UNIVERSITY OF BELGRADE
FACULTY OF MEDICINE

GORICA D. MARIC

ASSESSMENT OF COMORBIDITY BURDEN IN PERSONS WITH MULTIPLE SCLEROSIS: OBSERVATIONAL STUDY BASED ON BELGRADE POPULATION REGISTRY DATA

Doctoral Dissertation

Belgrade, 2020

UNIVERZITET U BEOGRADU
MEDICINSKI FAKULTET

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PROCENA OPTEREĆENJA KOMORBIDITETIMA KOD OSOBA SA MULTIPLOM SKLEROZOM: OPSERVACIONA STUDIJA ZASNOVANA NA PODACIMA POPULACIONOG REGISTRA BEOGRADA

doktorska disertacija

Beograd, 2020

Supervisor:

Full Professor Tatjana Pekmezović, MD, PhD, Faculty of Medicine, University of Belgrade, Institute of Epidemiology

Co-Supervisors:

1. Full Professor Katarina Lalić, MD, PhD, Faculty of Medicine, University of Belgrade, Clinic of Endocrinology, Diabetes and Metabolic disorders, Clinical Center Serbia

2. Associate professor Šarlota Mesaroš, MD, PhD, Faculty of Medicine, University of Belgrade, Clinic of Neurology, Clinical Center Serbia

Committee for reviewing:

1. Full Professor Dr Jelena Drulović, MD, PhD, Faculty of Medicine, University of Belgrade, Clinic of Neurology, Clinical Center Serbia

2. Associate Professor Darija Kisić Tepavčević, MD, PhD, Faculty of Medicine, University of Belgrade, Institute of Epidemiology

3. Full Professor Maura Pugliatti, MD, PhD, Department of Neuroscience and Rehabilitation, Section of Neurology, Psychiatry and Psychology, University of Ferrara, Ferrara, Italy

Date of defense of doctoral dissertation:

Mentor:

Prof. dr Tatjana Pekmezović, redovni profesor Medicinskog fakulteta Univerziteta u Beogradu, Institut za epidemiologiju

Komentori:

Prof. dr Katarina Lalić, redovni profesor Medicinskog fakulteta Univerziteta u Beogradu, Klinika za endokrinologiju, dijabetes i bolesti metabolizma, Klinički centar Srbije

Prof. dr Šarlota Mesaroš, vanredni profesor Medicinskog fakulteta Univerziteta u Beogradu, Klinika za neurologiju, Klinički centar Srbije

Članovi komisije za odbranu doktorske disertacije:

Prof. dr Jelena Drulović, redovni profesor Medicinskog fakulteta Univerziteta u Beogradu, Klinika za neurologiju, Klinički centar Srbije

Prof. dr Darija Kisić Tepavčević, vanredni profesor Medicinskog fakulteta Univerziteta u Beogradu, Institut za epidemiologiju

Prof. dr Maura Pugliatti, redovni profesor Univerziteta u Ferari, Italija

Datum odbrane:

Zahvalnica

Želim da se zahvalim svojoj mentorki, ***prof. dr Tatjani Pekmezović****, što je svaki deo ove doktorske disertacije prošla sa mnom, zbog čega sam se osećala sigurno u radu, i što je uvek verovala u mene usmeravajući me napred.*

Izuzetnu zahvalnost dugujem ***prof. dr Jeleni Drulović****, na idejama, strpljenju i vremenu koje je posvetila ovoj doktorskoj disertaciji od njenog početka.*

Zahvalnost upućem komentorima, ***prof. dr Katarini Lalić*** *i* ***prof. dr Šarloti Mesaroš*** *za pomoć i podršku u izradi doktorske disertacije.*

Zahvaljujem se članovima ***Komisije*** *na podršci tokom istraživanja i izrade doktorske disertacije.*

Veliku zahvalnost dugujem kolegama sa ***Klinike za neurologiju KCS****, posebno lekarima* ***V odeljenja****, na nesebičnoj pomoći u svim fazama istraživanja.*

Zahvaljujem se osoblju ***Klinike za neurologiju i Klinike za endokrinologiju, dijabetes i bolesti metabolizma KCS,*** *na izuzetno velikoj pomoći u prikupljanju podataka za izradu doktorske disertacije.*

Zahvaljujem se svojim koleginicama i kolegama sa ***Instituta za epidemiologiju,*** *na podršci i spremnosti za pomoć u svakom trenutku. Zadovoljstvo je biti deo takvog kolektiva.*

Zahvalnost pripada i članovima moje ***porodice*** *- najzaslužnijima za dosadašnji rad.*

ASSESSMENT OF COMORBIDITY BURDEN IN PERSONS WITH MULTIPLE SCLEROSIS: OBSERVATIONAL STUDY BASED ON BELGRADE POPULATION REGISTRY DATA

Abstract

Introduction: Comorbidity is one of major patients' characteristics relevant to the clinical presentation and management of multiple sclerosis (MS).

Aims: To estimate the prevalence of comorbidities in MS patients in Belgrade, Serbia, based on population-based registry data with special emphasis on cardiovascular disorders (CVD); and to determine their effects on MS course.

Methods: Observational studies were perfomed at the Clinic of Neurology and Clinic of Endocrinology, diabetes and metabolic disorders, Clinical Center of Serbia, in the period 2016-2019. The Belgrade population MS registry was used as a source of data.

Results: The most prevalent groups of comorbidities were psychiatric (20.59%) and CVD (15.23%) and most prevalent single comorbidities were depression (11.82%) and hypertension (11.41%). Progression index was significantly associated with the number of comorbidities ($p<0.001$). MS patients treated with disease-modifying therapies had a higher risk of comorbidity occurrence compared to those untreated ($p=0.001$). MS patients had a higher prevalence of insulin resistance (64.1%) compared to healthy individuals (30.8%), ($p=0.008$), and glucose level at 120' during oral glucose tolerance test was independently associated with MS. Expanded Disability Status Scale (EDSS) score, physical activity, body mass index and type 2 diabetes comorbidity explained 48% of the variance in difference between chronological and vascular age in MS patients. EDSS score and MS phenotype were independent predictors of occurrence of CVD comorbidity, coronary artery disease and type 2 diabetes.

Conclusion: Our findings implicate need for screening and early adequate management of different comorbidities in MS patients, having in mind its involvement in range of adverse outcomes in MS.

Key words: multiple sclerosis, comorbidity, prevalence, cardiovascular diseases, diabetes, insulin resistance, vascular age, population-based MS registry

Scientific field: Medicine
Scientific subfield: Epidemiology

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Sažetak

Uvod: Komorbiditeti predstavljaju jednu od ključnih karakteristika relevantnih za kliničku prezentaciju i ishod osoba sa multiplom sklerozom (MS).

Cilj: Odrediti prevalenciju različitih grupa komorbiditeta kod osoba sa MS u populaciji Beograda, Srbija, sa posebnim osvrtom na kardiovaskularne bolesti (KVB) na osnovu podataka populacionog MS registra Beograda, kao i njihov uticaj na tok MS.

Metod: Opservacione studije su sprovedene na Klinici za neurologiju i Klinici za endokrinologiju, dijabetes i bolesti metabolizma, Kliničkog centra Srbije, u periodu 2016-2019. godine. Kao izvor podataka korišćen je populacioni MS registar Beograda.

Rezultati: Najprevalentnije grupe komorbiditeta su bile psihijatrijske bolesti (20,59%) i KVB (15,23%), a najprevalentniji pojedinačni komorbiditeti depresija (11,82%) i hipertenzija (11,41%). Indeks progresije onesposobljenosti je bio statistički značajno povezan sa brojem komorbiditeta ($p<0.001$). Osobe sa MS na terapiji lekovima koji modifikuju tok bolesti su imale veći rizik za pojavu komorbiditeta u odnosu na nelečene ($p=0.001$). Kod osoba sa MS je registrovana značajno viša prevalencija insulinske rezistencije (64,1%) u poređenju sa zdravim kontrolama (30,8%), ($p=0,008$), a nivo glukoze u 120' oralnog testa tolerancije glukoze bio je nezavisno povezan sa MS. Skor proširene skale neurološke onesposobljenosti (EDSS), fizička aktivnost, indeks telesne mase i prisustvo tip 2 dijabetesa objasnili su 48% varijanse u razlici između hronološkog i vaskularnog uzrasta osoba sa MS. EDSS skor i MS fenotip predstavljaju nezavisne prediktore pojave KVB, koronarne arterijske bolesti i tip 2 dijabetesa kod osoba sa MS.

Zaključak: Naši rezultati upućuju na potrebu za skriningom i ranim i adekvatnim menadžmentom komorbiditeta kod osoba sa MS, posebno imajući u vidu njihov uticaj na veliki broj neželjenih ishoda u MS.

Ključne reči: multipla skleroza, komorbiditeti, prevalencija, kardiovaskularne bolesti, dijabetes, insulinska rezistencija, vaskularna starost, populacioni MS registar

Naučna oblast: Medicina
Uža naučna oblast: Epidemiologija

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1. INTRODUCTION

Multiple sclerosis (MS) is a progressive inflammatory and neurodegenerative chronic disease of the central nervous system (CNS) (1). Also, it is a leading reason of disability unrelated to the trauma in young people worldwide (2), with costs that overcome those of Alzheimer's disease and stroke (3).

It is estimated that more than 2.5 million people globally have MS (4). What is even more important, the incidence and prevalence of MS have an increasing tendency worldwide (5). The prevalence of MS rises with the distance from the Equator and is the highest in northern parts of Europe and America as well as Australasia (5). On the contrary, the prevalence is very low in countries around the Equator (5).

MS predominantly affects female population and disease onset is usually at age between 20 and 40 years. It is characterized by huge heterogeneity in clinical presentation and disease outcomes. Symptomatology varies significantly and includes different physical and mental deficits such as visual impairments, gait disturbances, spasticity, sensory deficits, ataxia, sphincter dysfunction and cognitive impairment. Exact explanations for these variations, however, are still missing. Disease may present in several various phenotypes including: relapsing-remitting (RRMS), primary progressive (PPMS) and secondary progressive (SPMS). Among them, RRMS is the most common MS phenotype (6). Disease usually begins as a RRMS with occurrence of relapses between stable phases of disease (remission). Over time, usually 10-15 years, RRMS often leads to SPMS, with continuous and slowly disease progression, which causes significant deterioration of their quality of life and represents a great burden for society, especially in the social and economic spheres (6). Only in a small percentage of MS patients, disease is progressive from the beginning (PPMS). It is believed that neuronal inflammation is "responsible" for relapsing form of the disease, and neuronal degeneration for progressive course of MS (7).

Despite MS affects women more frequently, difference is obvious in RRMS, where the female to male ratio is about 3:1, while in PPMS gender-specific rates are almost equal (8-10). Although MS is considered as chronic incurable disease, introduction of disease-modifying therapies in the last decade of XX century significantly contributed to improved prognosis of disease with slowing down course of the disease and decreasing relapse rate (11). Regardless of these improvements which provided better survival of persons with MS, it has been revealed that those patients live shorter compared to general population (12).

Although many studies in the field of MS have been performed, the etiology of the disease has not been completely clarified yet. However, there is evidence that etiology of MS is multifactorial including genetic susceptibility, environmental risk factors and gene-environment interactions (13). Research conducted so far indicate that insufficient serum levels of vitamin D,

obesity during childhood, history of Epstein–Barr virus infection and smoking are probably most responsible risk factors for MS occurrence (7).

1.1 HISTORY OF MS

MS has been known for centuries. Despite this, there is a little literature data about the disease before 19th century. Description of MS is usually considered as a merit of Jean-Martin Charcot and 19th century, although there were many other clinicians who substantially helped in discovery of many relevant aspects of MS.

First cases of MS are most likely registered in Middle Ages (6). According to the literature data, it was a girl Ludwina of Schiedam, from Holland, born in 1380, whose symptoms started at age of 16 (2). Her symptomatology (vision disturbances, walking problems, pain, etc.) was indicative of MS, and progressed over time until her death in 1433 (6).

The first published MS case is related to Charles-Prosper Ollivier d'Angers and 1824 year (14). Ollivier first introduced the term "myelitis"after diagnosing and describing spinal disease in a male 20 years old. First illustration of the disease was done by Robert Carswell in 1838 (15). His contemporary, Jean Cruveilhier, described disease as a "gray degeneration of a cord". Contribution of all of these physicians is very significant, however, it is well known that the major work on elucidating MS symptomatology and pathological characteristics was performed by JM Charcot. Together with his colleague Edme Vulpian, Charcot described MS for the first time as "sclerose en plaques disseminees" in 1866. He had a chance to follow the natural course of MS since his housemaid suffered from the disease. After progression of her symptomatology and death, Charcot performed autopsy and discovered patognomonic signs of MS – plaques of demyelination in CNS. Thanks to this and several similar cases Charcot was able to give a precise description of MS (6). Consequently, three common MS symptoms (nystagmus, intention tremor, and dysarthria) were named Charrcot's triad. Charcot also was the first clinician who noticed that cognitive impairment could also be associated with MS. Besides these, Charcot gave a numerous lectures worldwide on MS helping physicians and public to hear about the disease and understand it. Keeping in mind all of these facts, it is clear why MS was called Charcot's disease until 1921. After that, this term was intended for other disease, amyotrophic lateral sclerosis, also discovered by Charcot at the end of 19th century (6).

Pathophysiology characteristics of MS were described also in 19th century. Initially, it was considered that MS is a vascular disease, which led to inclusion of anticoagulant medications in treatment of MS. However, in 1936, Greenfield and King showed that in the basis of plaques present in CNS of patients with MS is demyelinating process, along with axons loss.

First attempts of administration of therapy for MS are linked to William Hammond and 1871, when he tried to treat MS patients using different substances including chloride of barium, iron, nitrate of silver, hyoscyamus and strychnine (6).

Significant contribution was also provided by Pierre Marie (1884) who suggested the first classification of MS courses and marked infection as a potential trigger of MS occurrence.

1.2. PATHOPHYSIOLOGY OF MS

The nature of MS has been studied for centuries. One of the first theories, suggested in the XIX century, that MS origin was vascular, according to which changes in blood vessels and perivascular regions were responsible for MS development (6). Charcot marked glial hypertrophy as an initial event in MS pathology, while Pierre Marrie stated that MS is a consequence of an infection (6). Although etiology of MS is not yet completely understood, nowadays evidence emerges that crucial steps in the development of MS are neuroinflammation and neurodegeneration (16). Neuroinflammation is assumed to be a result of an abnormal reaction of the immune system to antigens of CNS (16). It is important to note that both innate and acquired immune systems play role in MS occurrence. This process is leading to the reaction of autoreactive leukocytes with the inflamed cerebral endothelium, and consequent breakdown of the blood–brain barrier (BBB) and occurrence of leukocytes in the CNS tissue (16). Many studies have been conducted in order to reveal initial step in this process and it is most likely that some infectious agents are the triggers of the cascade of events resulting in the MS onset. It seems that infectious agents promote the sensibilization of T lymphocytes against different proteins of infectious agents which have similar structure as some CNS proteins including myelin basic protein. This process is followed by strong immune reaction by host. Penetration of BBB consequently leads to immune reaction to many CNS antigens and production of different inflammatory mediators among which the greatest attention is focused on IL-12, IL-17, and IL-23 (16). Significance of these findings is additionally supported by the results obtained in studies that initiation of IFN-beta can positively influence the course of MS by decreasing levels of those inflammatory mediators (16).

Except T lymphocytes, B lymphocytes are also involved in the pathogenesis of MS. Namely, B lymphocytes produce antibodies which can also cross BBB. Intratechal production of antibodies was strongly supported by the presence of oligoclonal bands in cerebrospinal fluid which was discovered in 1942. Oligoclonal bands may be found in patients with certain diseases of inflammatory and infectious nature, however if these conditions are excluded, there is high probability of MS diagnosis (16).

The crucial pathologic sign of MS is plaque of demyelination found in the CNS, along with relatively preserved axons (17). Presence of plaques in different CNS structures leads to its damage

and causes a spectrum of MS symptoms. Plaques are usually described as active or inactive depending on the specific constituents, especially macrophages (17). Their structure varies and they can be found in each part of white and grey matter, however, they are usually located in following CNS structures: periventricular regions, optic nerves, brain stem, cerebellum and spinal cord (17).

Mechanism of demyelination is one of the characteristics that is used for the classification of MS pathologies in four categories – patterns (17). According to this, in the pattern I, macrophages play a key role. These plaques are characterized by sharp borders as well as surviving oligodendrocytes and remyelination. In the pattern II, there is an antibody complement related demyelination, with similar edges like in pattern I and predomination of macrophages and T lymphocytes. In this pattern survival of oligodendrocytes and remyelination have also been observed. The key characteristic of the pattern III is distal oligodendrogliopathy caused by hypoxia, and the edges are unclear. Additional feature of this pattern is loss of oligodendrocytes. In the pattern IV, it is considered that the underlying process of demylination is a primary oligodendrocytes loss. The presence of pattern IV plaques is restricted to PPMS (17).

1.3. MS DIAGNOSIS

Diagnosing MS represents a complex process. The main reasons for this are various symptoms of disease and different imaging findings (18). As a consequence, misdiagnosis of MS is not a rare issue. Keeping in mind that there is no any specific clinical feature of disease or diagnostic test specific for the disease, crucial step in MS diagnose is fulfillment of two criteria: time and space dissemination which means that in an individual at least two episodes of neurological symptomatology have to be recorded including different CNS areas, after having excluded other CNS disorders MS. Nowadays diagnosis of MS requires integration of clinical parameters, imaging procedures and laboratory testing. Inclusion of different techniques contributes to an earlier detection of disease and consequently better prognosis.

1.3.1. DIAGNOSTIC CRITERIA

The criteria for diagnosing MS have undergone significant changes in recent decades, but they have always emphasized the need to exclude other diseases that should be considered as differential diagnoses, bearing in mind that MS is one of the greatest imitators in neurology (19-21). Recommendations for the exclusion of other diseases that can present similarly to MS resulted from the fact that many diseases according to the clinical presentation, magnetic resonance imaging, as well as cerebrospinal fluid findings, may present similar to the presentation in MS (22-24), while on the other hand, it is well known that MS can have atypical presentation (22).

From the historical point of view, in the era before imaging techniques were available, diagnosis of MS was based on clinical parameters exclusively and all patients were classified as having clinically proven, probable or possible MS (25). After that, Poser and colleagues suggested addition of imaging procedures together with cerebrospinal fluid and evoked potentials analyses, according to which four different scenarios were possible: proven and probable MS which are clinically or laboratory confirmed (19).

However, in 2001 International Panel on Diagnosis of Multiple Sclerosis defined set of criteria named McDonald criteria (20) which are, with following revisions in 2005 (21), 2010 (26) and 2017 (18), most often used criteria currently. The main objectives of these criteria were shortening time from disease onset to MS diagnosis and early treatment initiation as well as high validity of diagnosis. Moreover, McDonald criteria made distinction between relapsing-remitting and primary progressive MS phenotype for the first time. Initial version of McDonald criteria was made using data mostly from European and North American populations below 50 years of age and with only one episode of neurological symptomatology indicative of MS (20). After criteria development, its applicability was confirmed in many countries and populations (27-37). The current revised version of McDonald criteria 2017 are presented in Table 1.

Table 1. The 2017 revised McDonald criteria for MS diagnosis

Number of clinical attacks	Number of lesions with objective clinical evidence	Additional data needed for a diagnosis of multiple sclerosis
≥2 clinical attacks	≥2	None*
≥2 clinical attacks	1 (as well as clear-cut historical evidence of a previous attack involving a lesion in a distinct anatomical location†)	None*
≥2 clinical attacks	1	Dissemination in space demonstrated by an additional clinical attack implicating a different CNS site or by MRI‡
1 clinical attack	≥2	Dissemination in time demonstrated by an additional clinical attack or by MRI§ OR demonstration of CSF-specific oligoclonal bands¶
1 clinical attack	1	Dissemination in space demonstrated by an additional clinical attack implicating a different CNS site or by MRI‡ AND Dissemination in time demonstrated by an additional

		clinical attack or by MRI§ OR demonstration of CSF-specific oligoclonal bands¶

*No additional tests are required to demonstrate dissemination in space and time. However, unless MRI is not possible, brain MRI should be obtained in all patients in whom the diagnosis of multiple sclerosis is being considered. In addition, spinal cord MRI or CSF examination should be considered in patients with insufficient clinical and MRI evidence supporting multiple sclerosis, with a presentation other than a typical clinically isolated syndrome, or with atypical features. If imaging or other tests (eg, CSF) are undertaken and are negative, caution needs to be taken before making a diagnosis of multiple sclerosis, and alternative diagnoses should be considered.

†Clinical diagnosis based on objective clinical findings for two attacks is most secure. Reasonable historical evidence for one past attack, in the absence of documented objective neurological findings, can include historical events with symptoms and evolution characteristic for a previous inflammatory demyelinating attack; at least one attack, however, must be supported by objective findings. In the absence of residual objective evidence, caution is needed.

‡The MRI criteria for dissemination in space are described in panel 5. §The MRI criteria for dissemination in time are described in panel 5.

¶The presence of CSF-specific oligoclonal bands does not demonstrate dissemination in time per se but can substitute for the requirement for demonstration of this measure.

1.3.2. DIAGNOSTIC PROCEDURES

Nowadays, diagnosis of MS is based on combination of clinical findings and additional diagnostic techniques including MRI, CSF analysis, etc. First lumbar puncture and CSF analysis were performed in XIX century by Quincke (38). Since the changes detected in the cerebrospinal fluid in patients with MS support the assumption that the nature of the lesion on MRI is inflammatory, the information obtained by examination of the cerebrospinal fluid can be extremely useful in situations where the criteria for dissemination in time needed to confirm the diagnosis of RRMS are not met, as well as in cases when the brain MRI is not specific enough (elderly) or when the clinical presentation of the disease is atypical (39).

The cerebrospinal fluid findings that support MS are (39):

1. Detection of intrathecal synthesis of immunoglobulin G (IgG) by the method of isoelectric focusing (IEF) of cerebrospinal fluid and serum by immunofixation, which includes:
a) the presence of oligoclonal IgG bands in the cerebrospinal fluid but not in the serum, or
b) the presence of oligoclonal IgG bands in the cerebrospinal fluid, with the presence of a small number of oligoclonal IgG bands in the serum whose number, distribution and intensity differ
2. Detection of elevated IgG index
3. The number of cells in the cerebrospinal fluid in patients with MS does not exceed 50 lymphocytes in mm^3, and the protein level is not more than 1g/L.

Magnetic resonance imaging (MRI) is the most sensitive neuroradiological technique for detecting changes in the brain and spinal cord in patients with MS. Furthermore, it is the most supportive instrument which can forecast conversion to MS in patients with CIS (40). According to the literature data, MRI findings characteristic for MS are found in majority (50%-70%) of patients

with CIS (41). It is estimated that persons with MRI documented lesions have a chance of 60%–82% for clinically definite MS, compared to the chance of 8%–25% in persons without MRI findings (42).

A typical finding for MS is the existence of zones of increased signal intensity (hyperintensive zones) on T2W (T2-weighted) sequence and proton density sequence, whose predilection sites are periventricular white matter, corpus callosum, deep white matter, infratentorial regions of the brain (brain stem and cerebellum), juxtacortical, as well as spinal cord (43). High-intensity zones (HIZ) on the T sequence of the brain can vary in size from a few millimeters to more than one centimeter in diameter. They are usually circular or elliptical in shape, relatively well demarcated, but can also have a very irregular shape in case of confluent lesions, and they generally have an asymmetrical arrangement. In patients with MS, MRI shows changes typical of this disease in about 90-95% of patients, which indicates the high sensitivity of this method, but the specificity of these changes is small because they can reflect various pathological processes in the brain: demyelination, inflammation, edema, gliosis, ischemia and axonal loss that are seen not only in patients with MS but also in other diseases of the CNS, and their presence is also possible in 20-30% of healthy people older than 40 years (44).

1.4. CLINICAL PRESENTATION AND EVOLUTION OF MULTIPLE SCLEROSIS

The clinical symptoms and signs of MS are extremely diverse. The huge diversity in clinical manifestations at the beginning and during the disease course reflects the presence of significant number of predilection sites for demyelinating plaques, including optic nerves, periventricular regions, brainstem, cerebellum, and cervical spinal cord. Motor, sensitive, visual, as well as symptoms and signs of the brainstem and cerebellum lesions are the most common clinical manifestations of MS. In addition, bladder and bowel dysfunction, as well as sexual dysfunction, fatigue, cognitive and psychiatric disorders are also frequently present manifestations. It should be emphasized that the clinical manifestations that may occur, as well as the clinical course of MS, are unpredictable. Numerous symptoms of MS can negatively affect daily life activities, physical, emotional and psychological functioning, and the quality of life of MS patients, and some of them can lead to severe disability. Therefore, it is important to point out that early symptomatic treatment can alleviate the risk of later morbidity, especially because symptoms may worsen or precipitate the occurrence of new ones.

1.4.1. SYMPTOMS AND SIGNS OF MULTIPLE SCLEROSIS

Motor symptoms and signs arise from involvement of the corticobulbar and corticospinal tract. Involvement of the corticospinal tract occurs in the first attack of MS in 32-41% patients and in 62% of cases during the disease (45). Signs of the cortico-spinal tract impairement may be minimal, in the form of pathological reflex activity, but often progress to severe spastic paraparesis. Babinski's sign is often present, sometimes as the only manifestation of corticospinal tract dysfunction. The most common motor deficit is limb weakness. Patients may have weakness in all four extremities, with a relatively weaker group of extensor muscles of the hands, and the symptoms in the legs usually appear earlier (45, 46). Of the particular importance is the possibility of acute development of respiratory muscle weakness, usually caused by lesions in the upper parts of the cervical spinal cord (45).

Cerebellar involvement leads to cerebellar ataxia, lesions of the posterior columns of the spinal cord lead to sensory ataxia, and those in the vestibular system to vestibular ataxia. Gait, trunk and limb ataxia, ocular and bulbar ataxia, as well as intentional tremor and titubation are caused by lesions of the cerebellum or its afferent or efferent pathways (45, 46). Approximatelly 75% of MS patients have balance disturbances during disease course (46).

Sensitive symptoms are the most common symptoms that occur as an initial clinical manifestation in MS (43%) and are present in more than 90% of patients during the disease (47). The anatomical distribution of the disorder depends on the location of the lesion, but is often unique and does not correspond to standard dermatomes, impaired sensitivity in the distribution of innervation of peripheral nerves, or the “homuncular pattern”(46). Symptoms may be localized on several fingers, may involve the hand or foot, or may present as a hemisensitive syndrome, a spinal cord injury syndrome, while a dissociation of sensibility may also occur. In MS, sensory impairment may occur in any anatomical distribution and with any combination of sensory impairment for pain, temperature, fine touch, and vibrational and deep positional sensory disturbances. Decreased perception of pain and temperature occur less frequently and indicates involvement of the spinothalamic tract. A rather specific sensitive manifestation in MS is the Lhermitte’s sign, which occurs in about 33% of patients (48). Patients with this sign complain of a sudden feeling of electricity that descends through the back and legs and occurs after neck flexion. It has been shown that about 94% of patients with this disorder have lesions on the cervical spine on MR (48, 49).

Visual symptoms in MS occur as a consequence of various pathological processes involving inflammation, demyelination, and degenerative processes in the axons of the afferent optic tract (50). Autopsy studies have shown that 94% to 99% of patients with MS have histologically confirmed optic pathway lesions. Involvement of the optic tract in MS leads to clinically evident

manifestations as in optic neuritis, but subclinical manifestations are also common. Clinical examination can reveal reduced visual acuity, impaired color vision, as well as visual field disturbance. Even in cases where patients do not report any visual symptoms, neurophysiological and other examinations may reveal subclinical changes involving the optic nerve, chiasm, and posthiazmal regions of the optic tract. Optic neuritis is a common manifestation of MS and occurs in 14% -23% of patients (51). It occurs unilaterally in 95% of cases, with a characteristic subacute development in several days. The pain that patients most often describe as "pain in the eyes and behind the eyes" occurs due to stretching of the optic nerve affected by the inflammatory process through the bone structures. Clinical examination reveals reduced visual acuity of varying severity. A typical visual field defect is a central scotoma, with color vision impairment present (52).

Lesions involving brainstem structures can produce a number of clinical manifestations: eye movement disorders, sensory disturbances, mimic muscle weakness, dizziness, hearing impairment, dysarthria, and dysphagia. The most common manifestations of brainstem dysfunction that occur in MS are disorders of eyeball mobility, i.e. diplopia, and nystagmus, which is most often horizontal and occurs in 40-70% in some studies (46).

Cognitive disorders in MS are common and are detected in 40-65% of patients, with dominant disorders of memory, attention, and information processing speed (53). They can occur in all stages and forms of the clinical course of the disease, although they generally occur less frequently in patients with RRMS compared with those with SPMS and PPMS (54). Cognitive impairment also occurs in 20-30% of patients with clinically isolated syndrome (55). Cognitive disorders have a significant impact on the quality of life, activities of everyday life, professional and social life of patients with MS regardless of the degree of their physical disability (56). Prevalence of depression in persons with MS is about 50% (57). Risk factors that increase probability of depression occurrence are female gender, younger age, family history of depression and high level of stress (58).

Sphincter disorders are among the most disabling features of MS and occur in up to 78% of patients during the course of the disease. Patients may complain of frequent urination, urgency and incontinence, reluctance to urinate, urinary retention, and nocturnal urination (59).

Sexual dysfunction is also common in people with MS. The term primary sexual dysfunction refers to a disorder that occurs directly due to a demyelinating lesion in the spinal cord or brain and includes erectile dysfunction, genital paresthesias, and decreased libido. Secondary sexual dysfunction includes symptoms that are not directly related to the genital system, but play a role in sexual functioning. These symptoms include fatigue, muscle weakness, spasticity, gait disturbance, pain and other unpleasant sensations, cognitive dysfunction and side effects of medications.

Fatigue is one of the most common symptoms of MS, occuring in 50-84% of patients and has a significant impact on mood, sleep and quality of life (60). This symptom is more common in progressive versus relapsing form of the disease (61).

1.4.2. NATURAL HISTORY AND EVOLUTION OF MULTIPLE SCLEROSIS

Although many improvements in MS diagnosis and treatment have been made, heterogeneity in clinical presentation and outcomes remains one of the key features of MS (62).

MS usually initially presents with a clinically isolated syndrome (CIS). CIS can be characterized with various symptoms depending on affected CNS area, although most frequently it presents as optic neuritis (7). A study of 156 persons with CIS diagnosis revealed that after 7 years, almost half of them (42%) experienced transition to clinically definite MS (63). Additionally, it has been shown that some MRI findings correlate with a greater chance for transition to definite MS such as lesions in white matter tracts associated to motor function, lesions in spinal cord, occurrence of new lesions etc. (40).

On the other hand, in recent years, presence of clinically silent lesions of white matter, incidentally revealed on MRI has been reported and labeled as a radiologically isolated syndrome (RIS) in 2009 (40). Persons with RIS have not experienced any clinical symptoms or signs of demyelinating disease. It was found that an average time between RIS and clinical disease onset is about 5.4 years (64). The RIS is usually diagnosed in persons performing MRI for other reasons such as migraine (40). According to the literature data, these white matter lesions indicative of demyelinating disease can be found in approximately 0.1-0.7% of general population (65).

Relapsing form of disease is characterized with intermittent occurrence of relapses and remissions. Time interval between two distinct relapses is arbitrary set at one month. Definition of relapse i.e. clinical attack includes duration of at least one day (24 hours), and absence of association with infection and fever (18). It is very important to make a distinction between relapse and a pseudorelapse which is a consequence of a preceding symptomatic or asymptomatic relapse (62). Patients with RRMS experience relapses with usually complete recovery at the beginning. However, over time, recovery following relapses becomes incomplete, leading to sustained disability accumulation (7). Finally, after approximately 10-15 years, RRMS progresses to SPMS (7) with transformation of relapsing form of diseases into slowly progressive course. It is estimated that more than three quarters of MS patients (80%) convert to progressive MS (66).

In a small proportion of MS patients, disease has a progressive course from the beginning (PPMS) (7). This form is characterized by steady disability accumulation from the disease onset (7). This MS phenotype may present in several forms among which most frequent is a progressive spastic paraparesis (7). It was found that the aging is strongly associated with progressive form of

MS (62). A decrease in proportion of patients with PPMS has been noticed recently, however it is probably due to administrative reasons, since different treatment modalities are predominantly available for RRMS (7).

1.5. TREATMENT OF MS

The treatment of patients with MS includes therapy for relapses, disease modifying therapies (DMTs) and treatment of MS related symptoms.

Relapses in MS are typically treated with short-term, high-dose intravenous methylprednisolone (67). Other treatment option is therapeutic plasma exchange (67).

Although there is no causal treatment for MS and it is considered as an incurable disease, treatment modalities such as DMTs can substantially reduce relapse rate and slow the disease progression. The mechanisms of their effects are modulation and suppression of immune system and are primary indicated for persons with RRMS. As a result of their administration, patients usually experience reduction in relapse rate, decrease in number and activity of MRI lesions and eventually slight improvement of pre-existing disability to some extent. The treatment with DMTs in MS started with the application of interferons and glatiramer acetate, however, so far many additional treatment modalities have been alos approved.

One of the most commonly used DMTs nowadays is ocrelizumab, monoclonal antibody against B cells, included in MS therapy in 2017 (68). It has shown high effectiveness in preventing relapses and quieting progression of the disease in relapsing MS. At the same time it is the only currently available treatment for PPMS. Although it is generally well-tolerated, herpes viral infections have been identified as serious adverse effects (69). Patients receive ocrelizumab as an intravenous infusion in 24 weeks time intervals.

Natalizumab acts as an antagonist of alpha-4-beta-1 integrin which mediates passing of T-lymphocytes through BBB to different CNS areas (70). Similarly to ocrelizumab, it is administered intravenously, although in shorter time intervals, every four weeks. The greatest risk for MS patients receiving natalizumab is potential development of progressive multifocal leukoencephalopathy (PML) which depends of duration of treatment, titer of John Cunningham (JC) virus antibodies detected in serum and previous treatment with immunosuppressive drugs (71).

Fingolimod was the first oral medication approved for RRMS treatment. Different adverse reactions have been observed and among them most common are irregularities in markers of liver function (72-75). Also, it is suggested to pay special attention on patient, six hours following the administration of first dose of fingolimod, due to recognized adverse cardiovascular events (76).

Dimethyl-fumarate is another treatment option with proven anti-inflammatory activity (77), however it is also linked to slightly increased risk of PML (78).

Teriflunomide is another oral treatment for RRMS (79). Adverse events have also been noticed, however effects of teriflunomide can be rapidly neutralized by orally administered cholestyramine.

Interferon beta (IFN-beta) decreases frequency of relapse occurrence and slows MS progression ells (80). IFN-beta has some adverse effects such as flu-like symptoms (81), and majority of them can be occupied by anti-inflammatory drugs.

Glatiramer acetate is also frequently used in RRMS patients. Adverse reactions usually occur after application of glatiramer acetate and include skin reactions at the site of administration (82-83).

Besides aforementioned therapeutic options, alemtuzumab and cladribine are also used in treatment of RRMS, although less common compared to previous treatment modalities.

Treatment of SPMS is a specific issue in MS management. Recently, siponimod has been approved for therapy of this MS phenotype (84), while some treatment options for RRMS can also be used including ocrelizumab and cladribine.

Symptomatic therapy of MS depends on the presence of various MS symptoms and signs and includes a spectrum of drugs for treatment of spasticity, psychiatric disorders, fatigue, pain, sphyncterial dysfunction etc.

1.6. PROGNOSIS OF MULTIPLE SCLEROSIS

Prediction how MS will progress in every single patient is extremely difficult. One of the reasons is that the severity of the disease varies widely from one patient to another. However, a majority of people diagnosed with MS will experience a relatively normal life span (85). Still, although MS is rarely fatal disease, research has shown that persons with MS live approximately 7 years shorter than general population (86). This has been reported in Norwegian cohort study that included all MS patients whose disease started in time period from 1953-2012. In this study median longevity in MS patients was 74.7 years, compared to 81.8 years in general population (86). Furthermore, female MS patients were living longer (77.2 years) than men (72.2 years). In addition, it was observed that there is a difference in life expectancy between different MS phenotypes. Namely, patients with RRMS were living longer (77.8 years) in comparison to patients suffering from PPMS (71.4 years) (86). According to the results of this study, cause of death in more than a half of MS is a MS disease by itself (56.3%). Other reasons are cardiovascular and cerebrovascular diseases (14.8%), malignancies (14.1%), respiratory and infectious diseases (3.8%), accidents and suicide (4.4%), while 6.5% of MS patients die from other causes (86). Finally, authors concluded that in MS patients risk of death expressed through mortality rate is about three times higher compared to general population (86).

Many studies investigated the role of different factors in MS prognosis and their results are conflicting. Better understanding of these factors is substantial for patients, their families, doctors and researchers. Swedish study published in 1993, which presented results from 25-year follow-up period pointed out that, at the moment of disease onset, most valuable long-term prognostic factor for disease outcome is disease phenotype with worse prognosis in case of primary progressive course (87). Additionally, they analyzed which factors in situation of acute onset are associated with better prognosis, and revealed that they were: younger age at disease onset, first attack of disease followed by optimal recovery, presence of afferent nerve symptomatology and symptoms in only one area (87). Also, they found that five years following disease onset, factors associated with better prognosis were small number of affected CNS areas, lower disability score and optimal recovery after last relapse (87).

Later investigations (88) revealed that prognostic factors associated with poor prognosis in MS patients are male gender, older age at disease onset, initial motor, cerebellar, and sphincter symptomatology, progressive disease phenotype from the start, short time intervals between two relapses, frequent relapses at disease onset and absence of complete recovery after relapses at disease onset (88). Moreover, it was suggested that the most useful tool for prognosis of conversion to definite MS diagnosis is MRI (88).

Similar results were obtained in a more recent review (89). It was found that features that are negatively associated with disease prognosis regardless course of disease are progressive disease phenotype as well as disability level two and five years after disease onset (89). In patients with RRMS and SPMS unfavorable prognostic factors were conversion of relapsing to progressive disease course, high relapse frequency, high level of disability during first five years after disease onset, short time between first and second relapse as well as affection of different CNS areas (89). Specific prognostic factor in SPMS is time to transition from relapsing to progressive form, with shorter period being associated with poor prognosis. Specific prognostic factor for PPMS were higher disability level after two and after five years of disease duration (89). Based on all of these findings, authors concluded that in order to improve MS patients' prognosis, efforts should be directed to the relapse frequency, disability in first five years of disease and progression onset (89).

1.7. EPIDEMIOLOGY OF MULTIPLE SCLEROSIS

According to the Atlas of MS from MS International Federation a total of 2.8 million people worldwide live with MS in 2020 (90) with corresponding prevalence of 36 MS cases per 100,000 people at the global level. This is a significant increase from 2013 when 2.3 million people with MS were registered (90). An increasing tendency of MS prevalence has been noticed continuously (91), and there are different explanations for this, including change in diagnostic criteria, improved

diagnostic possibilities, increased prevalence of risk factors etc (18, 92). Additionally, experts in the field of MS were asked to list probable reasons for 0.5 million increase in MS prevalence from 2013 to 2018 and the most common explanations were improvement in MS diagnostic criteria, in MS treatment and better possibilities for data collection (90).

It is well known that the prevalence of MS is related to the latitude with increasing prevalence with higher latitude, however opposite results have also been demonstrated in Norway and United States (93). Worldwide, countries with the highest prevalence of MS are San Marino (337 cases per 100,000 people), Germany (303/100,000), USA (288/100,000), Canada (250/100,000), Sweden (218/100,000), Finland (218/100,000), Iceland (212/100,000) and Italy (208/100,000) (90). Prevalence of MS between 100 and 200 cases per 100,000 is found in Australia (104/100,000), and many European countries such as France (155/100,000), United Kingdom (196/100,000), Spain (120/100,000), Austria (153/100,000) and Serbia (136/100,000) (90). The lowest prevalence of disease has been reported for parts of Central and South America, Africa and South-Eastern Asia with the prevalence between 0 and 25 cases per 100,000 inhabitants (90). When prevalence is analyzed according to WHO regions, the highest prevalence is observed in Europe (133/100,000), followed by Americas (112/100,000), while the lowest prevalence was noticed in Western Pacific WHO region (5/100,000) (90).

At the same time, leading countries in incidence of MS are Austria (19.5 new cases annually /100,000), Germany (17.6/100,000), Canada (12.2/100,000), United Kingdom (10/100,000), Lithuania (12.4/100,000) and Egypt (10/100,000) (90). Among countries with MS incidence ranging from 6.0 to 9.99 new cases per 100,000 there are USA (7.9/100,000), Sweden (8.7/100,000), Iceland (7.8/100,000), Ireland (6/100,000), France (6.2/100,000), Netherlands (9/100,000), Czech Republic (6.5/100,000), Iran (6.1/100,000), Kuwait (6.4/100,000) and Australia (6.7/100,000). The lowest incidence values are again recorded for parts of Central and South America, Africa and South-Eastern Asia with prevalence between 0 and 1.99 newly diagnosed cases per 100,000 inhabitants (90). Based on data from 75 countries, it is estimated that the incidence at global level is 2.1/100,000 (90). In general, incidence rate is more precise estimate compared to disease prevalence, however, data on incidence is lacking for many countries. An important issue in determining incidence of MS is that, keeping in mind that disease can vary significantly in symptomatology, sometimes there is a long delay between disease onset and MS diagnose. Consequently, at the moment of making diagnose MS case is not incident any more, but prevalent. Due to these reasons, in reporting epidemiology of MS, prevalence is used rather than incidence rate. However, incidence estimates are very important since it tells about the risk of developing disease in certain population.

1.7.1. DEMOGRAPHIC CHARACTERISTICS OF PERSONS WITH MS

MS affects women more frequently. According to the last update of MS atlas, worldwide, 69% of persons with MS are females, while 31% are men (90). Female-to-male ratio is the greatest in the Western Pacific and South-East Asia regions. Among WHO regions percentage of women in total number of persons with MS ranges from 66% in Eastern Mediterranean region to 78% in the Western Pacific region. However, according to the literature data, differences between genders were not so evident before. At the beginning of 20th century proportions of females and men with MS were almost the same. Since that period, proportion of female patients with MS has started increasing up to nowadays, reaching female-to-male ratio of approximately 3:1 (94). The underlying causes for these differences in male-to female ratio are not completely understood, however, it is believed that genetic, physiological and lifestyle characteristics may play role in these differences (95). One of the key explanation is raise in prevalence of women who smoke (96). It is noteworthy that collecting data on MS according to the gender distribution will help to take insight into this issue.

MS diagnosis can be made at any age, however, it is usually diagnosed in young people. The average age of diagnosing MS is currently 32 years worldwide (90). Value differs for various WHO regions but is in range from 30 to 33 years (90). Variations are more pronounced at the country level, with age at diagnosis between 20 and 50 years (90).

Although MS usually affects adults, it can occur during the childhood as well. It is estimated that more than 30,000 children worldwide live with MS (90). Similarly to adult MS, an increase in total number of persons with MS has been observed in the population younger than 18 years (90).

1.7.2. ETIOLOGY OF MS

The etiology of MS is still unknown, and the results of numerous studies in various fields indicate the etiological heterogeneity, i.e. the importance of genetic and environmental factors, as well as their interactions.

Modern epidemiological studies of environmental risk factors for MS could open space for potentially ending processes that occur at the earliest stage of MS, before it becomes clinically evident. Identifying and researching people at high risk for developing MS would be useful for a better understanding of the causal cascade processes in MS, which is extremely important for potential strategies for preventing this disease (39).

A large number of studies explored genetic susceptibility for MS onset. Majority of these studies focused on HLA DRB1*15:01 and HLA A*02 as well as their interactions with different environmental factors such as smoking, viral infections and obesity (39).

Infections are the most commonly investigated environmental risk factor in the etiology of MS. The infectious hypothesis, first postulated at the end of the 19th century, is based on findings obtained in MS incidence and migrant studies, as well as the existence of possible clusters of disease (97). Based on the data obtained in these studies, as well as a large number of experimental studies in virology, immunology and genetics, several key facts have emerged that indicate the role of infections, especially those caused by viruses, in the development of MS: a) exposure to infectious agents in late childhood is often present in people with MS; b) viral infections are associated with the occurrence of disease exacerbation; c) the geographical distribution of MS may be related to differences in exposure to infectious agents; d) several possible MS clusters have been identified; e) the change in the magnitude of the risk of developing MS is associated with migrations from one geographical area to another at a younger age; f) more frequent occurrence of MS in environments with a high degree of urbanization, good hygienic conditions, higher socio-economic status, cold and humid climate; g) pathological immune response to infection caused by various viruses in persons with MS; h) the presence of a high concentration of oligoclonal immunoglobulin G (IgG) in the brain and cerebrospinal fluid of persons with MS; i) the possibility that in animal models, viruses cause diseases with a long incubation period, relapse-remittent course and demyelination (39).

There are a large number of infectious agents that have been studied as possible risk factors for the development of MS including Epstein-Barr virus (EBV), Human herpesvirus type 6 (HHV-6), Herpes simplex virus (HSV), Cytomegalovirus (CMV), Varicella zoster virus (VSV), Human endogenous retrovirus (HERV), Rubella virus, Measles virus, Mumps virus, Human T-cell, leukemia virus type 1 (HTLV-1) and different Corona viruses (39).

Highly consistent findings from different types of epidemiological studies (case and control studies, ecological, cohort, cluster analysis and migrant studies), as well as serological and experimental studies, indicate that EBV is the most significant "infectious" candidate in the etiology of MS (98). The contribution of an EBV infection to increased susceptibility for MS development is at least partially explained by impact of the virus on gene transcription (a), especially in combination with HLA-B*07 and DRB1 positive individuals (99, 100).

Insufficient UV radiation and vitamin D levels are also among most frequently studied risk factors associated with increased risk of MS. The distribution of MS is not unique worldwide, but raises with increasing distance from the equator (1). Sun exposure is an important source of vitamin D for most people and experimental and epidemiological data indicate that vitamin D is a mediator of the effect of sun exposure (101). A plethora of studies examined the association between sun exposure and MS and showed an increased risk in persons with insufficient sun exposure since childhood (102). Similar situation has been observed for lower 25OHD levels (103). It is well

known that vitamin D plays a role in neuroprotection and immunomodulation, as well as in the prevention of infections, potential triggers of autoimmune processes in MS (104). Therefore, hypotheses that increased sun exposure and adequate dietary intake of vitamin D could contribute to the prevention of MS are sustainable, as they are consistent with data on the geographical distribution of the disease.

Together with infections and lower sun exposure and vitamin D levels, smoking represents one of three most significant factors contributing to increased risk of MS. Although smoking cannot be linked to the prevalence gradient of MS and changes in the risk of disease due to migration, today it is considered a very significant risk factor for this disease, as evidenced by well-documented data and consideration of new causal links on the pathogenesis and natural course of MS. It has been shown that age at which person start smoking is not relevant, however there is an undoubted dose-response relationship between smoking and elevated risk of MS (105). Additionaly, it has been observed that the risk decrease after smoking cessation (106) and that passive smoking also increases risk of MS (107). There are numerous mechanisms that explain the influence of smoking on the development of MS. These include modulation of the immune response, damage to axons by nitrogen oxides from tobacco, demyelination caused by chronic cyanide intoxication, as well as more frequent respiratory infections (108).

Among other important risk factors for MS there are obesity during childhood and adolescency, diet and supplements, gut microbiota, reproductive factors, air pollution and others (1).

1.8. CONCEPT OF COMORBIDITY

The comorbidity can be defined as the presence of one or more co-occurring conditions or diseases, and it refers to the total burden of diseases other than the illness of interest (109, 110). On the other hand, term multimorbidity is used to describe existence of several chronic diseases in one individual (111). It is worth mentioning that comorbidity definition excludes complications that are a direct consequence of the disease (112). The comorbidity is frequently present in general population, and as it is expected, its prevalence rises with age (113). For example, in 2005, presence of at least one chronic condition was registered in over 130 million of American population (114). Having in mind that prevalence of chronic diseases raises with age, on one side and prolongation of lifespan on the other, it is apparent that the number of people living with some comorbidity will increase at the global level.

In recent years, more attention has been paid on comorbidity since evidence emerges that it can be associated with many adverse health outcomes such as lower health-related quality of life and increased mortality. Furthermore, it has been shown that presence of comrobidity is linked to

the additional costs for healthcare system (111). This is especially important bearing in mind that in many countries worldwide, hospitalizations are the greatest contributors to resource use (115). Examination of comorbidity may help to elucidate potential shared etiological factors of two or more distinct entities (116). However, assessment of the influence of comorbidities is a complex process since there are no unique definition and measurement criteria (111). Additionally, different sources of comorbidity data that can be used (self-report, medical records, administrative data) make it difficult to get a deep insight into this area (116).

1.8.1. MECHANISMS OF COMORBIDITY OCCURENCE

Valderas et al. in their review regarding comorbidity explained that presence of at least two different conditions in one person can occur in three situations: by chance (especially in case of diseases with high prevalence in general population), selection bias (people using healthcare services have greater chance to be diagnosed compared to those who do not seek medical care) and causal association (111). Further, they described that there are four potential models of causal association for the presence of comorbidity in one individual (111). In the first one, direct causation model, it is assumed that the one disorder is direct consequence of another disease or even its treatment. Associated risk factors model emphasizes relationship of the associated risk factors with occurrence of different diseases, i.e. association of risk factors makes it more possible for two diseases to occur together. Third model, heterogeneity model, involves different risk factors, which are not associated; however, one risk factor may lead to occurrence of other disease associated also with another risk factor. Finally, independence model assumes that the presence of two different health conditions is related to the presence of third one.

1.8.2. COMORBIDITY IN MS

It has been shown that the presence of comorbidity is common in patients with chronic disorders such as multiple sclerosis (MS) (97). Comorbidity is one of major patients' characteristics, which include genetics, age, gender, race, ethnicity, and socioeconomic status, relevant to the clinical presentation and management of MS (99).

The main goal of investigation of comorbidities in persons with MS is to improve the outcome of the disease and health of the patients (98). Previous studies (100) reported that more than a half of persons with MS have some comorbidity. Therefore, it is an area of increasing interest as evidence emerges that it can have impact on a range of outcomes in MS patients (104). An increasing amount of evidence suggests that comorbidities might affect the diagnostic delay between MS onset and diagnosis (102). Furthermore, comorbidities can affect treatment decisions, including the choice whether to start treatment, the specific choice of treatment, and its subsequent

effectiveness (98). Additionally, it has been shown that MS patients with one or more comorbidities have a two-fold higher all-cause hospitalization rate than MS patients without any comorbidity (101). Marrie et al. reported that MS patients with vascular comorbidities, any time during MS course, progressed to an EDSS of 6 on average 6 years faster than MS patients who have never had a vascular comorbidity (103). The presence of comorbidity has been associated with decreased health-related quality of life and progression of lesion burden on magnetic resonance imaging (98). Finally, within the MS population, comorbidity is associated with a two-fold increased mortality risk (105). Recent systematic reviews revealed that the five most prevalent comorbidities in MS are depression, anxiety, hypertension, hypercholesterolemia and chronic lung disease (98).

Although physical and mental comorbidities are increasingly recognized as relevant to clinical outcomes, gaps in the understanding of epidemiological aspects of comorbidity in MS are still present (106). Firstly, studies of the frequency of comorbidity in MS compared with the general population are rarely designed as population based (104). Moreover, there are huge variations in stated prevalence of different comorbidities in patients with MS, and those variations are attributed to number and category of comorbidities that are analyzed, and characteristics of population in which study is performed. Having this in mind, it is clear that variations are even more present in calculating incidence of comorbidities in MS patients. Although presence of comorbidities in MS is strongly influenced by population characteristics such as age structure, gender distribution etc, estimates of comorbidity frequency and distribution are hardly ever reported as age- and gender-specific rates (104). Precise estimates of comorbidity burden, including incidence and prevalence are significant as indicators of alterations in population characteristics of certain exposures (104). Finally, these measures can help in post-marketing pharmacovigilance (104).

Based on all aforementioned, it is obvious that investigation of comorbidities in MS population can potentially improve many aspects of this neurological condition such as prognosis, therapeutic approach, elucidating exposures associated with disease occurrence and its underlying mechanisms. Research of comorbidity could eventually explain phenomenon of extreme heterogeneity in health outcomes of MS patients. And as a final point, better understanding of comorbidity would probably enable its earlier diagnosis and improved treatment which in return could lead to slower disease progression and higher degree of health-related quality of life.

1.8.3. MOST COMMON GROUPS OF COMORBIDITIES IN MS

1.8.3.1. Psychiatric comorbidity

Psychiatric comorbidities are among most prevalent conditions occurring in persons with MS. While 1in 3 MS patient is suffering also from some kind of physical comorbidity, every second MS patient has some psychiatric comorbidity (117-120). Additionally, studies have revealed that

among all psychiatric comorbidities, MS patients have the highest chance for developing depression and anxiety (110). What is more important, it has been shown that MS patients who have already developed some somatic comorbidity, have a higher risk for occurrence of some psychiatric disease, compared to MS patients without any physical comorbidity (121, 122). Similarly, the association between physical disease and comorbid mental disease occurrence has been reported for rheumatoid arthritis (123).

In a study by Marrie et al. (124) it was found that prevalence of psychiatric disorders in MS population is higher when compared to general population. This observation was especially reported for depression, anxiety and bipolar disorder (125). Also, in another study it was reported that psychiatric disorders occur frequently during MS course, however, they can be present even at the moment of disease onset (125). Results of the study showed that anxiety is present in 1.24% - 36% of persons with MS (125). Prevalence of depression was 6.94% - 70.1%, based on questionnaire assessment, and 3.80% - 68.4% when other instruments for data collection were used (125).

1.8.3.2. Autoimmune comorbidity

Although many comorbidities in MS have been studied, special attention has been paid on autoimmune diseases. Since MS is considered as a disease of autoimmune nature, it is not surprising that researchers expect that investigation of autoimmune conditions occurring in these patients could put some light on MS etiology and reveal some unknown contributors to the disease onset (126). Marrie et. all published in 2015 a systematic review on prevalence of autoimmune disorders in people with MS and analyzed 61 single study examining this group of comorbidity. Their findings were that the most frequently occurring autoimmune diseases in MS patients were thyroid disease which was diagnosed in 2.08%-10% of observed MS patients and psoriasis (found in 0.39%-7.74% of sample) (126). Despite same nature of these comorbidities and of MS, prevalence of autoimmune disorders in MS is usually under 5% (126). However, there are some disorders with prevalence greater than 5% including abovementioned thyroid disease and psoriasis as well as type 1 diabetes and celiac disease (126).

1.8.3.3. Cancer comorbidity

Research has shown that persons with MS can also be affected by malignancies, similarly to general population. However, it was revealed that in comparison with general population, MS patients experience malignant comorbidities less often (127). In a systematic review of 38 studies of cancer comorbidity in MS it has been found that the leading malignant comorbidities are cervical (stated prevalence varying from 0.06% to 0.67%), followed by breast (found in 0.38-2.3% MS

patients) and gastrointestinal cancer comorbidity (prevalence of: esophageal cancer 0.01%, stomach cancer 0.02-0.48%, liver cancer 0.02-0.31%, colorectal cancer 0-0.7%) (127). Detailed analysis revealed that some malignancies including brain tumors and tumors of urinary tract occur more frequently in MS patients compared to general population, while, on the other side, some cancers such as ovarian, testicular, prostate and pancreatic are rarely present in MS population (127). It is assumed that research of cancer comorbidity in MS could be beneficial having in mind that immune system of an individual has a major role in development of both, MS and malignant diseases. Therefore, this could help in understanding role of continuous inflammation and administration of immunosuppressive therapeutics in development of malignant comorbidities (127).

1.8.3.4. Sleep disorders and seizure

Similarly to general population, presence of sleeping is frequently affected in MS population as well. Identification and appropriate management of this comorbidity in MS is of great importance due to its impact on every day activities and overall quality of life on one side, and duration on the other. Majority of research conducted so far was directed to investigation of restless legs syndrome and sleep apnea. It was found that the restless legs syndrome is quite common in MS population with reported prevalence in range from 14.4% to 57.5% (128). Estimates for sleep apnea are almost identical with values in range from 7.14% and 58.1% (128). When it comes to seizures, results of studies indicate that this disorder appear in 3.09% of MS patients, while the risk for their occurrence (incidence rate) is 2.28% (128). It has also been reported that in comparison with general population, seizures are more frequently present in persons with MS, which can be attributed to underlying processes in MS - inflammation and demyelization (128).

1.8.3.5. Cardiovascular comorbidity

Bearing in mind high prevalence of cardiovascular comorbidities in general population, it is not surprising that they are frequently found in MS population. Most common cardiovascular comorbidities are hypertension (with prevalence between 0% and 47.8%), hyperlipidemia (with prevalence between 3% and 47.8%) and diabetes (with prevalence between 0% and 27.1%) (129). Investigation of cardiovascular comorbidity burden in MS has been considered very important since evidence suggest that vascular risk factors may be involved in many unfavorable MS outcomes such as disability level and its progression, MRI findings and satisfaction with quality of life (130, 131). Additionally, it is assumed that endothelial status in an individual mirrors status of blood vessel health. However, this concept, called “vascular age” is not yet completely understood and implemented. Concept of vascular age has been initially proposed in study by D’Agostino et al. (132). Vascular age has been defined as age of some person corrected according to atherosclerotic

burden. This means that in some individual, chronological and vascular age may differ significantly (133).

Additionally, studies revealed that, when presence of cardiovascular comorbidities in MS population is analyzed, that they are more frequently observed in male MS patients as well as in older persons with MS (129). History of stroke or ischemic heart disease was identified in less than 5% of MS patients (129). Finally, when the risk of cardiovascular comorbidity occurrence was analyzed in comparison to general population, it was concluded that ischemic heart disease, congestive heart failure, ischemic stroke and peripheral vascular disease are entities more common in MS population compared to general population (129). This might be a consequence of a high prevalence of well-established cardiovascular risk factors in MS patients, including high prevalence of smoking, obesity and absence of physical activity (129).

1.8.3.6. Other comorbidities

Aside from abovementioned comorbidities, which have the highest observed prevalence in MS, other groups of co-occuring conditions may be present as well. Among them it is noteworthy to mention gastrointestinal, musculoskeletal and pulmonary diseases (134). Among gastrointestinal disease most prevalent is irritable bowel syndrome, and among pulmonary diseases, chronic lung disease has the highest prevalence (both conditions have prevalence greater than 10%) (135). Furthermore, arthritis is labeled as most frequent musculoskeletal comorbidity with prevalence estimates 2.97% - 26%, while glaucoma with prevalence 0.74% - 12.1% and cataracts (prevalence 1.24% to 3.5%) are leading ocular comorbidities in MS (134). Renal disease is present in 2.5% of MS patients (134). Nevertheless, all of these numbers should be carefully interpreted since number of studies investigating less common comorbidity in MS is still very small.

2. AIMS OF THE STUDY

The aims of this doctoral dissertation were:

1. To estimate the prevalence of groups of comorbidities in MS patients based on Belgrade Population Registry data with special emphasis on cardiovascular disorders;

2. To determine the difference between chronological and vascular age in patients with MS;

3. To investigate relationships of cardiovascular comorbidites with MS course.

3. MATERIAL AND METHOD

3.1. STUDY DESIGN

The research was organized through several observational study designs: cross-sectional, case-control and cohort study. Studies were conducted at the Clinic of Neurology, Clinical Center of Serbia, and at Clinic of Neurology and Clinic of Endocrinology, Diabetes and Metabolic diseases, Clinical Center of Serbia, Belgrade, in the period 2016-2019.

3.2 SELECTION OF PARTICIPANTS

a) Belgrade population MS Registry (source of data for cross-sectional study)

For the purposes of cross sectional study, Belgrade population MS Registry was used as a source of data. This registry was initiated in 1996 and since then has been regularly updated at the Clinic of Neurology, Clinical Center of Serbia, which is a national referral center for MS in the Republic of Serbia. It contains data on each person with MS living on the territory of Belgrade region which is Capital of Serbia with total of 1.7 million inhabitants. The last day of 2019, December 31, was used as the prevalence day. On that day there was a total of 2725 registered MS cases (with diagnose confirmed according to McDonald Criteria) that were alive and currently living in Belgrade region.

Registry contains all the relevant data related to demographic and clinic characteristics of included subjects. Among demographic variables, data on age, gender, highest educational level, current employment and marital status, address and district where person is living, home and mobile phone are being collected. Clinical characteristics included in this registry are the year when MS diagnosis was confirmed, results of diagnostic procedures (oligoclonal bands, MRI findings), family history of MS and relationship, treatment (current and past along with its duration), date of the last appointment at neurologist, actual MS phenotype and EDSS score (136), data on the presence of comorbidity, status (alive/died), name of doctor in charge and neurological clinic where patient is treated (137).

Presence of one or more comorbid condition was recorded in the registry only if it was diagnosed and documented with appropriate written report or/and laboratory findings. Included in the registry were all existing comorbidities that fulfilled these criteria, however, for the purposes of this study, a list of comorbidities for investigation was made according to the literature data (138). The list included several groups of comorbidities that are most relevant for MS cases in terms of their frequency in persons with MS and impact on different aspects of the disease. Those groups were cardiovascular diseases, malignant diseases, autoimmune diseases, epilepsy, type II diabetes and psychiatric disorders. Cardiovascular diseases group included ischemic heart disease,

cerebrovascular disease, cardiac disease, hypertension and hyperlipidemia. Malignant diseases group comprised any diagnosed malignancy in MS patients reported in the registry. Autoimmune diseases group had the highest number of included disease – 12: psoriasis, vitiligo, autoimmune thyroid disease, rheumatoid arthritis, systemic lupus erythematosus, uveitis, dermatomyositis, systemic sclerosis, pernicious anemia, Sjogren's syndrome, primary biliary cirrhosis and inflammatory bowel disease. Psychiatric disorders observed were anxiety, depression and all other psychiatric disorders together.

b) Selection of participants for case-control study investigating glucose metabolism in MS patients and healthy controls.

In the case-control study we included 78 patients with MS and a total of 26 healthy individuals. Patients' recruitment was performed in the period November 2017 – March 2018, during regular neurological examinations at the Clinic of Neurology, Clinical Center of Serbia. All patients were examined by one of three experienced neurologists (JD, SM or OT). During visit, patient EDSS was recorded. For this part of evaluation necessary demographic and clinical data were obtained from the Belgrade population-based MS registry. Besides EDSS, for each person with MS, Multiple sclerosis severity score (MSSS) and Progression index (PI) were determined. For the calculation of MSSS, current EDSS score (as recorded in participants' clinical records) and time since disease onset, expressed in years, were used. On the other side, dividing EDSS with duration of the disease resulted in PI (139). Disease duration was calculated as the time period from the first attack of neurological symptomatology suggestive of MS, to the prevalence day, December 31, 2019. EDSS scores were recorded either before the relapse or at least 2 months following the relapse. Inclusion criteria for persons with MS included in case-control study were no history of corticosteroid therapy or relapses in past three months (i.e. inclusion criteria was the stable phase of MS).

Inclusion criteria for healthy controls were place of residence in the same area as for cases and similarity to MS patients in terms of age, gender distribution and the body mass index (BMI) ($p>0.05$). In each MS patient following anthropometric measurements were performed: body weight, body height, waist circumference and fat mass percentage. All these measurements were done using standard WHO protocols (140). BMI was determined by dividing body weight (expressed in kilograms) with body height (expressed in squared meters). Body composition as well as the amount and the distribution of the body fat were determined by bioelectrical impedance (Tanita Body Composition Analyzer). Blood samples for each participant were taken after overnight fasting for the evaluation of plasma glucose values (measured by a glucose-oxidase method; Beckman Instruments, Fullerton, CA, USA), plasma insulin levels (determined with RIA,

INEP, Belgrade, Serbia), total cholesterol (Ch), high density lipoprotein (HDL-Ch) and triglycerides (analyzed using commercial enzymatic kit; Boehringer Mannheim GmbH Diagnostics), while low density lipoprotein (LDL-Ch) levels were calculated by using standard Friedewald formula. Additionally, HbA1c levels (commercial kit, SEBIA, France) were measured by spectrophotometry and alanine aminotransferase (ALT) by enzymatic method. Following collection of fasting blood samples, in both groups a 2 hour oral glucose tolerance test (OGTT) was conducted. Participants were asked to ingest glucose solution (75g of glucose) in two minutes and blood samples were collected five times, at 0, 30, 60, 90 and 120 minutes for determination of plasma glucose and insulin levels (141). Homeostasis model assessment of insulin resistance (HOMA-IR) was computed using following equation: fasting glucose (milimoles per liter) x fasting insulin (microunits per milliliter) / 22.5. If in one person value of HOMA-IR were greater than or equal to 2.5 this person was considered as having IR.

c) Selection of participants for case-control study investigating difference between chronological and vascular age in patients with MS

All MS patients from the Belgrade population MS Registry were considered for inclusion in the study. General exclusion criteria were: age below 18, heart failure and impaired renal and liver function. After evaluation of exclusion criteria, according to the presence of hyperlipidemia, hypertension, type 2 diabetes and history of coronary artery disease (CAD) participants were assigned to the following 5 groups:

1. Control group consisting of MS patients without evidence of hyperlipidemia, hypertension, type 2 diabetes and CAD
2. MS patients with hyperlipidemia
3. MS patients with hyperlipidemia and hypertension
4. MS patients with hyperlipidemia, hypertension and type 2 diabetes
5. MS patients with CAD

The groups were created in that manner that they range from a MS patients group with healthy endothelium to groups with gradually increasing degree of endothelial damage. The sample size was assessed keeping in mind the fact that it is conventionally estimated that multiple regression models require that the minimum ratio of valid cases to independent variables be at least 10 to 1.

For purposes of this study particular questionnaire was designed and used. It comprised questions regarding socio-demographic characteristic of participants (age, profession, occupation, place of residence), as well as the presence of cardiovascular risk factors: smoking status (current

and ex-smokers), presence of cardiovascular disease in family, body height and weight (BMI), as well as waist circumference, and physical activity. In all subgroups of patients, levels of biochemical parameters (fasting blood sugar, HDL, LDL, triglycerides) associated with presence of vascular conditions were determined.

d) Selection of participants for retrospective cohort study

In this study subgroup of MS patients with CAD represented exposed sub-cohort. Exclusion criteria was history of CAD before the time of confirmed MS diagnosis. Unexposed sub-cohort was selected among patients in MS population registry without history of CAD. Each participant in exposed sub-cohort was individually matched with corresponding unexposed respondent, according to gender and chronologic age and MS course at the time of diagnosis of CAD. All participants were evaluated in terms of their MS course and level of physical disability (EDSS) from the time point of inclusion in the study to the end of the follow-up or death.

3.3. STATISTICAL ANALYSIS

After designing and completing database with all comorbiities in MS patients in Belgrade region, statistical analysis was performed. The first step in statistical analysis was the calculation of the prevalence of comorbidities in persons with MS. The prevalence was determined by dividing number of certain comorbidity/comorbidities among all cases with MS. Additionally, each prevalence value was presented with corresponding 95% Confidence Interval (CI), calculated according to Poisson's frequency distribution for rarely occurring events (142).

In order to allow international comparisons of prevalence of comorbidities in Belgrade MS population, crude values were age- and gender-adjusted using European and World standard population (143). Moreover, the frequency of different comorbidities was calculated for different MS phenotypes (RRMS and SPMS versus PPMS) and compared using Chi-square test. The difference in average PI between sub-group of MS patients with no comorbidity and sub-group of MS patients with at least one comorbidity, was determined using t-test. The association between different variables was determined by Spearman's correlation analysis. Odds ratio (OR) with corresponding 95% CI was used as a measure of the effect of the association between variables.

In case-control study investigating glucose metabolism in patients with MS, values were reported as mean ± SD or median values with range for continuous data, according to the results of normality of distribution tests. Categorical variables were reported as frequency and percentage. Comparisons of mean levels of different biochemical parameters between MS and HC group were done using ANOVA. The relationship of level of disability and disability progression with clinical and anthropometric characteristics was evaluated by Spearman correlation coefficient (for EDSS) or

Pearson coefficient (for MSSS and PI). Univariable and multivariable logistic regression analyses were conducted for determination of factors that are independently associated with MS. Dependent variable was grouping variable (MS or control group), while independent factors included various demographic, anthropometric and biochemical characteristics. After performing univariable logistic regression analysis, all variables that were statistically significant were included in multivariable regression analysis model. Finally, sensitivity and specificity of HOMA-IR values were evaluated using Receiving Operating Curve (ROC) analysis.

The vascular age was calculated using SCORE project equations for different situations of the combination of CA, gender, smoking, total cholesterol serum and systolic blood pressure levels. For this assessment the SCORE project tables for high-risk countries were used. In order to investigate the independent predictive contribution of discrepancy between CA and VA on disability progression in MS patients the different models of regression analysis were performed.

To evaluate factors associated with presence of CVD comorbidity and diabetes among persons with MS we performed univariate and multivariate Cox proportional hazards regression with hazard ratios (HRs) and corresponding 95% confidence intervals (95% CI) as effect measure. The estimated time period in Cox model was considered as the total period of follow-up. Dependent variable in the model was the presence of event (dichotomized as yes/no) while independent variables were potential risk factors. All variables statistically significant in univariate regression analyses were included in the multivariate models. Finally, in order to explore cumulative risk for occurrence of cardiovascular diseases and type 2 diabetes according to MS phenotype, Kaplan-Meier curves were prerformed.

All analyses were done using The Statistical Package for Social Sciences (SPSS), version 17.0. Probability level of <0.05 was considered statistically significant.

3.4. ETHICAL APPROVAL

All participants included in case-control study and retrospective cohort study signed informed consent. The study was approved by the Ethics Committee of the Faculty of Medicine, University of Belgrade.

4. RESULTS

On the prevalence day, December 31, 2019, there were 1903 females and 822 men with MS in the Belgrade population MS Registry (Figure 1).

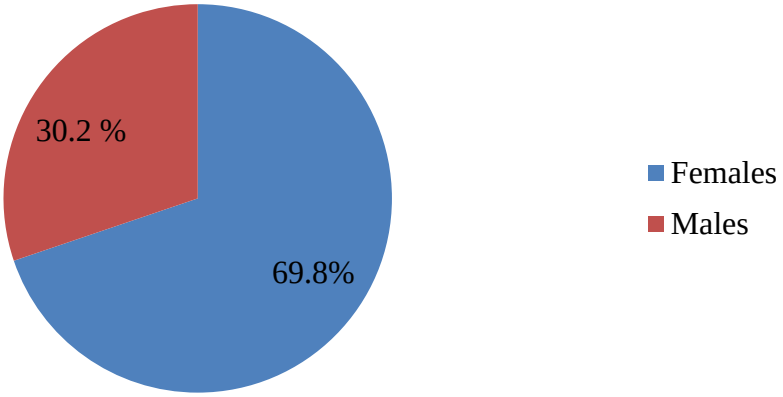

Figure1. Distribution of MS patients in Belgrade MS population Registry according to the gender

The average age of MS patients in the Registry was 55.8 ± 14.3 years (Figure 2).

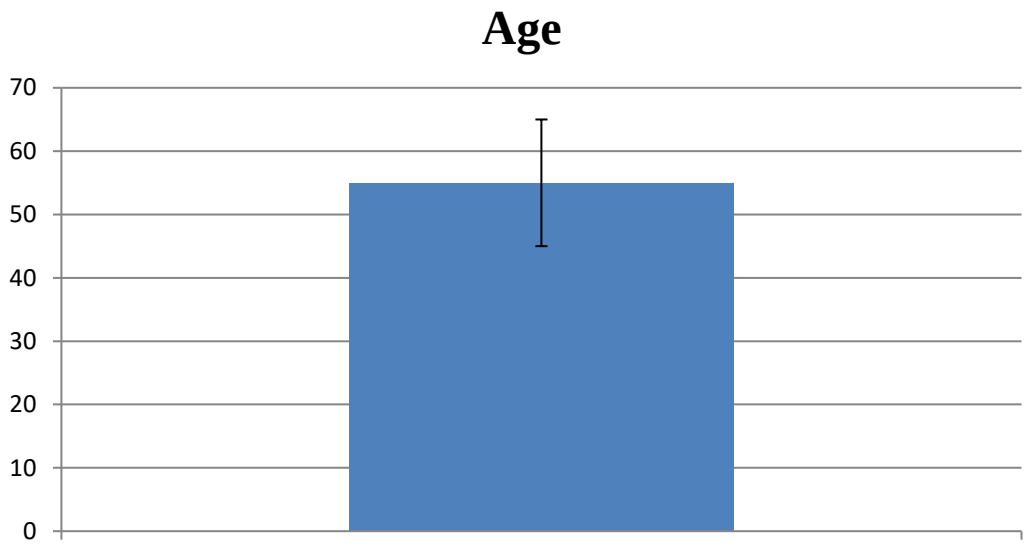

Figure 2. The mean age of MS patients in Belgrade population MS Registry

Distribution of patients according to the work status is summarized in Figure 3. The greatest proportion of patients was employed (54.8%).

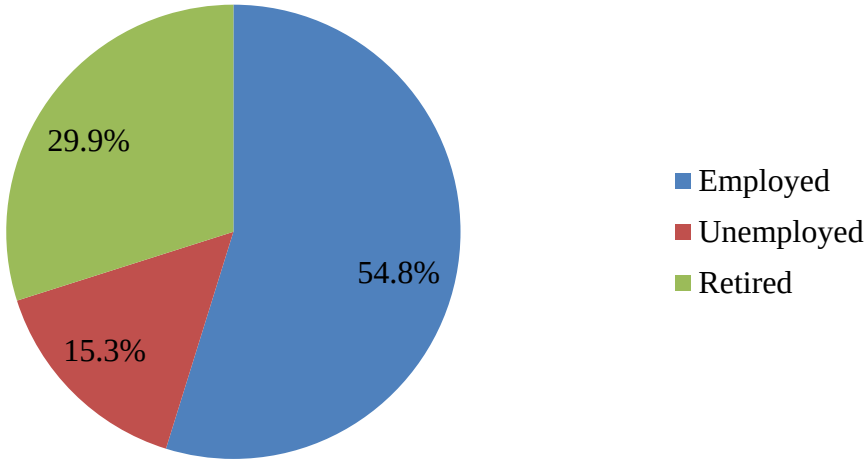

Figure 3. Distribution of MS patients according to work status

Distribution according to marital status is presented in Figure 4. Majority of MS patients were married (60.2%).

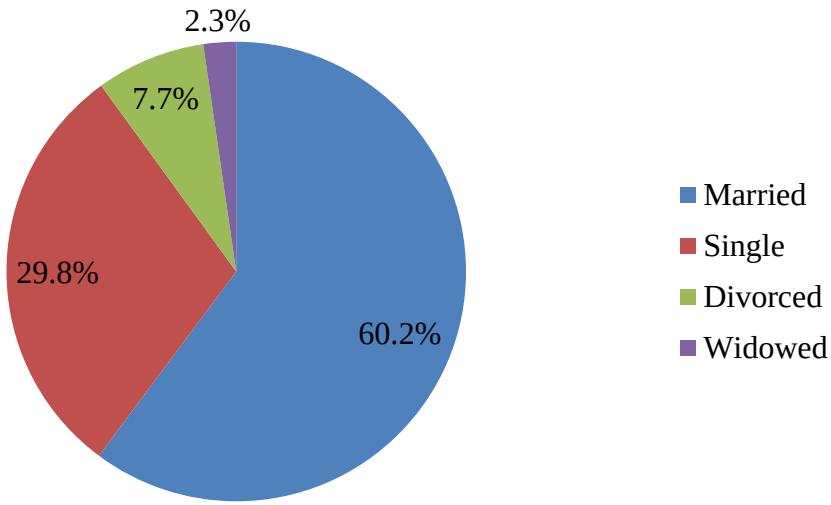

Figure 4. Distribution of MS patients according to marital status

Distribution of MS patients according to the disease phenotype is presented in Figure 5. The greatest proportion of patients had RRMS (62.9%).

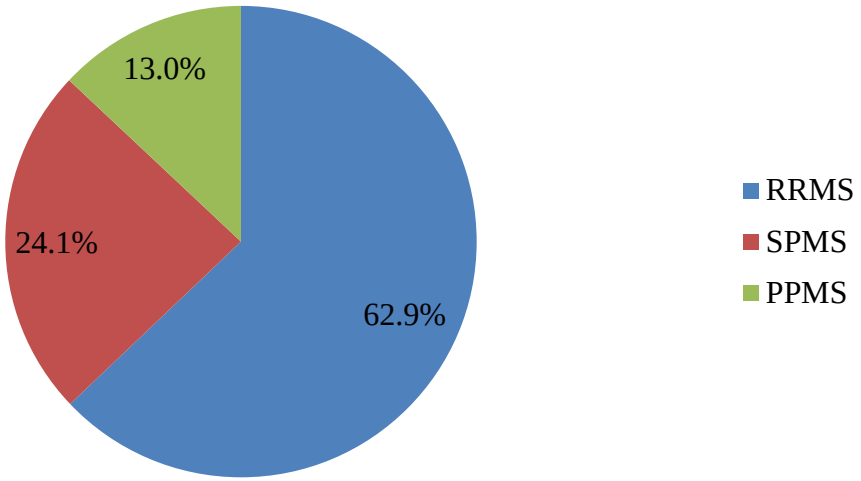

Figure 5. Distribution of MS patients according to disease phenotype

The average disease duration of patients in Belgrade population MS registry was 21.6 ± 12.5 years (Figure 6).

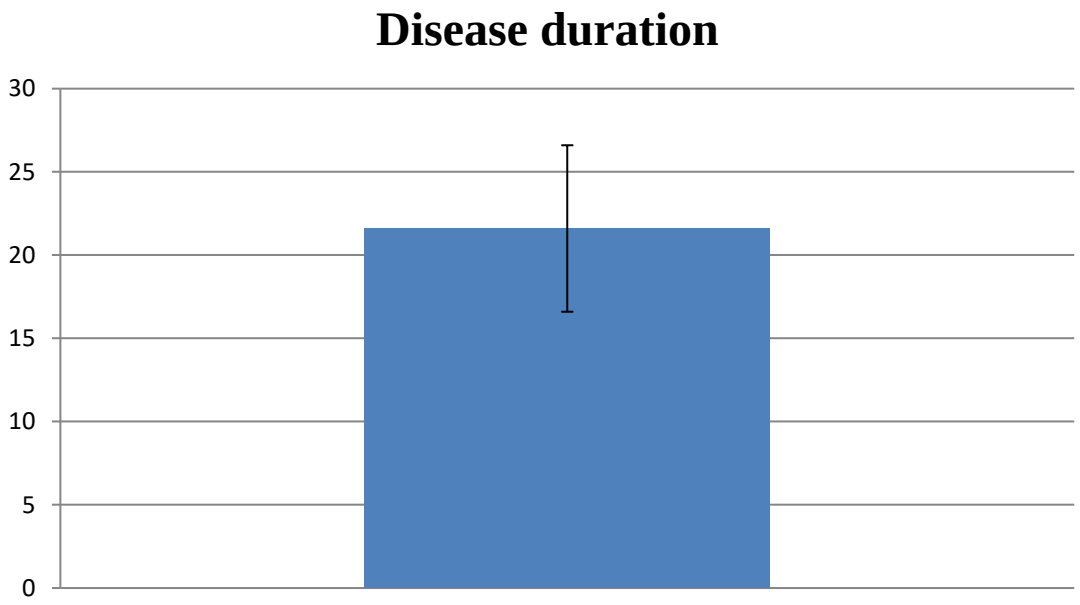

Figure 6. Average disease duration of MS patients in Belgrade population MS registry

The average disability level measured by EDSS was 4.00±4.00 (Figure 7).

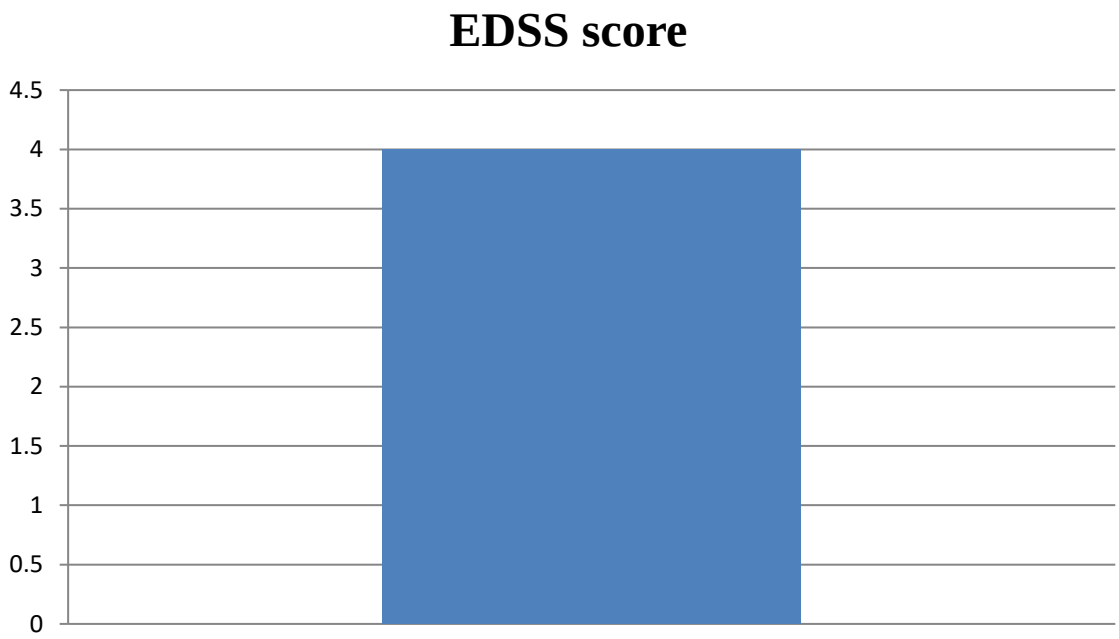

Figure 7. Median level of disability in MS patients in the Registry

Presence of family cluster of MS was noticed in 92 MS patients (3.4%) in the Belgrade population MS registry (Figure 8).

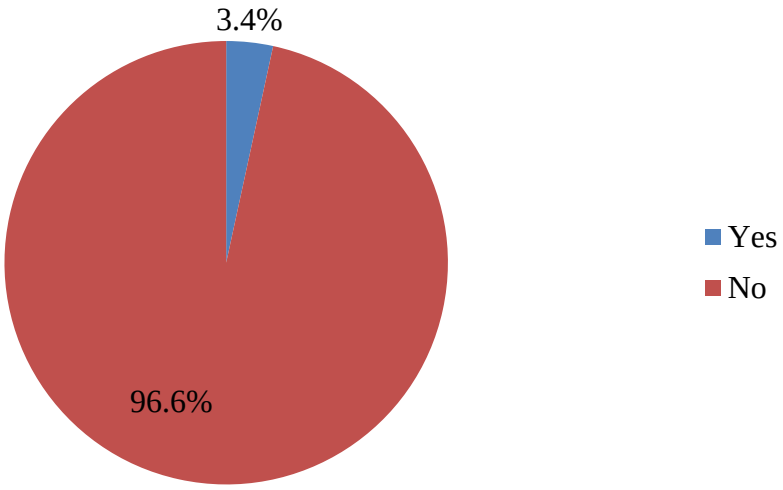

Figure 8. Distribution of MS patients according to the presence of family cluster of MS

Presence of any comorbidity was observed in 1331 MS patients in the Registry (48.8%) (Figure 9).

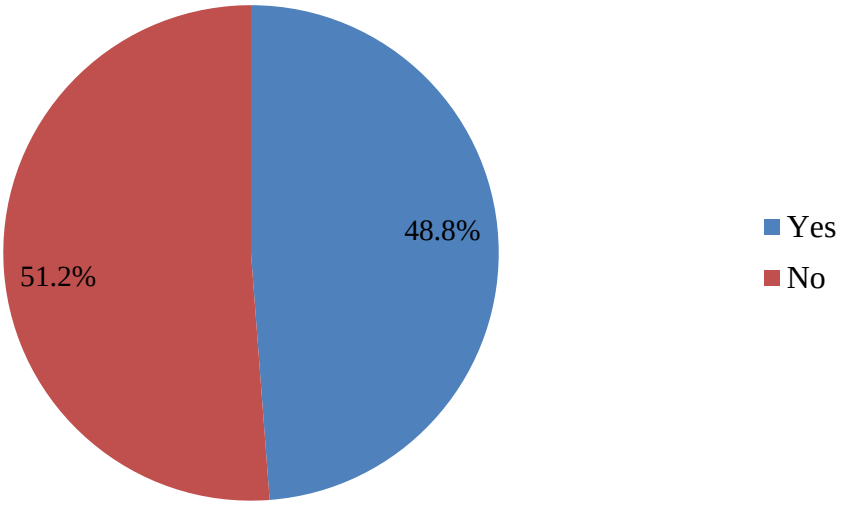

Figure 9. Distribution of MS patients according to the presence of comorbidity

The prevalence of various groups of comorbidities is reported in Table 2. Groups of comorbid conditions with the highest prevalence were psychiatric (prevalence (P) = 20.59%, 95% CI 19.10–22.17), cardiovascular comorbidities (P = 15.23%, 95% CI 13.93–16.63) and autoimmune disorders (P = 6.06%, 95% CI 5.22–7.02). Malignant comorbidities were less frequent, their prevalence was 2.53% (95% CI 1.99–3.21), while prevalence of epilepsy and diabetes type II were 2.60% (95% CI 2.06–3.30) and 2.06% (95% CI 1.57–2.69), respectively.

When the prevalence of single comorbidities is considered, the most frequent comorbidities were depression (P = 11.82%, 95% CI 10.64–13.11) and hypertension (P = 11.41%, 95% CI 10.25–12.68). Among malignant diseases, the most prevalent was the breast cancer (P = 0.95%, 95% CI 0.58–1.53). The most common autoimmune disease was the thyroid disease (P = 4.44%, 95% CI 3.71–5.30).

The presence of two or more comorbidities was found in 534 MS patients (19.6%).

Table 2. Prevalence of comorbidities in multiple sclerosis

Comorbidity	**Prevalence (%)**	**95% CI[a]**
Cardiovascular comorbidities	15.23	13.93-16.63
Hypertension	11.41	10.25-12.68
Hyperlipidemia	2.86	2.28-3.58
Ischemic heart disease	0.66	0.40-1.06

Cerebrovascular disease	0.15	0.05-0.41
Cardiac disease	0.15	0.05-0.41
Malignant diseases	2.53	1.99-3.21
Breast cancer	0.95	0.58-1.53
Ovarian cancer	0.57	0.31-1.07
Thyroid gland cancer	0.29	0.13-0.60
Uterine cancer	0.21	0.07-0.58
Colorectal cancer	0.15	0.05-0.41
Lung cancer	0.15	0.05-0.41
Melanoma	0.15	0.05-0.41
Prostate cancer	0.12	0.01-0.78
Non-melanoma skin cancer	0.11	0.03-0.35
Kidney cancer	0.07	0.01-0.29
Brain	0.04	0.00-0.24
Leukemia	0.04	0.00-0.24
Parathyroid gland cancer	0.04	0.00-0.24
Hodgkin disease	0.04	0.00-0.24
Nasal cancer	0.04	0.00-0.24
Spleen cancer	0.04	0.00-0.24
Autoimmune disorders	6.06	5.22-7.02
Autoimmune thyroid disease	4.44	3.71-5.30
Sjogren's syndrome	0.37	0.19-0.70
Inflammatory bowel disease	0.33	0.16-0.65
Vitiligo	0.22	0.09-0.50
Psoriasis	0.15	0.05-0.41
Rheumatoid arthritis	0.15	0.05-0.41
Uveitis	0.15	0.05-0.41
Systemic lupus erythematosus	0.07	0.01-0.29
Systemic sclerosis	0.07	0.01-0.29
Dermatomyositis	0.04	0.00-0.24
Pernicious anemia	0.04	0.00-0.24
Primary biliary cirrhosis	0.04	0.00-0.24
Epilepsy	2.60	2.06-3.30
Type 2 diabetes	2.06	1.57-2.69
Psychiatric disorders	20.59	19.10-22.17
Depression	11.82	10.64-13.11
Anxiety	5.32	4.52-6.25
Other psychiatric disorders	3.45	2.81-4.22

[a]CI – confidence interval

The prevalence of various comorbidity groups in different MS phenotypes is presented in Table 3. The only statistically significant difference in the frequency of various comorbidities was observed for type 2 diabetes which was more common in patients with PPMS (4.7%) compared to the patients with RRMS and SPMS (1.5%) ($\chi 2 = 15.618$, $p < 0.001$). For all other comorbidities difference in frequency between various MS phenotypes did not reach statistical significance.

Table 3. Distribution of comorbidities in various disease phenotypes

Comorbidity	Relapsing (%)	Primary progressive (%)	Chi-square	p-value
Cardiovascular comorbidity	12.5	16.4	6.201	0.102
Malignant diseases	2.3	3.2	7.521	0.057
Autoimmune disorders	5.6	5.0	5.345	0.148
Epilepsy	2.6	2.9	0.111	0.739
Type 2 diabetes	1.5	4.7	15.618	**<0.001**
Psychiatric disorders	17.5	20.4	5.183	0.159

When PI was analyzed in MS patients with comorbidity and those MS patients with no comorbidity, it was observed that MS patients with at least one comorbidity have statistically significantly higher PI (PI = 0.31 ± 0.28) compared to MS patients without any comorbidity (PI = 0.27 ± 0.25) ($t = -2.946$; $p = 0.003$, data not presented). Furthermore, PI statistically significantly correlated with the number of comorbidities ($\rho = 0.306$, $p < 0.001$).

For the evaluation of potential impact of the presence of one or more comorbidities on the treatment of MS patients, the association between comorbidities and DMTs treatment was analyzed. A total of 534 MS patients were receiving DMTs at some period of the disease (Figure 10). Among them, 457 received interferon beta preparations, 72 received glatiramer acetate and 80 patients received mitoxantrone. It is important to emphasize that some of patients received more than 1 treatment during their MS course.

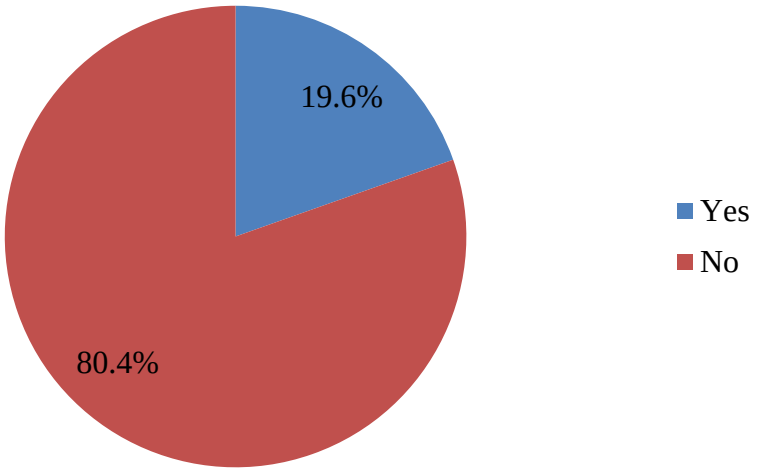

Figure 10. Distribution of MS patients based on receiving DMTs at any time during disease course

It was found that MS patients who had at least one comorbidity had greater chance to be in the group receiving DMTs in comparison with those patients that were without comorbidities (age-

adjusted OR = 1.40, 95% CI 1.15–1.69, p = 0.001). Furthermore, with increasing number of comorbidities, decreasing chance for treatment with DMTs was observed ($\chi 2$ for trend = 6.07, p = 0.014). More precisely, patient with two comorbidities had 9% less chance to be treated with DMTs compared to MS patient without comorbidity (OR = 0.91). Similarly, patient with 3 comorbidities had 45% less chance for DMTs treatment (OR = 0.55).

When the influence of exposure to DMTs on risk of development of comorbidity was analyzed, it was observed that MS patients receiving DMTs were at greater risk for comorbidity occurrence in comparison to MS patients without DMTs treatment (age-adjusted OR = 1.54, 95%CI 1.18–2.00, p = 0.001).

Table 4 presents age-adjusted prevalence (per 100,000 MS patients) of various comorbidity groups. Psychiatric disorders had the highest prevalence in both genders, followed by cardiovascular diseases.

Table 4 Age-adjusted (/100,000) prevalence of comorbidity groups in patients with MS

Comorbidity group	**Age-adjusted prevalence (WHO standard population)**			**Age-adjusted prevalence (Europe standard population)**		
	Men	**Females**	**Both sexes**	**Men**	**Females**	**Both sexes**
Cardiovascular diseases	0.13	0.09	0.10	0.14	0.11	0.12
Malignant diseases	0.01	0.02	0.02	0.01	0.02	0.02
Autoimmune disorders	0.03	0.07	0.06	0.03	0.07	0.06
Epilepsy	0.02	0.02	0.02	0.02	0.02	0.02
Type 2 diabetes	0.03	0.01	0.02	0.03	0.01	0.02
Psychiatric disorders	0.15	0.22	0.19	0.15	0.22	0.20

Prevalence of different comorbidity groups according to the gender (per 1000 MS patients) is shown in Table 5. Specific prevalence was highest for cardiovascular and psychiatric conditions. In case of cardiovascular diseases, prevalence was rising with the age, while for psychiatric disorders increasing tendency was noticed until 50-59 age group, with decrease in older ages. When comparing men to women, malignancies, psychiatric and autoimmune disorders were more common in female than in male MS patients, while cardiovascular diseases, epilepsy and type 2 diabetes were more frequent in male MS patients.

Table 5. Age- and sex-specific (/1000) prevalence of comorbidity

Comorbidity group	Age groups (years)					
	20-29	30-39	40-49	50-59	60-69	70+
Cardiovascular diseases						
- Men	3.45	7.48	12.34	22.58	24.83	22.61
- Females	1.96	4.23	6.42	16.31	26.35	18.75
- Both sexes	2.50	5.18	8.36	18.23	25.96	19.95
Malignant diseases						
- Men	0.00	0.00	0.00	2.15	2.01	0.00
- Females	0.00	1.15	2.48	3.55	5.41	3.52
- Both sexes	0.00	0.82	1.67	3.12	4.53	2.43
Autoimmune disorders						
- Men	3.45	2.80	5.11	3.23	1.34	1.74
- Females	5.88	5.38	7.25	9.22	9.18	3.52
- Both sexes	5.00	4.63	6.55	7.39	7.14	2.96
Epilepsy						
- Men	0.00	3.74	4.26	3.23	0.67	1.74
- Females	1.96	1.54	2.69	2.60	2.59	3.13
- Both sexes	1.25	2.18	3.20	2.79	2.09	2.70
Type 2 diabetes						
- Men	3.45	2.80	0.85	0.54	5.37	3.48
- Females	0.00	0.77	0.62	0.95	4.47	3.52
- Both sexes	1.25	1.36	0.70	0.82	4.70	3.50
Psychiatric disorders						
- Men	3.45	2.52	16.60	20.97	15.44	1.74
- Females	17.65	21.54	30.64	26.71	21.18	5.47
- Both sexes	12.50	22.62	26.04	24.96	19.69	4.31

Characteristic of sub-group of MS patients chosen for case control study investigating glucose metabolism are shown in Table 6 and Table 7. Almost three quarters of participants in MS group (74.4%) were females, while 25.6% were men. The mean age of MS group participants was 45.5±11.3 years.

Table 6. Baseline characteristics of MS group

	Mean ± SD or Frequency (%)	Median	Minimum	Maximum
Age (years)	41.7 ± 10.9	41.5	18.0	78.0
Gender (%)				
Men	20 (25.6)			
Females	58 (74.4)			
Weight (kg)	72.5 ± 15.0	69.7	48	119
Height (m)	1.7 ± 0.1	1.7	1.5	2.0
BMI (kg/m^2)	24.8 ± 4.5	24.1	16.7	37.7
WC (cm)	85.7 ± 11.8	85.5	66	123
FM (%)	30.0 ± 9.2	30.1	7.6	49.3
MS phenotype (%)				
Relapsing-remitting	63 (80.8)			
Primary progressive	9 (11.5)			
Secondary progressive	6 (7.7)			
EDSS	2.6 ± 1.7	2.0	0.0	6.5
Progression Index	0.6 ± 0.7	0.3	0.0	4.0
MS Severity Score	3.7 ± 2.7	2.9	0.0	9.4
Duration of MS (years)	10.2 ±9.0	8.0	1.0	38.0
DMTs (%)				
Yes	22 (28.2)			
No	56 (71.8)			

In the control group, 69.2% participants were females and 30.8% participants were men. Their average age was 45.5±11.3 years.

Table 7. Baseline characteristics of the control group

	Mean ± SD or Frequency (%)	Median	Minimum	Maximum
Age (years)	45.5 ± 11.3	45.0	30	65
Gender (%)				
Men	8 (30.8)			
Females	18 (69.2)			
BMI (kg/m^2)	24.7 ± 3.7	23.9	17.3	33.3

The prevalence of IR in compared groups, based on HOMA-IR value, was statistically significantly higher in MS patients (64.1%) than in controls (30.8%) ($\chi 2=7.084$, $p=0.008$) (data not presented). Additionally, in three MS patients presence of type 2 diabetes was registered (P=3.8%), while in 12 MS patients pre-diabetes (IGT and IFG) was found (P=15.4%). Presence of type 2 diabetes was not found among participants in the control group, while in one participant IFG condition was detected (data not presented).

Table 8 presents data on mean levels of biochemical parameters measured in MS and control group. It can be observed that for nearly all biochemical parameters values were statistically significantly higher in MS patients than in control group ($p<0.05$). Only for total cholesterol and LDL levels were significantly higher among controls ($p<0.05$).

Table 8. Mean levels of biochemical parameters in MS and control group

	Mean ± SD or Frequency (%)		Median		Minimum		Maximum		ANOVA	*p* value
	MS	Controls	MS	Controls	MS	Controls	MS	Controls		
Glucose 0'	5.3 ± 0.7	4.5 ± 0.9	5.2	4.4	4.1	3.0	9.3	6.1	12.549	**0.001**
Glucose 30'	8.7 ± 1.7	6.9 ± 2.5	8.5	6.5	5.5	4.1	16.5	12.4	14.348	**<0.001**
Glucose 60'	8.6 ± 2.6	6.6 ± 2.5	8.5	5.7	4.2	3.8	18.3	12.5	8.424	**0.005**
Glucose 90'	7.5 ± 2.5	5.3 ± 2.2	7.2	5.0	3.7	2.7	18.8	11.5	10.480	**0.002**
Glucose 120’	6.5 ± 2.3	4.4 ± 1.4	6.2	4.1	3.2	2.3	16.3	7.1	12.659	**0.001**
Insulin 0’	14.6 ± 7.3	9.3 ± 4.7	14.0	8.4	3.0	3.3	35.6	18.5	10.949	**0.001**
Insulinogenic index	18.3 ± 20.4		13.1		0.2		122.8			
HOMA IR	3.5 ± 2.1	2.0 ± 1.1	3.3	1.9	0.8	0.4	13.1	4.9	11.498	**0.001**
HbA1c (mmol/mol)	34.8 ± 4.4		35.0		27.0		58.0			
HbA1c (%)	5.3 ± 0.6		5.4		2.1		7.4			
Total cholesterol	5.3 ± 1.1	5.9 ± 1.3	5.3	5.9	3.3	3.6	9.5	8.0	5.500	**0.021**
HDL	1.5 ± 0.4	1.5 ± 0.3	1.4	1.6	0.8	1.0	2.9	2.1	0.176	0.676
LDL	3.2 ± 1.0	3.8 ± 1.2	3.0	3.8	0.9	2.0	7.0	5.5	5.156	**0.025**
TG	1.4 ± 0.7	1.4 ± 0.5	1.2	1.4	0.5	0.8	3.5	2.5	0.093	0.762
TG/HDL	1.1 ± 0.8	1.0 ± 0.5	0.8	1.0	0.3	0.4	4.1	2.3	0.079	0.780
ALT	23.4 ± 24.2		17.0		7.0		166.0			

In Table 9 correlations of different biochemical parameters with clinical disability and its progression are shown. It can be observed that EDSS score was statistically significantly associated with glucose levels at different time points: glucose 60' (ρ=0.245, p=0.030), glucose 90' (ρ=0.331, p=0.003), glucose 120' (ρ=0.379, p=0.001), and with HbA1c (in mmol/mol: ρ=0.294, p=0.025 and, in percentages: ρ=0.271, p=0.039) and TG levels (ρ=0.251, p=0.027). MSSS correlated significantly with glucose 30' (ρ=0.281, p=0.013), 60' (ρ=0.326, p=0.004), 90' (ρ=0.360, p=0.001) and 120' (ρ=0.343, p=0.002) while PI correlated significantly with HbA1c (%) (ρ=-0.414, p=0.001).

Table 9. Correlation between EDSS, PI, MSSS and different biochemical parameters

	EDSS		PI		MSSS	
	r-coefficient	*p* value	*r*-coefficient	*p* value	*r*-coefficient	*p* value
FM (%)	0.091	0.440	0.027	0.823	0.051	0.668
Waist circumference	0.128	0.264	-0.017	0.883	0.072	0.533
Glucose 0'	-0.054	0.639	-0.071	0.537	0.022	0.846
Glucose 30'	0.194	0.090	0.159	0.164	0.281	**0.013**
Glucose 60'	0.245	**0.030**	0.187	0.100	0.326	**0.004**
Glucose 90'	0.331	**0.003**	0.211	0.064	0.360	**0.001**
Glucose 120'	0.379	**0.001**	0.165	0.149	0.343	**0.002**
Insulin 0'	0.074	0.520	0.003	0.981	0.115	0.316
Insulinogenic index	0.095	0.408	0.019	0.870	-0.001	0.994
HOMA IR	0.068	0.554	-0.013	0.913	0.111	0.332
HbA1c (mmol/mol)	0.294	**0.025**	-0.109	0.414	0.067	0.618
HbA1c (%)	0.271	**0.039**	-0.414	**0.001**	-0.163	0.222
Total cholesterol	0.091	0.429	-0.040	0.728	0.037	0.750
HDL	-0.115	0.315	-0.141	0.220	-0.164	0.152
LDL	0.115	0.314	0.032	0.782	0.116	0.310
TG	0.251	**0.027**	0.151	0.186	0.173	0.130
TG/HDL ratio	0.200	0.079	0.169	0.138	0.190	0.097
ALT	0.054	0.647	0.024	0.838	0.007	0.949

Differences in levels of various biochemical parameters among different MS phenotypes are summarized in Table 10. Glucose levels at different time intervals (glucose 30' (p=0.038), glucose 60' (p=0.040), glucose 90' (p=0.019)) and TG levels differed between MS phenotypes, with higher values observed in RRMS compared to PPMS and SPMS.

Table 10. Levels of biochemical parameters according to MS phenotype

	Relapsing-remitting	Secondary progressive	Primary progressive	ANOVA	*p* value
FM (%)	29.9 ± 9.5	32.7 ± 7.3	29.1 ± 8.0	0.293	0.747
Waist circumference	85.3 ± 12.1	89.3 ± 12.9	86.1 ± 9.3	0.322	0.725
Glucose 0'	5.2 ± 0.7	5.1 ± 0.5	5.5 ± 0.9	0.739	0.481
Glucose 30'	8.5 ± 1.7*	9.2 ± 1.4	9.9 ± 0.7*	3.412	**0.038**
Glucose 60'	8.2 ± 2.6	9.9 ± 2.0	10.3 ± 2.1	3.364	**0.040**
Glucose 90'	7.1 ± 2.6	9.2 ± 1.2	9.1 ± 1.9	4.180	**0.019**
Glucose 120'	6.3 ± 2.4	7.9 ± 1.4	7.5 ± 1.9	2.354	0.102
Insulin	14.4 ± 7.7	15.3 ± 6.8	15.7 ± 4.9	0.163	0.850
Insulinogenic index	18.6 ± 21.9	15.9 ± 12.5	17.3 ± 14.5	0.058	0.944
HOMA IR	3.4 ± 2.2	3.5 ± 1.6	3.8 ± 1.0	0.108	0.898
HbA1c (mmol/mol)	34.7 ± 4.6	34.0 ± 1.6	35.3 ± 4.6	0.107	0.898
HbA1c (%)	5.3 ± 0.4	5.3 ± 0.2	4.9 ± 1.3	1.384	0.259
Total cholesterol	5.2 ± 1.2	5.6 ± 0.6	5.5 ± 0.9	0.506	0.605
HDL	1.5 ± 0.4	1.4 ± 0.5	1.5 ± 0.5	0.326	0.723
LDL	3.1 ± 1.1	3.4 ± 1.0	3.6 ± 0.7	1.014	0.368
TG	1.3 ± 0.6	1.9 ± 0.6	1.6 ± 0.8	3.199	**0.046**
TG/HDL	1.0 ± 0.7	1.6 ± 0.9	1.2 ± 0.9	2.306	0.107
ALT	23.6 ± 24.9	16.8 ± 7.8	26.4 ± 28.5	0.277	0.759

Results of logistic regression analyses (univariable and multivariable) can be found in Table 11. It can be seen that, in univariable logistic regression analysis, following variables reached statistical significance: glucose 0' (OR=6.010, 95% CI 2.122-17.024, p=0.001), glucose 30' (OR=1.975, 95% CI 1.338-2.916, p=0.001), glucose 60' (OR=1.512, 95% CI1.128-2.027, p=0.006), glucose 90'(OR=1.852, 95% CI 1.260-2.722,p=0.002), glucose 120'(OR=2.380, 95% CI 1.466-3.863, p<0.001), insulin 0' (OR=1.143, 95% CI 1.048-1.248, p=0.003), HOMA-IR (OR=1.881, 95% CI 1.279-2.767, p=0.001), total cholesterol (OR=0.625, 95% CI 0.412-0.947, p=0.027) and LDL (OR=0.602, 95%CI 0.379-0.956, p=0.032).

After inclusion of all variables statistically significant in univariable analysis into multivariable logistic regression analysis, it was found that glucose 120' was independently associated with MS (OR=3.937, 95% CI 1.178-13.159, p=0.026).

Table 11. Results of logistic regression analysis (MS vs. controls)

Variable	Univariable analysis		
	Odds ratio	95% CI	*p* vaule
Gender	1.289	0.486-3.419	0.610
Age	0.970	0.931-1.010	0.143
BMI	1.004	0.902-1.117	0.945
Glucose 0’	6.010	2.122-17.024	**0.001**
Glucose 30’	1.975	1.338-2.916	**0.001**
Glucose 60’	1.512	1.128-2.027	**0.006**
Glucose 90’	1.852	1.260-2.722	**0.002**
Glucose 120’	2.380	1.466-3.863	**<0.001***
Insulin	1.143	1.048-1.248	**0.003**
HOMA IR	1.881	1.279-2.767	**0.001**
Total cholesterol	0.625	0.412-0.947	**0.027**
HDL	0.784	0.254-2.418	0.672
LDL	0.602	0.379-0.956	**0.032**
TG	0.890	0.423-1.872	0.759
TG/HDL ratio	1.109	0.542-2.269	0.777

*Multivariable analysis: OR=3.937 (95%CI:1.178-13.159, p=0.026

In Figure 11, ROC curve for HOMA-IR in MS patients is shown. Based on this analysis, best cut-off value for HOMA-IR in our study is 2.3, which provides both sensitivity and specificity of 66.7% in discriminating persons with MS from controls.

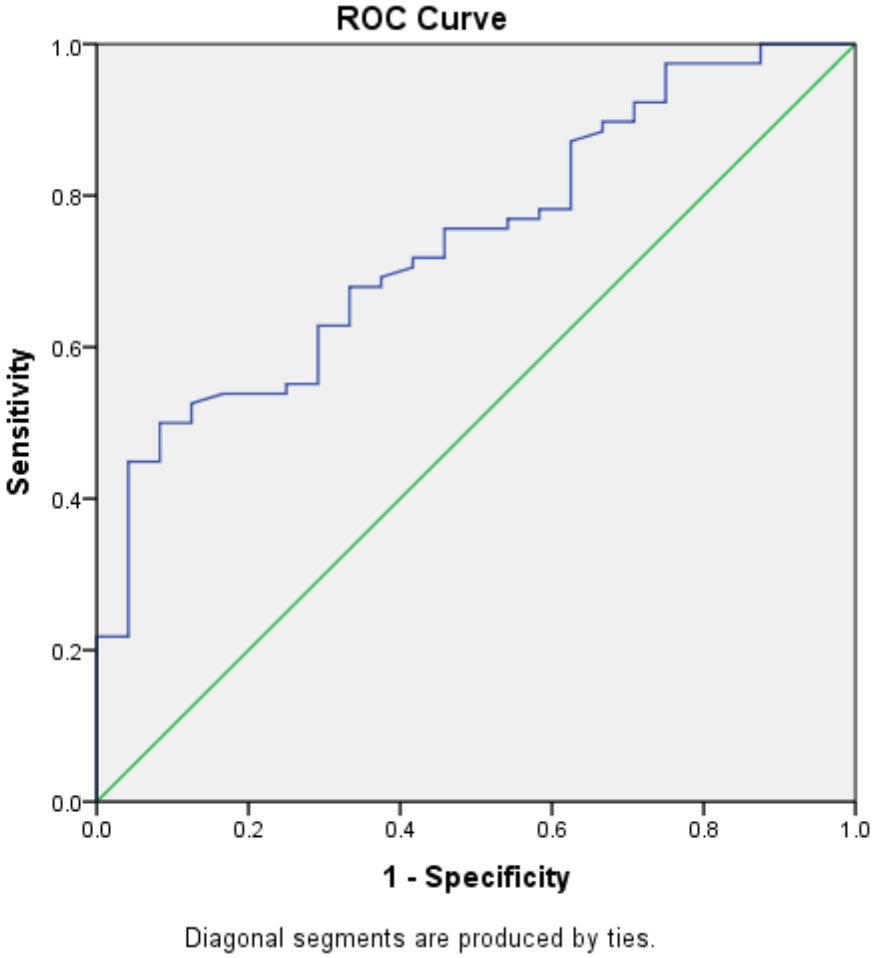

Figure 11. The ROC curve of HOMA IR

Demographical and clinical characteristic of study groups in the case control study investigating difference between chronological and vascular age in patients with MS are displayed in Table 12. As it can be observed, study groups did not differ according to gender (p=0.208), family history of cardiovascular diseases (p=0.651), prevalence of smoking (p=0.492), and physical activity (p=0.154). However, groups statistically significantly differed in terms of chronological (p=<0.001) and vascular (p=<0.001) age with highest values observed in MS and CAD group. Difference between chronological and vascular age also statistically significantly differed among compared groups (p=<0.001), with greatest discrepancy reported for MS, HLP, HTA and type 2 diabetes group (8.9±8.8 years). Further, average total cholesterol levels were highest in the MS and CAD group (6.3±0.0 mmol/L), with p<0.001 for intergroup comparison. Finally, groups significantly differed in body mass index values (p=0.001), with participants in MS, HLP, HTA and type 2 diabetes group being most obese (BMI=27.2±2.3kg/m^2)

Table 12. Demographic and clinical characteristic of study groups

Variable	Study group					p-value
	MS without any comorbidity n=108	MS and HLP n=84	MS, HLP and HTA n=26	MS, HLP, HTA and type 2 diabetes n=14	MS and CAD n=3	
Proportion of females (%)	68.5	76.2	65.4	50.0	100.0	0.208
Chronological age (years)	38.5±9.9*†‡§	44.9±9.2¶†‡§	56.8±10.1¶*	55.0±13.7¶*	64.7±111.0¶*	**<0.001**
Vascular age (years)	39.0±10.6*†‡§	46.0±10.8¶†‡§	63.8±16.1¶*	64.5±21.4¶*	68.7±11.7¶*	**<0.001**
Difference between chronological and vascular age (years)	0.5±1.0†‡	1.0±2.2†‡	7.0±7.7¶*	8.9±8.8¶*	4.0±2.6	**<0.001**
Total cholesterol (mmol/L)	4.3±0.6*†‡§	6.1±1.0¶‡	5.8±1.2¶	5.3±1.0¶*	6.3±0.0¶	**<0.001**
Body mass index (kg/m^2)	22.9±4.0‡	23.5±4.1‡	25.0±3.9	27.2±2.3¶*	25.8±5.5	**0.001**
Family history for CVD *N* (%)	86.9	88.1	96.2	92.9	100.0	0.651
Smoking *N* (%)	34.3	41.7	46.2	28.6	66.7	0.492
Physical activity *N* (%)	76.9	65.5	61.5	50.0	66.7	0.154

CVD - cardiovascular diseases; ¶Tukey's test $p < 0.05$ for MS without any comorbidity group; *Tukey's test $p < 0.05$ for MS and HLP group; †Tukey's test $p < 0.05$ for MS, HLP, HTA and type 2 diabetes group; ‡Tukey's test $p < 0.05$ for MS, HLP, HTA and type 2 diabetes group; §Tukey's test $p < 0.05$ for MS and CAD

The differences between chronological and vascular in each of the study group are presented in Figure 12. In each of five investigated groups, vascular age was higher compared to the chronological age, however, the greatest discrepancy was observed for MS, HLP, HTA and type 2 diabetes group (8.9±8.8 years) and for MS, HLP and HTA group (7.0±7.7 years).

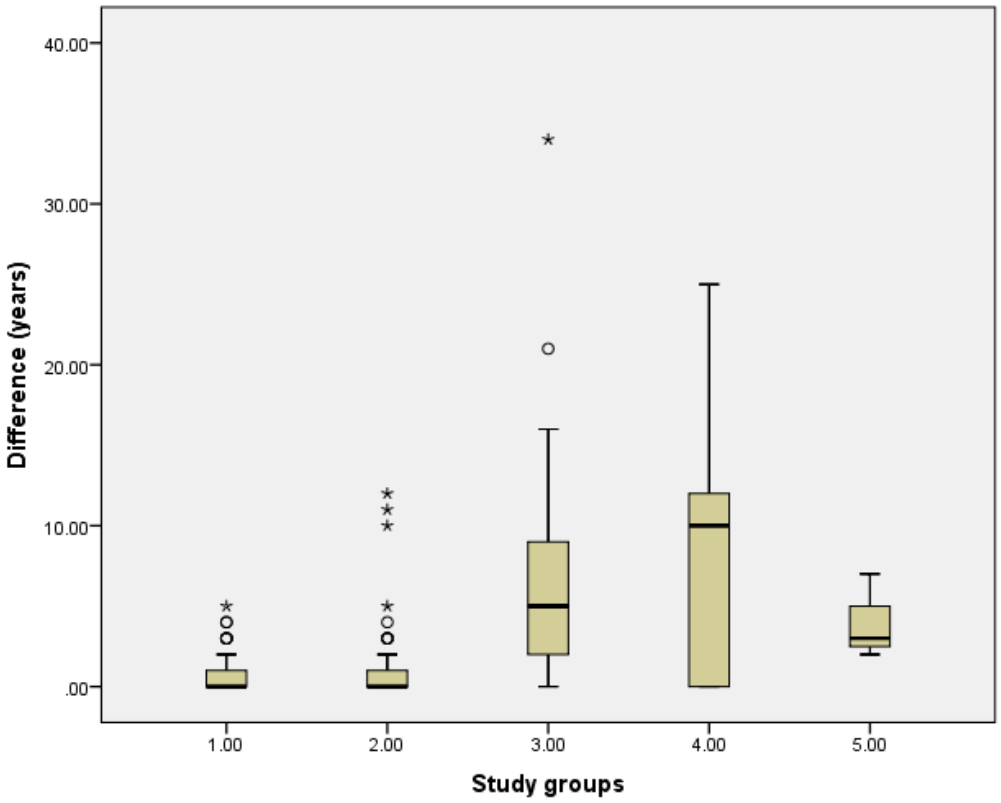

Figure 12. Difference between vascular and chronological age in the study groups

The results of hierarchical regression analysis are shown in Table 13. The hierarchical regression analysis showed that disability level in patients with MS, measured by EDSS score accounted for 27%% of the variance of discrepancy between CA and VA as a dependent variable ($p<0.01$) (Model 1). In the second model, when physical activity and body mass index were added, another 10% in the variance were explained ($p<0.05$). Finally, when the presence of type 2 diabetes comorbidity was included (Model 3), proportion of variance explained increased for additional 11% ($p<0.01$). This means that the final model showed that EDSS, physical activity, body mass index and presence of type 2 diabetes comorbidity explained a total of 48% of the variance in discrepancy between chronological age and vascular age.

Table 14. Summary of hierarchical regression analysis of variables predicting discrepancy between chronological and vascular age

Variable	Model 1			Model 2			Model 3		
	Unstandardized B	Standard E (B)	Standardized β	B	SE (B)	β	B	SE (B)	β
EDSS	0.59	0.20	**0.27****	0.55	0.24	**0.25***	0.50	0.23	**0.23***
Physical activity				0.02	1.07	0.01	-0.32	1.02	-0.03
Body mass index				0.28	0.10	**0.24****	0.20	0.10	**0.17***
Type 2 diabetes							-6.46	1.77	**-0.31****
R^2	0.27			0.37			0.48		
F for change in R^2	**9.104****			**3.797***			**13.310****		

*p < 0.05, **p < 0.01

Tables 15-17 display results of Cox regression analysis for predictors of cardiovascular disease, coronary artery disease and type 2 diabetes. In each of these analyses independent predictors of investigated comorbidity were EDSS score (HR=0.872, 95% CI 0.809-0.941, p<0.001) and primary-progressive MS phenotype (HR=1.799, 95% CI 1.099-2.945, p=0.019).

Table 15. Cox regression analysis for predictors of CVD comorbidity

Variable	Univariate analysis			Multivariate analysis		
	Hazard ratio	95% CI	*p* vaule	Hazard ratio	95% CI	*p* value
Age	0.983	0.970-0.995	**0.007**			
Gender	0.844	0.664-1.072	0.165			
EDSS score	0.888	0.843-0.936	**<0.001**	0.872	0.809-0.941	**<0.001**
MS phenotype	1.514	1.110-2.066	**0.009**	1.799	1.099-2.945	**0.019**

Table 16. Cox regression analysis for predictors of CAD comorbidity

Variable	Univariate analysis			Multivariate analysis		
	Hazard ratio	95% CI	*p* vaule	Hazard ratio	95% CI	*p* value
Age	0.983	0.970-0.995	**0.007**			
Gender	0.844	0.664-1.072	0.165			
EDSS score	0.888	0.843-0.936	**<0.001**	0.872	0.809-0.941	**<0.001**
MS phenotype	1.514	1.110-2.066	**0.009**	1.799	1.099-2.945	**0.019**

Table 17. Cox regression analysis for predictors of type 2 diabetes comorbidity

Variable	Univariate analysis			Multivariate analysis		
	Hazard ratio	95% CI	*p* vaule	Hazard ratio	95% CI	*p* value
Age	0.983	0.970-0.995	**0.007**			
Gender	0.844	0.664-1.072	0.165			
EDSS score	0.888	0.843-0.936	**<0.001**	0.872	0.809-0.941	**<0.001**
MS phenotype	1.514	1.110-2.066	**0.009**	1.799	1.099-2.945	**0.019**

According to Kaplan-Meier curves, higher risk for both, type 2 diabetes and cardiovascular disease comorbidity is statistically significantly associated with progressive MS phenotype ($p<0.001$) (Figure 13 and 14).

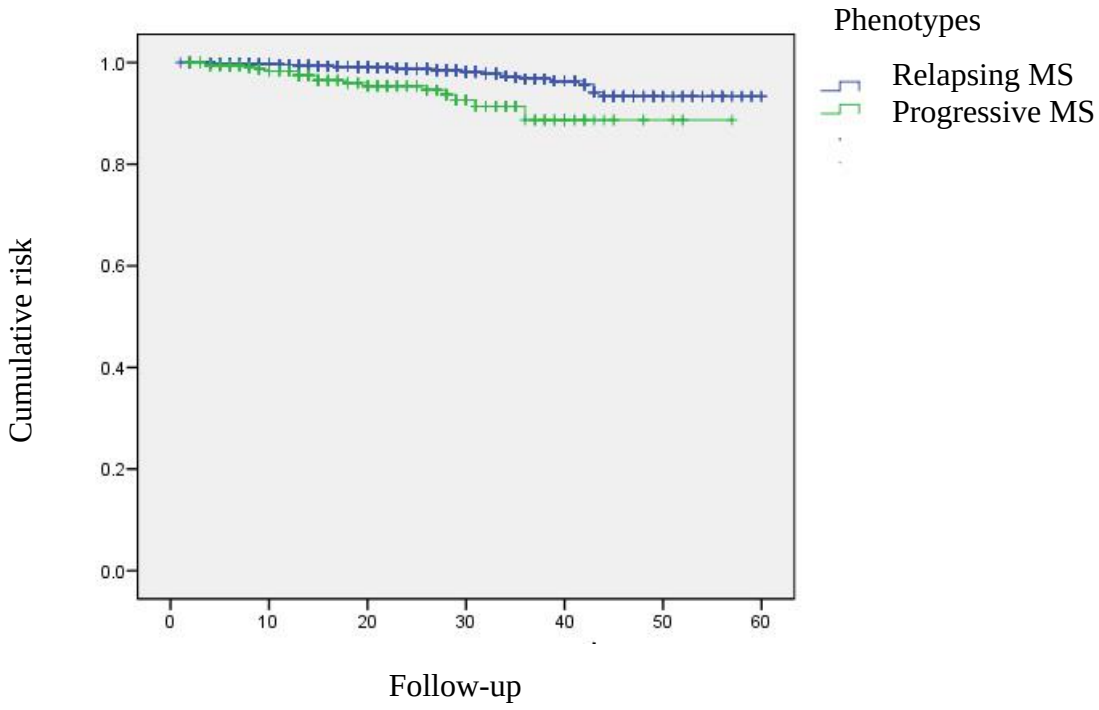

Figure 13. Cumulative risk for type 2 diabetes occurrence according to MS phenotype

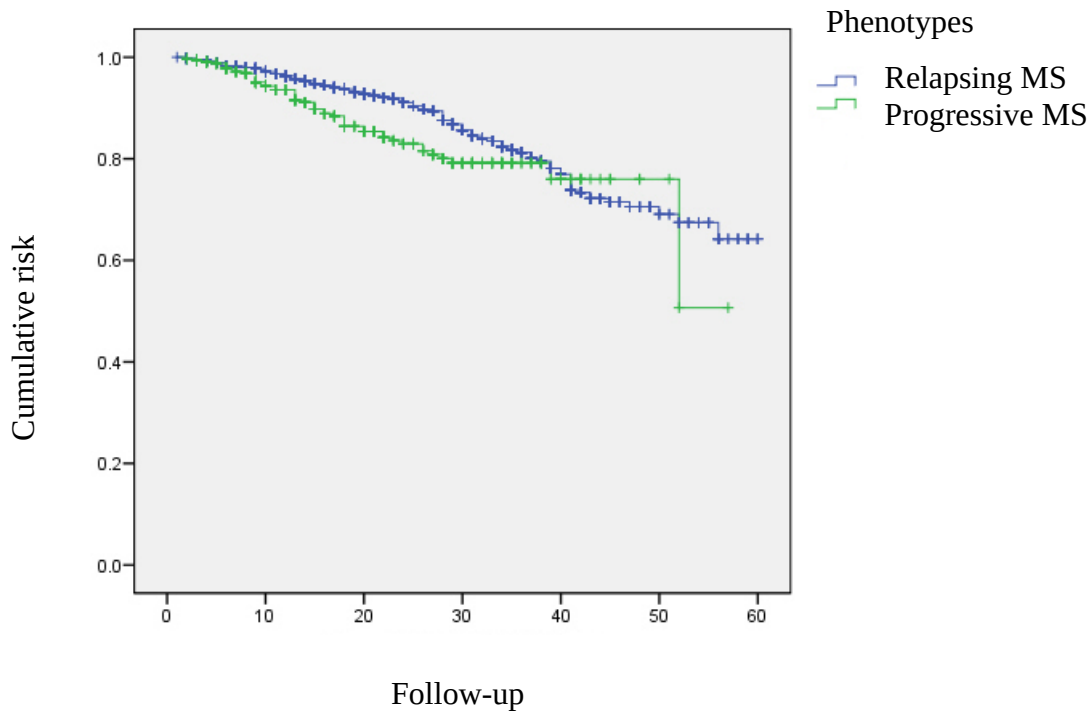

Figure 14. Cumulative risk for cardiovascular disease occurrence according to MS phenotype

5. DISCUSSION

Our study investigated comorbidity burden in patients with MS, using Belgrade population-based MS registry data. The most prevalent groups of comorbidities were psychiatric and cardiovascular diseases, while the most prevalent single comorbidities were depression and hypertension. When the prevalence of comorbidity was analyzed between relapsing and primary progressive MS phenotypes, it was shown that only prevalence of type 2 diabetes differed significantly, with higher prevalence among patients with primary progressive MS. Disability progression, measured by means of PI, was statistically significantly faster in MS patients with at least one comorbidity compared to those without. Furthermore, higher values of PI were associated with increasing number of comorbidities. Patients with at least one recorded comorbidity had higher probability of being treated with DMTs compared to MS patients without any comorbidity. This probability decreased with increasing number of co-occuring conditions. On the other hand, MS patients treated with DMTs had a higher risk of comorbidity occurrence compared to untreated MS patients.

Investigation of glucose metabolism patterns revealed that MS patients had a significantly higher prevalence of IR in comparison with healthy individuals. Further, disability level, expressed as EDSS, PI and MSSS, correlated significantly with glucose levels at different time points during OGTT, and other biochemical parameters. Also, measured levels of biochemical parameters differed significantly between relapsing and primary progressive course of MS. Finally, multivariable logistic regression analysis has shown that glucose level at 120’ was independently associated with MS.

Results of the case-control study exploring difference between chronological and vascular age in MS patients ranging from a group with healthy endothelium to groups with gradually increasing degree of endothelial damage have shown meaningful difference in chronological age, vascular age and difference between them among compared groups. The hierarchical linear regression analysis has revealed that EDSS, physical activity, body mass index and presence of type 2 diabetes comorbidity explained a total of 48% of the variance in discrepancy between chronological age and vascular age.

Additional finding of our study was that EDSS score and MS phenotype were independent predictors of occurrence of cardiovascular comorbidity, coronary artery disease and type 2 diabetes.

According to the analysis of frequency of different groups of comorbidities, it was observed that the most common comorbidities in Belgrade MS population are psychiatric disorders (20.59%) and cardiovascular comorbidities (15.23%). A study from Chile found that most prevalent comorbidities in total MS cohort were smoking (any time during life, prevalence = 42.2%), depression/anxiety (34.9%), thyroid disease (15.7%), hypertension (11.3%), insulin resistance/type

2 diabetes mellitus (11%), followed by migraine (7.9%), dyslipidemia (7.3%), epilepsy (4.6%) and bipolar disorder (4.4%) (144). Similar findings were observed in a systematic review which comprised 249 different studies investigating comorbidity in MS (138). It was found that anxiety is present in 21.9% (95% CI 8.76–35.0), depression in 23.7% (95% CI 17.4–30.0), hypertension in 18.6%, (95% CI 13.9– 23.2), while hyperlipidemia was noticed in 10.9% of MS patients (95% CI 5.6–16.1) (138). It is important to emphasize that the authors of this systematic review pointed out that all of these findings should be carefully interpreted having in mind that studies differ in many aspects including study design, sample size, source of data etc. and that 95% CI should be more trusted compared to meta-analysis summary values.

A recent study from Australia compared comorbidity prevalence in MS before and after disease onset and found that at the time of the first symptoms occurrence, most prevalent co-occurring conditions were allergies (29.2%), migraine (20.2%), anxiety (15.9%), depression (15.8%), hypertension (8.3%), elevated cholesterol levels (7.2%) and anaemia (7.2%) (145). At the time of investigation which was on average 20.5 years after disease onset, the most common co-occurring conditions were depression (42.7%), anxiety (39.0%), allergies (37.4%), hypertension (30.2%), migraine (28.1%), high cholesterol (23.5%) and osteoarthritis (23.3%) (145). Results of the study revealed that the comorbidities with the highest increase in prevalence during period between disease onset and time of investigation were depression (+ 26.9%), anxiety (+ 23.1%), hypertension (+ 21.9%), osteoarthritis (+ 17.1%), elevated cholesterol levels (+ 16.3%), eye diseases (+ 11.6%), osteoporosis (+ 10.9%) and cancer (+ 10.3%) (145).

In our analyses we also presented age and gender adjusted prevalence values. Adjustment was done in two ways, using European and World standard populations. This was performed because comparisons of results between countries or even between different regions of the same country are often influenced by demographic characteristics of compared populations. Given that populations all over the world differ significantly, it is clear that adjustment is necessary in order to make international comparisons possible and accurate. Since differences are predominantly affected by age and gender, adjustment is usually done for these two characteristics. In absence of adjustment, crude values could result in significant misinterpretation of obtained data (146).

In our study we put emphasis on investigation of the presence of vascular comorbidities in people with MS. Special attention has been directed to hypertension, hyperlipidemia, and type 2 diabetes keeping in mind that those conditions are very prevalent in general population. We observed that 11.41% of our sample had hypertension, 2.86% had hyperlipidemia, while diabetes was present in 2.06% of MS patients in the registry. This is pretty lower when compared to a systematic review on incidence and prevalence on comorbidity in MS, in which 18.6% (95% CI

13.9–23.2) of MS population suffered from hypertension, 10.9% (95% CI 5.6–16.1) from hyperlipidemia and 8.57% from diabetes (138).

A systematic review published this year, which included 121 studies on comorbidity in MS, revealed that cardiovascular disorders are second or third leading cause of death in this population (147). Authors emphasized that all included studies which investigated cardiovascular comorbidity in MS population found an increased incidence, prevalence or risk in persons with MS compared to MS-free population (147). Furthermore, it has been shown that the presence of cardiovascular comorbidity is associated with DMTs treatment (147). Another study has shown that increased cardiovascular risk is correlated with greater risk of relapses, disability, and DMTs escalation in MS (148).

In 2016, Thormann et al. published paper on the prevalence of cardiovascular and cerebrovascular comorbidities in MS population in Denmark, before and after the clinical onset of disease (149). This study is of particular interest since it was conducted in a sample which comprised all MS patients with disease onset registered in period 1980-2015 in this country. This was possible because of the existence of population-based MS registry (The Danish Multiple Sclerosis Registry) which is considered as the best MS registry in the world. When occurrence of investigated comorbidities was compared before and after disease onset it was observed that before the disease onset, MS patients had a lower chance for presence of cerebrovascular disorders compared to MS-free population (OR 0.69, 95 % CI 0.48–0.99, p=0.043), while after the disease onset, precisely, after one year after disease onset, probability for cerebrovascular disease occurrence was higher compared to MS-free population (HR=1.84, 95 % CI 1.69–2.00, p=0.0005) (149). The hazard ratio for stroke was 1.28 (95% CI 1.16–1.41, p<0.0005). Similarly when the same analysis was done for cardiovascular diseases, it resulted in decreased chance for presence of this comorbidity before disease onset compared to MS-free population (OR 0.87, 95 % CI 0.71–1.07, p = 0.188) (149). Following disease onset, risk for cardiovascular comorbidity occurrence increased (HR 1.08, 95 % CI 1.02–1.15, p = 0.013) (149).

Recently, in Sweden, study on noncommunicable comorbid diseases in MS population has been published (150). Study was designed as a population-based, national cohort study, and included 6602 new MS cases diagnosed in period 2008-2016, along with 61,828 persons without MS. Prior to observed time interval, prevalence of cardiovascular disease in persons with and without MS was between 0% and 2% (150). Nevertheless, prevalence of certain cardiovascular conditions was higher in MS population, including stroke and transient ischemic attack as well as venous tromboembolism and peripheral vascular disease (150). During the investigated time frame (2008-2016), incidence rates for major adverse cardiovascular events (Incidence rate ratio (IRR) =1.42, 95% CI 1.12-1.82), stroke (IRR=1.46, 95% CI 1.05-2.02), transient ischemic attack

(IRR=1.65, 1.09-2.50), heart disease (IRR=1.55, 95% CI 1.15-2.10) and venous tromboembolism (IRR=1.42, 95% CI 1.14-1.77) were greater in MS patients than in matched non-MS participants (150). Additionally, when the risk for these comorbidities was stratified according to the age it was found that the risk was higher in all ages in MS population, particularly in those younger than 40 years (150).

One of the most recently published papers investigated hypertension in MS population, including 37 million individual electronic health records from United States, has been published (151). This study found crude prevalence of hypertension of 41.5% in MS population, and 22.7% in persons without MS. Further, when these values were adjusted according to the age and gender, prevalence values changed to 32.3% for MS population and 22.8% for persons without MS (151). Finally, when race of patients was taken into consideration, prevalence of hypertension in MS patients was 33.0%, while in those without it was 26.4% (151). Although reasons for high prevalence of hypertension in MS patients are not completely understood, it is believed that high prevalence of risk factors such as smoking and physical inactivity strongly contributes to these findings. Additionally, it was suggested that MS treatment may also play an important role in development of hypertension in this population since it has been shown that it can be adverse effect of some treatment modalities, for example glucocorticoids (151). It was also confirmed that the prevalence of hypertension has an increasing tendency towards age in both, MS population and non-MS population. Also, when same age groups of MS and non-MS population were compared it was observed that the hypertension was more common in MS population in nearly all age groups (151). Also, data on gender or race did not influence these findings (151). When authors analyzed distribution of hypertension on relation to the gender, it was revealed that hypertension is more frequently present in men with MS than in female MS patients (151). This finding was explained by estrogen effects on the one side, and women's behavior on the other side, precisely, more frequent use of healthcare services and better adherence to hypertension treatment (151).

An additional study from Canada, which was focused on management of hypertension and diabetes in persons with MS, reported prevalence for both conditions in MS and general population and according to the gender for each population. It was found that 22.0% of female MS patients and 24.9% of male MS patients have hypertension diagnosis, along with 21.3% of females and 24.4% of men without MS (152). For type 2 diabetes, it was observed in 9.6% of females and 11.8% of men with MS, and in 9.31% of females and 13.0% of men without MS (152). Final remark of the authors was that hypertension and diabetes do not differ in prevalence between general population and MS population (152). On the contrary, a study combining electronic databases from USA and UK revealed that MS population has two times higher rate of venous thromboembolism and peripheral

vascular disease compared to those without MS, while the rates of myocardial infarction were 2.5 times higher in female MS patients in comparison with females without MS (153).

In our study we found statistically significant difference in vascular age and in discrepancy between chronological and vascular age, among MS patients with various levels of endothelium damage, ranging from MS patients with no history of cardiovascular diseases to MS patients with coronary artery disease. Furthermore, it was observed that the presence of type 2 diabetes explained 11% of variance in difference between chronological and vascular age, while physical activity and body mass index explained 10% of variance. The difference in chronological and vascular age can be explained, at least partially, by higher prevalence of cardiovascular risk factors in MS population. The hypothesis that vascular ageing could be more rapid, if cardiovascular risk factors are present, has been already postulated (154, 155). Łoboz-Rudnicka et al. in their investigation have also shown that type 2 diabetes mellitus as a vascular risk factor was associated with an 5-year discrepancy between chronological and vascular age (156). Additionally, in this study it was also observed that body mass index is associated with advanced VA, but only in women (156).

Investigation of vascular comorbidity in MS population is of great importance and it can be explained in several ways. First, these conditions are among leading causes of morbidity and mortality in general population. Second, having in mind that their prevalence is highest in older age groups as well as disability in MS population, it is clear that it represents additional burden for MS patients (157). Moreover, vascular conditions represent frequent cause of mortality in MS population (158).What is even more important, it has been shown that presence of this comorbidity can cause more rapid disability progression in MS patients in comparison to MS patients without vascular comorbidity (159). In line with this, findings suggest that changes in small vessels in CNS are the crucial feature of MS besides demyelinating plaques (160). Therefore, it is assumed that small vessel disease is the main contributor among vascular comorbidities to worsening of disability level in MS (160). Based on all of this information it is evident that identification and treatment of vascular comorbidity in persons with MS should be among priorities, as it could prevent disability progression to at least some extent.

When psychiatric disorders were discussed, systematic review based on 41 research concluded that anxiety is found in 1.24-36.0%, while the depression was found in 6.94 to 70.1% MS population (125). In another study, online research that included participants from several different countries, observed prevalence of anxiety and depression of 29.1% (95% CI 27.4–31.0) and 31.7% (95% CI 29.9–33.6), respectively (161). In addition, it has been shown that the point prevalence of depressive symptoms in MS population is approximately 30% (57). According to the results of another systematic review, reported prevalence of depression and anxiety in included studies varied from 21.1 to 59.4% and from 28.1 to 57.0%, respectively (147).

It should be pointed out that psychiatric comorbidities, especially depression and anxiety are the leading group of comorbidity that has been investigated in MS population, since evidence emerges that they can be associated with certain adverse outcomes in these patients. In more details, it has been shown that the presence of these comorbidities may negatively affect quality of life of MS patients and level of disability which is a key determinant of disease (162, 163). This relationship was also confirmed in a previously mentioned systematic review (147). Additionally, in the CombiRx trial, a double-blind, randomized controlled clinical trial, exploring the combination of different combination of DMTs in treatment of MS patients, it was revealed that anxiety (hazard ratio [HR]: 1.25; 95%CI: 1.01-1.55), as well as hyperlipidemia were (HR 1.32; 95% CI: 1.01-1.72) related to higher risk of disease activity, while opposite results were found for migraine (HR 0.80; 95%CI: 0.67-0.96) (164). The significance of depression in MS is even more important bearing in mind its relationship with suicide, given that rates of suicide in MS population are two-fold higher than in general population (165).

Study investigating impact of comorbidity on quality of life in MS population has shown that psychiatric and musculoskeletal comorbidities are the leading contributors of lower quality of life in MS (166). When impact of single comorbidities was analyzed it was revealed that depression had a strong impact on total score of quality of life as well as on psychosocial functioning in MS population (166).

Another important finding of these studies is that depression is more frequently present in persons with MS than in general population (167, 147). Similar results were obtained for anxiety and bipolar disorder (147). What is also important, it was found that the presence of psychiatric disorders is common in MS patients even before the disease onset (147).

Results of our study indicated that any type of cancer was present in 2.53% (95% CI 1.99–3.21) of MS population. Among those MS patients with cancer, the most prevalent localization was the breast cancer (Prev = 0.95%, 95% CI 0.58–1.53). Similar findings were obtained in a systematic review examining occurrence of malignant diseases in MS population where the meta-analysis summary estimate was that prevalence of this comorbidity in MS population is 2.23% (95% CI 1.18–3.29) (138). However, in this systematic review, breast cancer was more frequently observed with the estimated prevalence of 2.01% (138). Another systematic review revealed that in included studies association of MS and cancer was not observed, and even some slightly decrease in cancer occurrence in MS was reported in some studies (147). Increased risk for cancer in MS population was only shown for breast cancer in Sweden and for several cancer localizations in MS population in Taiwan (147). Further, it has been observed that treatment of MS patients with DMTs may increase the chance for cancer development (168), while another study suggested that switching DMTs in MS patients is associated with increased risk of cancer (169). Investigation of cancer

occurrence in MS population is of particular interest as the both type of diseases are related to immune system functioning and consequently research on this topic could provide some deep insight into mechanisms for their etiology.

Overall prevalence of autoimmune diseases in our study was 6.06% (95% CI 5.22–7.02). Further, when the prevalence of autoimmune comorbidities was analyzed separately for each condition, it was found that the thyroid disease is the most prevalent autoimmune disease in our cohort of MS patients (4.44%, 95% CI 3.71–5.30). Marrie et al. in their research collected data from all previously published studies on prevalence of autoimmune disease in MS which resulted in overall prevalence of 3.0%-26.1% and prevalence of thyroid disease of 6.44% (95% CI 0.19–12.7), which is higher compared to our MS cohort (138). A previously mentioned cohort study from Sweden revealed that prior to investigated time period (2008-2016), autoimmune disorders were more frequent in MS population compared to those without MS (150). Additionally, during study period it was found that persons with MS have greater risk for development of autoimmune disease (IRR=3.83, 95% CI 3.01-4.87) (150). This was also confirmed in study by Hauer et al. in which it was found that persons with MS have greater prevalence of all autoimmune disease than general population, with special emphasis on psoriasis, rheumatoid arthritis, and inflammatory bowel disease (147).

Our research identified presence of epilepsy in 2.60% (95% CI 2.06–3.30) of MS patients in the Belgrade MS population. Marrie et al. in their research found that the epilepsy is more frequently observed in MS population compared to the general population (128). In the recently published systematic review, it was reported that the prevalence of epilepsy ranges between 1.9% and 7.6% in MS population, while the highest values (8.5%) were observed in the early-onset MS (147). Additional finding of this study was that epilepsy is associated with the disability level in persons with MS (147).

Additional analysis in our study referred to the comparison of frequency of different comorbidity according to the MS phenotype. The only statistically significant difference was observed for prevalence of diabetes which was more common in patients with PPMS compared to patients with RRMS ($p< 0.001$). One of the potential explanations for this finding is age. Namely, participants in our cohort with PPMS were statistically significantly older (59.84 ± 13.35 years) compared to the patients with RRMS (52.84 ± 14.22 years) ($p < 0.001$). However, the association of MS and type 2 diabetes has been a subject of investigation for a long time. It has been even shown that the presence of type 2 diabetes increases chance for MS development (170). Although etiology and exact mechanisms of co-occurrence of these conditions is yet to be discovered, so far performed research suggest potential role of adaptive immune system and insulin resistance (171, 172). Moreover, diagnosis of type 2 diabetes has been recently associated with the occurrence brain

atrophy, short time after diagnosis (173). Based on all these facts, it is evident that additional studies revealing connection between MS and type 2 diabetes should be performed in the near future.

A recently published multicenter study from Chile investigated also prevalence of comorbidity in MS patients with various phenotypes (PPMS, RRMS, SPMS and CIS) (144). Study sample consisted of 453 MS patients, of which 87 had a progressive course of disease. Average age of the MS cohort was 41±12 years and disease duration 10.3±7.2 years. The median EDSS in inflammatory group was 1.0 (range 0-10) and in progressive 6.0 (range 2-10) (144). Authors compared inflammatory and progressive group of MS patients in terms of prevalence of smoking any time in life, depression/anxiety, bipolar disorder, epilepsy, migraine, thyroid disease, hypertension, insulin resistance/type 2 diabetes, dyslipidemia, polycystic ovary syndrome and cancer. The only statistically significant difference in prevalence of comorbidity according to disease phenotype was registered for hypertension ($p=0.004$), which occurred in 9% of MS patients in inflammatory group and 20.7% of patients in progressive group (144).

Our study revealed that there is a relationship between the presence of comorbid conditions and disability evolution in MS patients. Namely, PI was significantly higher in MS patients with history of comorbidity compared to MS patients without co-occuring conditions ($p=0.003$). Furthermore, as the number of comorbidities increased, PI had a higher value ($p< 0.001$). Our findings were confirmed in many other investigations (159, 162, 174). It is worth mentioning that for both somatic and psychiatric comorbidities relationship with disability worsening has been documented (162, 174).

Impact of presence of different comorbidity on disability level was discussed in the already mentioned Chilean study (144). They performed these analyses separately for the inflammatory MS group and progressive MS group. Results showed that in the inflammatory group, factors associated with higher disability degree (EDSS≥6.0) were older age at the beginning of disease ($p=0.008$), longer time period between disease onset and observed study period ($p=0.039$), and co-occurring epilepsy ($p=0.018$) (144). For the progressive MS group, factors associated with an EDSS≥6.0 was only a longer time period between disease onset and observed study period ($p=0.014$). On the contrary, it was found that in progressive MS cohort shorter time between symptoms onset and MS diagnosis ($p=0.025$) as well as co-occurring insulin resistance/type 2 diabetes ($p=0.028$) lowered chance for EDSS≥6.0 in this group, i.e. they were associated with decreased chance for achieving this disability level (144).

Additional step in our analysis of presence of comorbidity in Belgrade MS population was investigation of relationship with DMTs treatment. MS patients with any comorbid condition were more frequently treated with DMTs in comparison with MS patients without comorbidity. Also,

with higher number of registered comorbidities in MS patients, likelihood for administration of DMTs treatment decreased (p = 0.014). This observation was also present in a Canadian study, where authors concluded that MS patients with highest number of registered comorbidities have the lowest chance for starting DMTs treatment (175). Another important issue is related to the influence of DMTs therapy on comorbidity occurrence. It was observed that in our MS cohort, MS patients who are already treated with DMTs more frequently experience presence of comorbidity compared to treatment-naïve MS patients (p = 0.001). It is apparent that this information should be taken into consideration when making decisions on initiating DMTs treatment in MS patients.

Another important finding of our study was the high prevalence of IR in MS population (64.1%), in comparison with healthy controls (30.8%) (p=0.008). These finding were already observed in previous research (176, 177), although they are controversial. Namely, some research has shown that prevalence of IR in MS and non-MS populations are comparable (178-180). This may be a consequence of some study design characteristics, such as study sample comprising only incident MS cases (178) or small number of participants (180). Additionally, in one study meaningfully elevated insulin levels as well as HOMA indexes in MS group compared to healthy controls, have been reported. However, opposite to our results, authors did not find statistically significant difference in glucose levels between compared groups.

HOMA-IR has been most commonly used for determination of IR presence, however, opinions in terms of its use are different. It is suggested that some other measure, such as Quantose score should be used (181-183). This score is calculated based on the levels of fasting insulin, α-hydroxybutyrate, linoleoylglycerophosphocholine and oleate. In an investigation of IR prevalence in MS population, in which Quantose score was used, more than a half of MS patients (51%) had IR (181). Additional findings in this research were that IR, determined through Quantose score use, contributes to disability progression and is associated with SPMS course (181).

In our study we also reported the association of glucose levels during an OGTT and disability degree as well as its evolution. We revealed statistically significant relationship between PI and HbA1c in percentages. Furthermore, results of univariable and multivariable logistic regression analyses have shown that an independent association was observed between MS and glucose level at 120’ during OGTT. Having this in mind, it has been shown that persons with MS have 3.937 times greater risk of glucose metabolism disturbances compared to healthy controls. It should be pointed out that, due to unavailability of causal treatment for MS patients, early treatment with DMTs may substantially contribute to slower disease progression, through decreased disease activity and brain preservation (184, 185). Also, it has been shown that, in USA, 66.7% newly diagnosed persons with MS do not start their DMTs treatment following diagnosis, which results in greater comorbidity burden (186). In that light, new brain health concept emphasizes the importance

of early detection of MS, immediate initiation of DMTs treatment and switching of DMTs if necessary, together with inclusion of certain lifestyle habits such as regular physical activity, optimal diet, smoking cessation and adequate and early management of comorbidities. Appropriate application of these recommendations could significantly contribute to prolonged brain health in MS patients. Keeping in mind these facts, results of our study implicate that inclusion of an OGTT could be considered as a part of brain health-focused care in MS patients (187).

6. CONCLUSIONS

Based on the all aforementioned data on comorbidity burden in Belgrade MS population, following conclusions can be drawn:

1. On the prevalence day, December 31, 2019, a total of 1903 females and 822 men with MS were registered in the Belgrade population MS Registry, with an average age of 55.8 ± 14.3 years. Majority of MS patients were employed (54.8%) and married (60.2%).

2. The majority of patients had RRMS (62.9%), followed by SPMS (24.1%) and PPMS (13.0%). Median EDSS score was 4.0. The average disease duration was 21.6 ± 12.5 years. A total of 534 MS patients were receiving DMTs at some period of the disease

3. Presence of at least one comorbidity was observed in 1331 MS patients in the Registry (48.8%). Groups of comorbid conditions with the highest prevalence were psychiatric (20.59%) and cardiovascular diseases (15.23%). Malignant comorbidities were observed in 2.53% of our cohort while prevalence of epilepsy and type 2 diabetes were 2.60% and 2.06%, respectively.

4. The most frequent single comorbidities were depression (11.82%) and hypertension (11.41%). The most prevalent malignant disease was the breast cancer (0.95%), while the most common autoimmune disease was the thyroid disease (4.44%).

5. The prevalence of investigated comorbidity groups did not differ between relapsing and primary progressive MS phenotypes, except in case of type 2 diabetes which was more common in patients with PPMS (4.7%) compared to the patients with RRMS and SPMS (1.5%).

6. MS patients with at least one comorbidity have statistically significantly higher PI (0.31 ± 0.28) compared to MS patients without any comorbidity (0.27 ± 0.25). Furthermore, PI statistically significantly correlated with increasing number of comorbidities.

7. MS patients who had at least one comorbidity had greater chance to be in the group receiving DMTs in comparison with those patients that were without comorbidities (age-adjusted OR = 1.40, 95% CI 1.15–1.69, p = 0.001). Furthermore, with increasing number of comorbidities, decreasing chance for treatment with DMTs was observed. Patient with two comorbidities had 9% less chance

to be treated with DMTs compared to MS patient without comorbidity (OR = 0.91). Patients with three comorbidities had 45% less chance for DMTs treatment (OR = 0.55).

8. MS patients receiving DMTs were at greater risk for comorbidity occurrence in comparison to MS patients without DMTs treatment (age-adjusted OR = 1.54, 95%CI 1.18–2.00, p = 0.001).

9. A case-control study comparing MS patients and healthy controls have shown that the prevalence of IR, based on HOMA-IR value, was statistically significantly higher in MS patients (64.1%) than in controls (30.8%). Additionally, in three MS patients presence of type 2 diabetes was registered (P=3.8%), while in 12 MS patients pre-diabetes (IGT and IFG) was found (P=15.4%). Presence of type 2 diabetes was not found among participants in the control group, while in one participant, IFG condition was detected.

10. EDSS score was statistically significantly associated with glucose levels at different time points and with HbA1c and TG levels. MSSS correlated significantly with glucose levels at different time points while PI correlated significantly with HbA1c.

11. Glucose levels at different time points and TG levels differed between MS phenotypes, with higher values observed in RRMS compared to PPMS and SPMS.

12. Results of univariable and multivariable logistic regression analysis revealed that glucose 120' was independently associated with MS (OR=3.937, 95% CI 1.178-13.159, p=0.026).

13. Based on the ROC analysis for HOMA-IR, best cut-off value for HOMA-IR in our study was 2.3, which provided both sensitivity and specificity of 66.7% in discriminating persons with MS from controls.

14. Results of the case-control study exploring difference between chronological and vascular age in MS patients ranging from a group with healthy endothelium to groups with gradually increasing degree of endothelial damage have shown statistically significant difference in chronological age, vascular age and difference between chronological and vascular age among compared groups.

15. The hierarchical regression analysis showed that EDSS score, physical activity, body mass index and presence of type 2 diabetes comorbidity accounted for 48% of the variance of discrepancy between chronological age and vascular age as a dependent variable.

16. In the retrospective cohort study investigating impact of comorbidity on MS course, Cox regression analysis for predictors of cardiovascular disease, coronary artery disease and type 2 diabetes have shown that independent predictors of these comorbidities were EDSS score and primary-progressive MS phenotype.

17. According to Kaplan-Meier curves, higher risk for both, type 2 diabetes and cardiovascular disease occurrence, was statistically significantly associated with primary progressive MS phenotype.

18. Our findings implicate the need of screening and early and adequate management of different comorbidities in MS patients, especially having in mind its association with range of adverse outcomes in these persons.

19. Additionally, based on our results that persons with MS have almost four times greater risk of disturbed glucose metabolism compared to healthy controls, it can be hypothesized that, it might be very useful to incorporate the performance of OGTT in the brain health-focused care in these patients.

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BIOGRAPHY

Gorica Maric is a Teaching Assistant at Institute of Epidemiology, Faculty of Medicine, University of Belgrade. She obtained MD degree at Faculty of Medicine, University of Belgrade (2012). She finished Specialist Academic Studies Module: Cytology, immunohistochemistry and electron microscopy also at Faculty of Medicine, University of Belgrade (2013). In 2013 she started PHD studies in field of Epidemiology and PhD thesis regarding comorbidities in patients with multiple sclerosis. In 2016 she started specialization in Epidemiology and finished it in 2019. TA Gorica Maric is currently involved in one domestic research project supported by Ministry of Education, Science and Technological Development of Republic of Serbia: "Epidemiological investigation of neurological disorders: global measurement of disease impact" as well as two international projects “Autonomic dysfunction in persons affected by neuromyelitis optica spectrum disorders”, bilateral project with Croatian party, Ministry of Education, Science and Technological Development of the Republic of Serbia, 2019-2020, and “Early life events and the risk for neuroinflamatory diseases in adulthood: a population-specific epidemiological approach on multiple sclerosis”, bilateral project with Italian party, Ministry of Education, Science and Technological Development of the Republic of Serbia, 2019-2021. She is a Member of Initial Board of PhD Students Association at the Faculty of Medicine, University of Belgrade (2016 - present) and a member of Ethics Committee at Faculty of Medicine, University of Belgrade. She has published 32 papers in journals from JCR list and has reviewed for several international journals. She finished several international educational courses, among them OMI Seminar in Salzburg, Austria.

BIOGRAFIJA

Asistent dr Gorica Marić je zaposlena na Institutu za epidemiologiju Medicinskog fakulteta, Univerziteta u Beogradu. Diplomirala je na Medicinskom fakultetu u Beogradu 2012. godine. Završila je specijalističke akademske studije, modul: Citologija, imunohistohemija i elektronska mikroskopija na Medicinskom fakultetu u Beogradu 2013. godine. Iste godine, započela je doktorske akademske studije, smer Epidemiologija i rad na doktorskoj disertaciji o komorbiditetima u multiploj sklerozi. Tokom 2016. godine, upisala je specijalizaciju iz Epidemiologije, koju je završila 2019. godine. Asist. dr Gorica Marić je trenutno uključena u projekat Ministarstva prosvete, nauke i tehnološkog razvoja Republike Srbije "Epidemiološka istraživanja neuroloških bolesti: sveobuhvatna procena uticaja bolesti", kao i dva međunarodna projekta "Autonomni poremećaji kod osoba sa oboljenjima iz spektra neuromijelitis optika", bilateralni projekat istraživača iz Hrvatske i Ministarstva prosvete, nauke i tehnološkog razvoja Republike Srbije, 2019-2020, i "Rani životni događaji i rzik za neuroinflamatorne bolesti u odraslom dobu: epidemiološki pritup multiploj sklerozi", bilateralni projekat istraživača iz Italije i Ministarstva prosvete, nauke i tehnološkog razvoja Republike Srbije, 2019-2021. Član je Inicijativnog odbora za osnivanje Sekcije studenata doktorskih akademskih studija Medicinskog fakulteta u Beogradu (2016 - 2020) i član Etičkog komiteta Medicinskog fakulteta Univerziteta u Beogradu. Publikovala je 32 rada u časopisima sa *JCR* liste i bila recenzent u više časopisa. Završila je nekoliko međunarodnih edukacija, među njima i OMI seminar u Salzburgu, Austrija.

образац изјаве о ауторству

Изјава о ауторству

Име и презиме аутора ГОРИЦА МАРИЋ

Број индекса ЕП-15/13

Изјављујем

да је докторска дисертација под насловом

ASSESSMENT OF COMORBIDITY BURDEN IN PERSONS WITH MULTIPLE SCLEROSIS: OBSERVATIONAL STUDY BASED ON BELGRADE POPULATION REGISTRY DATA

- резултат сопственог истраживачког рада;
- да дисертација у целини ни у деловима није била предложена за стицање друге дипломе према студијским програмима других високошколских установа;
- да су резултати коректно наведени и
- да нисам кршио/ла ауторска права и користио/ла интелектуалну својину других лица.

Потпис аутора

У Београду, 2.11.2020.

ГоницаМарић

образац изјаве о истоветности штампане и електронске верзије докторског рада

Изјава о истоветности штампане и електронске верзије докторског рада

Име и презиме аутора ГОРИЦА МАРИЋ

Број индекса ЕП-15/13

Студијски програм ЕПИДЕМИОЛОГИЈА

Наслов рада ASSESSMENT OF COMORBIDITY BURDEN IN PERSONS WITH MULTIPLE SCLEROSIS: OBSERVATIONAL STUDY BASED ON BELGRADE POPULATION REGISTRY DATA

Ментор ПРОФ. ДР ТАТЈАНА ПЕКМЕЗОВИЋ

Изјављујем да је штампана верзија мог докторског рада истоветна електронској верзији коју сам предао/ла ради похрањивања у **Дигиталном репозиторијуму Универзитета у Београду.**

Дозвољавам да се објаве моји лични подаци везани за добијање академског назива доктора наука, као што су име и презиме, година и место рођења и датум одбране рада.

Ови лични подаци могу се објавити на мрежним страницама дигиталне библиотеке, у електронском каталогу и у публикацијама Универзитета у Београду.

Потпис аутора

У Београду, 2. 11. 2020.

Горица Марић

образац изјаве о коришћењу

Изјава о коришћењу

Овлашћујем Универзитетску библиотеку „Светозар Марковић" да у Дигитални репозиторијум Универзитета у Београду унесе моју докторску дисертацију под насловом:

ASSESSMENT OF COMORBIDITY BURDEN IN PERSONS WITH MULTIPLE SCLEROSIS: OBSERVATIONAL STUDY BASED ON BELGRADE POPULATION REGISTRY DATA

која је моје ауторско дело.

Дисертацију са свим прилозима предао/ла сам у електронском формату погодном за трајно архивирање.

Моју докторску дисертацију похрањену у Дигиталном репозиторијуму Универзитета у Београду и доступну у отвореном приступу могу да користе сви који поштују одредбе садржане у одабраном типу лиценце Креативне заједнице (Creative Commons) за коју сам се одлучио/ла.

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