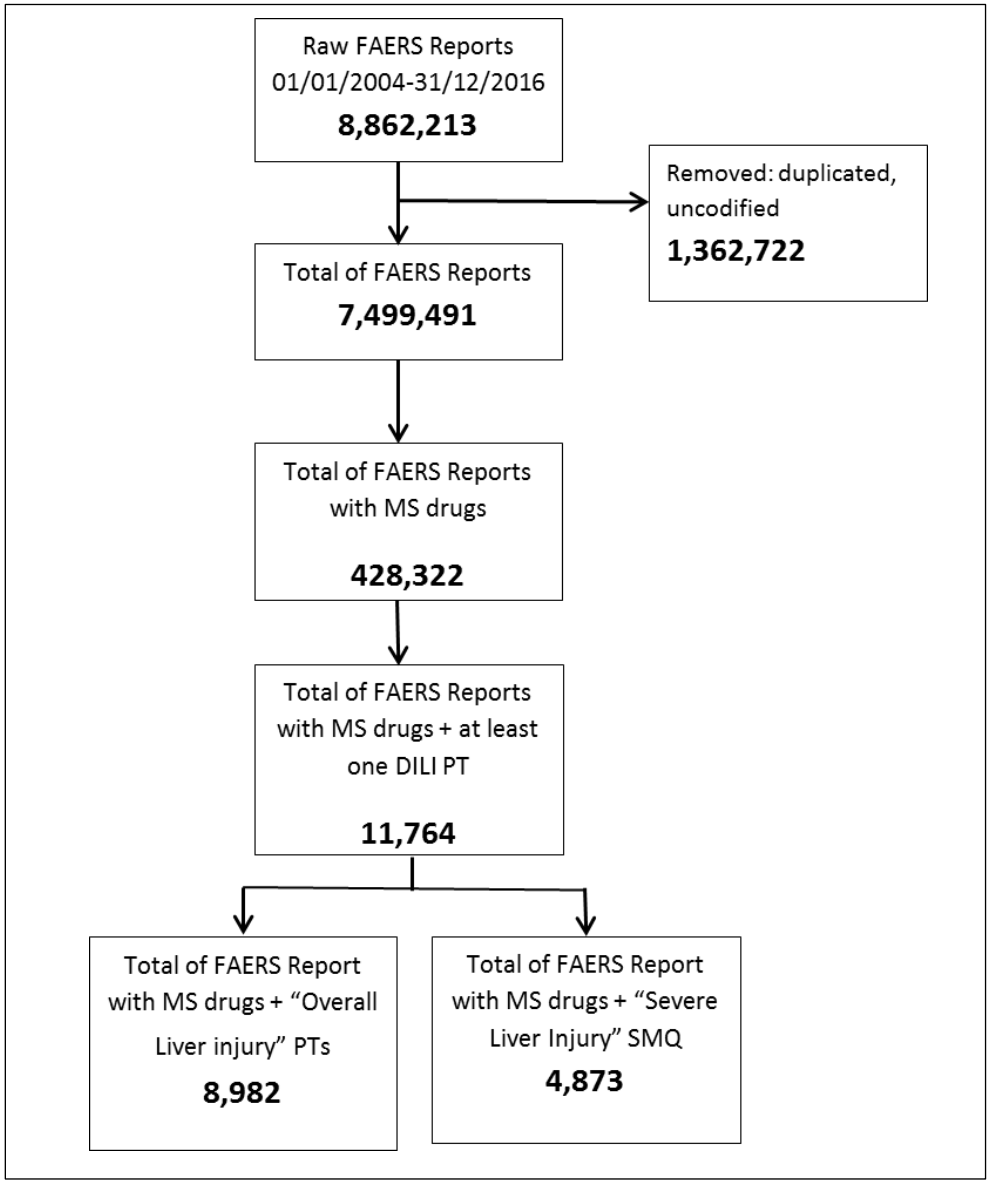
Substance	Female N	ROR (95%CI)	Male N	ROR (95%CI)
ANTINEOPLASTIC AND IMMUNOMODULANTING AGENTS				
Etanercept	**291**	**1.85 (1.64 - 2.1)**	**111**	**4.16 (3.40 - 5.10)**
Adalimumab	**214**	**1.56 (1.36 - 1.8)**	**94**	**3.15 (2.54 - 3.92)**
Infliximab	**79**	**2.06 (1.65 - 2.58)**	**33**	**2.80 (1.97 - 3.97)**
Ustekinumab	**16**	**3.87 (2.36 - 6.33)**	**16**	**7.57 (4.61 - 12.44)**
Abatacept	**21**	**2.60 (1.69 – 4.00)**	**7**	**8.56 (4.06 - 18.04)**
Golimumab	**26**	**3.92 (2.66 - 5.77)**	1	0.80 (0.11 - 5.70)
Tocilizumab	11	1.51 (0.84 - 2.73)	2	1.76 (0.44 - 7.05)
Mycophenolic acid	**9**	**2.86 (1.48 - 5.50)**	2	1.08 (0.27 - 4.34)
Methotrexate	**32**	**2.04 (1.44 - 2.89)**	4	1.01 (0.38 - 2.71)
Interferon alfa-2b	**8**	**8.05 (4.01 - 16.15)**	2	3.36 (0.84 - 13.48)
NERVOUS SYSTEM				
Varenicline	**59**	**1.98 (1.53 - 2.56)**	**14**	**1.72 (1.01 - 2.92)**
Clozapine	**20**	**1.80 (1.16 - 2.80)**	13	1.59 (0.92 - 2.75)
Desvenlafaxine	**11**	**1.81 (1.00 - 3.27)**	2	2.27 (0.57 - 9.08)
Caffeine	**7**	**3.11 (1.48 - 6.55)**	**4**	**8.39 (3.14 - 22.46)**
Eletriptan	**6**	**4.63 (2.07 - 10.33)**	**4**	**36.42 (13.54 - 97.95)**
ALIMENTARY TRACT AND METABOLISM				
Esomeprazole	**70**	**3.20 (2.52 - 4.06)**	**9**	**2.00 (1.04 - 3.87)**
Insulin lispro	**24**	**1.54 (1.03 - 2.30)**	9	1.76 (0.91 - 3.39)
Insulin (human)	12	1.61 (0.91 - 2.83)	**8**	**3.08 (1.53 - 6.18)**
MUSCULO-SKELETAL SYSTEM				
Alendronic acid	**43**	**2.01 (1.49 - 2.72)**	1	1.18 (0.17 - 8.42)
Alendronate sodium	**14**	**2.39 (1.41 - 4.05)**	--	---
GENITO-URINARY SYSTEM AND SEX HORMONES				
Etonogestrel	41	**1.68 (1.23 - 2.29)**	--	---
Sildenafil	--	---	26	**4.18 (2.82 - 6.19)**
OTHER DRUGS				
Isotretinoin	13	1.13 (0.65 - 1.94)	**16**	**3.54 (2.16 - 5.81)**
Rosuvastatin	**22**	**1.56 (1.02 - 2.37)**	6	1.24 (0.55 - 2.77)
Omalizumab	**19**	**2.25 (1.43 - 3.54)**	1	0.58 (0.08 - 4.11)
Levofloxacin	14	1.65 (0.97 - 2.79)	1	0.38 (0.05 - 2.73)
Octreotide	**15**	**2.78 (1.67 - 4.63)**	1	0.50 (0.07 - 3.52)
Naloxone	**10**	**2.65 (1.42 - 4.94)**	2	1.29 (0.32 - 5.16)

4.3 Part 3: MS therapies and idiosyncratic liver injury (DILI) events

Results showed in this in this section were published in relevant article (206).

In the third part of the thesis, from the curated FAERS database, we selected 11,764 cases of DILI and we grouped them as follows: 8,982 overall liver injury (OLI) and 4,873 severe liver injury (SLI) (Figure 4.3a). Cases were mainly female (75%), aged 30 years or older, and most of them reported only one MS therapy as suspected drug (99%).

Figure 4.3a. Synopsis of cases selection



Interferons and mitoxantrone had been already associated with DILI. In fact, they were already classified in class A (interferons) and D (mitoxantrone) according to Bjornsson et al., based on the number of published reports of liver injury (199). In our analyses they showed significant RORs with high ROR in almost all performed analyses (Table 4.3a and table 4.3b).

As for OLI analysis, a more generic DILI classification, several drugs had significant results (Table 4.3a). In this case, some drugs showed disproportionality signals among the rest of MS therapies (right part of table). Additionally, results performed among the entire database FAERS had similar results (left part of table). Among these therapies fingolimod and teriflunomide showed significant results (RORadj: 2.53; 95%CI: 2.40-2.66) and (2.31; 2.12-2.52) respectively, followed by mitoxantrone (2.10; 1.81-2.42) and alemtuzumab (1.34; 1.09-1.65).

The only therapy different from the disease modifying ones, approved specifically for MS symptoms, reported signal of disproportionality with significant ROR (1.58; 1.15-2.17; Table 4.3a) in OLI analysis and (2.27; 1.59-3.26; Table 4.3b) in SLI analysis.

Analysis focused on only severe events had slightly different pattern of disproportionality (Table 4.3.b). Fingolimod showed a signal of disproportionality in non-adjusted ROR (RORraw: 1.13; 95%CI:1.03-1.24) that disappeared in adjusted analysis (RORadj: 1.09; 0.99-1.20). Also mitoxantrone had similar pattern, but in this case it still maintained statistically significant values (RORraw: 3.04; 2.54-3.62) and (RORadj: 2.51; 2.10-3.00).

Table 4.3a. Disproportionality analyses according with Overall Liver Injury classification. In bold: statistically significant disproportionality (i.e., the lower limit of the 95%CI of ROR>1).

			Overall Liver Injury (OLI)		
Drug			**General database(A)**	**Multiple sclerosis database(B)**	
	Total reports	**N cases**	**ROR (95%CI)**	**ROR (95%CI)**	**ROR_{adj} (95%CI)**
Moderate efficacy or standard initial disease modifying therapies					
Interferon beta-1a	146,470	3,368	0,9 (0,87-0,93)	**1.10 (1.05-1.15)**	**1.15 (1.10-1.2)**
Glatiramer acetate	18,591	274	0,57 (0,51-0,65)	0.70 (0.62-0.79)	0.69 (0.61-0.78)
Interferon beta-1b	22,053	878	**1,59 (1,48-1,7)**	**1.94 (1.8-2.08)**	**1.86 (1.73-1.99)**
Fingolimod	38,563	1,823	**1,9 (1,81-1,99)**	**2.32 (2.20-2.44)**	**2.53 (2.40-2.66)**
Teriflunomide	11,945	578	**1,95 (1,79-2,12)**	**2.37 (2.18-2.59)**	**2.31 (2.12-2.52)**
Dimethyl fumarate	50,182	397	0,31 (0,28-0,34)	0.37 (0.34-0.41)	0.34 (0.31-0.37)
High efficacy or later disease modifying therapies					
Mitoxantrone	3,880	199	**2,07 (1,79-2,39)**	**2.52 (2.19-2.92)**	**2.10 (1.81-2.42)**
Natalizumab	136,180	1,452	0,41 (0,39-0,43)	0.50 (0.48-0.53)	0.44 (0.42-0.47)
Alemtuzumab	2,822	93	**1,30 (1,06-1,6)**	**1.59 (1.29-1.96)**	**1.34 (1.09-1.65)**
Daclizumab(C)	166	3	0,7 (0,22-2,21)	0.86 (0.27-2.69)	0.81 (0.26-2.53)
Symptomatic therapy					
Fampridine	1,145	40	**2,07 (1,79-2,39)**	**1.69 (1.23-2.32)**	**1.58 (1.15-2.17)**

ROR: Reporting odds Ratio; RORadj: ROR adjusted for concomitant hepatotoxic drugs; NA: not applicable (number of cases<3).

(A) Analysis performed considering the entire FAERS database.

(B) Analysis performed only within Multiple sclerosis drugs.

(C) Withdrawn by the European Medicines Agency for serious drug-induced events (liver injury and encephalitis).

Table 4.3b Disproportionality analyses based on the Standardized MedDRA Query "Drug-related hepatic disorders – severe events only".
In bold: statistically significant disproportionality (i.e., the lower limit of the 95%CI of ROR>1).

			Severe Liver injury (SLI)		
Drug			**General database[(A)]**	**Multiple sclerosis database[(B)]**	
	Total reports	**N cases**	**ROR (95%CI)**	**ROR (95%CI)**	**ROR_{adj} (95%CI)**
Moderate efficacy or standard initial disease modifying therapies					
Interferon beta-1a	146,470	2,343	0,76 (0,73-0,79)	**1.41 (1.33-1.49)**	**1.78 (1.68-1.88)**
Glatiramer acetate	18,591	212	0,54 (0,47-0,62)	1.00 (0.87-1.15)	1.01 (0.88-1.16)
Interferon beta-1b	22,053	535	**1,16 (1,06-1,26)**	**2.16 (1.97-2.37)**	**2.09 (1.91-2.29)**
Fingolimod	38,563	496	0,61 (0,56-0,66)	**1.13 (1.03-1.24)**	1.09 (0.99-1.20)
Teriflunomide	11,945	158	0,63 (0,53-0,73)	1.16 (0.99-1.37)	1.09 (0.93-1.28)
Dimethyl fumarate	50,182	223	0,21 (0,18-0,24)	0.39 (0.34-0.44)	0.35 (0.31-0.40)
High efficacy or later disease modifying therapies					
Mitoxantrone	3,880	131	**1,63 (1,37-1,94)**	**3.04 (2.54-3.62)**	**2.51 (2.10-3.00)**
Natalizumab	136,180	759	0,26 (0,24-0,28)	0.49 (0.45-0.53)	0.43 (0.39-0.46)
Alemtuzumab	2,822	33	0,55 (0,39-0,78)	1.03 (0.73-1.45)	0.85 (0.60-1.21)
Daclizumab[(C)]	166	1	NA	NA	NA
Symptomatic therapy					
Fampridine	1,145	31	1,30 (0,91-1,86)	**2.42 (1.69-3.46)**	**2.27 (1.59-3.26)**

ROR: Reporting odds Ratio; RORadj: ROR adjusted for concomitant hepatotoxic drugs; NA: not applicable (number of cases<3).

(A) Analysis performed considering the entire FAERS database.

(B) Analysis performed only within Multiple sclerosis drugs.

(C) Withdrawn by the European Medicines Agency for serious drug-induced events (liver injury and encephalitis).

According to Bjornsson (199), 4 out of 11 studied drugs had categorized as hepatotoxic drugs: interferons (class A), natalizumab (C) and mitoxantrone (D).

When concomitant therapies were analyzed in the case-by-case analysis, only in a few cases drugs specifically used to treat hepatitis infections were found; in particular OLI cases indicated only drugs used to treat HBV whereas SLI cases both HBV and HCV drugs (Table 4.3c). As regards the rest of drugs, about 40% of cases reported concomitant use of drugs potentially involved in DILI events. However, only a minority of cases had drugs classified as class A (with huge number of published cases). Analysis of concomitant drugs pointed also out that the most reported drugs were therapies generally used during first symptoms and relapse of disease such as baclofen (546 cases), gabapentin (523) ibuprofen (500), and other more generic and largely used in the general population such as clonazepam (243) and omeprazole (216).

Table 4.3c. Case by case analysis of DILI reports.

Drug		Overall Liver Injury (OLI)							Severe Liver Injury (SLI)[A]						
			N. of cases with concomitant hepatotoxic agents[B], with relevant DILI categorization					Reported use of anti-hepatitis drug[C]		N. of cases with concomitant hepatotoxic agents[B], with relevant DILI categorization					Reported use of anti-hepatitis drug[C]
	DILI risk [B]	N cases	N (%)	A	B	C	D		N cases	N (%)	A	B	C	D	
Moderate efficacy or standard initial disease modifying therapies															
Interferon beta-1a	A	3,368	1,332 (40)	541 (16)	641 (19)	751 (22)	582 (17)	N	2,343	849 (36)	329 (14)	454 (19)	475 (20)	411 (18)	Y (2)
Glatiramer acetate		274	97 (35)	40 (15)	39 (14)	48 (18)	41 (15)	N	212	67 (32)	18 (8)	35 (17)	37 (17)	32 (15)	N
Interferon beta-1b	A	878	394 (45)	191 (22)	182 (21)	205 (23)	209 (24)	N	535	225 (42)	98 (18)	110 (21)	124 (23)	101 (19)	N
Fingolimod		1,823	746 (40)	213 (12)	372 (20)	476 (26)	338 (19)	N	496	210 (42)	64 (13)	108 (22)	123 (26)	101 (20)	Y (1)
Teriflunomide		578	230 (40)	64 (11)	127 (22)	135 23)	121 (21)	N	158	51 (32)	12 (8)	22 (14)	38 (24)	28 (18)	N
Dimethyl fumarate		397	103 (26)	28 (7)	58 (15)	68 (17)	65 (16)	N	223	58 (26)	15 (7)	33 (15)	42 (19)	38 (17)	N
High efficacy or later disease modifying therapies															
Mitoxantrone	D	199	180 (90)	43 (22)	98 (49)	161 (81)	70 (35)	Y (2)	131	117 (89)	22 (17)	60 (46)	103 (79)	48 (37)	Y (4)
Natalizumab	C	1,452	284 (16)	86 (6)	144 (10)	179 (12)	151 (10)	Y (1)	759	208 (27)	68 (9)	108 (14)	122 (16)	110 (14)	Y (1)
Alemtuzumab		93	55 (59)	13 (14)	32 (34)	38 (41)	23 (25)	N	33	20 (61)	---	14 (42)	17 (51)	5 (15)	N
Daclizumab[D]		3	1 (33)	---	---	1 (33)	---	N	1	1 (100)	---	---	1 (100)	---	N
Symptomatic therapy															
Fampridine		40	18 (45)	3 (8)	7 (18)	9 (23)	11 (28)	N	31	20 (65)	8 (26)	9 (29)	9 (29)	10 (32)	N

(A) Based on the Standardized MedDRA Query "Drug-related hepatic disorders – severe events only".

(B) Categorization based on Bjornsson et al. (199)

(C) Including boceprevir, telaprevir, ribavirin, lamivudine, interferon alfa, simeprevir, daclatasvir, sofosbuvir and multiple combinations; in parenthesis number of cases; Y=yes; N=no.

(D) Withdrawn by the European Medicines Agency for serious drug-induced events (liver injury and encephalitis).

5. DISCUSSIONS

Here below, we discuss the overall research activity by highlighting both strengths and limitations of the methods, as well as possible clinical implications of the main findings (202, 205, 206).

5.1 Drugs as possible risk factors for MS occurrence

In the part 1 and 2 (and relevant articles) we detected the role of drugs in MS onset. In particular, first we conducted a review of literature in order to evaluate the available evidence and its quality (part 1) (205). Then, we explored signals of MS after drug exposure by using the largest pharmacovigilance database FAERS (part 2) (202).

From the analysis of the literature, specific classes of drugs more than others seemed to have a detrimental effect on MS onset and its worsening. For example, immunomodulating agents were the most retrieved drugs in case reports, case series and longitudinal studies. They provided also significant results in FAERS database: they were the most reported drugs and disproportionality signals were found for many of them, especially for infliximab, adalimumab and etanercept. It should be recognized that possible exacerbation of demyelinating disease and more generic neurological adverse events are reported in the Summary of Product Characteristics (SPC) of some TNF-antagonist drugs (e.g., the European SPC of adalimumab (207)). However, this information sometime is very generic, frequently derived from indirect evidence, and therefore scarcely useful for both clinicians and researchers. Pharmacovigilance studies maintain an important role in defining possible impact of all TNF-antagonists agents on occurrence of MS. For this class of drugs, a dual mechanism of actions can be suggested; albeit their role in MS disease is

still debated, they 1) may represent *per se* cause of disease by dysregulating immune system or 2) may be *proxy* of previous or concomitant diseases (i.e., chohn's disease or other autoimmune disease), which can trigger the development of a second autoimmune disease (208, 209).

Drugs acting on hormonal homeostasis were emerged in evidence mapping (contraceptive and assisted reproduction treatment). Moreover, FAERS analysis pointed out also other drugs of the same area such as insulin (in the article it is classified into alimentary tract metabolism group, according to ATC code), etonorgestrel and sildenafil. As regards insulin, it should be acknowledged that some evidence suggest an interplay between MS and diabetes (210, 211). A possible hypothesis is that the two diseases may share common risk factors such as low vitamin D level as well pathological pathways; this raises concerns on possible increased risk to develop a second one of these autoimmune disease in patients already affected by the other one (212). Concerning contraceptives, etonorgestrel has been tested in an ongoing clinical trials as possible MS treatment (213). However, as highlighted in evidence mapping, contraceptives (usually, estrogens and progestinin in combinations) could have misleading results in both evidence mapping and FAERS due to increased number of relapses during pill-free period (214). Therefore, MS events could be erroneously associated with contraceptive rather their discontinuation. Another typical female drug class is represented by bisphosphonates, which showed statistically significant results in FAERS analysis. This class of drugs is generally used to treat osteoporosis, a disease that affects adult women. The two diseases (MS and osteoporosis), share several risk factors such as smoking, geographical distribution and estrogens levels (215). Therefore, a mutual influence cannot be completely excluded in their pathogenesis, although their apparent relationship could also be due to common risk factors.

Overall evidence on antimicrobials shows high uncertainty on the association with MS. The published studies have contrasting findings and FAERS analysis provided signal only for

levofloxacin. It should be recognized that antimicrobials treatments are usually brief cycles and therefore rarely associated by potential reporter with the occurrence of chronic neurodegenerative disorders (such as MS) for which latency between cellular damage and disease onset can be several years long. As a matter of fact, it should be remembered that, generally, reporters are more confident to signal adverse events that occur after few hours or days from treatment. This can generate a misleading results for some disorders when studied as possible ADR of drug exposure such as MS. For this reason, antimicrobial and similar agents are more prone to be studied with longitudinal studies, which should integrate inherent limitations of PhV studies. As general hypothesis, these agents can also be considered as proxy of infections, which could be the actual responsible for immune system dysregulation and consequent MS development, as some studies have already suggested for virus infections (216). These drugs are able to influence also intestinal microbiome during their therapeutic activity. A recent review, highlighted a possible link between intestinal microbiome perturbations and neurological/neurodegenerative diseases (i.e., MS) (217). The role of microbiome in humans is still far to be completely elucidated, but it is known that this “organ” produces mediators, which are able to influence immune system functions. Generally, antibiotic therapies generate depletion of some species with consequent growth of other ones; this disequilibrium in microbiota composition, can cause change in mediator productions, which are able to influence immunity system, which can trigger MS development (218).

As regards the rest of drugs emerged from FAERS analysis, lack evidence are available to support biologic hypotheses on their role in MS onset. In fact, some of them can represent a spurious signal of disproportionality, due to large use of these drugs in the general population (e.g. esomeprazole) or possible use of medication in extreme early stage of disease (i.e., sildenafil).

It should be recognized that FAERS analysis showed new signals for several classes of drugs, which have never been previously investigated. In addition, FAERS provided signals also for drugs previously detected but with contrasting results. New studies on possible role of drugs in MS development should be planned in order to confirm or reject hypothesis generated with FAERS analysis. These studies, should also take into account weakness emerged from quality assessment of longitudinal studies identified by evidence mapping. In particular, they should include: more detailed inclusion criteria; longer follow-up; and analysis of other parameters with potential impact on disease. Finally, also case reports and case series, generally used to plan new PhV studies (and longitudinal ones), should indicate data on clinical history, concomitant therapies, clinical tests and other useful information that can be used in PhV analysis designs (although we are aware that not all variables are included in PhV databases) and studies on Health-care databases as well.

Improvements in case report description together with adequate longitudinal study designs are essential to assess the real role of drugs in MS pathogenesis (cause Vs *proxy* of disease). These approaches can contribute in the achievement of a sort of personalized medicine, which allows to avoid some drugs (associated to disease) for patients potentially prone to develop MS. On the other hand, analysis of previous drug exposures (*proxies*) can help clinicians to detect potential diseases or pathogens, which could have a role in new MS cases. Finally, all data retrieved by using case reports, pharmacovigilance and longitudinal studies could provide a continuous updating of drugs' SPC. These integrate approaches can contribute to highlight possible occurrence of demyelinating events during therapies which apparently have no relation with neurological ADRs.

5.2 MS therapies and DILI events

In the third part of the thesis, we explored FAERS database detecting signals of DILI events during MS treatments (206). New signals of disproportionality were found for some MS therapies, whereas for other drugs our results confirmed their hepatotoxic effects.

The first treatments approved specifically for MS were interferons and mitoxantrone. For these agents, our results confirmed their potential hepatotoxic effects. In fact, liver tests are recommended before new cycles of treatment and periodically during the therapy with recommendation to discontinue it if abnormalities of liver enzymes are observed (112-114, 121, 155). Our findings for these drugs can be interpreted as “positive controls” in this study, adding weight to the reliability of adopted strategy of analysis.

For the first time, we observed signal of DILI in patients treated with alemtuzumab, teriflunomide and fingolimod. Teriflunomide already received a warning for potential liver adverse effect based on leflunomide (its precursor) data (149, 150), and now we provide further for this concern. In light of different mechanisms of action of above drugs, several hypotheses of liver damage can be supposed. These include: 1) block of receptors on liver cell surface with consequent loss of function that can also stimulate auto-repair ability dysfunctions with consequent cellular death; 2) decreasing immune system reactivity, that can trigger new infection or reactivation of previous ones, with consequent liver damage; 3) finally, these modulating immune system drugs can create unbalance between immunity cells with over expression of specific mediators, such as cytokines, that influence liver cell life inducing apoptosis (219, 220).

The only symptomatic drug specifically approved for treatment of MS symptoms is fampridine, which showed significant disproportion in FAERS analysis, thus increasing concerns on its safety profile. In clinical practice, fampridine can be co-prescribed with other MS disease

modifying therapies, however most cases no other MS therapies were reported (55% of cases). From basic research, new evidence of possible effect of fampridine on liver has been found: an animal study showed that rats exposed to fampridine can develop liver cell dysfunction (221). The mechanism is still far to be elucidated, however, the blockage of K+ channels or the Na+/K+ ATPase and modulation of pro- and anti-apoptotic gene expression can have a crucial role in the observed adverse effect.

Our study did not provide significant results for daclizumab, that was authorized for MS in summer 2016 and was withdrawn by Biogen from the market in the subsequent winter due to fatal adverse events including liver damage (222). Several factors might influence our results. The short time on market and early restriction of its use by EMA can have impacted on its use in the general population and therefore on the occurrence of ADRs. In addition, we cannot completely excluded that the adopted strategy of analysis (drug + brand name and drug + therapeutic indication), allows to include in the analysis only complete records. In fact, we found only 1 case for OLI and no SLI. However, it should be noted that we adopted the same strategy of analysis for alemtuzumab, that on the contrary had signal of disproportionality in our analysis. This finding cannot be disregarded and further studies on possible effects on liver enzymes should be planned by also including the new approved drug ocrelizumab.

The case-by-case analysis performed on OLI and SLI events highlighted a concomitant use of symptomatic drugs such as baclofen, gabapentin, pregabalin and others, which have already been associated with DILI events by Bjornsson et al., (199). Presence of disease modifying treatments (DMTs) plus other symptomatic drugs raise concerns on possible synergic effects between therapies involved in DILI events: interactions between therapies as well as alterations of therapeutic effect cannot be completely excluded. In this complex scenario, another aspect that

should be investigated is represented by the general downregulation of immune system generated by MS therapies that may *per se* trigger the infections potentially responsible for liver damage.

The complexity of DILI events deserves also evaluation of environmental and social habits. In fact, alcoholism and previous infections can influence future liver damage. These aspects were only partially covered in FAERS database so far. In fact, we were only able to detect previous hepatitis by assessing presence of specific drugs, and in our cases only few patients (at most 4) had HBV or HCV infection. New algorithm specifically devoted to assess DILI events in PhV database has been recently published in the literature, however additional validation is needed before applying this strategy on FAERS data (223). In the meanwhile, reporters should be as accurate as possible when they submit a new case reports to the FDA or after regulatory agencies. In fact, application of complex algorithms require as much as possible information that generally are not reported or are affected by incompleteness.

Our results call for future studies in order to assess the role of MS therapies in DILI events. These studies should evaluate the role of other factors such as clinical history, concomitant therapies, comorbidities, and social habits in order to characterized the patients who experience liver damage, and define the role of different exposure in DILI occurrence.

5.3 Methodological consideration and limitations

5.3.1 Evidence mapping strategy

The first part explored available evidence on drug exposure and possible MS risk and some limitations should be recognized. Firstly, the search strategy developed to perform literature review could be not able to retrieve all published items. However, this weakness was partially

resolved by snowballing of selected reviews, which allowed to include some previously excluded records. Another limit in this filed is represented by the absence of MeSH terms specifically devote to study “MS drug induced” in both Pubmed and Embase databases. This together to the prior limit can affect our analysis. This limit was figure out by applying no type of study restrictions, this strategy allows to collects as much as possible articles. Additionally, it should be noted that concerns on possible role of drugs in neurodegenerative disorders is a relatively new area of research therefore the amount of missing records can be considered very low. We encourage authors to be as specific as possible in article classification in order to increase sensibility of future update of this review, meanwhile new specific MeSH strategies for this topic should be included in the Mesh list of above databases.

Analysis of pooled data from different studies was not possible due to variability of study designs included in the review. As showed by the quality assessments, many studies present several weaknesses, which should be taken into account in future studies in the same area of research. In particular, absence or incomplete data on patients selection, exposure assessment and study design did not allow comparison between the studies. Therefore, future studies on the same class of drugs should take into account weakness of previous studies trying to level out previously discrepancies. In addition, according with relevance of this possible “risk factor” authors should provide a minimum set of data which can be used for future meta-analysis or at least to compare results in accordance with peculiarities of each study. The last remark is about the importance of open-data. Recently, an increased number of editors required publication of the anonymous database used for the analysis together with relevant article. Sharing of data can contribute to reanalyze them by other authors clarify adopted strategy of analysis and may contribute to test new ones.

Nature of retrieved case reports and case series allowed only a general description of phenomenon. Also these piece of literature showed several missing data that have negative impact on evaluation of final association. Future case reports and case series should be more detailed, including previous therapies (also therapies not apparently involved in observed adverse event), co-morbidities and laboratory tests in order to pool case reports highlighting possible factors involved in the observed events.

Nevertheless this study point out a new prospective in MS risk factor detection, giving an overview of available literature and providing some recommendations that should be taken into account in future studies. This effort can help future update of the present review and allows to pool data from single study in a more powerful studies. In addition, this review adds weight on importance to study the role of drugs in MS development. Finally, we would encourage authors to classify in a proper way their work by using more specific keywords. In the meantime scientific community should create new suitable MeSH more specific for this topic in order to increase the sensitivity of future research in the same area.

5.3.2 Signal detection on FAERS database

After having mapped already available clinical evidence on a possible role of drugs in MS occurrence, we explored the largest source of freely available adverse drug reaction reports namely FAERS database. The same source of data was used also to explore possible signal of DILI events during MS treatments. Results included in the part 2 and 3 of this thesis should be read taking into account some general and project-specific considerations according to the database characteristics. Firstly, the impact of Weber effect on our analysis. The Weber effect is a well-known issue in pharmacovigilance studies. It is characterized by an increased number of reports of

adverse events during the first years of marketing of new agents (224). However, a recent publication focused on FAERS database highlighted the scarce impact of Weber effect in the last analysis due to huge number of reports included in the database during the last years (225).

Secondly, under reporting or over reporting can generate a misclassification of signals, increasing risk of notoriety bias (226). We developed *ad hoc* strategies of analysis taking in mind the specific characteristics of each study. As regards evaluation of MS after drug exposure we created *ad hoc* exclusion criteria in order to minimize misleading signals (i.e., signals for drugs used to treat patients already affected by MS). For this reason we excluded from the analysis: 1) cases with drugs specifically approved for MS treatment (i.e. interferons); 2) cases reported other drugs but with "Multiple Sclerosis" as reason for their use; 3) cases with vaccines because those are better studied in a dedicated database (VAERS). In addition, in order to prioritized the signals we focused only on drugs with: 1) significant ROR, 2) at least 10 cases, and 3) adequate cleaning index (≥70%). In pharmacovigilance studies, significant ROR with at least 3 cases is the minimum requirement to claim for a new signal (227), in this study we adopted strictly criteria in order to avoid misclassification of signals focusing on new potential signals that should be further investigated in future studies. As regards the study on DILI events during MS treatments, we were aware that DILI is generally a rare and severe condition that requires specific laboratory tests and clinical evaluation and that has several ways to be classified even if all of them indicate a severe medical condition. For this reason we planned two different strategies of analyses, one more inclusive and generic that include several preferred terms (OLI) and one that included only severe clinical conditions (SLI) by using few PTs.

Misspelling errors can affect ability to codify drugs as well medical terms or other relevant information in pharmacovigilance databases. It should be noted that generally this does not represent a real issue for medical events, which are codify by using MedDRA dictionary. On the

contrary several errors can be detected in drugs information dataset. In fact, this dataset include a free text variable and a complex datamining procedure is needed in order to make suitable this information. Our data mining approach includes: a) elimination of word which do not indicate a drug (i.e., articles, verbs, unspecific words); b) application of Leveshtein distance strategy in order to replace misspelling error in specific letter with right one; c) codification of retrieved drugs by applying an "home-made" substance vocabulary (derived from previously codification of FAERS database); d) manually identification and codification of drugs (presenting at least 100 cases) which are still not codified with subsequent update of home-made vocabulary. The use of mentioned vocabulary is essential for several reasons, among those the most important are: fast codification of drugs in new version of FAERS, link each substance with relevant ATC code and possibility to perform grouped ATC code analyses (i.e., ATCIII level analysis instead of ATCV level). The vocabulary is updated accordingly to the new FAERS quartiles database. However, sometime selection of cases by using only substance name can generate an overestimation of phenomenon, therefore additional criteria in order to avoid false signal should be considered. For example, in the last study among the included drugs, there were two of them which had different indications (daclizumab and alemtuzumab). In this case we used also other variables to increase sensitivity of our case selection, so we included only reports with 1) substance (alemtuzumab/daclizumab)+brand name or 2) substance + MS as indication.

Finally, it should remember that other factors can be involved in observed adverse events, unfortunately, so far, evaluation of their role could be challenging in PhV studies. However, we can use the already included variables to adjust or stratify analyses performed by using FAERS database (sex, country, age, concomitant therapies, sometime indicated comorbidities, reporter, challenge and dechallenge information). In spite of the huge number of information included in the database application of specific algorithm such as Naranjo algorithm is almost impossible or

misleading due to incompleteness of data (188). This is true also for other algorithms generally used to test presence of other risk factors. For example, in the DILI study could be useful to indicate concomitant presence of other risk factors able to trigger DILI events and should be recognized that a specific tool (PV-RUCAM) has been already developed. However, more studies need in order to test its applicability in PhV database (223). Although a proper algorithm was not applicable in our data, we decide to investigate concomitant presence of drugs already classified as potentially DILI inducer or drugs usually used to treat hepatic infections. Future studies by including more detailed information for each report can allow a proper application of algorithms which will be useful to evaluate also the impact of other lifestyle factors such as alcoholism or other ones.

We would to remark that pharmacovigilance studies do not represent risk estimation but only a signal evaluation that deserves further evaluations. In fact, pharmacovigilance findings can be used to drive further studies able to include more detailed data in order to confirm or disclaim previously signals from PhV reports.

Pharmacovigilance studies and in particular studies based on FAERS database have also several strengths. FAERS database is a freely available source of data that allows to perform analysis with relatively low cost budget. In addition, this source of data collects reports worldwide without any restriction criteria therefore is less prone to have a selected population as happen during clinical trials. This database is a suitable source to conduct pharmacovigilance studies on rare and unpredictable ADR such as DILI, MS and long QT syndrome. Finally, characteristics of data allow to generate findings which can be generalized worldwide.

As last remark considering the importance of continuous surveillance of new approved drugs, quality of records is essential. As we said before, everyone (clinicians, consumers and

pharmaceutical companies) can submit the reports, however usually they have several lacking or missing data. This does not represent a real problem in FAERS database due to huge amount of data, however can limit application of specific algorithms. Reporters should include as detailed information as possible in order to improve above weakness. Therefore we encourage people to submit complete reports including also lifestyle information together with already well filled drugs, indication and ADRs information.

6. FEATURE PERSPECTIVES

Our findings add weight to hypothesis of possible role of drugs in multiple sclerosis development. In particular, by evidence mapping we retrieved and analyzed available evidence on this topic, in order to take the best of evidence from the already available findings and by the FAERS analysis we have new signals of possible association between previous drug exposure and new MS cases. Although both strategies of analysis have some limitations they should represent the first step to explore the role of drugs in adverse effect because of their availability to scientific community. Future studies by using drugs as cause of disease or proxy of previous and concomitant disease can provide new data on this controversy association contributing to clarify also possible mechanism of pathogenesis, which are still under-detected in this very complex mechanism of disease. Use of longitudinal studies, which take into account differences or weaknesses of previous studies, can help researcher to define a minimum of requirement necessary in order to conduct studies on drugs as MS risk factor. An example of this delivery is represented by the ongoing study conducted in Emilia-Romagna on antibiotic exposure and MS risk. Results from this study and similar ones are going to contribute also to identifyother possible causes of disease not yet detected.

As regards safety of authorized and generally used therapies in MS, post marketing phase is crucial in order to detect possible ADR which were not properly detected during pre-marketing phase studies. This aspect is important for both clinicians and patients, in fact ADRs occurrence have also an impact on treatment adherence and wellness of patients and therefore should be continuously evaluated. In particular we aimed to assess possible signals of liver injury during MS treatments. Our results on 11 authorized drugs suggested a possible risk of DILI by MS drug treatments. Future studies can detect new signal associated with adverse effect in treated

individuals by using an update FAERS database with most recent months data in order to include in the analysis also new marketed drugs: ocrelizumab and cladibrine. Unexplored ADRs can be assessed by using original strategies of analysis such as selection of specific Preferred terms (PTs), as well as use of available standardized queries that are called SMQ in MedDRA dictionary. In fact, the huge number of data collected in the last years in FAERS and other databases and the raised awareness of clinicians and consumers about the importance of pharmacovigilance analysis contribute to generate more and more data on still unknown ADRs as well as rare ADRs. Results of pharmacovigilance concerning MS therapies can drive stakeholders during ongoing clinical trials on possible new MS therapies (i.e., on ublituximab, ponesimod and anti-aquaporin-4 antibody), towards an early detection of safety profile of new drugs, specifically on emerged signals of risk for similar already marketed drugs.

7. CONCLUSION

In this thesis, we explored the role of drugs as possible risk factor in MS development by performing both literature review and pharmacovigilance analysis. From literature review contrasting results emerged, whereas new signals were generated by exploring FAERS database. In particular, drugs acting on immune and endocrine system as well as drug used to treat microbial infections showed interesting relationship with MS development and they should be further investigated in order to assess their role in MS (*per se* cause of disease or proxy of concomitant disease).

As regard to MS therapies, we aimed to assess occurrence of DILI events. New signals have been found for some disease modifying treatments (alemtuzumab, fingolimod, teriflunomide) and symptomatic agent (farmpridine). Additionally, concomitant presence of other drugs already associated with hepatic adverse events raise concern on possible synergic effect between therapies. Therefore future studies should assess the role of MS therapies in DILI occurrence taking into account concomitant therapies and lifestyle conditions (i.e., smoking and alcohol) that can trigger the studied phenomenon. Finally, liver tests should be planned in treated patients especially in those who already experienced hepatic dysfunctions.

This thesis highlighted the importance of pharmacovigilance studies in the field of neurodegenerative diseases. Signal detection findings can drive future longitudinal studies with more appropriate designs in order to confirm or disprove them. We encourage clinicians and consumers to submit as detailed and complete information as possible for each report in order to better characterizing the signals and to allow sensitivity analyses, which could be useful for clinical and regulatory decisions.

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Article 1

"Occurrence of Multiple Sclerosis After Drug Exposure: Insights From Evidence Mapping"

Abstract

Introduction:

The role of drugs in the occurrence of multiple sclerosis (MS) is perceived to be insufficiently investigated.

Objective:

The aim of this study was to map and assess the evidence on MS occurrence after drug exposure, in order to identify possible signals of causal association.

Methods:

A search strategy was performed in MEDLINE and Embase as of July 2016; references consistent with the aim of the study were analysed to extract relevant measures of causal association between drugs and MS. The Newcastle-Ottawa Scale and appropriate guidelines from the International Society for Pharmacoepidemiology (ISPE) and the International Society of Pharmacovigilance (ISoP) were used to assess the quality of included studies.

Results:

After screening 832 articles, 58 were selected (of which 14 were found by checking the reference lists of reviews): 30 case reports and case series, 24 longitudinal studies and four randomized controlled trials. Seven longitudinal studies had good (at least 7 out of 9) quality scores, whereas case reports/case series presented several limitations. Half of included articles focused on immunomodulatory drugs (etanercept, infliximab and adalimumab), especially in case reports/series, suggesting an association with MS occurrence. Contraceptives and antibacterials were investigated in some population-based studies, without definite results.

Conclusion:

A heterogeneous pharmacological profile of identified classes emerged. Low strength of evidence and conflicting results highlighted the difficulties in addressing the possible contribution of drugs in MS occurrence. Methodological advances are needed, especially to control the confounding role of underlying disease for specific drug classes.

Article 2

"Multiple sclerosis as an adverse drug reaction: clues from the FDA Adverse Event Reporting System"

Abstract

Background:

Possible relationship between drug exposure and multiple sclerosis (MS) development is insufficiently investigated, and further challenged by the incomplete understanding of MS etiopathogenesis. The study aims to investigate whether drug exposure could contribute to MS, by analyzing worldwide spontaneous reporting archives of adverse drug reaction (ADRs).

Research design and methods:

We retrieved information from the US Food and Drug Administration Adverse Event Reporting System (FAERS) over a 13-year period. Reporting odds ratio (ROR) for MS was calculated for each single substance. Disproportionality signals were considered when at least 10 cases were retrieved with a lower limit of the 95% confidence interval (CI) >1.

Results:

After a customized data-mining process, 3,226 reports of MS were retrieved. 'Antineoplastic and immunomodulating drugs' (33% of total reports) were the most frequently reported, with 10 disproportionality signals, including etanercept (445 cases; ROR: 2.48; 95% CI: 2.24-2.74), adalimumab (329; 2.05; 1.83-2.30), and infliximab (119; 2.25; 1.87-2.70). We also observed signals for drugs acting on hormone balance, bone density, and central nervous system.

Conclusion:

Our findings suggest that immunomodulatory drugs increase the risk of MS and point out that some other drug classes should be further investigated for this risk.

Article 3

"Liver injury with drugs used for multiple sclerosis: A contemporary analysis of the FDA Adverse Event Reporting System"

Abstract

Background:

Drug-induced liver injury (DILI) has been observed in patients with multiple sclerosis (MS), raising concerns on the liver safety of MS drugs.

Objective:

To describe DILI events with MS drugs by analyzing the FDA Adverse Event Reporting System.

Methods:

DILI reports were extracted and classified in overall liver injury (OLI), including asymptomatic elevation of liver enzymes, and severe liver injury (SLI). We performed disproportionality analysis by calculating adjusted reporting odds ratios (RORs) with 95% confidence interval (CI) and case-by-case evaluation for concomitant drugs with hepatotoxic potential.

Results:

Fampridine showed statistically significant ROR for both OLI and SLI, whereas teriflunomide and fingolimod generated solid disproportionality (ROR > 2) only for OLI (ROR, 2.31; 95% CI, 2.12-2.52; and 2.53; 2.40-2.66, respectively). Among monoclonal antibodies, only alemtuzumab generated higher-than-expected ROR for OLI (1.34; 1.09-1.65). We also detected the expected hepatotoxic potential of beta interferon and mitoxantrone. Concomitant reporting of hepatotoxic drugs ranged from 26% (dimethyl fumarate) to 90% (mitoxantrone).

Conclusion:

These real-world pharmacovigilance findings suggest that DILI might be a common feature of MS drugs and call for (1) formal population-based study to verify the risk of fampridine and (2) awareness by clinicians, who should assess the possible responsibility of MS drugs when they diagnose DILI.