İSTANBUL TECHNICAL UNIVERSITY ★ INSTITUTE OF SCIENCE AND TECHNOLOGY

CLASSIFICATION OF CLINICALLY DIFFERENT SUBTYPES OF MULTIPLE SCLEROSIS

M.Sc. Thesis by
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Programme : Molecular Biology-Genetics and Biotechnology

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MULTİPL SKLEROZ HASTALIĞININ FARKLI KLİNİK ALTTİPLERİNİN SINIFLANDIRILMASI

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FOREWORD

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December 2010

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TABLE OF CONTENTS

Page

ABBREVIATIONS

AdaBoost : Adaptive Boosting
ANOVA : Analysis of Variance
AUC : Area under ROC curve
BayesNet : Bayesian Networks
CIS : Clinically Isolated Syndrome
CISRR : CIS patients that became RRMS
CNS : Central Nervous System
CSF : Cerebrospinal Fluid
GFAP : Glial Fibrillary Acidic Protein
HC : Healthy Controls
J48 : Decision Tree
MBP : Myelin Basic Protein
MOG : Myelin Oligodendrocyte Glycoprotein
MRI : Magnetic Resonance Imaging
MS : Multiple Sclerosis
NFL : Neurofilament Light Chain
OCB : Oligoclonal Band
OND : Other Neurological Disease
PPMS : Primary – Progressive MS
PRMS : Prograssive – Relapsing MS
ROC : Receiver Operating Characteristic
RRMS : Relapsing – Remitting Multiple Sclerosis
SPMS : Secondary – Progressive MS
kNN : k Nearest Neighbor

LIST OF TABLES

Page

LIST OF FIGURES

Page

CLASSIFICATION OF CLINICALLY DIFFERENT SUBTYPES OF MULTIPLE SCLEROSIS

SUMMARY

Multiple sclerosis(MS) is an immune-mediated disease of the central nervous system (CNS) with heterogeneous clinical presentation and course. Today, revised McDonald's criteria is the gold standard for MS diagnosis. MS can be confused with other neurological diseases. Moreover, there is no absolute criteria for the prediction of prognosis of the disease.

This study focuses on the classification of different clinical subtypes of MS using TAU,GFAP,NFL and MOG proteins and clinical data. The aim of this study are summarized as follows:

- To investigate different candidate protein and clinical data patterns among the MS subtypes, CIS samples and control samples.
- To show that clinical subtypes of MS can be classified using protein data and clinical data.
- To predict the transition between CIS and MS. This study aims to show that the prognosis of MS can be predicted using protein and clinical data.

Protein findings and clinical data of 67 Relapsing Remitting MS (RRMS), 46 Clinically Isolated Syndrome (CIS), 22 Primary Progressive MS (PPMS) patients and 22 control subjects were analyzed in this study. CSFs of patients were collected by lumbar puncture (LP) within 3 days of an acute attack. LP was performed before the medication. TAU,GFAP,NFL, MOG and MBP protein concentrations of samples were determined by Western Blot analysis. Protein bands were scanned by using densitometer and scanned protein bands were analyzed by using ImageJ analysis software to obtain quantitative measurement [1]. Quantities of proteins were taken as colorimetric unit (CU). CU is a numerical value showing the insensitivity of protein band concentration, ranged between 0 (most) and 255 (least). Analyzed values were linearized and normalized due to loaded total protein concentration. All samples were scanned and analyzed with the same standard procedure. After classical statistical analysis such as ANOVA, TAU,GFAP, NFL and MOG protein results found to be significantly different among subtypes and control samples ($p<0.001$). Using different classification methods, different clinical subtypes of multiple sclerosis were classified according to their protein and clinical data patterns.

To the best of our knowledge, there are no other studies in the literature that uses these patterns to predict the transition from Clinically Isolated Syndrome (CIS) to Multiple Sclerosis. The clasification results of protein data showed that when the proteins are used together for classification of MS and control samples, $94.25\% \pm 6.44$ accuracy and 0.97 ± 0.08 area under curve (AUC) was obtained. It is also found that control group and CIS patients can be classified using these proteins together

with 87.31% ± 12.02 accuracy and 0.93 ± 0.09 AUC. The overall accuracy obtained using GFAP-MOG is 74.12% ± 10.77 (AUC=0.79 ± 0.13) between control group, CIS patients and MS patients. In addition, when used for discriminating PPMS from RRMS, TAU-GFAP and MOG provided 93.65% ± 8.35 accuracy and 0.96 ± 0.11 AUC.

Although the sample size is limited, it has been also shown in this study for the first time that the transition from CIS to RRMS can be predicted by using TAU protein concentration in CSF. The level of TAU protein gave the 76.22% ± 17.15 (AUC = 0.77 ± 0.24) accuracy for the differentiation of CIS from CIS/RRMS, whereas GFAP levels provided the 67.07% ± 11.77 (AUC =0.81 ± 0.13) accuracy for the overall classification of CIS, CIS/RRMS and RRMS.

The overall results are listed as follows:

1. MS patients, CIS patients, and control group were classified with 71.43%± 10.95 accuracy (AUC: 0.82± 0.12),
2. CIS and control group were classified with accuracy: 87.31%±12.02 (AUC: 0.93±0.09),
3. MS and CIS were clasified with 76.51% ±11.15 (AUC: 0.83 ±0.12) accuracy,
4. RRMS and PPMS were classified with 95.77% ±6.63 accuracy (AUC: 0.97±0.08),
5. MS and control group were classified with 92.64% ±7.15 (AUC: 0.97±0.06) accuracy.
6. Transition from CIS to RRMS was predicted with 86.45% ±12.6 (AUC: 0.89±0.19) accuracy.

This is a novel study using computer aided classification methods with protein and clinical data for diagnostic and prognostic purposes in predicting clinical subtypes of MS and predicting transition between subtypes. In future studies, sample size should be increased, and new biomarkers should be tested. For better classification results, other classification methods can be used. In addition, the parameters of classification algorithms can be fine-tuned for better classification performance. A hierarchical model can be applied for overall classification of clinical subtypes of MS/CIS patients and control group.

MULTIPL SKLEROZ HASTALIĞININ FARKLI KLİNİK ALTTİPLERİNİN SINIFLANDIRILMASI

ÖZET

Multipl Skleroz farklı klinik özelliklere sahip farklı altgrupları olan, merkezi sinir sisteminin bağışıklık sistemi merkezli bir hastalığıdır. Günümüzde MS teşhisi koymak için gözden geçirilmiş McDonalds Kriterleri yaygın bir biçimde kullanılmaktadır. Ancak MS diğer sinir sistemi hastalıklarıyla karıştırılabilmektedir. Ayrıca, hastalığın prognozunu tayin etmekte kullanılan geçerli bir kriter listesi yoktur.

Bu çalışma TAU, GFAP, NFL ve MOG proteinlerini ve klinik verileri kullanarak MS'in farklı klinik alttiplerinin sınıflandırılmasına odaklanmaktadır. Bu çalışmada yapılması amaçlananlar aşağıdaki şekilde özetlenebilir:

- MS örnekleri, CIS örnekleri ve kontrol grubu arasında farklı aday proteinlerin ve klinik veri örüntülerinin araştırılması,
- MS'in farklı klinik alttiplerinin protein verileri kullanılarak sınıflandırılabileceğinin gösterilmesi,
- CIS'dan kesin MS'e geçişin (prognoz) tahmin edilmesi. Bu çalışma, bu tahminle MS'in prognozunun protein verileri ve klinik veriler kullanılarak tahmin edilebileceğini göstermeyi amaçlamaktadır.

Bu çalışmada 67 RRMS, 46 CIS, 22 PPMS ve 22 kontrol (MS olmayan) örneğinin protein ve klinik verileri incelenmiştir. Bu çalışma için kullanılan protein verileri, hastaların BOS örneklerinden elde edilmiştir. Hastaların BOS örnekleri bir ataktan sonraki 3 gün içinde lomber ponksiyon (LP) yöntemiyle elde edilmiştir. LP ilaç kullanımından önce gerçekleştirilmiştir. Örneklerin TAU, GFAP, NFL, MOG ve MBP protein konsantrasyonları Western Blot yöntemiyle tayin edilmiştir. Protein bantları densitometre kullanılarak taranmıştır ve taranan protein bantları, niceliksel bir ölçüm elde edilebilmesi için ImageJ programı ile analiz edilmiştir. Protein miktarları kolorimetrik birim (CU) olarak elde edilmiştir. CU protein bant konsantrasyonunun yoğunluğunu gösteren ve 0 ile 255 arasında değer alan bir sayısal değerdir. Analiz edilmiş değerler yüklenen toplam protein konsantrasyonuna göre doğrusallaştırılmış ve normalize edilmiştir. Tüm proteinler aynı prosedür kullanılarak taranmış ve analiz edilmiştir. ANOVA gibi klasik istatistiksel analizler sonucunda TAU, GFAP, NFL ve MOG protein seviyelerinin farklı alttipler ve kontrol örnekleri arasında anlamlı bir farklılık gösterdiği bulunmuştur ($p<0.001$). Farklı sınıflandırma yöntemleri kullanılarak, MS'in farklı klinik alttpleri protein verileri ve klinik verilere göre sınıflandırılmışlardır.

Ayrıca, bu çalışmada literatürde ilk kez CIS'tan MS'e geçiş bu klinik veri ve protein verilerinin örüntüleri kullanılarak gösterilmiştir.

Sınıflandırma için, 6 yöntem karşılaştırılmıştır: kNN, Bayes Ağları, Decorate, Karar Ağaçları, Rasgele Ağaç ve AdaBoost. Ayrıca sınıflandırmalar aşağıdaki veri altgruplarıyla gerçekleştirilmiştir:

- Sadece protein verileriyle,
- Protein verileri üzerinde temel bileşenler analizi uygulandıktan sonra,
- Protein verileri ve klinik verilerle,
- Tüm veriler üzerinde temel bileşenler analizi uygulandıktan sonra,
- Bilgi Kazancı yöntemiyle özellik seçimi yapıldıktan sonra.

Protein verileriyle yapılan testlerin sonuçlarına göre, tüm proteinler kullanılarak MS hastaları ve Kontrol grubu 94.25% ± 6.44 (AUC=0.97 ± 0.08) doğrulukla sınıflandırılmıştır. Kontrol grubu ve CIS hastalarının sınıflandırılması ise aynı protein grubuyla 87.31% ± 12 (AUC= 0.93 ± 0.09) doğrulukla gerçekleşmiştir.

GFAP-MOG proteinleri kullanılarak, MS hastaları, CIS hastaları ve kontrol grubu %74.66 (AUC = 0.73) doğrulukla sınıflandırılmıştır. Buna ek olarak, PPMS ve RRMS 'in sınıflandırılması TAU-GFAP ve MOG proteinleri tarafından 93.65% ± 8.35 (AUC= 0.96 ± 0.11) doğrulukla elde edilmiştir.

Bu çalışmada veri boyutunun sınırlı olmasına karşın, CIS'tan RRMS'e geçişin TAU proteini kullanılarak öngörülebileceği gösterilmiştir. TAU protein seviyesi CIS'tan CISRR'ye geçişi 76.22% ± 17.15 (AUC = 0.77 ± 0.24) doğrulukla tahmin etmiştir. CIS, CISRR ve RRMS'in sınıflandırılması ise GFAP proteini kullanılarak 67.07% ± 11.77 (AUC =0.81 ± 0.13) doğrulukla elde edilmiştir.

Tüm sınıflandırma yöntemlerinin ve tüm veri altgruplarının sonuçlarına bakıldığında:

1. MS hastaları, CIS hastaları ve kontrol grubu arasındaki 71.43%± 10.95 (AUC: 0.82± 0.12) doğrulukla,
2. CIS ve Kontrol grubu arasındaki sınıflandırma 87.31%±12.02 (AUC: 0.93±0.09) doğrulukla,
3. MS ve CIS arasındaki sınıflandırma 76.51% ±11.15 (AUC: 0.83 ±0.12) doğrulukla,
4. RRMS ve PPMS arasındaki sınıflandırma 95.77% ±6.63 (AUC: 0.97±0.08) doğrulukla,
5. MS ve Kontrol grubu arasındaki sınıflandırma 92.64% ±7.15 (AUC: 0.97±0.06) doğrulukla,
6. CIS grubundan RRMS grubuna geçiş 86.45% ±12.6 (AUC: 0.89±0.19) doğrulukla tahmin edilmiştir.

Bu çalışma, MS'in klinik alttiplerinin tanısı ve prognozunu ve farklı alttipler arası geçişi tahmin etmek için bu protein ve klinik verileri ve bilgisayar destekli sınıflandırma yöntemlerini kullanan ilk çalışmadır. Çalışmaların devamında örnek sayısı arttırılmalıdır. Ayrıca farklı sınıflandırma yöntemlerinin denenmesi de gereklidir. Sınıflandırma yöntemlerinin parametrelerinin optimizasyonu da daha iyi sonuçlar vermesi beklenmektedir. MS hastaları, CIS hastaları ve kontrol grubunun sınıflandırılması için hiyerarşik bir model uygulanabilir.

1. INTRODUCTION

Multiple Sclerosis (MS) is a neuroinflammatory, demyelinating disease with an unknown etiology. MS is a very complex and hard-to-diagnose disease. To cope with that, several diagnostic criteria are proposed. Today, revised McDonald's criteria is the gold standard for diagnosis of MS. In recent years, there are extensive studies aiming the discovery of novel biomarker(s) for MS diagnosis. Yet, there is no biomarker with sufficient specificity or sensitivity for MS diagnosis

MS has an autoimmune nature which is caused by both genetic and environmental factors, and it is clinically highly heterogeneous with respect to both clinical course and pathological mechanisms [2-3]. There are different subtypes of MS which may transform from one subtype to another over time depending on the patterns of progressions and frequency of symptoms [4]. Complex nature of the disease requires reliable diagnostic tools to identify and characterize MS subtypes [5]

The symptoms of MS can be easily confused by the symptoms of other neurological diseases such as Neurobehcet's Disease, Lyme disease [6-7]. In addition, it is not possible to predict whether a CIS patient will become a MS patient. Furthermore, there is no certain way to determine the prognosis of disease, i.e. whether it will become progressive. Early prediction of prognosis is important because early prediction of outcome can help to the modification of the treatment process on behalf of patient.

Machine learning and pattern recognition methods provide a wide set of tools in the area of medical decision making, solution of diagnostic and prognostic problems in medicine. In addition, there are various biological applications where machine learning methods are applied for information extraction from data [8].

The primary aims of this study are as follows:

1. To investigate the different protein and clinical data patterns among the MS subtypes, CIS samples and control samples.

2. To show that clinical subtypes of MS can be classified using protein data and clinical data.
3. To predict the CIS-MS transition.
4. To show that the MS prognosis can be predicted using protein and clinical data.

In this study, CSF findings and clinical data of 67 RRMS, 46 CIS, 22 PPMS patients and 22 control subjects were analyzed for the classification of clinically different subtypes of MS. The accuracy of the classification is investigated by ROC analysis using 10-fold cross validation method.

This thesis is organized as follows:

- Second chapter covers information about Multiple Sclerosis
- Third chapter covers information about properties of data, preprocessing methods applied to data, and classification methods used for the classification of different clinical subtypes of MS
- Fourth chapter gives results of statistical analysis and classification methods
- Fifth chapter discusses the findings from this work and discusses future improvements.

2. MULTIPLE SCLEROSIS

Multiple sclerosis (MS) is an immune-mediated disease of the central nervous system (CNS) with heterogeneous clinical presentation and course. Not only MS may change between various forms over time, but also the clinical symptoms of these forms may be very similar. According to current data, MS is an immune-mediated disease of the CNS, with both inflammatory and degenerative features [9]. It is characterized by recurring relapses and progression that appear multifocal white matter and within the lesions [9-11]. The destruction of oligodendrocytes, neurons and axons play important role in the pathogenesis of MS [12-15].

Studies on MS shows that different patient groups may have different disease courses and onset of irreversible disability change. Onset of irreversible disability may be later for: females, younger patients, patients with an onset of RR course, patients with complete recovery from the first neurological episode; with a low number of relapses during the first years of the disease; and those with longer periods of time between the first two attacks. In RR patients there are three parameters that shows the higher probability for rapid progression to irreversible disability: 1) the late onset MS, 2) an incomplete recovery from the first relapse, and 3) a high number of relapses during the first 5 years of MS [16].

RR and Progressive MS show differences in gender, onset age, initial symptoms, and time from onset to irreversible disability. But RR and progressive MS show no difference in time course of disability accumulation from assignment to a given disability score to a higher score [17].

2.1 Immunopathogenesis of MS

Recent studies have showed the role of immune cells other than $CD4^+$ type-1 T helper cells in MS, causing a change in the idea that MS is a $CD4^+$ type 1 T helper cells mediated autoimmune disorder. Now it is known that the immune response in MS is mediated by various immune cells that target brain antigens and the clonal

expansion of lymphocytes and the antigen-driven maturation of the B-cell receptors are also a part of T- and B-cell responses in MS patients' brains [18].

Environmental and genetic factors could effect the permeability of Blood-Brain-Barrier to the T cells and demyelinating antibodies. Activated T cells in the CNS begin to produce proinflammatory cytokines like IFN-γ and TNF-α, that increase the expression of surface molecules of lymphocytes and antigen presenting cells [19].

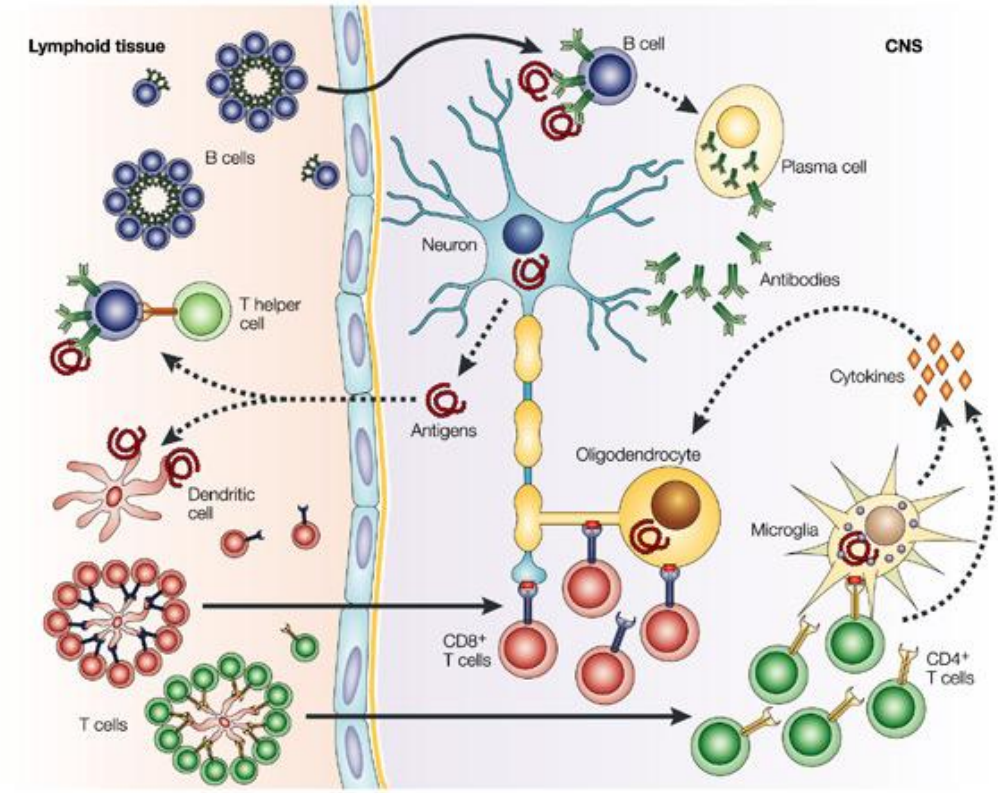

Figure 2.1: Immunopathogenesis of MS[18]

2.2 Symptoms of MS

The first symptoms of MS are usually visual loss or double vision, nystagmus, sensory, and motor signs and symptoms, but a variety of symptoms can be seen. Some cases may show no symptoms and/or no disability, others may have a mild prognosis or have full-symptomatic MS and severe disability. In progressive cases, some cognitive impairment may be observed. This variety of symptomatic changes makes MS very difficult to diagnose and predict its prognosis [20].

2.3 Diagnosis of MS

Diagnosis of MS is a very complicated and difficult issue because of the variety of symptoms. Furthermore, similar symptoms can be observed in other neurological diseases. In addition, there is not a single test to confirm MS, but there are series of criteria that are accepted by MS Society. These criteria include a group of clinical and radiological findings. Before 2001, Poser Criteria was used and in 2001 McDonald's Criteria was accepted [21].

2.4 Epidemiology of MS

MS is more common in northern Europe. The ratio of MS patients in Turkey is estimated as 34 per 100000 [22]. Female: Male ratio is two to three times. The disease onset age is typically early adulthood (ages between 20- 40) [23]. For Europe, the total estimated prevalence rate of MS is 83 per 100000 with higher rates in northern countries, and mean annual MS incidence rate is 4.3 cases per 100 000 [24].

2.5 Subtypes of MS

There are different clinical MS subtypes that may show different progression and symptoms of the disease, shown in Figure 2.2. In addition, disease course can change from a subtype to another in years, according to the progression of symptoms.

2.5.1 Relapsing – Remitting MS (RRMS)

RRMS is the most common form of MS in the onset of disease. RRMS is characterized by the acute attacks (relapses) and following total or partial remissions. The disease is continuous between the attacks, and relapses are unpredictable. Furthermore, full remission may not be obtained after some relapses. RRMS usually turn into secondary progressive MS form as the duration of disease increases [25].

2.5.2 Primary Progressive MS (PPMS)

Progression in PPMS is continuous from the beginning. There can be stable time periods, in which no new signs of disease activity is seen. 10–15% of all MS patients are in this group, and it tends to occur in late onset. Usually disease progression

continues until death. The female to male ratio is equal in this group, unlike other forms [25].

2.5.3 Secondary Progressive MS (SPMS)

This form of MS starts as a RRMS and becomes progressive after 5-6 years. Attack increases the level of disability [25].

2.5.4 Progressive – Relapsing MS (PRMS)

This uncommon form (about 5%) is progressive from the onset with superimposed relapses [25].

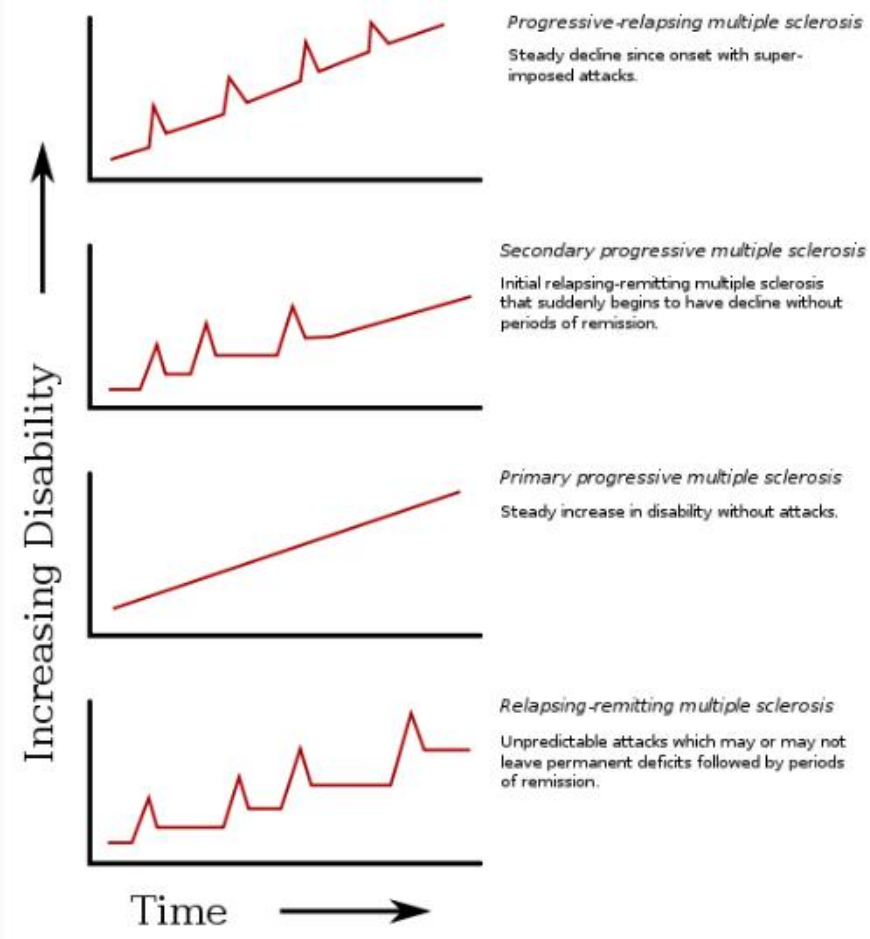

Figure 2.2: MS Subtypes

2.6 Clinically Isolated Syndrome (CIS)

In some patients, MS-like symptoms occur but they do not fulfill the diagnostic criteria. Some of these patients develop typical MS later on (5 years).The clinical

onset starts with a monoregional involvement of CNS. In some cases of CIS, MRI may reveal polyregional involvement of the CNS, in others; the disease will be limited to the corresponding anatomic site, remaining monoregional [9].

2.7 Prognostic Factors in MS

There are different prognostic factors that have different predictive values for the diagnosis and prognosis of MS. In this study, the dataset used has 4 proteins and 17 clinical features of different subtypes of MS.

2.8 Biomarkers in MS

Complex diseases are hard to diagnose, and their diagnosis requires specific biomarkers. In MS, proteomic studies aim finding new biomarkers in order to help the clinicians to diagnosis and predict prognosis of MS. Here, TAU, MOG, GFAP and NFL were used as potential biomarkers for the classification of clinical MS subtypes.

2.8.1 TAU Protein in MS

TAU play an important role in assembly of microtubules of axons. TAU can be used as a biomarker for monitoring neuroaxonal damage. The combination of increased NFH and TAU protein levels was more specific than MRI changes for the prediction of transition from CIS to RRMS [26]. Also, TAU protein levels can be used to predict of disease progression or transition from RRMS to PPMS [27].

2.8.2 Myelin Oligodendrocyte Glycoprotein (MOG) in MS

MOG plays a role in the structure of myelin sheath and oligodendrocyte. Antibodies of myelin-oligodendrocyte-glycoprotein (MOG), which is exclusively localized on the surface of myelin sheaths and oligodendrocytes, and myelin basic protein (MBP), have been suggested to predict future disease progression in patients with CIS [28].

2.8.3 Glial Fibrillary Acidic Protein (GFAP) in MS

GFAP is an intermediate filament protein expressed in CNS cells. It was reported that patients with major disability showed higher GFAP concentrations in the CSF

than patients with low disability [29]. Therefore, GFAP may serve as a biomarker for disease progression, probably showing the increasing rate of astrogliosis [26].

2.8.4 Neurofilament Light Chain (NFL) in MS

Neurofilaments consist of three parts: a light chain (NFL), an intermediate chain (NF-M), and a heavy chain (NF-H). The levels of CSF neurofilaments may have some predictive value in patients with CIS (light chain) and RRMS (heavy chain) [30].

2.8.5 Myelin Basic Protein (MBG) in MS

MBP is a main functional protein in the myelination process of nerves in the CNS. Various forms of MBP with splice forms and post translational modifications are found in CSF and CNS space [31-33]. In this study, 14 patients in RRMS, 7 patients in CIS, 1 patient in PPMS group and 6 control samples have MBP in their CSF samples. These results did not show any significant difference ($p>0.05$). There may be post transitionally modified variants of MBP, which is more abundant in CSF. In addition to this MBP isoform, other MBP forms should be studied and their differences can be better investigated in future studies.

2.9 Clinical Data in MS

In this part, the clinical features in the dataset used in this study and their differences between different clinical subtypes of MS are explained:

2.9.1 MR/T1:

Black holes on T1 represent lesions with extensive structural loss. They develop if lesions are larger, have a lower MT ratio during enhancement or are ring-enhancing [34]. Truyen and van Walderveen described a significant correlation of change in the EDSS and change in hypo intense-lesion volume in T1-weighted scans in SPMS, but no correlation was found in RRMS [35].

2.9.2 MR/T2:

It is known that all new lesions go through a phase of enhancement for 2 - 8 weeks and although most lesions get smaller by time, almost all the time a T2 abnormality

persists. Several studies have shown that the number and volume of enhancing tissue predicts the onset and severity of relapses [34].

2.9.3 Gadolinium Enhancement:

Gadolinium enhanced magnetic resonance imaging (MRI) of the brain shows the development of inflammatory lesions in MS by reflecting the blood-brain-barrier disturbances [35].

2.9.4 Atrophy (cortical and corpus callosum):

Brain atrophy is a common finding in MS patients. There is a significant correlation between brain atrophy and EDSS score in SPMS, but not in RRMS. Furthermore, it was found that total brain atrophy was significantly greater in MS patients than in healthy controls [36]. Cortical thinning is an early phenomenon in MS that is already detectable at clinical onset. It correlates with clinical disability [37].

2.9.5 Family history (MS in family):

Familial and twin studies showed that, risk of MS development increases if there is any MS patient among parents or siblings [38]. In addition, familial aggregation of MS is genetically determined, not by environmental factors [39]. However, the category of MS suffered by the patient is not predictive of the MS phenotype of an affected relative [40].

2.9.6 Family history (autoimmune diseases in family):

Broadley et. al. showed an excess rate of autoimmune disease within first-degree relatives of probands with multiple sclerosis [41].

2.9.7 Autoimmune diseases in self:

There was no increase in autoimmune disease within patients with multiple sclerosis themselves when compared with the controls or population data [41].

2.9.8 Gender:

The prevalence of multiple sclerosis (MS) is much greater in women [42]. However, women had a significantly longer survival time in the disease [43]. When comparing RRMS and SPMS patients, gender distribution showed difference; a higher

proportion of females RRMS than in SPMS [17]. The female propensity seen in RRMS is absent in PPMS[44].

2.9.9 Onset age:

Progressive onset patients tend to be older than patients with RRMS onset [40]. PPMS tends to have a later onset [44]. The prognosis was significantly worse in patients with the age at onset over 25. Also, median survival time was 11 years shorter in patients with the age at onset over 25 than the patients with earlier onset. Later onset age was also a predictor of a poor outcome in RRMS patients [43] .

2.9.10 Duration of MS:

The cumulative probabilities of survival over 40 years' period were 22.2% in patients with PP and 44.7% in patients with RR disease course. Median survival time in RR patients is 38 years whereas progressive patients have survived 19 and 21 years shorter [43].

2.9.11 EDSS:

EDSS score at 5 years in patients with PPMS is a strong predictor of the disease outcome. The shorter time to reach EDSS 6 was found to be related to the worse outcome in patients with RR [43]. Patients developing a progressive disease course had significantly higher EDSS scores at baseline than patients who remained RR [45]

2.9.12 CSF/Serum protein and glucose:

Low CSF glucose (CSF/serum glucose ratio) and high total CSF protein content shows an infectious situation [46]. For this reason, glucose (CSF-to-serum ratio) and Total CSF protein by are used for confirmation MS [26].

2.9.13 Oligoclonal Band:

The proportion of being OCB-positive and OCB-negative, or the number of OCB show no difference between progressive and RRMS patients [45].

3. METHODS

In computer-aided diagnosis, machine learning techniques have been widely applied to learn hypothesis from diagnosed samples in order to assist the medical experts in making diagnosis [47]. Methods for obtaining the results in machine learning approaches used various classifications for medical reasoning.

In this section, statistical/classification methods used in this study and data characteristics are explained.

3.1 Statistical Methods

Classical statistical methods were applied for the analysis of given proteins for the classification significance among different clinical subtypes of MS, CIS and control subjects. In this work, Weka 3.6 software was used for data preprocessing and classification [48], and SPSS (v.18.0) software was used for statistical analysis [49].

3.2 Data Characteristics

This thesis is a part of an ongoing research project of our group, which was supported by Istanbul Technical University and Marmara University scientific research projects grant (Grant No: SAG-B-030408-0065). CSF and serum samples were obtained during routine diagnostic evaluation of 67 RRMS, 46 CIS, 22 PPMS patients at Istanbul University, Cerrahpaşa Faculty of Medicine (CTF), Neuroimmunology and Demyelination Service. Patients were diagnosed according to McDonald's (2001) and revised McDonald's criteria (2005). Diagnosis was based on radiological findings (brain MRI and CT), clinical findings and oligoclonal band formation in the CSFs of patients. Samples were collected before any treatment and medication. Female to male ratio was 1.9:1 (104:53). Control group included 22 patients suffering from other neurological diseases (OND) like neurobehçet's disease, polyneuropaty, sarcoidosis, apoplexy (n=11), and a non-inflammatory subgroup suffering from migraine (n=11). Ages and genders of the control group

were matched with the patient groups. The CSFs were obtained from the patients by lumbar puncture (LP). CIS group comprised of two additional subgroups; CIS subgroup (remaining as CIS in five years) and CIS/RR subgroup(transition from CIS to RRMS within five years). The protocol was approved by the ethics review committee of the CTF, Istanbul University for research ethics, oral and written information was given to the patients and confirmed consent in writing was received before inclusion into the study.

CSFs of patients were collected by LP within 3 days of an acute attack. LP was performed before the medication. TAU,GFAP,NFL, MOG and MBP protein concentrations of samples were determined by Western Blot analysis. Protein bands were scanned by using densitometer and scanned protein bands were analyzed by using ImageJ analysis software to obtain quantitative measurement. Quantities of proteins were taken as colorimetric unit (CU). CU is a numerical value showing the insensitivity of protein band concentration, ranged between 0 (most) and 255 (least). Analyzed values were linearized and normalized due to loaded total protein concentration. All samples were scanned and analyzed with the same standard procedure.

3.3 Preprocessing

Data contained missing values, and features were in different scales. Different methods for handling missing values such as Multiple Imputation or using median were investigated. Since they gave similar results, using mean values were preferred for handling missing values due to easiness of application.

3.3.1 Handling Missing Data

A common problem in medical data analysis is missing values, and obtaining valid estimates a major issue [33]. In data processing, missing values were replaced using “ReplaceMissingValues” filter Weka 3.6 [50]. This filter replaces missing values with the modes and mean.

3.3.2 Normalization of Data

In addition to the replacement of missing values, data were normalized in order to compare the real characteristics of the data sets by bringing them to a common scale. “Normalize” filter was used in Weka for normalization of values within [0,1] range.

3.3.3 Feature Selection

For feature selection, information gain method was used [51]. For this purpose, “InfoGainFeatureEval” feature selection method was used in Weka. “Ranker” was selected as a search method. Default settings in Weka were used.

3.3.4 Principal Component Analysis(PCA)

Principal component analysis was applied to data and classification results of PCA-applied data and original data were compared.

3.4 Machine Learning Methods

Computational methods are required to assess the statistical significance of biomarkers with the phenotypes of different diseases. Several classification methods can be used in this context. Computational methods are also required for reducing the biological variation so that, only significant and relevant proteins can be validated by biological methods.

Ensemble learning paradigms train multiple component learners and then combine their predictions. Ensemble techniques can significantly improve the generalization ability of single learners, and therefore ensemble learning has been a hot topic during the past years. An ensemble is usually built in two steps: The first step is to generate multiple component classifiers, and the second step is to combine their predictions [47].

3.4.1 Decision Tree

In some fields such as medicine, it is preferable not to use black box approaches because it is important for the user to understand the classifier and evaluate its results [34]. Decision tree divides a complex decision making process into a collection of simpler decisions [52]. J48 is a standard decision tree classifier. It is the

implementation of C4.5 algorithm in Weka. J48 uses greedy approach for inducing the decision trees for the classification problem given [53].

A decision tree offers a representation of the relevant decisions and outcomes. Every path in a decision tree from its root to a leaf represents a result, and only meaningful results can be kept by pruning [54].

3.4.2 Random Forests

Random forests are a combination of tree predictors such that each tree depends on the values of a random vector sampled independently and with the same distribution for all trees in the forest [55].

Random forest is an ensemble method, which uses two powerful machine-learning techniques: bagging and random feature selection adds an additional layer of randomness to these techniques. Bagging, which means bootstrap aggregating, uses resampling to improve accuracy of predictions [56]. This randomness results in better performance of the classifier when compared to other well known classifiers such discriminant analysis, support vector machines and neural networks, and also improves the robustness of the classifier against overfitting [55].

Random forests consist of using randomly selected inputs or combinations of inputs at each node to grow each tree while constructing each tree using a different bootstrap sample of the data. The simplest random forest with random features is formed by selecting a small random group of input variables at each node to split on [55].

3.4.3 AdaBoost

Adaboost (Adaptive Boosting) is a very popular boosting algorithm. Boosting is a general method for improving the accuracy of classifiers [57]. The main idea of Adaboost is focusing on the weak classifiers more than the strong ones.

3.4.4 kNN

kNN (k- Nearest Neighbor) algorithm takes the k nearest examples from a reference training set and determines the class of the new example according to the majority vote of these examples[58]. In this study, k was considered as 5 for all classification tests.

3.4.5 DECORATE

Decorate (Diverse Ensemble Creation by Oppositional Relabeling of Artificial Training Examples) is an ensemble learner proposed by [59] that uses an existing "strong"(giving high accuracy) learner to build an effective diverse sample subset.

3.4.6 Bayesian Networks

Bayesian Networks (Bayesnet for short), which are used for modeling relations between parameters, are generally used in uncertain data environments. If the output value of some parameters are known (this is called evidence), Bayesian networks provide the probability distribution of the other parameters in the system [60]. Bayesian networks (BNs) are a kind of probabilistic graphical models (GMs), which are used to represent knowledge about an uncertain domain. The nodes represent a random variable whereas the edges represent probabilistic dependencies of the corresponding variables. As a result, Bayesian networks combine different theories such as graph theory, probability theory, computer science, and statistic [8].

3.5 Evaluation Methods

10-fold cross-validation was used for evaluation of the accuracy and area under ROC curve (AUC) [8] analysis. In 10-fold cross-validation, data was partitioned into 10 folds and each fold was left out of the training process and used as a test set. The resulting accuracy was the overall proportion of the accuracies on all folds [8]. AUC curve is typically used as a performance measure for machine learning algorithms, and higher AUC values correspond to better classification performance [61]. Each classifier was run 1000 times using 10-fold cross validation in order to obtain a distribution of accuracy and AUC.

AUC shows hit rate versus false alarm rate. There is a threshold for deciding the number of true positives versus false positive in each classification method, such that, increasing true positives also increased false alarms. A point on this curve is decided depending on the cost of false positives in a given classification method [8].

4. RESULTS

In this section, results of 13 classifiers, which are obtained using different feature subsets, are given and explained.

The accuracy reported here is the percentage of correctly classified instances. Since the class sizes are not balanced, AUC results are used for further evaluation. A good classifier should result in a range of AUC index between 0.5 (chance behavior) and 1.0 (perfect classification performance) for 2 classes [62]. Our study showed that, concentrations of TAU, GFAP, NFL, and MOG proteins in CSF can be used as biomarkers of MS for prognosis and diagnosis. Here, our aim is not only to compare classification methods and results, but also to show that these selected proteins have a predictive value per different subtypes of multiple sclerosis.A general view of demographic information for patient records are shown in table 4.1.

Table 4.1: Demographic information of different subtypes of MS, CIS samples and control samples. D indicates the duration of the disease, EDSS, expanded disease status scale, MR/T1 and MR/T2 indicates the T1 weighted and T2 weighted magnetic resonance score of patients showing the lesion counts of the patients when the CSF samples obtained. OCB, indicates the oligoclonal band formation score of the patient groups. CSF [protein] and CSF [glucose] indicates the level of total protein and total glucose in the CSF of sample

Subtype	D	Age	EDSS	MR/T1	MR/T2	OCB	CSF [protein]	CSF [glucose]
CIS	1.7±2	31.7±10.3	0.7 ±0.8	0,0.3±0.6	2.2±1.2	1.7±0.8	42.6±17.5	62.7 ±16.7
CTRL(total)	-	39.4±15.1	-	-	-	-	33.8	51.3
PPMS	10.7 ± 7.6	40.3± 8	4.4±2.2	0, 1±1.3	2.8±1.3	1.9 ±0.3	36.4	62.9
RRMS	4.5 ±4.7	33.9±10.1	1.4±1.3	0.4±0.8	2.4±1.2	1.8±0.4	33.1±9.2	63.5±12.4
CISRR	1.11±0.8	33.1±11.1	0.9±0.7	0.6±0.9	2.8±1	1.9±0.3	50.7 ±23.2	68.3±27.3
HC	-	51	-	-	-	-	32	79
OND	-	38±15.5	-	-	-	-	34.4	42

4.1 Results of Statistical Analysis of Clinical Data

The results of analysis with each feature is given in this section. For this purpose, the mean value +/- standard deviation is given per each clinical subset of MS. If meaningful, mean value +/- standard deviation of control groups is also given.

In figure 4.1, mean value and standard deviation of onset age among different subtypes are shown.

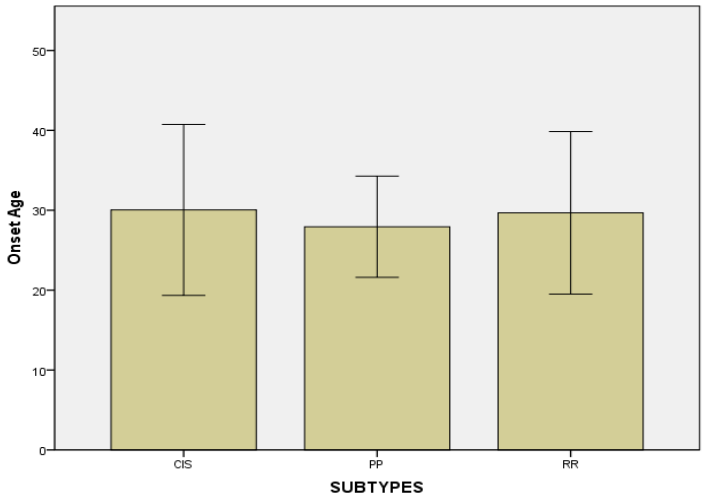

Figure 4. 1: Mean value of onset age according to different subtypes.

In figure 4.2, mean value and standard deviation of disease duration among CIS,RRMS and PPMS are shown.

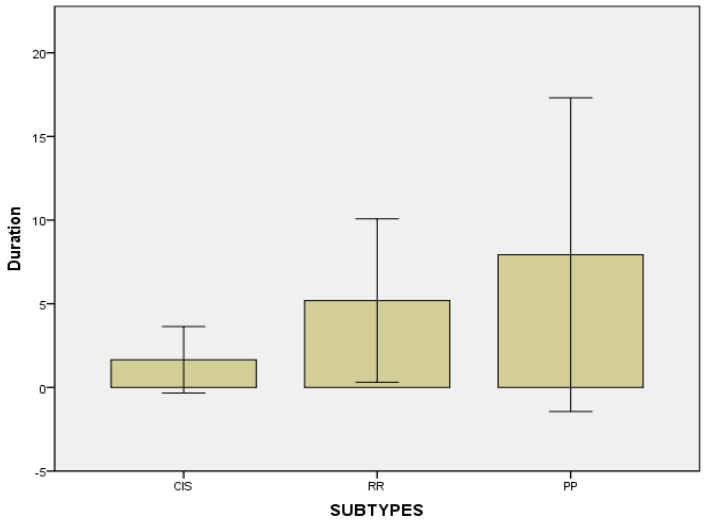

Figure 4. 2: Mean value of disease duration according to different subtypes.

In figure 4.3, mean value and standard deviation of disease duration among CIS,RRMS and PPMS are shown. EDSS tends to increase as the severity of disease increases.

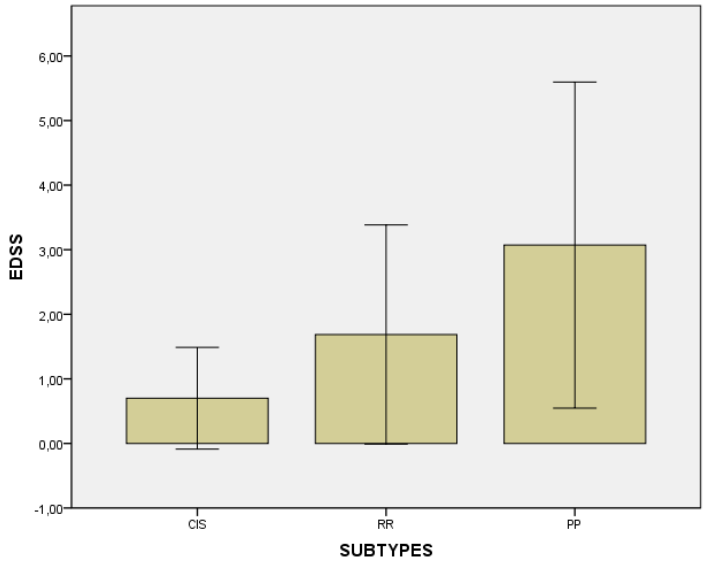

Figure 4. 3: Mean value of EDSS scores according to different subtypes.

In figure 4.4, mean value and standard deviation of MR/T1 scores among CIS,RRMS and PPMS are shown. MR/T1 findings tend to increase similar to the severity of disease.

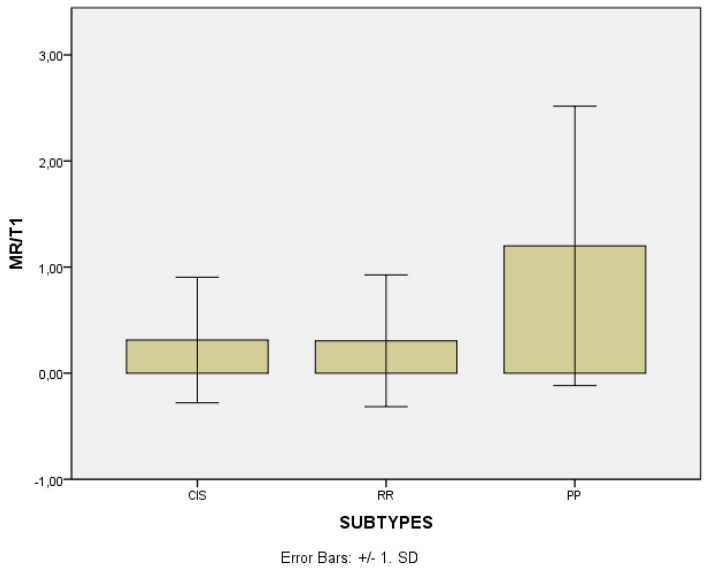

Figure 4. 4: Mean value of MR/T1 scores according to different subtypes.

In figure 4.5, mean value and standard deviation of MR/T2 scores among CIS,RRMS and PPMS are shown.

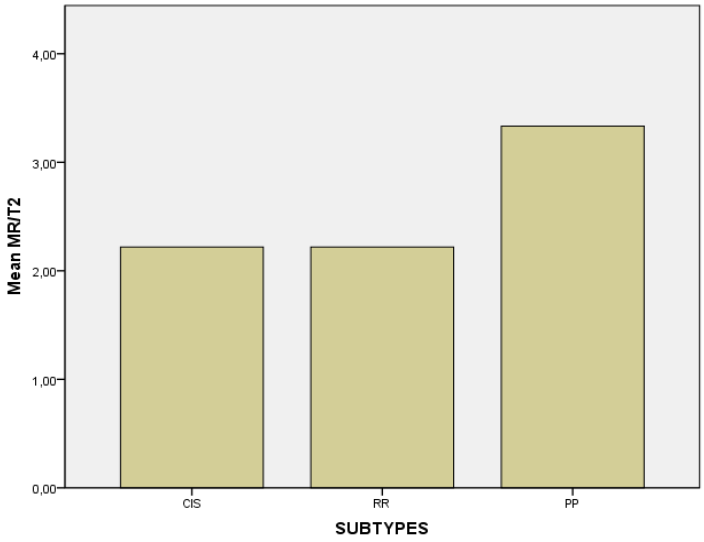

Figure 4. 5: Mean value of MR/T2 scores according to different subtypes.

In figure 4.6, mean value and standard deviation of cortical atrophy scores among CIS,RRMS and PPMS are shown.

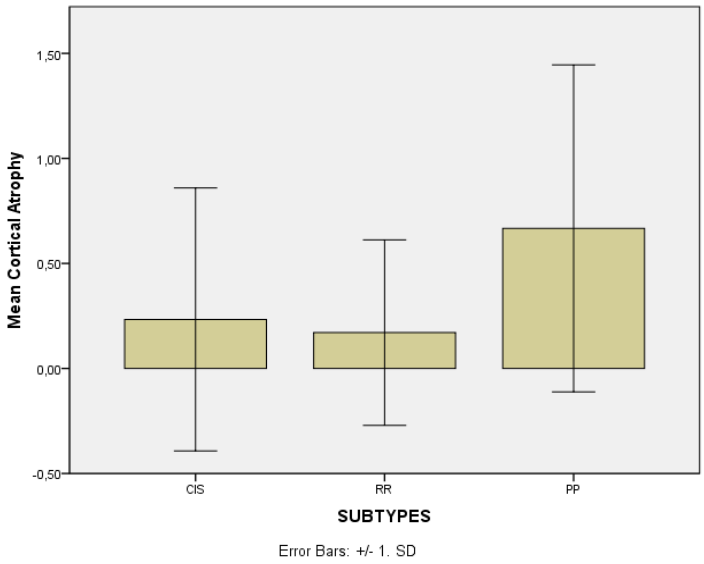

Figure 4. 6: Mean value of Cortical Atrophy scores according to different subtypes.

In figure 4.7, mean value and standard deviation of corpus callosum atrophy scores among CIS,RRMS and PPMS are shown. In figure 4.8, mean value and standard deviation of gadolinium enhancement scores among CIS,RRMS and PPMS are shown. In figure 4.9, mean value and standard deviation of OCB scores among CIS,RRMS and PPMS and control groups (HC, OND and total control) are shown.

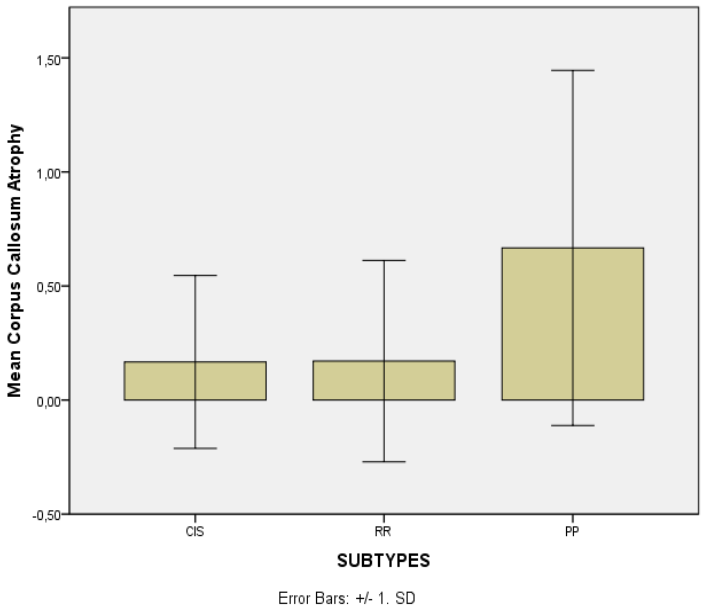

Figure 4. 7: Mean value of Corpus Callosum Atrophy scores according to different subtypes.

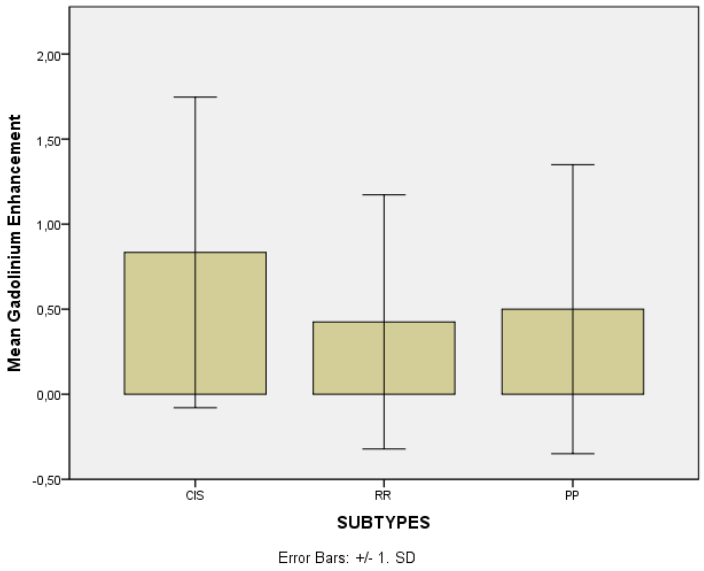

Figure 4. 8: Mean value of Gadolinium Enhancement scores according to different subtypes.

In figure 4.10, mean value and standard deviation of CSF protein levels among CIS,RRMS and PPMS and control groups (HC, OND and total control) are shown. In figure 4.11, mean value and standard deviation of CSF glucose levels among CIS,RRMS and PPMS and control groups (HC and OND) are shown. In figure 4.12, mean value and standard deviation of serum protein levels among CIS,RRMS and PPMS and control groups (HC and OND) are shown. In figure 4.13, mean value and standard deviation of serum glucose levels among CIS,RRMS and PPMS and control groups (HC and OND) are shown.

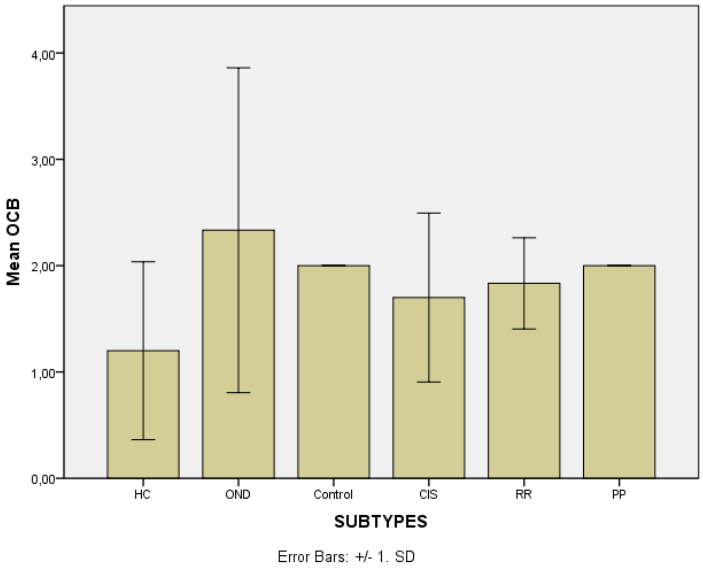

Figure 4. 9: Mean value of OCB scores according to different subtypes.

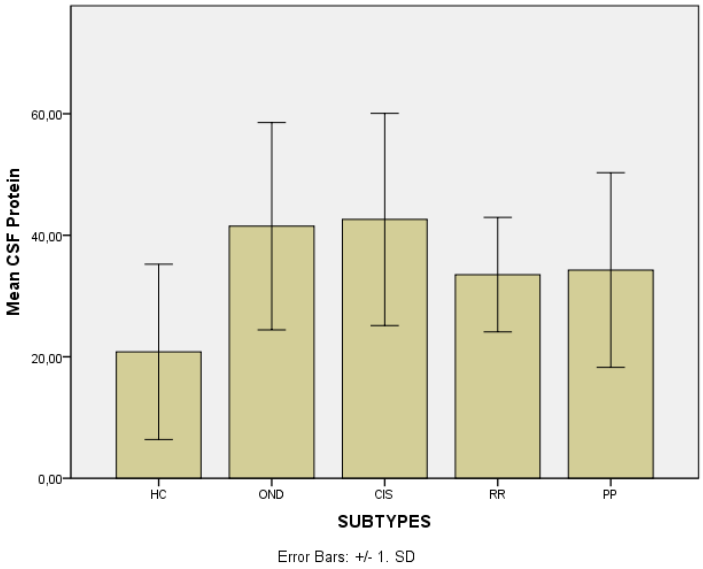

Figure 4. 10: Mean value of CSF protein levels according to different subtypes.

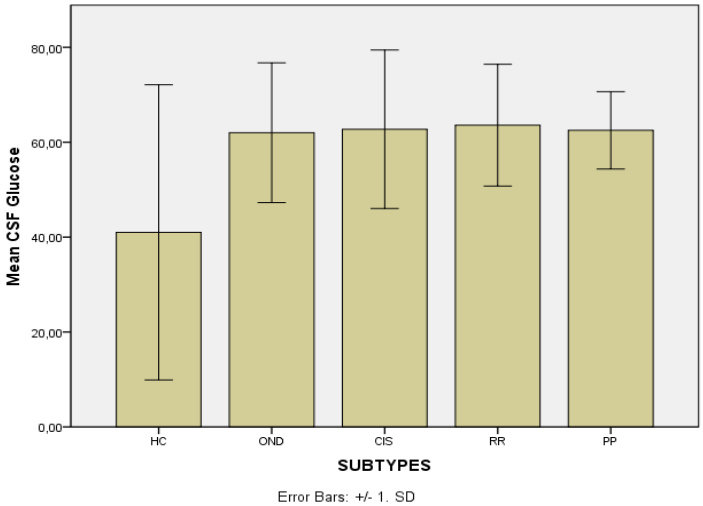

Figure 4. 11: Mean value of CSF glucose levels according to different subtypes.

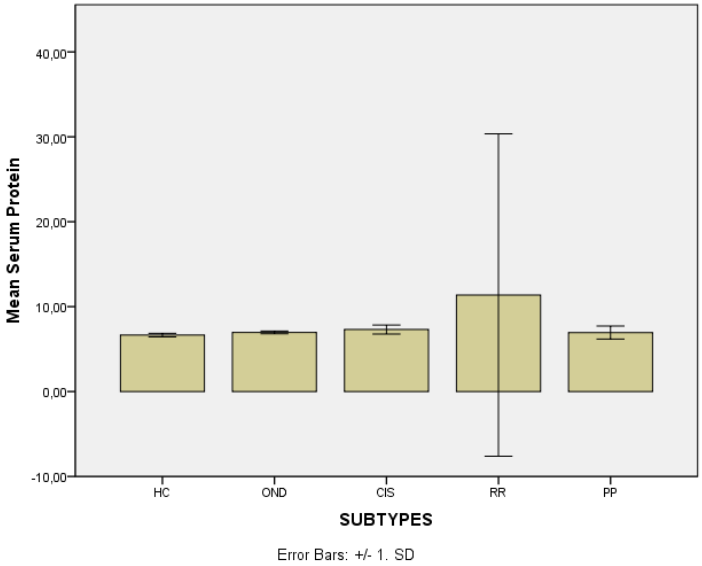

Figure 4. 12: Mean value of serum protein levels according to different subtypes.

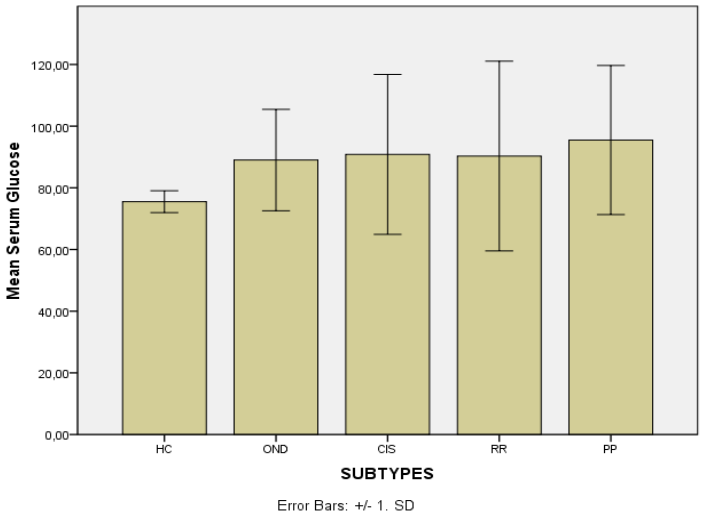

Figure 4. 13: Mean value of serum glucose levels according to different subtypes.

4.2 Results of Statistical Analysis of Protein Data

The results of statistical analysis for proteins (ie. ANOVA and PostHoc tests) are given in this section. In table 4.2, mean/standard deviation, standard error and confidence interval of TAU protein levels among different clinical subtypes, CIS and control groups are given.

In table 4.3, homogenity test results for TAU is given. This Levene's test results are not significant (p=0.979). So, the variances are not significantly different, they are homogenous.

Table 4.2: Descriptives for TAU levels

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
CIS	37	47,8452	18,61744	3,06069	41,6378	54,0525
CIS/RR	9	49,5878	16,11484	5,37161	37,2008	61,9747
OND	11	32,3857	18,10448	5,45871	20,2230	44,5485
HC	11	37,1254	20,66999	6,23224	23,2391	51,0116
PP	16	75,5487	16,69612	4,17403	66,6520	84,4454
RR	66	55,4156	20,92675	2,57590	50,2712	60,5600
Total	150	52,3159	21,94210	1,79156	48,7758	55,8561

Table 4.3: Test of homogenity for TAU levels

Levene Statistic	df1	df2	Sig.
,152	5	144	,979

Table 4.4 shows the ANOVA results for TAU levels. There was a significant effect of TAU on the classification of subtypes of Multiple Sclerosis, $F(5,149) = 8,934$, $p < .001$.

Table 4.4: ANOVA for TAU levels

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	16984,466	5	3396,893	8,934	,000
Within Groups	54752,416	144	380,225		
Total	71736,882	149			

In table 4.5, Brown-Forsythe and Welch forms of F-ratio are shown. But since the assumption of homogeneity of variance is not broken, these results only approve the previous F-ratio.

Table 4.5: Robust Tests of Equality of Means for TAU levels

	Statistic[a]	df1	df2	Sig.
Welch	10,010	5	33,590	,000
Brown-Forsythe	9,996	5	73,458	,000

a. Asymptotically F distributed.

In table C.1, PostHoc tests for TAU levels are given. A post-hoc test is needed after we complete an ANOVA in order to determine which groups differ from each other. In Table 4.6, Tukey post-hoc comparisons of the six subtypes indicate that the PPMS

group gave significantly higher TAU levels than all of the other subtypes, $p < .001$.Also, RRMS group is significantly different than OND group according to TAU levels (p=0.005). In Table 4.7, mean, standard deviation, standard error and confidence interval of GFAP protein levels among different clinical subtypes, CIS and control groups are given. In Table 4.8, homogenity test results for GFAP is given. This Levene's test results are not significant (p=0.645). So, the variances are not significantly different, they are homogenous. Table 4.9 shows the ANOVA results for GFAP levels. There was a significant effect of GFAP on the classification of subtypes of Multiple Sclerosis, F(6,147) = 11,831, p< .001.

Table 4.6: Homogeneous Subsets for TAU levels (Tukey HSD[a,b])

SUBTYPES	N	Subset for alpha = 0.05		
		1	2	3
OND	11	32,3857		
HC	11	37,1254	37,1254	
CIS	37	47,8452	47,8452	
CIS/RR	9	49,5878	49,5878	
RR	66		55,4156	55,4156
PP	16			75,5487
Sig.		,155	,110	,057

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 15,090.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

In Table 4.10, Brown-Forsythe and Welch forms of F-ratio are shown. Since the assumption of homogeneity of variance is still valid, these results only approve the previous F-ratio. In Table 4.12, mean, standard deviation, standard error and confidence interval of NFL protein levels among different clinical subtypes, CIS and control groups are given. In Table 4.13, homogenity test results for NFL is given. This Levene's test results are not significant (p=0.540). So, the variances are not significantly different, they are homogenous. Table 4.14 shows the ANOVA results for NFL levels. There was a significant effect of NFL on the classification of subtypes of Multiple Sclerosis, F(5,141) = 9,399, p< .001.

Table 4.7: Descriptives for GFAP levels

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
CIS	36	24,3389	12,57111	2,09518	20,0854	28,5923
CIS/RR	9	26,9944	10,16711	3,38904	19,1793	34,8096
OND	11	17,8855	16,43760	4,95612	6,8426	28,9285
HC	11	22,0532	18,96609	5,71849	9,3116	34,7948
PP	16	54,6781	14,10928	3,52732	47,1598	62,1964
RR	65	32,4468	16,30719	2,02266	28,4061	36,4875
Total	148	30,6917	17,74134	1,45833	27,8097	33,5737

Table 4.8: Test of homogenity for GFAP levels

Levene Statistic	df1	df2	Sig.
,672	5	142	,645

Table 4.9: ANOVA for GFAP levels

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	13606,575	5	2721,315	11,831	,000
Within Groups	32662,422	142	230,017		
Total	46268,997	147			

Table 4.10: Robust Tests of Equality of Means for GFAP levels

	Statistic[a]	df1	df2	Sig.
Welch	12,277	5	33,421	,000
Brown-Forsythe	12,079	5	59,352	,000

a. Asymptotically F distributed.

In Table 4.15, Brown-Forsythe and Welch forms of F-ratio are shown. Since the assumption of homogeneity of variance is still valid, these results only approve the previous F-ratio. In Table C.3, PostHoc tests for NFL levels are given. In Table 4.16, Tukey post-hoc comparisons of the six subtypes indicate that the control groups gave significantly lower NFL levels than all of the other subtypes, $p < .001$. In Table 4.17, mean, standard deviation, standard error and confidence interval of MOG protein levels among different clinical subtypes, CIS and control groups are given.

Table 4.11: Homogeneous Subsets for GFAP levels (Tukey HSD[a,b])

SUBTYPES	N	Subset for alpha = 0.05	
		1	2
OND	11	17,8855	
HC	11	22,0532	
CIS	36	24,3389	
CIS/RR	9	26,9944	
RR	65	32,4468	
PP	16		54,6781
Sig.		,096	CIS0

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 15,053.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Table 4.12: Descriptives for NFL levels

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
CIS	34	72,9670	31,61600	5,42210	61,9357	83,9984
CIS/RR	9	71,1459	18,89123	6,29708	56,6248	85,6670
OND	11	29,1336	23,15598	6,98179	13,5772	44,6900
HC	11	36,5886	27,37403	8,25358	18,1985	54,9788
PP	16	76,7922	16,42632	4,10658	68,0392	85,5452
RR	61	75,0726	26,40930	3,38136	68,3088	81,8363
Total	142	67,9735	30,04448	2,52128	62,9891	72,9579

Table 4.13: Test of homogenity for NFL levels

Levene Statistic	df1	df2	Sig.
,817	5	136	,540

Table 4.14: ANOVA for NFL levels

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	32685,885	5	6537,177	9,399	,000
Within Groups	94590,687	136	695,520		
Total	127276,572	141			

In Table 4.18, homogenity test results for MOG is given. This Levene's test results are not significant (p=0.874). So, the variances are not significantly different, they are homogenous. Table 4.19 shows the ANOVA results for MOG levels. There was a

significant effect of MOG on the classification of subtypes of Multiple Sclerosis, $F(5,142) = 13,799$, $p < .001$.

Table 4.15: Robust Tests of Equality of Means for NFL levels

	Statistic[a]	df1	df2	Sig.
Welch	10,292	5	34,397	,000
Brown-Forsythe	11,203	5	77,253	,000

a. Asymptotically F distributed.

Table 4.16: Homogeneous Subsets for NFL levels (Tukey HSD[a,b])

SUBTYPES	N	Subset for alpha = 0.05	
		1	2
OND	11	29,1336	
HC	11	36,5886	
CIS/RR	9		71,1459
CIS	34		72,9670
RR	61		75,0726
PP	16		76,7922
Sig.		,972	,992
OND	11	29,1336	

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 14,954.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Table 4.17: Descriptives for MOG levels

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
CIS	36	53,4517	21,90234	3,65039	46,0410	60,8624
CIS/RR	8	62,8995	16,79427	5,93767	48,8591	76,9399
OND	10	16,7144	19,06278	6,02818	3,0777	30,3511
HC	11	21,4639	13,51502	4,07493	12,3844	30,5434
PP	16	71,1574	17,87176	4,46794	61,6342	80,6806
RR	62	56,3437	23,06427	2,92916	50,4865	62,2009
Total	143	52,1856	25,43604	2,12707	47,9807	56,3904

Table 4.18: Test of homogenity for MOG levels

Levene Statistic	df1	df2	Sig.
,361	5	137	,874

Table 4.19: ANOVA for MOG levels

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	30770,979	5	6154,196	13,799	,000
Within Groups	61101,914	137	445,999		
Total	91872,893	142			

In Table 4.20, Brown-Forsythe and Welch forms of F-ratio are shown. But since the assumption of homogeneity of variance is not broken, these results only approve the previous F-ratio. In Table C.4, PostHoc tests for MOG levels are given. In Table 4.21, Tukey post-hoc comparisons of the six subtypes indicate that the control group gave significantly lower MOG levels than all of the other subtypes, $p < .001$.

Table 4.20: Robust Tests of Equality of Means for MOG levels

	Statistic[a]	df1	df2	Sig.
Welch	20,723	5	32,448	,000
Brown-Forsythe	17,924	5	81,424	,000

a. Asymptotically F distributed.

Table 4.21: Homogeneous Subsets for MOG levels (Tukey HSD[a,b])

SUBTYPES	N	Subset for alpha = 0.05	
		1	2
OND	10	16,7144	
HC	11	21,4639	
CIS	36		53,4517
RR	62		56,3437
CIS/RR	8		62,8995
PP	16		71,1574
Sig.		,991	,229

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 14,207.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Figure 4.14 shows the mean and standard deviation of TAU levels among the CIS group and CIS/RR group.

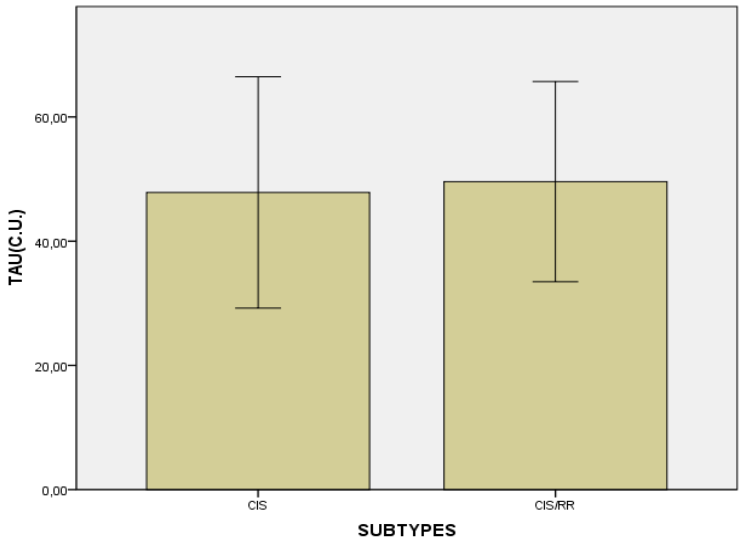

Figure 4. 14: Mean value of TAU levels between CIS and CIS/RR.

Figure 4.15 shows the mean and standard deviation of GFAP levels among the CIS group and CIS/RR group. Figure 4.16 shows the mean and standard deviation of NFL levels among the CIS group and CIS/RR group. Figure 4.17 shows the mean and standard deviation of MOG levels among the CIS group and CIS/RR group. Figure 4.18 shows the mean and standard deviation of TAU levels among different clinical subtypes and control groups.

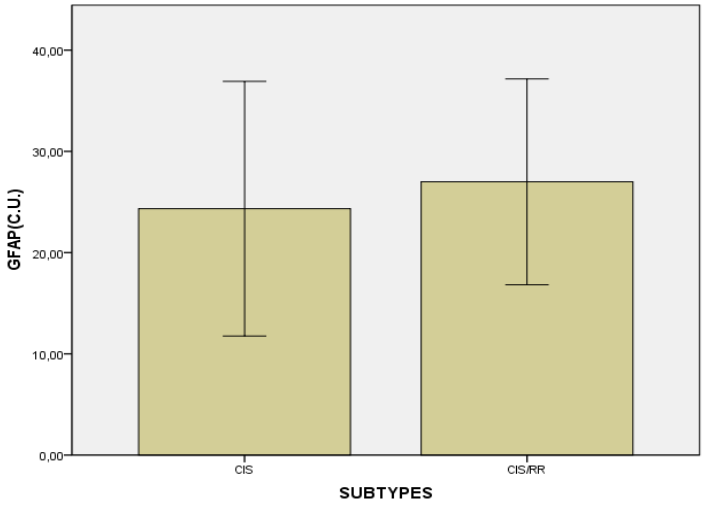

Figure 4. 15: Mean value of serum GFAP between CIS and CIS/RR.

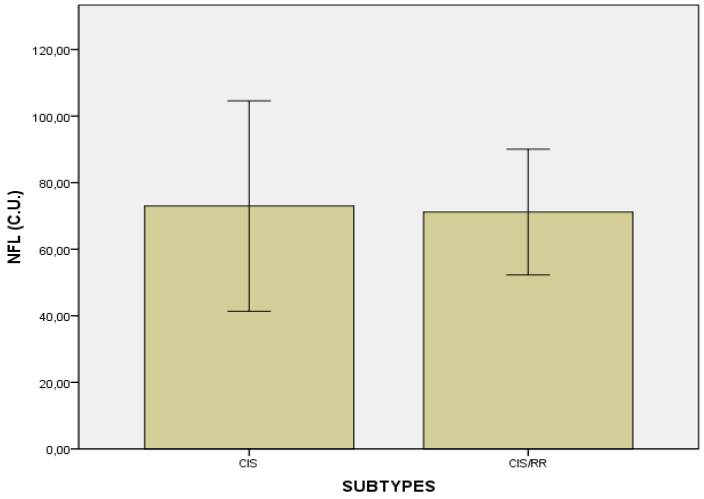

Figure 4. 16: Mean value of NFL levels between CIS and CIS/RR.

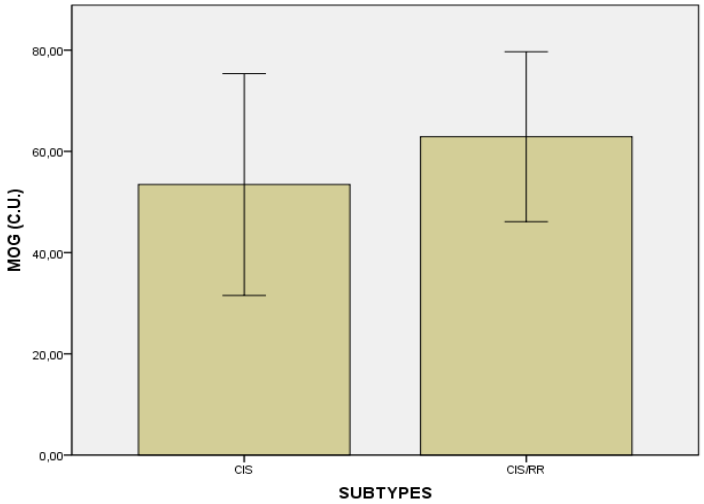

Figure 4. 17: Mean value of MOG levels between CIS and CIS/RR.

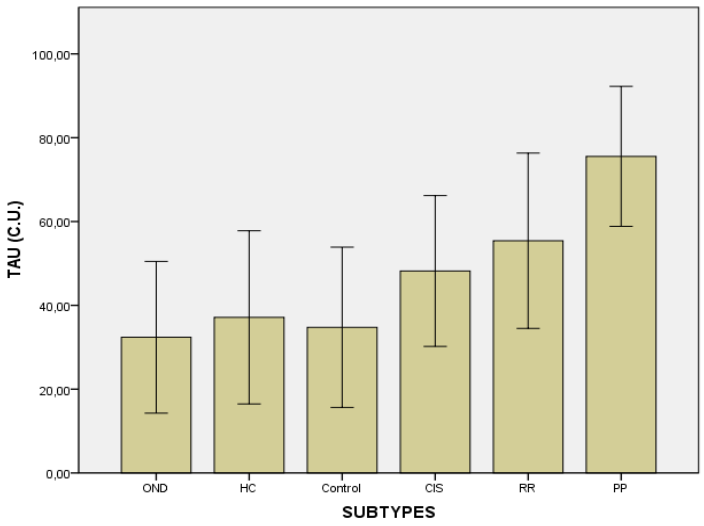

Figure 4. 18: Mean value of TAU levels according to different subtypes.

Figure 4.19 shows the mean and standard deviation of GFAP levels among different clinical subtypes and control groups.

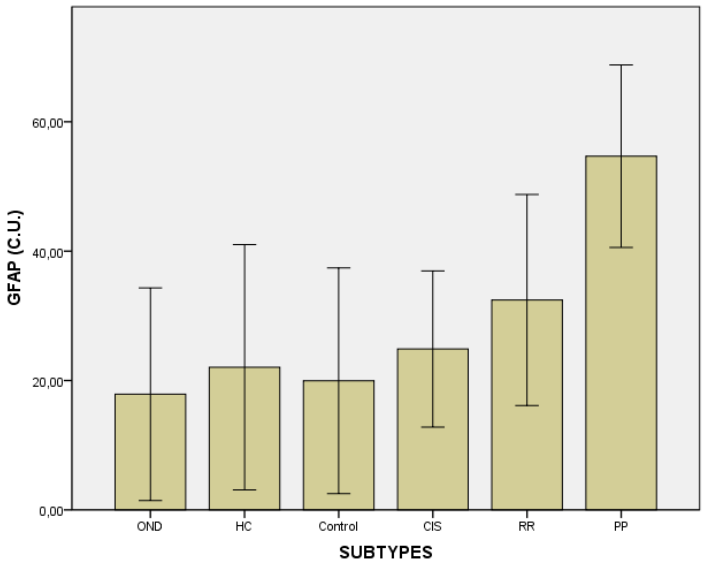

Figure 4. 19: Mean value of GFAP levels according to different subtypes.

Figure 4.20 shows the mean and standard deviation of NFL levels among different clinical subtypes and control groups. Figure 4.21 shows the mean and standard deviation of MOG levels among different clinical subtypes and control groups.

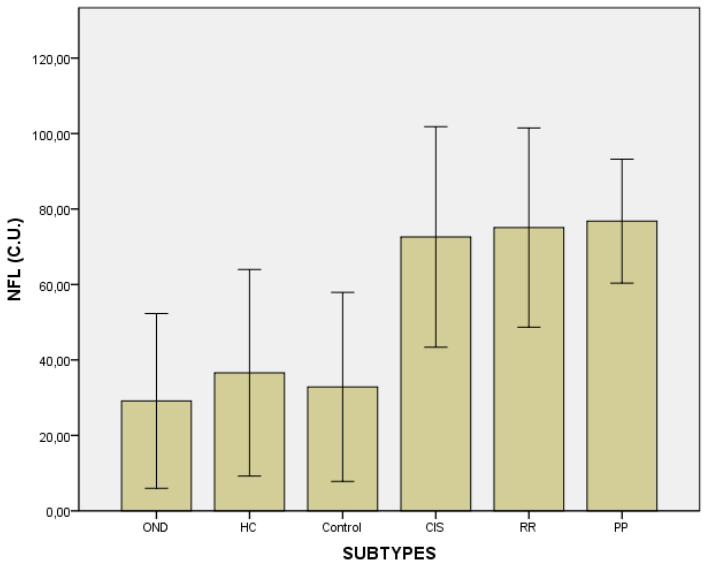

Figure 4. 20: Mean value of NFL levels according to different subtypes.

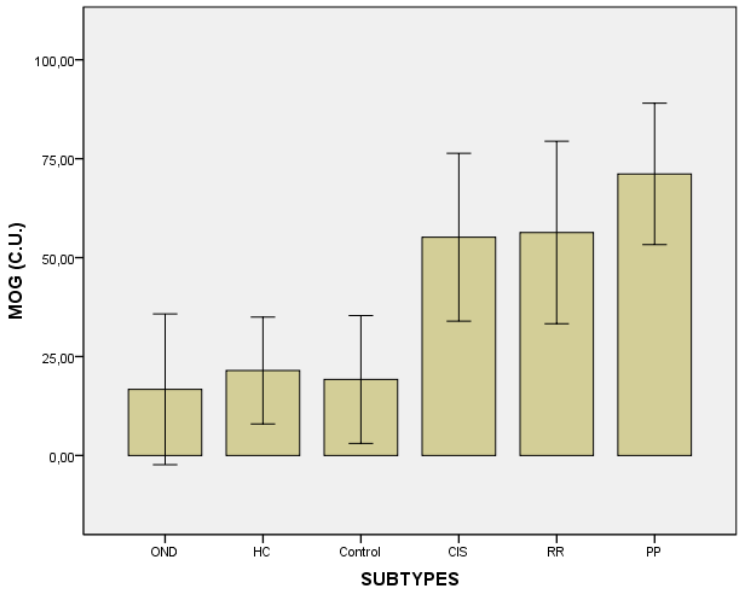

Figure 4. 21: Mean value of MOG levels according to different subtypes.

4.3 Results of Protein Data

Different combinations of TAU, GFAP, NFL and MOG were tested and for each classifier, only combination that gives the best AUC index is considered. These AUC indexes are calculated via accuracy of that classifier for the given combination of proteins. When the AUC indexes were the same, protein combination giving AUC with smaller variance value is shown here. Different protein combinations being best in different classifiers could be interpreted as that each protein has a different classification significance for different MS subgroups and/or control groups.

This study showed that, TAU, GFAP, NFL, MOG proteins can be used together for classification of prognosis and diagnosis stages in clinically different subtype of MS depending on their concentrations in the CSF. The difference between the mean values of these proteins for different MS subtypes can be seen in figure.4.22.

It is found that control group and CIS patients (Table D.1, Table D.2,Table D.3) can be differentiated using these proteins together by with 87.31% ± 12.02 accuracy and 0.93 ± 0.09 AUC.

Although our sample size is limited, it was also shown that the transition from CIS to RRMS can be best predicted using TAU protein. The CSF samples of these patients were taken when they were diagnosed as CIS patients, so the classification results proves that TAU protein level in CSF, differentiates the CIS and CIS/RRMS

subgroup even the CSF sample obtained when they are diagnosed as CIS. The level of TAU protein gives the best accuracy for the differentiation of CIS from CIS/RRMS patients (accuracy=76.22% ± 17.15, AUC = 0.77 ± 0.24 (Table 29).

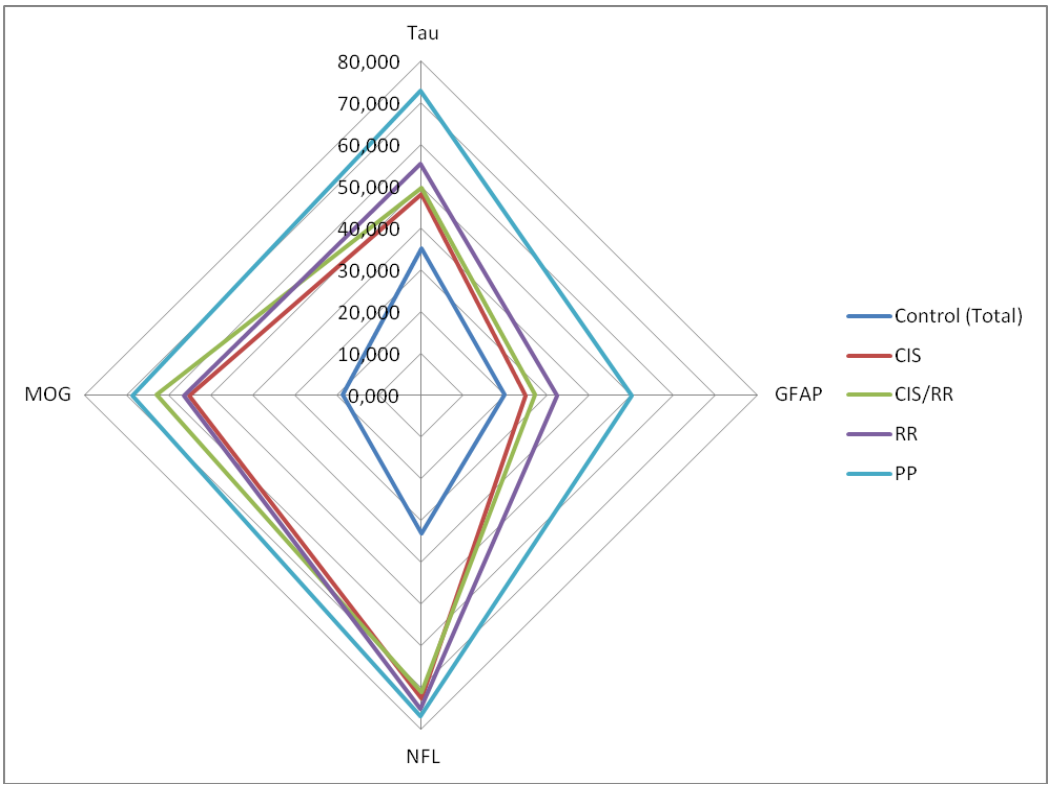

Figure 4. 22: Mean value of biomarkers according to different subtypes shown by a radar chart.

The classification results show that TAU, GFAP and MOG protein levels in CSF give the best accuracy for the differentiation of RRMS from CIS/RRMS patients (accuracy=84.28% ± 8.21, AUC 0.72±0.26) (Table 30).In addition, GFAP protein levels in CSF give the best accuracy for the differentiation of RRMS from CIS patients (accuracy = 70.57% ± 12.22, AUC 0.80±0.12) (Table 31). GFAP and NFL protein levels in CSF provided the best accuracy for the classification of CIS and MS (accuracy =76.72% ± 10.52, AUC =0.82±0.12) (Table 32). GFAP levels provided the best accuracy for the classification of CIS, CIS/RRMS and RRMS (accuracy =67.07% ± 11.77, AUC =0.81 ± 0.13) (Table 33).

When these proteins are used together for classification of MS and control samples, 94.25% ± 6.44 accuracy and 0.97 ± 0.08 AUC was obtained (Table D.9, Table D.10, Table D.11). In addition, with these proteins PPMS and RRMS subtypes can be

classified with 96,4% accuracy (AUC (0.96)) when all the protein data are used (Table D.12). The overall accuracy, obtained using GFAP-MOG, is 74.12% ± 10.77 (AUC=0.79 ± 0.13) between control group, CIS patients and MS patients (Table D.13).

When the classification results of TAU, GFAP, NFL and MOG are considered separately, using these proteins provided better results in general (Table 4.22). Therefore, using these proteins together gives better results in different groups of comparison.

Table 4.22: Classification Results of protein combinations resulting best AUC

Classifier	AUC (ROC area)	Accuracy	Proteins used
CIS vs. CIS/RR	0.77 ± 0.24	76.22 ± 17.15	TAU
CIS vs. CTRL	0.93 ± 0.09	87.31 ± 12.02	TAU-GFAP-NFL-MOG
CIS vs. HC	0.90 ± 0.17	90.96 ± 11.62	NFL-MOG
CIS vs. OND	0.93 ± 0.11	86.30 ± 13.22	TAU-GFAP-MOG
CIS vs. MS	0.82 ± 0.12	76.72 ± 10.52	GFAP-NFL
CIS vs. CIS/RR vs. RR	0.81 ± 0.13	67.07 ± 11.77	GFAP
MS vs. CTRL	0.97 ± 0.08	94.25 ± 6.44	TAU-GFAP-NFL-MOG
MS vs. HC	0.95 ± 0.14	96.65 ± 5.59	TAU-NFL-MOG
MS vs. OND	0.98 ± 0.05	95.80 ± 5.94	TAU-GFAP-NFL-MOG
MS vs. CTRL vs. CIS	0.79 ± 0.13	74.12 ± 10.77	GFAP-MOG
PP vs. RR	0.96 ± 0.11	93.65 ± 8.35	TAU-GFAP-MOG
RR vs. CIS	0.80 ± 0.12	70.57 ± 12.22	GFAP
RR vs. CISRR	0.80 ± 0.20	83.42 ± 8.52	GFAP-NFL

4.4 Results of Protein Data and Clinical Data

In this part, classification results of only protein data (proteins), results of Principle Components of Protein Data, Results of all features (protein data and clinical data), results of principal components of all features and results of a group of features that are selected using Information Gain Method are presented for each classifier. While selecting the features, all features that give positive information gain (that are >0) are selected. Six different classification methods are used for each classifier; K-nearest neighbors, Decision Tree, Random Forest, AdaBoost, Decorate and Bayesian Network. For all results, accuracy and AUC (AUC) are given together. Highest accuracy and AUC values are shown as bold. In addition, resulting features of Information Gain Feature Selection method are shown.

4.4.1 Classification of MS, Control and CIS samples

In Table E.1, Classification results of MS patients, Total Control group and CIS patients are given. Best accuracy is provided by selected features (using InfoGain), using Bayesian networks classification method (accuracy: 73.01%± 10.51, AUC: 0.77±0.13). Best AUC is achieved by selected features using Random Forest classification method (accuracy: 71.43± 10.95, AUC:0.82± 0.12). It is important to note that the results of feature selection contained protein data. This also shows the predictive and differentiative significance of proteins.

4.4.2 Differentiation of CIS from Control

In Table E.2, classification results of CIS patients, total control group are given Best accuracy is provided by principal components of protein data using kNN classification method (accuracy: 87.45%±12.02, AUC: 0.93±0.10). Best AUC is achieved by protein data using kNN classification method(accuracy: 87.31%±12.02, AUC: 0.93±0.09) and by resulting features of feature selection method, using kNN classification method (accuracy 86.06±12.14, AUC: 0.93±0.09). The results of feature selection contained protein data. In Table E.3, Classification results of OND Control Subgroup and CIS patients are given. For this classifier, results of feature selection only contained one of the proteins. In Table E.4, Classification results of Healthy Control group and CIS patients are given. The results of feature selection contained protein data.

4.4.3 Differentiation of MS from CIS

In Table E.5, Classification results of MS patients and CIS patients are given.

4.4.4 Differentiation of MS from Control

In Table E.6, Classification results of MS patients and Total Control group are given. It is important to note that the results of feature selection contained protein data. This also shows the predictive and differentiative significance of proteins. In Table E.7, Classification results of MS patients and OND Control subgroup are given. The results of feature selection contained only one of the proteins. It is important to note that the results of feature selection consists of protein data. So, these proteins are solely enough for the differentiation of MS from other neurological diseases. In Table E.8, Classification results of MS patients and Healthy Control group are given.

4.4.5 Classification of MS Subtypes: RR vs. PP

In Table E.9, Classification results of PPMS patients and RRMS patients are given. The results of feature selection contained 3 features of protein data

4.4.6 Transition from CIS to MS

In Table E.10, Classification results of CIS patients and CISRR patients are given. Here, the transition from CIS to MS is shown. It is important to note that the results of feature selection did not contain any protein data. This shows that protein data are not the best features for the differentiation of transition from CIS to MS. In Table E.11, Classification results of CISRR patients, RR patients and CIS patients are given

It is important to note that when looked at the confusion matrix (Table 4.23),CISRR patients were not classified correctly (there were no true positive). Six of them were classified as RR patients whereas 3 of them were classified as CIS patients. The majority of them being classified as RR patients supports the results of transition from CIS to MS. Although they were classified as CIS, they would be ‘misclassified’ as RR at the initial diagnosis. In Table E.12, Classification results of RRMS patients and CISRR patients are given. The results of feature selection contained no protein data. In Table 4.24, confusion matrix of classification of CISRR and RR is shown (accuracy: 88.16% , AUC:0.89).Although there are no false positives, there

are no true positives neither for CISRR patients. This shows that it is difficult to differentiate CISRR patients from RR patients using these data.

In Table 4.25, confusion matrix of classification method giving the best accuracy is shown. Here, there are false positives and false negatives for CISRR patients.

Table 4.23: Confusion Matrix of CIS vs. CISRR vs. RR, all features, Random Forest Classification Method (accuracy: 71.68% , AUC:0.79).

CIS	CISRR	RR	
26	0	11	CIS
3	0	6	CISRR
10	2	55	RR

Table 4.24: Confusion Matrix of CISRR vs. RR,feature selection applied, kNN Classification Method. (accuracy: 88.16% , AUC:0.89)

CISRR	RR	
0	9	CISRR
0	67	RR

Table 4.25: Confusion Matrix of CISRR vs. RR,feature selection applied, Random Forest Classification Method(accuracy: 90.79 %, AUC: 0.83).

CISRR	RR	
5	4	CISRR
3	64	RR

In Table E.13, classification results of RRMS patients and CIS patients are given. Six different classification methods are used for this classifier; K-nearest neighbors, Decision Tree, Random Forest, AdaBoost, Decorate and Bayesian Network. Also, here are presented the results of different feature sets: protein data, principle components of protein data, all features (protein data and clinical data), principal components of all data and features selected using Information Gain method. While selecting the features, all features that give positive information gain (that are >0) are selected.

In Table 4.26, a sumary of classification results is given. Here, for each clasifier, the best AUC is selected and used features and methods are shown.

Table 4.26: Classification results of features giving best AUC

Classifier	AUC (ROC area)	Accuracy	Features used
CIS vs. CIS/RR	0.89±0.19	86.45±12.62	Autoimmune Disease in Family, MR/T1, OCB, CSF Protein Level
CIS vs. CTRL	0.93±0.09	86.06±12.14	MR/T2, Gadolinium Enhancement, TAU, GFAP, NFL, MOG
CIS vs. HC	0.98±0.07	89.47±11.72	Duration of MS, Onset Age, Autoimmune Disease in Self, Autoimmune Disease in Family, Atrophy/Cortical, Atrophy/Corpus Callosum, Gadolinium Enhancement, TAU, NFL, MOG
CIS vs. OND	0.95±0.12	89.06±12.06	TAU,GFAP,NFL,MOG (PCA)
CIS vs. MS	0.83 ±0.12	76.51 ±11.15	All features
CIS vs. CIS/RR vs. RR	0.81±0.13	63.79±12.17	Duration of MS , OCB, GFAP
MS vs. CTRL	0.97±0.06	92.64±7.15	TAU,GFAP,NFL,MOG (PCA)
MS vs. HC	0.96 ±0.09	90.04 ±9.98	All features
MS vs. OND	0.99±0.04	95.02±5.91	TAU,GFAP,NFL,MOG (PCA)
MS vs. CTRL vs. CIS	0.82± 0.12	71.43± 10.95	Duration of MS, EDSS, OCB, TAU , GFAP, NFL , MOG
PP vs. RR	0.97±0.08	95.77±6.63	TAU,GFAP,NFL,MOG (PCA)
RR vs. CIS	0.80±0.13	75.35±12.05	Duration of MS, GFAP
RR vs. CISRR	0.92±0.12	89.77±7.00	Duration of MS, MS in Family, Gadolinium Enhancement, CSF Protein Level

5. DISCUSSION AND CONCLUSION

In this study, different clinical subtypes of multiple sclerosis are classified according to their protein and clinical data patterns with different classification methods.

To the best of our knowledge, it is the first study in the literature where the transition from Clinically Isolated Syndrome to Multiple Sclerosis is predicted using these patterns.

For the classification, 6 different methods were compared: KNN, Bayesian Networks, DECORATE, Decision Tree, Adaboost and Random Forest. Furthermore, following features are used for classification;

- Only protein data
- Principle Component Analysis on protein data
- All Features including protein data and clinical data
- Principal Component Analysis on all features
- Feature Selection according to Information Gain.

Here, each classification problem gives best results in different classification methods and usually using different features. This shows that each classification problem has different distribution for the features, and these classification problems should be handled separately. A hierarchical model should be applied for overall classification of clinical subtypes of MS and CIS patients and control group. Of course, number of samples is one of the most important criteria. Since the number of samples is relatively small, making a generalization would be difficult.

The results of PCA do not differ very much from the original results (even sometimes worse than the original results). This shows that features are independent from each other and correlation between features is low. In addition, the information gain based feature selection method selects the proteins as relevant features. It can be deduced that these selected proteins are good candidate biomarkers for the classification of clinically different subtypes of MS.

The most remarkable point for the clasification using proteins is that the candidate protein proteins gave more significant results when they were investigated together

in a sample. The results of classification showed that concentration levels of TAU, GFAP, NFL and MOG proteins in CSF should be considered together to use as biomarker for the prediction of diagnosis and prognosis of MS. In addition, the patients whose diagnose changes CIS to RRMS depending on the new attacks and lesions in the brain can be predicted by analyzing TAU protein level in CSF. This is a novel study using computer aided classification methods and these protein and clinical data together for diagnostic and prognostic purposes in predicting clinical subtypes of MS and predicting transition between subtypes.

In conclusion, this is the first study predicting transition from CIS to definite MS using TAU, GFAP, NFL and MOG proteins and clinical data patterns. Furthermore, this is the first study classifying the different subtypes of multiple sclerosis applying computer aided methods to given subset of proteins and clinical data.

For future studies, sample size should be increased for the generalization of classifier model to be implemented. In addition, different classification methods should be applied. The optimization of parameters of classification methods could give better results. Outlier detection and looking at the properties of data could be applied.

In addition, new identified protein biomarkers from proteome studies should be tested and these results need the comparison with other MS patient groups.

In order to compare the classification results, the error rates should be reduced. For this purpose, bootstrapping or leave-one-out method should be applied as cross-validation method instead of 10-fold cross validation.

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APPENDICES

APPENDIX A. Explanation of scoring system of parameters

APPENDIX B. Explanation of features

APPENDIX C. Results of PostHoc Analysis

APPENDIX D. Results of Classification of Protein Data

APPENDIX E. Results of Classification of Protein Data and Clinical Data

APPENDIX A. Explanation of scoring system of parameters

Gender: 1:male 0:Female

Autoimmune Disease in self: 1: Yes 0:No x:not known

Autoimmune Disease in family: 1: Yes 0:No x:not known

Autoimmune Disease in family: 1: Yes 0:No x:not known

Oligoclonal Band: 1:positive 2:negative 3:not checked 4:checked,but no data 5:other 6:not known

EDSS:

The functional systems(FS) are: pyramidal, cerebellar, brainstem, sensory, bowel and bladder, visual, cerebral and other.

0.0: Normal Neurological Exam

1.0: No disability, minimal signs on 1 FS

1.5: No disability, minimal signs on 2 of 7 FS

2.0: Minimal disability in 1 of 7 FS

2.5: Minimal disability in 2 FS

3.0: Moderate disability in 1 FS; or mild disability in 3 - 4 FS, though fully ambulatory

3.5: Fully ambulatory but with moderate disability in 1 FS and mild disability in 1 or 2 FS; or moderate disability in 2 FS; or mild disability in 5 FS

4.0: Fully ambulatory without aid, up and about 12hrs a day despite relatively severe disability. Able to walk without aid 500 meters

4.5: Fully ambulatory without aid, up and about much of day, able to work a full day, may otherwise have some limitations of full activity or require minimal assistance. Relatively severe disability. Able to walk without aid 300 meters

5.0: Ambulatory without aid for about 200 meters. Disability impairs full daily activities

5.5: Ambulatory for 100 meters, disability precludes full daily activities

6.0: Intermittent or unilateral constant assistance (cane, crutch or brace) required to walk 100 meters with or without resting

6.5: Constant bilateral support (cane, crutch or braces) required to walk 20 meters without resting

7.0: Unable to walk beyond 5 meters even with aid, essentially restricted to wheelchair, wheels self, transfers alone; active in wheelchair about 12 hours a day

7.5: Unable to take more than a few steps, restricted to wheelchair, may need aid to transfer; wheels self, but may require motorized chair for full day's activities

8.0: Essentially restricted to bed, chair, or wheelchair, but may be out of bed much of day; retains self care functions, generally effective use of arms

8.5: Essentially restricted to bed much of day, some effective use of arms, retains some self care functions

9.0: Helpless bed patient, can communicate and eat

9.5: Unable to communicate effectively or eat/swallow

10.0: Death due to MS

APPENDIX B. List of features

Feature1: Gender
Feature2:Duration of MS
Feature3:Onset age
Feature4:MS in Family
Feature5:Autoimmune Disease in self
Feature6:Autoimmune Disease in family
Feature7:EDSS
Feature8:MR/T1
Feature9:MR/T2
Feature10: Atrophy / Cortical
Feature11: Atrophy / Corpus Callosum
Feature12: Gadolinium Enhancement
Feature13: OCB
Feature14: CSF Protein Level
Feature15: CSF Glucose Level
Feature16:Serum Protein Level
Feature17: Serum Glucose Level
Feature18:CSF TAU
Feature19:CSF GFAP
Feature20:CSF NFL
Feature21:CSF MOG

APPENDIX C. Results of PostHoc Analysis

Table C.1: PostHoc Tests for TAU levels (Tukey HSD)

(I) SUBTYPES	(J) SUBTYPES	Mean Difference (I-J)	Std. Error	Sig.
CIS	CIS/RR	-1,74262	7,24732	1,000
	OND	15,45943	6,69644	,197
	HC	10,71980	6,69644	,599
	PP	-27,70353*	5,83442	,000
	RR	-7,57044	HC466	,412
CIS/RR	CIS	1,74262	7,24732	1,000
	OND	17,20205	8,76431	,369
	HC	12,46241	8,76431	,714
	PP	-25,96091*	8,12473	,021
	RR	-5,82783	6,92880	,959
OND	CIS	-15,45943	6,69644	,197
	CIS/RR	-17,20205	8,76431	,369
	HC	-4,73964	8,31456	,993
	PP	-43,16296*	7,63741	,000
	RR	-23,02988*	6,35035	,005
HC	CIS	-10,71980	6,69644	,599
	CIS/RR	-12,46241	8,76431	,714
	OND	4,73964	8,31456	,993
	PP	-38,42332*	7,63741	,000
	RR	-18,29024	6,35035	,051
PP	CIS	27,70353*	5,83442	,000
	CIS/RR	25,96091*	8,12473	,021
	OND	43,16296*	7,63741	,000
	HC	38,42332*	7,63741	,000
	RR	20,13308*	5,43370	,004
RR	CIS	7,57044	HC466	,412
	CIS/RR	5,82783	6,92880	,959
	OND	23,02988*	6,35035	,005
	HC	18,29024	6,35035	,051
	PP	-20,13308*	5,43370	,004

*. The mean difference is significant at the 0.05 level.

Table C.2: PostHoc Tests for GFAP levels (Tukey HSD)

(I) SUBTYPES	(J) SUBTYPES	Mean Difference (I-J)	Std. Error	Sig.
CIS	CIS/RR	-2,65558	5,65215	,997
	OND	6,45332	5,22494	,819
	HC	2,28568	5,22494	,998
	PP	-30,33926*	4,55691	,000
	RR	-8,10794	3,15089	,111
CIS/RR	CIS	2,65558	5,65215	,997
	OND	9,10890	6,81675	,764
	HC	4,94126	6,81675	,979
	PP	-27,68368*	6,31930	,000
	RR	-5,45236	5,39409	,914
OND	CIS	-6,45332	5,22494	,819
	CIS/RR	-9,10890	6,81675	,764
	HC	-4,16764	6,46694	,987
	PP	-36,79258*	5,94026	,000
	RR	-14,56125*	4,94463	,043
HC	CIS	-2,28568	5,22494	,998
	CIS/RR	-4,94126	6,81675	,979
	OND	4,16764	6,46694	,987
	PP	-32,62494*	5,94026	,000
	RR	-10,39362	4,94463	,292
PP	CIS	30,33926*	4,55691	,000
	CIS/RR	27,68368*	6,31930	,000
	OND	36,79258*	5,94026	,000
	HC	32,62494*	5,94026	,000
	RR	22,23133*	4,23259	,000
RR	CIS	8,10794	3,15089	,111
	CIS/RR	5,45236	5,39409	,914
	OND	14,56125*	4,94463	,043
	HC	10,39362	4,94463	,292
	PP	-22,23133*	4,23259	,000

*. The mean difference is significant at the 0.05 level.

Table C.3: PostHoc Tests for NFL levels (Tukey HSD)

(I) SUBTYPES	(J) SUBTYPES	Mean Difference (I-J)	Std. Error	Sig.
CIS	CIS/RR	1,82114	9,88617	1,000
	OND	43,83339*	9,14798	,000
	HC	36,37839*	9,14798	,002
	PP	-3,82516	7,99540	,997
	RR	-2,10554	5,64433	,999
CIS/RR	CIS	-1,82114	9,88617	1,000
	OND	42,01225*	11,85365	,007
	HC	34,55725*	11,85365	,047
	PP	-5,64630	10,98863	,996
	RR	-3,92668	9,41711	,998
OND	CIS	-43,83339*	9,14798	,000
	CIS/RR	-42,01225*	11,85365	,007
	HC	-7,45500	11,24536	,986
	PP	-47,65855*	10,32952	,000
	RR	-45,93894*	8,63893	,000
HC	CIS	-36,37839*	9,14798	,002
	CIS/RR	-34,55725*	11,85365	,047
	OND	7,45500	11,24536	,986
	PP	-40,20355*	10,32952	,002
	RR	-38,48394*	8,63893	,000
PP	CIS	3,82516	7,99540	,997
	CIS/RR	5,64630	10,98863	,996
	OND	47,65855*	10,32952	,000
	HC	40,20355*	10,32952	,002
	RR	1,71961	7,40756	1,000
RR	CIS	2,10554	5,64433	,999
	CIS/RR	3,92668	9,41711	,998
	OND	45,93894*	8,63893	,000
	HC	38,48394*	8,63893	,000
	PP	-1,71961	7,40756	1,000

*. The mean difference is significant at the 0.05 level.

Table C.4: PostHoc Tests for MOG levels (Tukey HSD)

(I) SUBTYPES	(J) SUBTYPES	Mean Difference (I-J)	Std. Error	Sig.
CIS	CIS/RR	-9,44778	8,25462	,862
	OND	36,73732*	7,54909	,000
	HC	31,98781*	7,27559	,000
	PP	-17,70572	6,34538	,065
	RR	-2,89199	4,42520	,987
CIS/RR	CIS	9,44778	8,25462	,862
	OND	46,18510*	10,01748	,000
	HC	41,43559*	9,81302	,001
	PP	-8,25794	9,14466	,945
	RR	6,55579	7,93369	,962
OND	CIS	-36,73732*	7,54909	,000
	CIS/RR	-46,18510*	10,01748	,000
	HC	-4,74951	9,22742	,996
	PP	-54,44304*	8,51322	,000
	RR	-39,62931*	7,19677	,000
HC	CIS	-31,98781*	7,27559	,000
	CIS/RR	-41,43559*	9,81302	,001
	OND	4,74951	9,22742	,996
	PP	-49,69353*	8,27166	,000
	RR	-34,87980*	6,90934	,000
PP	CIS	17,70572	6,34538	,065
	CIS/RR	8,25794	9,14466	,945
	OND	54,44304*	8,51322	,000
	HC	49,69353*	8,27166	,000
	RR	14,81373	5,92187	,131
RR	CIS	2,89199	4,42520	,987
	CIS/RR	-6,55579	7,93369	,962
	OND	39,62931*	7,19677	,000
	HC	34,87980*	6,90934	,000
	PP	-14,81373	5,92187	,131

*. The mean difference is significant at the 0.05 level.

APPENDIX D. Results of Classification of Protein Data

Table D.1: Classification Results of CIS vs. Total Control

5NN 10 fold cross validation, 1000 run p<0.05 two tailed, paired t-test (corrected)	ROC Area (AUC)	Accuracy
All proteins	**0.93±0.09**	**87.31±12.02**
TAU	0.82±0.15	67.78±15.92
GFAP	0.69±0.21	68.69±15.64
NFL	0.77±0.22	82.76±13.95
MOG	0.83±0.18	87.64±12.38
GFAP-MOG	0.91±0.12	86.36±12.23
TAU-GFAP	0.82±0.15	75.18±15.62
GFAP-NFL	0.85±0.14	76.30±14.60
GFAP-NFL-MOG	0.93±0.10	86.30±11.88
NFL-MOG	0.86±0.16	90.01±11.10
TAU-GFAP-MOG	0.90±0.12	85.50±13.30
TAU-GFAP-NFL	0.86±0.13	78.11±14.18
TAU-MOG	0.89±0.13	85.91±12.24
TAU-NFL	0.86±0.14	80.04±13.98
TAU-NFL-MOG	0.93±0.10	87.50±11.64

Table D.2: Classification Results of CIS vs. Healthy Control

5NN 10 fold cross validation, 1000 run p<0.05 two tailed, paired t-test (corrected)	ROC Area (AUC)	Accuracy
All proteins	0.90±0.17	87.79±12.18
TAU	0.78±0.20	78.62±10.39
GFAP	0.74±0.24	75.14±13.40
NFL	0.81±0.28	82.15±14.14
MOG	0.85±0.24	88.59±11.92
GFAP-MOG	0.85±0.23	90.93±11.80
TAU-GFAP	0.77±0.23	72.99±14.40
GFAP-NFL	0.77±0.26	82.09±14.37
GFAP-NFL-MOG	0.89±0.17	88.53±12.44
NFL-MOG	0.90±0.17	90.96±11.62
TAU-GFAP-MOG	0.86±0.22	89.06±12.54
TAU-GFAP-NFL	0.80±0.22	78.31±14.12
TAU-MOG	0.86±0.22	91.17±11.55
TAU-NFL	0.83±0.22	81.57±14.42
TAU-NFL-MOG	**0.90±0.17**	**89.82±12.11**

Table D.3: Classification Results of CIS vs OND

5NN 10 fold cross validation, 1000 run p<0.05 two tailed, paired t-test (corrected)	ROC Area (AUC)	Accuracy
All proteins	0.92±0.11	82.29±12.31
TAU	0.80±0.20	83.21±11.45
GFAP	0.55±0.28	75.71±11.22
NFL	0.74±0.32	79.68±14.37
MOG	0.88±0.18	88.45±13.26
GFAP-MOG	0.92±0.11	85.45±12.09
TAU-GFAP	0.77±0.18	70.79±13.92
GFAP-NFL	0.82±0.16	72.90±13.69
GFAP-NFL-MOG	0.92±0.11	85.85±12.49
NFL-MOG	0.86±0.23	90.50±11.70
TAU-GFAP-MOG	**0.93±0.11**	**86.30±13.22**
TAU-GFAP-NFL	0.86±0.14	74.83±13.60
TAU-MOG	0.89±0.21	95.52±6.55
TAU-NFL	0.85±0.20	81.51±13.86
TAU-NFL-MOG	0.91±0.12	85.59±12.38

Table D.4: Classification results for the differentiation of CIS and CIS/RRMS

5NN 10 fold cross validation, 1000 run p<0.05 two tailed, paired t-test (corrected)	ROC Area (AUC)	Accuracy
All proteins	0.68±0.32	83.24±14.19
TAU	**0.77±0.24**	**76.22±17.15**
GFAP	0.64±0.32	79.52±16.69
NFL	0.72±0.32	81.13±16.48
MOG	0.63±0.28	77.01±12.51
GFAP-MOG	0.69±0.36	79.99±15.64
TAU-GFAP	0.66±0.34	80.40±17.19
GFAP-NFL	0.69±0.36	79.99±15.64
GFAP-NFL-MOG	0.58±0.34	80.46±14.11
NFL-MOG	0.60±0.32	74.86±12.52
TAU-GFAP-MOG	0.63±0.35	80.32±15.08
TAU-GFAP-NFL	0.68±0.33	88.19±12.01
TAU-MOG	0.73±0.27	78.56±15.62
TAU-NFL	0.69±0.32	85.74±13.68
TAU-NFL-MOG	0.68±0.32	83.15±14.12

Table D.5: Classification Results of CIS/RRMS vs. RRMS

5NN 10 fold cross validation, 1000 run p<0.05 two tailed, paired t-test (corrected)	ROC Area (AUC)	Accuracy
All proteins	0.58±0.23	87.19±5.25
TAU	0.60±0.20	88.18±4.07
GFAP	0.66±0.28	84.17±8.54
NFL	0.51±0.27	88.19±4.05
MOG	0.65±0.28	85.31±7.24
GFAP-MOG	0.57±0.25	87.27±6.01
TAU-GFAP	0.63±0.25	87.97±4.34
GFAP-NFL	0.80±0.20	83.42±8.52
GFAP-NFL-MOG	0.61±0.22	84.98±7.17
NFL-MOG	0.59±0.29	87.10±6.16
TAU-GFAP-MOG	**0.72±0.26**	**84.28±8.21**
TAU-GFAP-NFL	0.62±0.28	87.28±5.77
TAU-MOG	0.60±0.29	84.95±7.39
TAU-NFL	0.56±0.32	86.08±7.16
TAU-NFL-MOG	0.53±0.25	86.69±5.95

Table D.6: Classification Results of CIS vs RRMS

5NN 10 fold cross validation, 1000 run p<0.05 two tailed, paired t-test (corrected)	ROC Area (AUC)	Accuracy
All proteins	0.75±0.14	70.76±12.42
TAU	0.55±0.16	61.74±13.12
GFAP	**0.80±0.12**	**70.57±12.22**
NFL	0.50±0.17	50.48±13.46
MOG	0.63±0.15	62.01±12.46
GFAP-MOG	0.78±0.13	75.43±12.22
TAU-GFAP	0.75±0.14	69.41±12.11
GFAP-NFL	0.78±0.13	75.43±12.22
GFAP-NFL-MOG	0.75±0.14	69.34±12.81
NFL-MOG	0.51±0.16	57.76±12.98
TAU-GFAP-MOG	0.76±0.14	69.35±13.03
TAU-GFAP-NFL	0.73±0.15	67.86±12.70
TAU-MOG	0.65±0.16	62.54±13.13
TAU-NFL	0.69±0.15	63.66±13.07
TAU-NFL-MOG	0.67±0.16	65.58±13.26

Table D.7: Classification Results of CIS vs. MS

5NN 10 fold cross validation, 1000 run p<0.05 two tailed, paired t-test (corrected)	ROC Area (AUC)	Accuracy
All proteins	0.78±0.13	75.25±10.70
TAU	0.60±0.15	68.10±11.60
GFAP	0.79±0.13	73.67±10.69
NFL	0.53±0.16	61.48±11.79
MOG	0.66±0.15	69.73±10.85
GFAP-MOG	0.82±0.12	76.72±10.52
TAU-GFAP	0.75±0.14	73.28±10.87
GFAP-NFL	**0.82±0.12**	**76.72±10.52**
GFAP-NFL-MOG	0.77±0.13	72.79±11.28
NFL-MOG	0.49±0.15	60.10±10.94
TAU-GFAP-MOG	0.79±0.13	71.99±11.36
TAU-GFAP-NFL	0.78±0.13	72.87±11.29
TAU-MOG	0.68±0.15	67.76±11.32
TAU-NFL	0.73±0.14	68.38±11.55
TAU-NFL-MOG	0.69±0.15	71.63±11.32

Table D.8: Classification Results of CIS vs CIS/RR vs. RRMS

5NN 10 fold cross validation, 1000 run p<0.05 two tailed, paired t-test (corrected)	ROC Area (AUC)	Accuracy
All proteins	0.81±0.14	69.87±11.61
TAU	0.63±0.17	59.00±12.74
GFAP	**0.81±0.13**	**67.07±11.77**
NFL	0.58±0.19	49.72±13.59
MOG	0.65±0.16	59.37±12.46
GFAP-MOG	0.77±0.15	71.26±12.53
TAU-GFAP	0.78±0.14	67.24±11.59
GFAP-NFL	0.77±0.15	71.26±12.53
GFAP-NFL-MOG	0.75±0.15	64.58±12.50
NFL-MOG	0.53±0.17	54.39±12.72
TAU-GFAP-MOG	0.78±0.14	67.14±12.72
TAU-GFAP-NFL	0.80±0.13	66.15±12.24
TAU-MOG	0.68±0.17	60.92±12.84
TAU-NFL	0.74±0.15	61.79±12.88
TAU-NFL-MOG	0.73±0.16	63.94±12.81

Table D.9: Classification Results of MS vs. CTRL

5NN 10 fold cross validation, 1000 run p<0.05 two tailed, paired t-test (corrected)	ROC Area (AUC)	Accuracy
All proteins	**0.97±0.08**	**94.25±6.44**
TAU	0.84±0.12	77.68±10.87
GFAP	0.82±0.15	83.51±9.65
NFL	0.83±0.18	90.94±7.83
MOG	0.88±0.16	92.35±7.63
GFAP-MOG	0.90±0.15	91.86±7.69
TAU-GFAP	0.88±0.13	87.72±9.75
GFAP-NFL	0.89±0.14	90.52±8.34
GFAP-NFL-MOG	0.94±0.11	90.99±7.77
NFL-MOG	0.91±0.14	93.01±7.38
TAU-GFAP-MOG	0.93±0.12	91.35±7.86
TAU-GFAP-NFL	0.93±0.11	92.26±7.42
TAU-MOG	0.90±0.14	92.38±7.77
TAU-NFL	0.95±0.09	90.74±8.31
TAU-NFL-MOG	0.94±0.11	93.86±6.53

Table D.10: Classification Results of MS vs OND

5NN 10 fold cross validation, 1000 run p<0.05 two tailed, paired t-test (corrected)	ROC Area (AUC)	Accuracy
All proteins	**0.98±0.05**	**95.80±5.94**
TAU	0.79±0.19	82.97±8.45
GFAP	0.76±0.25	88.53±8.23
NFL	0.81±0.28	92.61±7.58
MOG	0.86±0.28	93.49±7.50
GFAP-MOG	0.93±0.16	91.05±7.87
TAU-GFAP	0.84±0.22	87.76±9.36
GFAP-NFL	0.83±0.24	91.73±7.16
GFAP-NFL-MOG	0.94±0.16	95.09±6.59
NFL-MOG	0.90±0.21	95.40±6.62
TAU-GFAP-MOG	0.94±0.13	90.96±7.98
TAU-GFAP-NFL	0.94±0.14	94.34±6.57
TAU-MOG	0.93±0.15	92.15±7.82
TAU-NFL	0.98±0.06	93.83±7.32
TAU-NFL-MOG	0.94±0.15	94.95±6.28

Table D.11: Classification Results of MS vs. HC

5NN 10 fold cross validation, 1000 run p<0.05 two tailed, paired t-test (corrected)	ROC Area (AUC)	Accuracy
All proteins	0.95±0.15	97.64±4.58
TAU	0.85±0.16	82.50±8.07
GFAP	0.78±0.25	89.00±7.36
NFL	0.86±0.21	94.52±6.81
MOG	0.89±0.21	96.78±5.32
GFAP-MOG	0.88±0.22	93.66±7.49
TAU-GFAP	0.86±0.22	90.60±9.29
GFAP-NFL	0.84±0.26	94.17±7.04
GFAP-NFL-MOG	0.94±0.15	96.81±5.05
NFL-MOG	0.94±0.15	95.11±6.46
TAU-GFAP-MOG	0.89±0.21	93.65±7.49
TAU-GFAP-NFL	0.89±0.21	96.60±5.24
TAU-MOG	0.89±0.21	95.52±6.55
TAU-NFL	0.90±0.21	95.03±6.18
TAU-NFL-MOG	**0.95±0.14**	**96.65±5.59**

Table D.12: Classification Results of PPMS vs RRMS

5NN 10 fold cross validation, 1000 run p<0.05 two tailed, paired t-test (corrected)	ROC Area (AUC)	Accuracy
All proteins	0.95±0.11	95.91±6.85
TAU	0.77±0.22	87.73±7.63
GFAP	0.89±0.16	88.02±10.58
NFL	0.60±0.23	67.78±13.21
MOG	0.74±0.22	84.31±11.04
GFAP-MOG	0.95±0.12	92.86±8.52
TAU-GFAP	0.95±0.11	92.85±8.63
GFAP-NFL	0.95±0.12	92.86±8.52
GFAP-NFL-MOG	0.95±0.11	93.54±8.41
NFL-MOG	0.82±0.21	87.10±10.22
TAU-GFAP-MOG	0.96±0.11	93.39±8.37
TAU-GFAP-NFL	**0.96±0.11**	**93.65±8.35**
TAU-MOG	0.93±0.12	89.13±10.51
TAU-NFL	0.90±0.16	88.02±11.25
TAU-NFL-MOG	0.93±0.12	91.94±9.43

Table D.13: Classification Results of CIS vs MS vs. Control

5NN 10 fold cross validation, 1000 run p<0.05 two tailed, paired t-test (corrected)	ROC Area (AUC)	Accuracy
All proteins	0.75±0.13	71.62±10.21
TAU	0.57±0.14	51.03±11.21
GFAP	0.74±0.13	64.41±10.41
NFL	0.47±0.16	56.87±11.17
MOG	0.62±0.16	66.15±10.56
GFAP-MOG	**0.79±0.13**	**74.12±10.77**
TAU-GFAP	0.71±0.14	66.76±10.76
GFAP-NFL	0.73±0.14	64.01±11.18
GFAP-NFL-MOG	0.75±0.13	70.18±10.61
NFL-MOG	0.48±0.15	61.11±10.45
TAU-GFAP-MOG	0.76±0.13	70.17±10.82
TAU-GFAP-NFL	0.73±0.14	66.97±10.88
TAU-MOG	0.65±0.15	67.00±10.92
TAU-NFL	0.66±0.15	65.73±10.93
TAU-NFL-MOG	0.66±0.15	66.53±10.58

APPENDIX E. Results of Classification of Protein Data and Clinical Data

Table E.1: Classification results of MS patients, CIS patients and Total Control group using proteins only, PCA Applied to protein data, using all features, PCA Applied to all features and using information gain based feature selection methods.Applied classification methods are KNN, J48(Decision Tree), Random Forest, Adaptive Boosting(AdaBoost M1), DECORATE, Bayesian Networks (BayesNet). (10 fold cross validation, 1000 run $p<0.05$ two tailed, paired t-test (corrected))

MS vs. CTRL vs. CIS		**Knn (5-NN)**	**J48**	**Random Forest**	**AdaBoost M1**	**DECORATE**	**BayesNet**
Proteins	**Accuracy %**	71.62±10.21	72.27±10.73	69.13±11.21	67.22±9.57	71.51±11.02	71.36±11.29
	AUC	0.75±0.13	0.69±0.16	0.74±0.14	0.74±0.13	0.77±0.14	0.73±0.14
All Proteins-PCA	**Accuracy %**	71.18±10.37	67.41±11.48	64.46±11.28	63.89±6.93	66.84±11.38	69.95±9.88
	AUC	0.77±0.13	0.66±0.15	0.72±0.14	0.56±0.11	0.69±0.16	0.69±0.13
All Features	**Accuracy %**	63.13 ±11.61	70.66 ±10.99	72.09 ±10.99	67.09 ±9.57	72.23 ±10.90	72.80 ±10.56
	AUC	0.66 ±0.15	0.72 ±0.16	0.81 ±0.12	0.73 ±0.14	0.82 ±0.12	0.76 ±0.13
All Features-PCA	**Accuracy %**	65.17±11.60	64.84±11.61	65.52±11.48	61.52±7.07	61.40± 12.27	60.74± 10.37
	AUC	0.71±0.14	0.67±0.14	0.71±0.14	0.66±0.13	0.70±0.15	0.68±0.14
Feature Selection-InfoGain	**Accuracy %**	68.62±10.83	68.88±10.63	71.43± 10.95	67.09± 9.57	70.90±11.04	**73.01± 10.51**
	AUC	0.74±0.12	0.72±0.15	**0.82± 0.12**	0.73±0.14	**0.82±0.12**	0.77±0.13
	Selected Features	Duration of MS, EDSS, OCB, TAU , GFAP, NFL , MOG					

Table E.2: Classification results CIS patients and Total Control group using proteins only, PCA Applied to protein data, using all features, PCA Applied to all features and using information gain based feature selection methods.Applied classification methods are KNN, J48(Decision Tree), Random Forest, Adaptive Boosting(AdaBoost M1), DECORATE, Bayesian Networks (BayesNet).). (10 fold cross validation, 1000 run $p<0.05$ two tailed, paired t-test (corrected))

CIS vs Total CTRL		kNN (5-NN)	J48	Random Forest	AdaBoost M1	DECORATE	BayesNet
Proteins	AUC	**0.93±0.09**	0.84±0.16	0.87±0.14	0.81±0.20	0.89±0.15	0.86±0.17
	Accuracy %	87.31±12.02	83.38±14.18	82.82±13.77	79.78±14.99	83.76±13.85	84.58±13.32
All Proteins-PCA	AUC	0.93±0.10	0.85±0.15	0.92±0.11	0.91±0.13	0.91±0.12	0.92±0.10
	Accuracy %	**87.45±12.02**	84.47±13.64	83.44±13.28	83.68±12.66	83.39±13.22	81.36±13.79
All Features	AUC	0.87 ±0.14	0.79 ±0.19	0.91 ±0.13	0.89 ±0.14	0.90 ±0.14	0.85 ±0.18
	Accuracy %	82.29 ±13.96	81.86 ±13.73	82.93 ±13.07	80.46 ±14.56	83.76 ±13.38	83.41 ±13.75
All Features-PCA	AUC	0.84±0.15	0.73±0.18	0.88±0.15	0.86±0.15	0.86±0.17	0.86±0.16
	Accuracy %	72.55±16.43	75.25±15.63	82.11±13.39	78.22±14.80	78.66±15.04	82.53±13.88
Feature Selection-InfoGain	AUC	**0.93±0.09**	0.80±0.19	0.88±0.14	0.81±0.18	0.89±0.15	0.86±0.17
	Accuracy %	86.06±12.14	82.00±14.55	82.58±13.56	76.83±14.89	82.91±14.17	84.91±13.31
	Selected Features	MR/T2, Gadolinium Enhancement, TAU, GFAP, NFL, MOG					

Table E.3: Classification results CIS patients and OND Control group subset(Other Neurological Diseases) using proteins only, PCA Applied to protein data, using all features, PCA Applied to all features and using information gain based feature selection methods.Applied classification methods are KNN, J48(Decision Tree), Random Forest, Adaptive Boosting(AdaBoost M1), DECORATE, Bayesian Networks (BayesNet).). (10 fold cross validation, 1000 run $p<0.05$ two tailed, paired t-test (corrected))

CIS vs OND		**kNN (5-NN)**	**J48**	**Random Forest**	**AdaBoost M1**	**DECORATE**	**BayesNet**
Proteins	**AUC**	0.92±0.11	0.74±0.29	0.82±0.21	0.78±0.23	0.80±0.27	0.84±0.25
	Accuracy %	82.29±12.31	83.28±14.39	78.93±13.84	77.40±13.90	82.08±14.68	87.52±13.31
All Proteins-PCA	**AUC**	0.93±0.11	0.80±0.23	0.93±0.13	**0.95±0.12**	0.91±0.15	0.94±0.11
	Accuracy %	82.65±11.79	88.36±12.81	87.47±12.30	89.06±12.06	86.92±13.52	**90.91±11.51**
All Features	**AUC**	0.70 ±0.28	0.59 ±0.37	0.85 ±0.23	0.76 ±0.25	0.84 ±0.25	0.84 ±0.25
	Accuracy %	81.35 ±12.14	80.73 ±12.01	84.81 ±12.69	79.27 ±14.13	84.54 ±13.29	87.52 ±13.32
All Features-PCA	**AUC**	0.74±0.26	0.61±0.27	0.72±0.29	0.77±0.27	0.71±0.30	0.44±0.12
	Accuracy %	78.75±13.16	76.08±17.82	80.98±11.42	81.79±13.59	78.11±15.60	78.33±10.18
Feature Selection-InfoGain	**AUC**	0.87±0.18	0.80±0.23	0.78±0.23	0.83±0.19	0.86±0.21	0.81±0.21
	Accuracy %	87.19±13.61	84.78±13.90	76.42±14.38	78.82±14.42	83.69±14.02	84.90±13.69
	Selected Features	Autoimmune Disease in Self, MOG					

Table E.4: Classification results CIS patients and Healty Control group subset using proteins only, PCA Applied to protein data, using all features, PCA Applied to all features and using information gain based feature selection methods.Applied classification methods are KNN, J48(Decision Tree), Random Forest, Adaptive Boosting(AdaBoost M1), DECORATE, Bayesian Networks (BayesNet).). (10 fold cross validation, 1000 run $p<0.05$ two tailed, paired t-test (corrected))

CIS vs HC		kNN (5-NN)	J48	Random Forest	AdaBoost M1	DECORATE	BayesNet
Proteins	AUC	0.90±0.17	0.77±0.26	0.82±0.22	0.69±0.33	0.84±0.25	0.86±0.21
	Accuracy %	87.79±12.18	86.89±13.20	81.92±14.17	78.30±14.74	83.28±14.77	85.07±13.16
All Proteins-PCA	AUC	0.87±0.21	0.75±0.33	0.85±0.20	0.90±0.15	0.88±0.19	0.84±0.20
	Accuracy %	85.74±11.52	83.46±12.84	83.24±13.54	82.13±13.46	83.54±13.33	82.95±13.26
All Features	AUC	0.87 ±0.20	0.75 ±0.27	0.93 ±0.18	0.93 ±0.15	0.92 ±0.19	0.96 ±0.10
	Accuracy %	84.42 ±14.89	86.96 ±12.36	**92.04 ±10.31**	89.00 ±13.31	91.54 ±10.80	89.04 ±12.28
All Features-PCA	AUC	0.83±0.24	0.83±0.22	0.90±0.21	0.91±0.20	0.89±0.24	0.86±0.24
	Accuracy %	76.44±18.11	89.88±12.36	91.57±10.54	91.06±11.60	86.08±14.56	89.07±11.67
Feature Selection-InfoGain	AUC	0.94±0.16	0.75±0.27	0.92±0.18	0.96±0.13	0.93±0.18	**0.98±0.07**
	Accuracy %	91.22±11.51	86.99±12.32	91.53±10.70	91.73±11.57	91.98±10.62	89.47±11.72
	Selected Features	Duration of MS, Onset Age, Autoimmune Disease in Self, Autoimmune Disease in Family, Atrophy/Cortical, Atrophy/Corpus, Callosum, Gadolinium Enhancement, TAU, NFL, MOG					

Table E.5: Classification results of CIS patients and MS patients using proteins only, PCA Applied to protein data, using all features, PCA Applied to all features and using information gain based feature selection methods.Applied classification methods are KNN, J48(Decision Tree), Random Forest, Adaptive Boosting(AdaBoost M1), DECORATE, Bayesian Networks (BayesNet).). (10 fold cross validation, 1000 run $p<0.05$ two tailed, paired t-test (corrected))

MS vs. CIS		kNN (5-NN)	J48	Random Forest	AdaBoost M1	DECORATE	BayesNet
Proteins	AUC	0.78±0.13	0.72±0.15	0.78±0.13	0.78±0.14	0.77±0.14	0.76±0.13
	Accuracy %	75.25±10.70	71.79±10.63	71.64±11.95	74.77±11.45	73.42±10.93	74.36±12.36
All Proteins-PCA	AUC	0.79±0.12	0.66±0.14	0.73±0.14	0.72±0.15	0.72±0.14	0.76±0.13
	Accuracy %	74.99±10.68	68.48±12.49	66.25±12.24	70.15±11.64	67.82±11.67	73.13±10.62
All Features	AUC	0.68 ±0.15	0.73 ±0.16	0.82 ±0.12	**0.83 ±0.12**	0.82 ±0.12	0.81 ±0.13
	Accuracy %	65.09 ±12.55	75.75 ±11.94	75.80 ±11.46	76.51 ±11.15	75.75 ±11.29	77.14 ±11.36
All Features-PCA	AUC	0.70±0.14	0.59±0.15	0.68±0.15	0.68±0.14	0.60±0.17	0.49±0.06
	Accuracy %	68.75±11.95	61.68±12.56	65.55±12.47	65.35±11.97	61.59±12.55	61.35±7.09
Feature Selection-InfoGain	AUC	0.76±0.13	0.78±0.14	0.77±0.14	0.81±0.12	0.82±0.13	0.82±0.12
	Accuracy %	71.91±11.39	77.54±10.65	74.30±11.74	75.48±11.28	77.88±10.46	**78.31±10.76**
	Selected Features	Duration of MS, EDSS, GFAP					

Table E.6: Classification results of total Control Group and MS patients using proteins only, PCA Applied to protein data, using all features, PCA Applied to all features and using information gain based feature selection methods.Applied classification methods are KNN, J48(Decision Tree), Random Forest, Adaptive Boosting(AdaBoost M1), DECORATE, Bayesian Networks (BayesNet).). (10 fold cross validation, 1000 run $p<0.05$ two tailed, paired t-test (corrected))

MS vs.total	CTRL	kNN (5-NN)	J48	Random Forest	AdaBoost M1	DECORATE	BayesNet
Proteins	AUC	0.97±0.08	0.86±0.16	0.93±0.11	0.92±0.14	0.91±0.16	0.91±0.15
	Accuracy %	94.25±6.44	92.40±7.62	91.62±8.17	90.49±8.20	93.09±8.03	94.75±6.32
All Proteins-PCA	AUC	0.98±0.06	0.89±0.16	0.95±0.10	**0.97±0.06**	0.94±0.10	0.93±0.12
	Accuracy %	**94.79±5.93**	92.01±7.45	92.74±7.41	92.64±7.15	93.19±7.37	93.24±7.65
All Features	AUC	0.90 ±0.13	0.82 ±0.18	0.93 ±0.12	0.94 ±0.12	0.93 ±0.12	0.94 ±0.10
	Accuracy %	87.97 ±10.02	91.71 ±7.65	92.17 ±7.86	92.29 ±7.99	92.14 ±7.76	93.47 ±7.05
All Features-PCA	AUC	0.85±0.18	0.85±0.15	0.90±0.15	0.88±0.16	0.88±0.17	0.92±0.11
	Accuracy %	86.50±9.96	90.66±8.44	92.57±7.21	88.91±8.62	87.99±10.17	90.28±8.11
Feature Selection-InfoGain	AUC	0.97±0.06	0.86±0.16	0.94±0.11	0.94±0.12	0.93±0.12	0.94±0.10
	Accuracy %	92.93±7.59	92.33±7.75	91.97±7.94	91.04±8.21	92.22±8.09	93.51±7.01
	Selected Features	MR/T2, OCB, TAU, GFAP, NFL, MOG					

Table E.7: Classification results of OND (Other Neurological Diseases) Control subgroup and MS patients using proteins only, PCA Applied to protein data, using all features, PCA Applied to all features and using information gain based feature selection methods.Applied classification methods are KNN, J48(Decision Tree), Random Forest, Adaptive Boosting(AdaBoost M1), DECORATE, Bayesian Networks (BayesNet).). (10 fold cross validation, 1000 run $p<0.05$ two tailed, paired t-test (corrected))

MS vs. OND		kNN (5-NN)	J48	Random Forest	AdaBoost M1	DECORATE	BayesNet
Proteins	AUC	0.98±0.05	0.78±0.29	0.92±0.18	0.85±0.27	0.89±0.23	0.88±0.25
	Accuracy %	95.80±5.94	93.17±7.27	93.11±7.62	92.12±7.92	94.25±7.16	95.91±6.38
All Proteins-PCA	AUC	0.90±0.20	0.77±0.32	0.94±0.16	**0.99±0.04**	0.92±0.18	0.86±0.19
	Accuracy %	**96.81±4.97**	95.13±6.44	95.27±6.38	95.02±5.91	94.60±6.91	91.95±7.59
All Features	AUC	0.78 ±0.23	0.71 ±0.34	0.92 ±0.18	0.88 ±0.24	0.91 ±0.22	0.88 ±0.25
	Accuracy %	89.57 ±4.61	92.19 ±7.42	94.54 ±6.68	93.15 ±7.48	94.46 ±6.64	95.81 ±6.42
All Features-PCA	AUC	0.79±0.27	0.84±0.21	0.86±0.23	0.83±0.28	0.83±0.26	0.81±0.24
	Accuracy %	90.77±7.57	93.31±7.57	92.54±6.74	90.88±7.66	91.27±8.38	90.53±7.91
Feature Selection-InfoGain	AUC	0.98±0.05	0.78±0.29	0.92±0.18	0.85±0.27	0.87±0.26	0.88±0.25
	Accuracy %	95.80±5.94	93.17±7.27	93.11±7.62	92.12±7.92	93.97±7.34	95.91±6.38
	Selected Features	TAU ,GFAP, NFL, MOG					

Table E.8: Classification results of Healty Control subgroup and MS patients using proteins only, PCA Applied to protein data, using all features, PCA Applied to all features and using information gain based feature selection methods.Applied classification methods are KNN, J48(Decision Tree), Random Forest, Adaptive Boosting(AdaBoost M1), DECORATE, Bayesian Networks (BayesNet).). (10 fold cross validation, 1000 run $p<0.05$ two tailed, paired t-test (corrected))

MS vs. HC		kNN (5-NN)	J48	Random Forest	AdaBoost M1	DECORATE	BayesNet
Proteins	AUC	0.95±0.15	0.81±0.23	0.94±0.16	0.93±0.19	0.92±0.21	0.93±0.18
	Accuracy %	**97.64±4.58**	93.62±6.93	94.66±6.51	94.82±6.49	95.41±6.59	97.40±5.01
All Proteins-PCA	AUC	0.92±0.18	0.73±0.37	0.93±0.16	0.96±0.10	0.94±0.18	0.90±0.18
	Accuracy %	96.81±5.01	95.01±6.65	94.62±6.75	94.69±6.53	95.21±6.60	93.02±7.28
All Features	AUC	0.91 ±0.17	0.81 ±0.23	0.94 ±0.16	0.96 ±0.11	0.95 ±0.15	**0.96 ±0.09**
	Accuracy %	91.63 ±9.25	93.58 ±6.98	94.50 ±6.92	95.63 ±6.24	94.30 ±6.69	90.04 ±9.98
All Features-PCA	AUC	0.87±0.25	0.89±0.20	0.93±0.17	0.95±0.12	0.92±0.22	0.93±0.15
	Accuracy %	93.92±7.80	96.20±6.50	96.00±5.92	93.81±6.90	94.87±7.70	96.60±5.50
Feature Selection-InfoGain	AUC	0.95±0.15	0.81±0.23	0.94±0.16	0.96±0.12	0.95±0.12	0.96±0.09
	Accuracy %	97.53±5.14	93.58±6.99	94.45±6.76	95.80±6.14	93.68±6.89	90.02±10.11
	Selected Features	Onset Age, MS in Family, Autoimmune Disease in Self, Autoimmune Disease in Family, EDSS, Atrophy/Cortical, Atrophy/Corpus Callosum, OCB, TAU, GFAP, NFL, MOG					

Table E.9: Classification results PPMS patients and RRMS patients using proteins only, PCA Applied to protein data, using all features, PCA Applied to all features and using information gain based feature selection methods.Applied classification methods are KNN, J48(Decision Tree), Random Forest, Adaptive Boosting(AdaBoost M1), DECORATE, Bayesian Networks (BayesNet). (10 fold cross validation, 1000 run $p<0.05$ two tailed, paired t-test (corrected))

PP vs. RR		kNN (5-NN)	J48	Random Forest	AdaBoost M1	DECORATE	BayesNet
Proteins	AUC	0.95±0.11	0.86±0.21	0.92±0.15	0.90±0.16	0.93±0.17	0.93±0.15
	Accuracy %	95.91±6.85	91.90±8.78	91.38±9.16	88.88±9.58	92.37±9.07	93.72±8.26
All Proteins-PCA	AUC	0.95±0.11	0.93±0.11	0.96±0.11	0.96±0.09	0.95±0.13	**0.97±0.08**
	Accuracy %	**95.98±6.68**	93.03±8.18	93.99±8.14	95.02±7.33	93.51±8.19	95.77±6.63
All Features	AUC	0.84 ±0.18	0.85 ±0.22	0.93 ±0.14	0.95 ±0.13	0.93 ±0.15	0.94 ±0.14
	Accuracy %	80.79 ±8.47	91.12 ±9.30	91.13 ±9.23	91.52 ±9.15	90.96 ±9.30	92.60 ±8.42
All Features-PCA	AUC	0.84±0.17	0.72±0.29	0.84±0.20	0.90±0.15	0.81±0.22	0.81±0.19
	Accuracy %	84.01±8.49	84.72±10.54	85.86±10.41	85.50±10.29	82.44±12.10	83.58±11.92
Feature Selection-InfoGain	AUC	0.93±0.12	0.87±0.19	0.93±0.13	0.94±0.13	0.93±0.14	0.94±0.14
	Accuracy %	91.38±9.30	91.79±9.11	91.48±8.98	91.04±9.61	91.21±9.52	92.60±8.42
	Selected Features	Duration of MS, EDSS, CSF Glucose Level, TAU, GFAP, MOG					

Table E.10: Classification results CIS patients and CISRR patients who firsly diagnosed as CIS and became RR within 5 years using proteins only, PCA Applied to protein data, using all features, PCA Applied to all features and using information gain based feature selection methods.Applied classification methods are KNN, J48(Decision Tree), Random Forest, Adaptive Boosting(AdaBoost M1), DECORATE, Bayesian Networks (BayesNet).). (10 fold cross validation, 1000 run $p<0.05$ two tailed, paired t-test (corrected))

CIS vs. CISRR		kNN (5-NN)	J48	Random Forest	AdaBoost M1	DECORATE	BayesNet
Proteins	AUC	0.68±0.32	0.46±0.11	0.73±0.32	0.69±0.37	0.67±0.33	0.50±0.04
	Accuracy %	83.24±14.19	76.33±12.32	83.01±15.05	82.57±15.80	77.96±16.18	80.36±7.34
All Proteins-PCA	AUC	0.67±0.36	0.50±0.00	0.45±0.24	0.50±0.31	0.60±0.35	0.50±0.00
	Accuracy %	82.15±15.54	**88.21±4.04**	79.14±11.35	81.42±10.83	77.82±16.74	78.82±6.87
All Features	AUC	0.36 ±0.25	0.67 ±0.27	0.77 ±0.29	0.70 ±0.30	0.80 ±0.28	0.47 ±0.12
	Accuracy %	80.49 ±6.88	77.39 ±15.20	82.37 ±11.58	77.36 ±16.02	82.34 ±13.97	77.14 ±12.06
All Features-PCA	AUC	0.62±0.28	0.46±0.19	0.60±0.34	0.69±0.35	0.60±0.35	0.48±0.06
	Accuracy %	80.50±6.87	69.70±16.61	79.84±10.27	83.82±14.24	74.55±17.24	78.95±9.68
Feature Selection-InfoGain	AUC	0.67±0.30	0.63±0.24	0.74±0.28	0.78±0.31	**0.89±0.19**	0.49±0.09
	Accuracy %	80.05±7.70	83.22±13.20	78.82±16.50	80.92±15.73	86.45±12.62	78.91±9.02
	Selected Features	Autoimmune Disease in Family, MR/T1, OCB, CSF Protein Level					

Table E.11: Classification results of RR patients, CIS patients and CISRR patients who firsly diagnosed as CIS and became RR within 5 years using proteins only, PCA Applied to protein data, using all features, PCA Applied to all features and using information gain based feature selection methods.Applied classification methods are KNN, J48(Decision Tree), Random Forest, Adaptive Boosting(AdaBoost M1), DECORATE, Bayesian Networks (BayesNet).). (10 fold cross validation, 1000 run $p<0.05$ two tailed, paired t-test (corrected))

CIS vs. CISRR vs. RR		**kNN (5-NN)**	**J48**	**Random Forest**	**AdaBoost M1**	**DECORATE**	**BayesNet**
Proteins	**AUC**	0.81±0.14	0.68±0.17	0.74±0.16	0.72±0.13	0.75±0.16	0.73±0.12
	Accuracy %	69.87±11.61	62.42±12.38	63.63±13.13	66.25±12.00	62.83±13.00	66.81±12.19
All Proteins-PCA	**AUC**	**0.81±0.13**	0.75±0.18	0.79±0.15	0.70±0.15	0.78±0.16	0.53±0.08
	Accuracy %	68.47±11.83	67.81±13.74	64.76±12.92	64.48±12.23	65.16±13.09	56.84±8.04
All Features	**AUC**	0.65 ±0.17	0.70 ±0.16	0.80 ±0.14	0.80 ±0.14	0.79 ±0.14	0.74 ±0.14
	Accuracy %	59.81 ±13.40	63.79 ±12.36	68.72 ±12.33	66.01 ±11.85	67.62 ±12.38	66.57 ±11.65
All Features-PCA	**AUC**	0.67±0.16	0.51±0.17	0.63±0.18	0.54±0.14	0.63±0.18	0.51±0.13
	Accuracy %	63.81±12.75	51.08±13.24	58.37±12.93	54.74±9.69	54.48±14.08	53.69±8.54
Feature Selection-InfoGain	**AUC**	**0.81±0.13**	0.74±0.15	0.76±0.15	0.80±0.13	0.79±0.14	0.74±0.13
	Accuracy %	63.79±12.17	**69.87±11.59**	64.24±12.92	66.86±12.49	65.01±12.23	67.37±11.48
	Selected Features	Duration of MS , OCB, GFAP					

Table E.12: Classification results of RR patients and CISRR patients who firsly diagnosed as CIS and became RR within 5 years using proteins only, PCA Applied to protein data, using all features, PCA Applied to all features and using information gain based feature selection methods.Applied classification methods are KNN, J48(Decision Tree), Random Forest, Adaptive Boosting(AdaBoost M1), DECORATE, Bayesian Networks (BayesNet).). (10 fold cross validation, 1000 run $p<0.05$ two tailed, paired t-test (corrected))

RR v s. CISRR		kNN (5-NN)	J48	Random Forest	AdaBoost M1	DECORATE	BayesNet
Proteins	AUC	0.58±0.23	0.50±0.01	0.68±0.28	0.72±0.29	0.52±0.28	0.50±0.00
	Accuracy %	87.19±5.25	88.07±4.58	83.58±10.25	85.26±9.55	86.40±7.24	88.21±4.01
All Proteins-PCA	AUC	0.60±0.23	0.50±0.00	0.45±0.24	0.50±0.31	0.46±0.25	0.50±0.00
	Accuracy %	87.01±5.41	88.21±4.04	79.14±11.35	81.42±10.83	86.90±6.06	88.21±4.01
All Features	AUC	0.51 ±0.23	0.62 ±0.22	0.80 ±0.23	0.76 ±0.24	0.78 ±0.24	0.49 ±0.10
	Accuracy %	87.98 ±4.36	82.62 ±9.71	87.17 ±8.37	86.08 ±8.64	86.94 ±8.49	86.13 ±6.48
All Features-PCA	AUC	0.60±0.27	0.61±0.25	0.68±0.28	0.66±0.29	0.65±0.30	0.45±0.09
	Accuracy %	88.21±4.01	81.50±12.27	85.66±8.42	84.68±10.01	83.12±10.97	87.65±5.68
Feature Selection-InfoGain	AUC	**0.92±0.12**	0.71±0.24	0.88±0.19	0.89±0.16	0.88±0.16	0.49±0.10
	Accuracy %	89.77±7.00	84.51±9.37	**90.49±9.47**	89.98±9.27	87.89±9.17	86.13±6.48
	Selected Features	Duration of MS, MS in Family, Gadolinium Enhancement, CSF Protein Level					

Table E.13: Classification results of CIS patients and RR patients using proteins only, PCA Applied to protein data, using all features, PCA Applied to all features and using information gain based feature selection methods.Applied classification methods are KNN, J48(Decision Tree), Random Forest, Adaptive Boosting(AdaBoost M1), DECORATE, Bayesian Networks (BayesNet).). (10 fold cross validation, 1000 run $p<0.05$ two tailed, paired t-test (corrected))

RR v s. CIS		kNN (5-NN)	J48	Random Forest	AdaBoost M1	DECORATE	BayesNet
Proteins	Accuracy %	70.76±12.42	67.40±12.35	67.55±13.19	70.99±12.47	69.31±12.71	72.05±12.77
	AUC	0.75±0.14	0.68±0.16	0.74±0.15	0.75±0.15	0.73±0.15	0.73±0.13
All Proteins-PCA	Accuracy %	70.23±12.27	71.63±14.23	64.15±13.43	63.58±13.12	71.87±13.30	63.91±10.77
	AUC	0.76±0.14	0.67±0.17	0.71±0.15	0.66±0.16	0.72±0.16	0.62±0.12
All Features	Accuracy %	63.89 ±13.80	70.21 ±13.02	72.83 ±12.67	73.96 ±12.21	71.95 ±12.65	74.80 ±11.16
	AUC	0.67 ±0.16	0.72 ±0.16	0.80 ±0.13	0.81 ±0.13	0.78 ±0.14	0.78 ±0.13
All Features-PCA	Accuracy %	69.57±13.33	54.79±13.63	60.84±13.98	65.61±13.35	58.77±13.79	57.01±7.72
	AUC	0.72±0.15	0.53±0.16	0.64±0.16	0.69±0.16	0.59±0.17	0.48±0.05
Feature Selection-InfoGain	Accuracy %	71.28±12.59	**77.00±11.82**	72.82±12.72	72.57±12.47	75.35±12.05	75.13±11.15
	AUC	**0.80±0.13**	0.78±0.14	0.78±0.14	0.79±0.14	**0.80±0.13**	0.78±0.13
	Selected Features	Duration of MS, GFAP					

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