Heterogeneity of the immunopathology in advanced multiple sclerosis

An autopsy cohort analysis

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CHAPTER 1

Introduction and aim of thesis
**MULTIPLE SCLEROSIS**

Multiple sclerosis (MS) is the most prevalent chronic inflammatory disease of the central nervous system. It presents mostly in young adults and the mean age at diagnosis is around 30 years.\(^1,2\) Its world-wide prevalence is 30 patients per 100,000 people and between 1990 and 2016 the world-wide prevalence increased with 10.4%. There is a strong latitudinal gradient for prevalence, with a 3% relative increase for each degree increase of latitude.\(^3\) The geographical location before the onset of MS is a risk factor for susceptibility.\(^4\) These observations illustrate the importance of environmental risk factors in the development of MS.\(^5\) It has been proposed that the strong latitudinal gradient in MS prevalence can be explained by regional UVB exposure and vitamin D deficiency.\(^5–9\) The strongest environmental risk factor is infection with Epstein-Barr virus, however the mechanisms underlying this association still remain to be determined.\(^5,10,11\)

MS is a typical example of a common complex disease that results from a combination of environmental and genetic risk factors. The cause of MS and whether this varies from one patient to the other still remains elusive. The disease arises in genetically susceptible individuals, where genetic risk factors influence the penetrance of environmental factors.\(^12\) GWAS studies have identified over two hundred genetic risk loci for MS with the MHC region being the main genetic determinant for the susceptibility of MS.\(^13,14\) Most of the susceptibility associated genes are related to immunological pathways and there is notable overlap with other auto-immune diseases, suggesting common predisposing immunological processes.\(^15,16\) Pathway analysis and few functional studies have been performed for genetically associated genes to identify the related disease relevant pathways. These studies indicate central tolerance mechanisms (the negative selection of developing T and B cells in the primary lymphoid organs), peripheral differences in effector T cell function due to altered cytokine responsiveness, cytokine production and homeostatic proliferation and alterations in microglial function in the predisposition for multiple sclerosis.\(^14,17,18\) Overall there are strikingly few genetic associations shared with neurodegenerative conditions indicating that non-immunological, from a genetic perspective, primary neurodegenerative processes are less likely to contribute to the susceptibility of MS.\(^17\)

**Heterogeneity in MS clinical disease course**

The clinical presentation of MS is dependent on the location of the lesions. Patients typically present with monocular visual loss due to unilateral optic neuritis or facial sensory loss due to brainstem dysfunction, ataxia and nystagmus due to cerebellar lesions, limb weakness or sensory loss due to partial myelopathy. Clinical features that are suggestive for demyelination as a cause of these symptoms are age younger than 40, acute or subacute onset over hours or days, maximal neurological deficit within 4 weeks after onset and spontaneous remission. The diagnosis MS requires the dissemination of clinical symptoms and/or MRI lesions in time and space.\(^19\)
In general practice, three clinical disease phenotypes are distinguished; relapsing remitting, secondary progressive and primary progressive MS, these are illustrated in Figure 1. In 78% of the MS patients the disease starts with a relapsing onset, while in 22% of the MS patients the disease starts with a progressive onset. MS patients that show a progressive onset of the disease are older (38.5 years vs 29.5 years) compared to the MS patients with a relapsing onset. MS patients with a relapsing onset mostly show conversion to a secondary progressive phase, generally this conversion occurs after a median time of 16 years from onset and at a median age of 40 years. In the more recent definitions of MS, the clinical course of primary progressive MS is not considered pathophysiological distinct from relapsing forms of MS, that have entered a progressive course (SPMS). Therefore in recent years secondary and primary progressive MS subgroups are often combined and described as progressive and/or advanced MS.

Most MS patients accumulate disability over time, however the rate of disability progression is variable between patients. There is limited information regarding the predictors of the disease course. The time from first symptoms until the time a patient needed a walking aid (Expanded Disability Status Score 6) was longer in patients with a relapsing onset compared to a primary progressive onset. Differences in the rate of disability progression between MS cases have been described as either benign or malignant MS, although such a clinical classification and its definitions are controversial since MRI lesion load and cognitive decline are neglected. Benign MS has been defined as EDSS 2 or lower, which means minimal disability in only one of the functional systems, after a disease duration of more than 10 years. Benign MS cases have a higher chance to

**Figure 1. Different clinical disease courses in multiple sclerosis.**
Illustrating the development of clinical symptoms over time in the relapsing-remitting, secondary progressive and primary progressive disease course.
stay stable compared to non-benign MS patients. Malignant MS has been used when the need to walk continuously with a walking aid is reached within 5 years. Over 10% of the MS patients develop a malignant form of MS. Patients with an older age at onset, motor symptoms at onset and a progressive disease course, male gender and positive smoking history are more likely to develop malignant MS. Furthermore, patients with a motor pathway deficit at onset were less likely to have a benign MS disease course.

Sex differences in MS susceptibility and clinical course

In MS both the susceptibility and the clinical disease course shows sex differences. The global prevalence of MS in adults differs significantly by sex, with overall a higher prevalence in females compared to males, with a sex ratio of 2:1. Interestingly, in a progressive onset of MS, the male-female ratio is significantly lower compared to the relapsing onset MS patients. In MS patients with a relapsing onset males show a faster disability progression compared to females, while the disability progression is comparable between males and females in patients that show a progressive onset. Imaging studies showed that males show more destructive white matter lesions and more often cortical grey matter lesions compared to females. Altogether these observations demonstrate that males are more susceptible to develop a severe and often progressive disease course. Interestingly the incidence of multiple sclerosis in females doubled between 1950 and 2009, whereas an increase among males has been modest. This may be attributed to environmental changes affecting the behavior of females more compared to men, which include rise in obesity, increased cigarette smoking in females and changes in the frequency of breastfeeding. The hypothesis that sex steroids are also contributing to the differences between males and females is supported by the observation that MS relapses decrease during pregnancy and increase post-partum, when estrogen and progesterone levels rapidly decrease. Both sex steroids produced outside the CNS as well as “neurosteroids” produced within the CNS are potentially impacting on the MS disease activity. Especially progestogens and androgens show inhibition of demyelination and promotion of remyelination, anti-inflammatory and neuroprotective effects in models for MS. In human MS white matter lesions it has been demonstrated that there is altered expression of progesterone synthetic enzymes and their receptors; showing in females there is an increased progesterone signaling while this was not increased in males. This all suggests that sex steroids are contributing to the differences in susceptibility and clinical disease course between males and females.

Imaging and biomarkers monitoring MS disease activity

Several imaging and biomarkers have improved the monitoring of disease activity, the prediction of the clinical disease course and response to immunomodulatory therapies in MS patients. Furthermore they provide insight in disease mechanisms that contribute to the development of relapses and disability progression in MS. A role of blood brain barrier damage in the development of relapses is suggested by two meta-analysis showing that the best predictor of the effect of immunomodulatory therapies on relapses is the number of new or enlarging T2 lesions or
gadolinium enhancing lesions in the white matter. In a study of 3635 RRMS patients disability progression could be predicted by brain volume loss which is a marker for brain atrophy.\textsuperscript{39} Furthermore, imaging measures that reflect the extend of brain tissue damage in lesions and also outside lesions improve the prediction of disability progression.\textsuperscript{40,41} These observations indicate that neurodegenerative changes in the MS lesions correlate with the accumulation of disability in MS patients. Interestingly, disability progression was inversely correlated with remyelination of white matter lesions as shown by combining PET and MRI in longitudinal imaging studies.\textsuperscript{42} Therefore, besides the occurrence of focal inflammation and neurodegeneration, differences in remyelination capacity between MS patients appears to impact on disability progression.

Recently, mixed active/inactive lesions (also referred to as chronic active or smoldering lesions) were defined on MRI as non-gadolinium enhancing lesions with rims. These lesions are suspected to feature ongoing demyelination, remyelination failure and axonal degeneration. A longitudinal imaging study showed that lesions without a rim shrunk over time, while the lesions with a rim stayed stable or expanded over time. Rim lesions had a longer T\textsubscript{1} time suggesting more tissue destruction compared to lesions without rim. Finally, the presence of rim lesions associated with a more aggressive disease course with faster disability progression.\textsuperscript{43} Altogether, these observation suggest that ongoing demyelination, remyelination failure and axonal degeneration are contributing to disability progression.

Biomarkers in CSF and circulation improve the monitoring of disease activity and the prediction of disability progression. The presence of CSF-unique oligoclonal bands is a well-known feature of MS at diagnosis, with only 10\% of patients lacking this biomarker. The latter small proportion of MS patients develop more often a benign disease course.\textsuperscript{44} Recently, it was confirmed that the presence of oligoclonal bands at first symptoms is an independent predictor of disability progression.\textsuperscript{45} In radiologically isolated syndrome, where there are incidental radiological findings highly suggestive for MS in patients who are asymptomatic, the presence of oligoclonal bands and also high neurofilament light chain levels are associated with a faster progression to the clinical diagnosis MS.\textsuperscript{46} In large studies in MS patients the serum neurofilament light chain level is associated with new and enlarging lesions, contrast enhancement of lesions and brain and spinal cord volume loss on MRI, illustrating that this is an objective surrogate of ongoing disease activity.\textsuperscript{47,48} These observations illustrate that biomarkers of intrathecal immune activation and axonal loss reflect biological processes impacting on the disease course of people with MS.

The clinical disease course and disability progression is variable between MS cases. Longitudinal radiological studies show that MS patients with more inflammatory disease activity, less remyelination and more neurodegenerative changes show a faster disability progression in follow-up. However, the molecular and cellular mechanisms that underly these differences remain poorly understood. Several GWAS studies have identified genes associated with clinical disability progression and/or MRI outcome measures, but the functional pathways implicated in disability progression still remain to be determined.\textsuperscript{49–51} Since current immunomodulatory therapies only
show modest effect on disability progression a better understanding of pathological mechanisms that contribute to disability progression is warranted and the aim of this thesis.

Multiple sclerosis lesion pathology

Histologically MS is characterized by sharply demarcated focal inflammatory lesions with demyelination, variable axonal loss and gliosis in the white and grey matter. The lesions can be present in any location in the brain, including the white matter, the deep grey matter and the cortical grey matter. In the cortex the lesions are classified based on their location as subpial, intracortical and leukocortical lesions. Subpial cortical demyelination is spatially associated with meningeal T cell infiltrates, however the general inflammatory and demyelinating activity in cortical MS lesions is lower compared to the adjacent white matter. Inflammatory lesions in the white matter are dominated by T cells and activated microglia and/or macrophages. Blood brain barrier damage is classically seen in inflammatory active white matter lesions.

The formation of MS lesions is a dynamic process and the neuropathological appearance of lesions is determined by the development of myelin phagocytosis at the time of biopsy or autopsy. Since microglia/macrophages migrate slowly from the lesions, the presence or absence of microglia/macrophages provides an identification for the age of a lesion. In MS autopsy tissues different types of MS lesions can be distinguished and over the past decades a number of classification systems have been introduced. Recently, a consensus characterization of MS lesions has been described by Kuhlmann et al 2017 based on Luxol Fast Blue (LFB) histological staining and immunohistochemistry for MBP or PLP for myelin proteins, CD68 for microglia/macrophage activity, and CD3 for the presence of T cells. In this classification system active, mixed active/inactive (mixed) and inactive lesions are distinguished in the white matter. Active lesions are hypercellular, characterized by infiltration of CD68⁺ cells most of them with a foamy morphology and a loss of myelin. T cells are localized perivascular, but they are also encountered throughout the lesion in the brain parenchyma. It has been suggested that the presence of foamy microglia/macrophages in MS lesions indicates that they are formed within days or weeks before pathological analysis, however their presence is not a measure of ongoing myelin destruction since they do not always show myelin degradation products inside. Demyelinating and post-demyelinating lesions can be distinguished based on the MBP, PLP or Luxol fast blue positive myelin degradation products inside the microglia/macrophages. Active demyelinating lesions show microglia/macrophages that show myelin degradation products inside while the active and post-demyelinating lesions are infiltrated with foamy lipid containing microglia/macrophages lacking LFB or other myelin degradation products.

In contrast to the active lesions, mixed lesions are characterized by a hypocellular lesion center and a rim of activated microglia/macrophages at the lesion border. The center of the lesion is depleted of microglia/macrophages. To classify it as a mixed lesion, the rim of active microglia/macrophages does not need to surround the entire lesion. In these active and mixed lesions
Moderate T cell infiltrates are present perivascular and also in the brain parenchyma. In the mixed lesions, the same subdivision into demyelinating and post-demyelinating is made as in active lesions, based on myelin degradation products inside the microglia/macrophages. Frequently, the mixed lesions show a narrow rim of microglia/macrophages containing MBP or PLP degradation products, reflecting ongoing demyelination, these lesions have been called slowly-expanding or smoldering lesions.52

Inactive lesions are sharply demarcated, gliotic and hypocellular, only few T cells and microglia/macrophages are present, and axonal loss is evident in these lesions.

Remyelination in MS lesions has been proven at the ultra-structural level.60,61 MS lesions can be partly or completely remyelinated, and using immunohistochemical myelin staining, remyelinated axons can be identified having thinner myelin sheaths resulting in a paler myelin staining intensity. Remyelination is present in all lesion types, and also the active and demyelinating lesions contain remyelinated lesion areas. Inactive shadow plaques can be distinguished that show extensive remyelination with only few microglia/macrophages. The extent of remyelinated shadow plaques has been shown to be heterogenous between MS patients, where the presence of more shadow plaques is related to a longer disease duration, suggesting that potentially genetic and environmental factors may influence the ability to remyelinate in MS patients.52,62 A schematic overview of the stages of MS lesions that are distinguished in the Kuhlmann et al. 2017 consensus are shown in Figure 2 together with the staging system used by the NBB.

Characterization of MS lesions in the NHB MS cohort

At the Netherlands Brain Bank (NBB) all MS tissue samples have been histologically characterized over the past few decades. From MS autopsy cases at NBB tissue is dissected from standardized locations from the brainstem and the spinal cord, and macroscopically visible MS plaques (PLA) from white and grey matter are dissected. In addition, since 2001, MS lesions are dissected on post-mortem MRI guidance (MRI) on 1 cm thick coronal brain slices.63 The characterization of MS lesions at NBB uses PLP immunohistochemistry for myelin and HLA-DR immunohistochemistry for microglia/macrophages. The characterization of MS lesions is comparable to the Kuhlmann consensus, except that in active and mixed lesions the NBB does not distinguish demyelinating and post-demyelinating but uses the morphological appearance of microglia/macrophages to characterize the microglial/macrophage activity. Active lesions are characterized by a demarcated area of partial or paler PLP staining and HLA-DR+ microglia/macrophages that are identified throughout the lesion area. The mixed lesions contain a completely demyelinated and hypocellular center, with a hypercellular rim of HLA-DR+ cells. The morphology of the microglia/macrophages in both active and mixed lesions were classified as either ramified (suggestive for a resting, trophic state), rounded (suggestive for activation, or infiltration of peripheral macrophages) or foamy (suggestive for demyelination).64 Inactive lesions were sharply demarcated demyelinated areas that are hypocellular, with little HLA-DR+ cells. The inactive shadow plaques show paler PLP
staining compared to the normal appearing white matter with little HLA-DR⁺ cells, suggestive for a remyelinated area. Demyelinating and post-demyelinating lesions were not distinguished based on myelin degradation products inside microglia/macrophages, however the presence of foamy microglia/macrophages is suggestive for relatively recent demyelination. The mixed lesions, the inactive lesions and most of the active lesions are comparable in the NBB characterization and the Kuhlmann consensus characterization. However, the subset of active lesions with a ramified microglia/macrophage morphology at the NBB would not be considered as active lesions in the Kuhlmann consensus since the microglia throughout these lesions are not considered activated. These lesions are rather considered as (active) remyelinated areas since they show a paler myelin

Figure 2. Histopathological staging of multiple sclerosis lesions.
In active and mixed lesions the Kuhlmann et al. consensus distinguishes demyelinating and post-demyelinating based on the myelin degradation products inside microglia/macrophages, while the NBB uses microglia/macrophage morphology to characterize the microglia/macrophage activity. Image from Kuhlmann et al. consensus derived with permission of Kuhlmann et al. (2017) Acta Neuropathologica.
staining and lack evident ongoing demyelination. The stages of MS lesions that are distinguished by the NBB are included in Figure 2.

At the NBB it has since long been appreciated that the characteristics of MS lesions in the post-mortem MS brain are relatively consistent within a brain donor, while there are clear differences in for example the number of active lesions or the number of inactive remyelinated shadow plaques between MS brain donors.65,66 These differences between donors may represent the dynamic process of MS lesion formation and is potentially related to the clinical disease stage at time of autopsy. However, they might also reflect differences in genetic or environmental factors that influence the inflammatory lesion activity or the capacity to remyelinate in subsets of MS patients.

The immunopathology of early MS

In biopsy samples of early MS or in autopsy cases of acute MS with a very rapid disease course (died within 1 years), the dominant MS lesion type is the active and demyelinating white matter lesion.58 In these early MS biopsy lesions heterogeneity in immunoglobulin and complement depositions or oligodendrocyte apoptosis have been described.67 Therefore, it has been considered that these early MS lesions may arise from different etiologies. Initially four, but more recently three different immunopathological patterns of MS biopsy lesions have been proposed.1,67 Pattern I lesions show demyelination and the presence of activated microglia/macrophages and T cells. Pattern II lesions show next to activated microglia/macrophages and T cells also complement activation and antibody depositions, suggesting more involvement of the humoral immune response. Finally, pattern III lesions are characterized by the presence of oligodendrocytes with nuclear condensation and fragmentation, resembling apoptotic cell death. This is associated with a selective loss of MAG, a myelin antigen that is located in the most distal (peri-axonal) oligodendrocyte processes. Since in each patient only 1 pattern of MS lesions was identified, this suggests that different pathological mechanisms may lead to demyelination.68 However, whether these different patterns represent different etiologies or simply reflect temporal stages of MS lesion development remains to be determined. Furthermore, it needs to be considered that the cases used for the identification of the lesion patterns do not have a typical clinical presentation of MS, otherwise a brain biopsy would not have been performed. Finally, some of the antibodies required to identify these different patterns are not commercially available, therefore the identification of patterns is not considered within the scope of neuropathological analysis at the NBB and in the Kuhlmann et al. consensus.52 The characterization of the different patterns is illustrated in Figure 3, derived from Reich et al.1

Interestingly, it has been shown that cases with pattern II MS lesions did show a better effect on plasma-exchange compared to the patients with pattern I and pattern III MS lesions.69 Which illustrates that the potential differences in the involvement of the humoral immune response between MS patients, has potential clinical and therapeutic implications. Also in post-mortem autopsy lesions differences in the presence of IgG deposits have been described, where both the presence and absence of IgG deposits in MS lesions has been described.70,71 Whether this
represents different etiologies or donor specific differences in immune response due to genetic or environmental factors remains to be determined.

The immunopathology of advanced MS

The pathology of secondary progressive and primary progressive MS in autopsy cases is different compared to the early MS lesion pathology in biopsy lesions, since active demyelinating lesions with disruption of the blood brain barrier are less frequent. However in advanced MS substantial numbers of the lesions show inflammatory activity at their margin composed of T cells and microglial cells. As described previously the microglial cells in the lesion margins contain myelin degradation products and are therefore suggestive for a slow rate of ongoing demyelination in the progressive stages of the disease. It has been shown that these mixed active/inactive lesions occur more often in the progressive cases compared to the early and acute MS cases. In progressive MS also the normal appearing white matter is abnormal, where there is diffuse microglial activation which is suspected to be associated with diffuse axonal injury and destruction.
There are several pathological studies that describe the infiltration of T-cells, B cells and plasma cells in autopsy tissue derived from advanced MS patients.\textsuperscript{74–79} Which suggests these cells potentially contribute to MS lesion progression, also in the advanced stages of the disease. Lymphocytes in advanced MS lesions are mostly found in the perivascular space and meninges,\textsuperscript{52,57,80} which are since decades two compartments that are considered connected with each other and since a few years also to the meningeal lymphatic vessels.\textsuperscript{81–86} These compartments are considered relevant for the immune cell reactivation potentially driven by CNS derived antigens.\textsuperscript{86} This is illustrated in Figure 4 derived from Esiri et al. 1990.\textsuperscript{86}

![Diagram of neuroinflammation in the peri-vascular Virchow Robin space and meninges.](image-url)

\textbf{Figure 4. Neuroinflammation in the peri-vascular Virchow Robin space and meninges.}
A. Illustration of the relationship of the Virchow-Robin space to the subpial and subarachnoid space.
B. Diagram illustrating the view put forward that the Virchow-Robin space is an immunological space that can become expanded and filled with immune competent cells interacting together under conditions of immune stimulation in the brain. Image derived with permission of Esiri et al. (1990) \textit{Journal of Neurological Sciences}.

Interestingly, due to the ineffectiveness of current immunomodulatory therapies which target lymphocytes outside the CNS to reduce progression of disease, a large role for neurodegenerative mechanisms has been advocated in progressive MS. Although cortical demyelination has been
repeatedly related to the presence of meningeal infiltrates by several research groups, the inflammatory activity in cortical grey matter lesions is limited compared to the adjacent white matter. This raised the hypothesis that neurodegeneration in progressive MS is potentially independent of the inflammatory response.

However, pathological studies in progressive MS show that in line with the early phases of the disease, neurodegeneration occurs on the background of inflammation consisting of microglia, T cells and B cells. And both primary and secondary progressive MS shows substantial lymphocytic infiltrates. Figure 5 shows the hypercellular rims and the perivascular infiltrates that are considered characteristic for secondary progressive MS, derived from Revesz et al 1994.

Figure 5. Lymphocyte involvement in progressive MS pathology. H&E staining showing hypercellular lesion rim (C,D) and perivascular lymphocytic infiltrates (E-G). Image derived with permission of Revesz et al. (1994) Brain.

All together these observations illustrate that neuroinflammation in advanced MS lesions, comprising both the innate and adaptive immune response, is different from ‘neuroinflammation’ as described in classical neurodegenerative diseases as Alzheimer’s or Parkinson’s disease. Neuroinflammation has been classically defined as immune-mediated pathology in the central nervous system (CNS). Classically neuroinflammation in the CNS is similar to that seen in
other organs and it shows the same tissue characteristics;\textsuperscript{92} the elevation in pro-inflammatory cytokines and chemokines, activation of macrophages, recruitment of leukocytes and local tissue damage.\textsuperscript{93} In the CNS this is seen in MS and also acute and chronic infections, stroke and trauma. However the changes observed in Alzheimer’s and Parkinson’s disease comprise complement and microglial activation without the recruitment of leukocytes and therefore do not equal the classical definition of neuroinflammation.\textsuperscript{94} Because it has been shown that microglial cells can detect, process and respond to signals also in a non-inflammatory way.\textsuperscript{92,95} This non-inflammatory type of microglia activation seen in neurodegenerative diseases is different from the microglial activation in advanced MS lesions, where they show a foamy morphology and contribute to the ongoing demyelination. Furthermore the activated microglial cells in the advanced MS lesions are accompanied by pronounced activation of T cells and to lesser extend B cells.\textsuperscript{52,94} The pathological phenomena of immune cell infiltrates in the meninges in the advanced stages of MS also mimic the pathology of chronic auto-immune diseases in other organs, for example Sjogren’s disease and Rheumatoid Arthritis.\textsuperscript{96} This all corroborates the idea that advanced MS lesion pathology is immune mediated and differs principally from ‘neuroinflammation’ as it occurs in classical neurodegenerative diseases.

Although advanced MS can be considered an immune mediated disease with involvement of T and B cells there is surprisingly limited data available on T and B cells in the MS white matter lesions from MS autopsy cases. Quantifications of T cells in limited numbers of MS autopsy cases showed that they are slightly increased in active and mixed lesions compared to the inactive lesions,\textsuperscript{74–77} suggesting that the inflammatory and demyelinating activity in the MS lesions in the progressive phase is related to the presence of T cells. However, the characteristics of these T cells and to what extend also B cells and plasma cells contribute to the immune response in MS lesions remains to be further characterized in the advanced stage of MS. Furthermore, the heterogeneity in inflammatory activity and lymphocytes infiltration between MS autopsy cases and how this correlates with the clinical disease severity remains to be analyzed in a clinically and pathologically well-characterized MS autopsy cohort.

Clinically and pathologically well-characterized MS autopsy cohorts with substantial numbers of advanced MS cases with a progressive disease course are very limited available world-wide.\textsuperscript{72–97} Possibly the fact that progressive MS patients often die at home or in nursery homes, explains why relatively few progressive MS cases are presented to the neuropathologists in the hospital. At the NBB MS patients register as brain donor during life. Over the past 30 years almost 200 MS brain donors with a neuropathologically confirmed diagnosis of MS came to autopsy at the NBB. Extensive clinical information of the donors was collected retrospectively. Over the past ten years the MS lesions of the MS tissue collection at the NBB have been systematically characterized. This allows the study of the heterogeneity of the immunopathology of MS in relation to clinical characteristics in an autopsy cohort of advanced MS cases.
AIM OF THESIS

The aim of the thesis is to characterize the heterogeneity of the immunopathology in advanced MS in an autopsy cohort and identify mechanisms that contribute to the heterogeneity and sex differences in the clinical course of MS.

In Part 1 – entitled substantial inflammatory lesion activity in advanced MS – we aim to characterize the immune cells that are involved in advanced MS lesion pathology in autopsy tissue and their correlation with the clinical disease course and sex.

In Chapter 2 we ask the question whether neurodegenerative changes in progressive MS brainstem lesions relate to the innate and adaptive immune response. We used post-mortem MS brainstem tissue where we first staged lesions based on demyelination – detected with the proteolipid protein (PLP) marker of myelin – and microglia/macrophage accumulation, distribution and morphology – detected with the human leukocyte antigen (HLA-DR) marker of myeloid cells. Across all lesion stages in the MS brainstem we analyzed neurodegenerative changes including metabolic stress (mtHSP70), axonal transection/impaired transport (SMI312, APP) and synaptic alterations (synaptophysin) as well as the localization and extent of deposits of early (C1q, C3d) and terminal (MAC) complement factors, and the localization and density of T (CD3+, CD4+, CD8+) and B (CD20+, CD138+) lymphocytes. Findings in the MS brainstem were compared to brainstem tissue of non-neurological controls and controls with other neurodegenerative diseases.

In Chapter 3 we ask the question what are the pathological correlates of MS clinical disease course and sex in the autopsy cohort of the Netherlands Brain Bank, containing 182 MS brain donors. Using the standardized autopsy procedures from the Netherlands Brain Bank including systematic dissection from standard location, 3188 tissue blocks containing 7562 MS lesions were dissected. Based on previously proposed criteria, MS lesions in white matter, cortical grey matter and deep grey matter were categorized. Lesion demyelinating and innate inflammatory activity were visualized by immunohistochemistry for proteolipid protein (PLP) and human leukocyte antigen (HLA-DR). Lesions in the white matter and deep grey matter were classified into active, mixed active/inactive, inactive or remyelinated, while microglia/macrophage morphology was classified as ramified, amoeboid or foamy. Lesions in the cortical grey matter were characterized as leukocortical, intracortical or subpial based on their location. Lesion load, lesion type prevalence and microglia/macrophage morphology were analyzed in relation to clinical course, disease severity and sex, and in relation to each other.

In Chapter 4 we ask the question whether T cells that were previously identified in brain autopsy tissue represent a brain specific population of tissue resident memory T cells (T_RM). We performed flow-cytometric phenotyping of human T cells isolated from the post-mortem brain tissue. We analyze the expression profiles of molecules associated with cellular differentiation, migration,
effector functions, and transcriptional control in these cells as well as cytokine profiles after stimulation. We analyzed the existence and characteristics of two CD69^+ subsets distinguished by the surface presence of CD103. Furthermore we explore the characteristics of the lesser abundant brain CD4^+ T cell fraction and analyze whether these also are enriched for T_Rm cell associated surface markers.

In Chapter 5 we ask the question whether T cells are involved in the ongoing inflammatory lesion activity in advanced MS autopsy cases and whether they show a T_Rm cell phenotype. We used a combination of immunohistochemistry and flow cytometry to study localization, quantity, and phenotypic profile of T cells in control white matter, MS normal-appearing white matter and MS white matter lesions. We quantified T cells and perivascular T-cell cuffing at a standardly dissected location in 146 MS, 10 neurodegenerative control and 20 non-neurological control brain donors. In addition, we quantified CD3^+, CD4^+, and CD8^+ T cells in 141 subcortical white matter lesions. The location of CD8^+ cells, either in the perivascular space or in the brain parenchyma was determined using CD8/laminin staining and confocal imaging. We compared early MS biopsy and late MS autopsy lesions for the presence of CD103^- and S1P1^- T cells. Finally, we analyzed CD8^+ T cells, isolated from fresh autopsy tissues from subcortical MS white matter lesions (n=8), MS normal-appearing white matter (n=7), and control white matter (n=10), by flow cytometry. The CD8^+ T cells were phenotyped for CD69, CD103, CD44, CD49a, CXCR6, PD-1, GPR56, Ki67, and granzyme B.

In Part 2 – entitled heterogeneity of the immunopathology in advanced multiple sclerosis – we aim to identify pathophysiological mechanisms that contribute to the heterogeneity and sex differences in the immunopathology and clinical disease course of MS.

In Chapter 6 we ask the question whether differences in the presence of B cells and plasma cells in MS are correlated with clinical and pathological characteristics, the IgG ratio and presence of OCBs in the cerebrospinal fluid (CSF). Autopsy tissue from 140 MS and 24 non-neurological controls and early MS biopsy lesions from 24 MS patients were stained for CD20^+ and CD138^+ to detect B cells and CD138^+ plasma cells, respectively. The presence of B cells and CD138^+ plasma cells in white matter lesions and in the standardly dissected brainstem was correlated with pathological and clinical donor characteristics. In corresponding CSF and plasma, immunoglobulin (Ig)G ratio and oligoclonal band patterns were determined. Additionally, to determine whether oligoclonal bands could disappear over time in advanced MS the presence of oligoclonal bands was determined in a clinical cohort of 73 patients, at diagnosis and during follow-up.

In Chapter 7 we ask the question whether altered progesterone and androgen synthesis in the normal appearing cortical grey matter of males and females contributes to the increased susceptibility of males for the development of cortical grey matter lesions. In the standardly dissected superior temporal gyrus from 40 MS (20F/20M) and 35 non-neurological controls (20F/15M) cortical grey matter lesions were characterized. We analyzed if there were sex differences in gene expression of the progestogen and androgen synthetic enzymes and the progesterone receptor in normal
appearing cortical grey matter. Secondly this was correlated with neuroprotective and anti-inflammatory effects by measuring gene expression of GABA/Glutamate synthesis and re-uptake, the neuroprotective protein BDNF, the anti-inflammatory cytokines and the cytotoxic T cell response.

In Chapter 8 we ask the question whether genotype correlates with MS lesion characteristics in autopsy tissue. We genotyped 179 MS brain donors from the Netherlands Brain Bank MS autopsy cohort for 102 SNPs, selected based on their reported associations with clinical outcome or their associations with genes that show differential gene expression in MS lesions. In order to link genotype to pathological parameters we analyzed the correlation of allelic distributions for each SNP with the proportion of lesion subtypes that was scored for each donor. Total lesion load, reactive site load, presence of cortical grey matter lesions and the proportions of lesion subtypes, either active, mixed active/inactive, inactive or remyelinated were tested for correlation with genotype for each SNP. For the SNPs that showed significant association after multiple-testing correction the effect on gene expression levels in brain autopsy tissue was analyzed using eQTL database validated by qPCR from MS brain autopsy tissue. An immunohistochemical examination of MS lesions and flow-cytometric analysis of T cells derived from blood and brain was performed for the related proteins.
REFERENCES


