Childhood onset MS and MS during Pregnancy

Rinze Frederik Neuteboom

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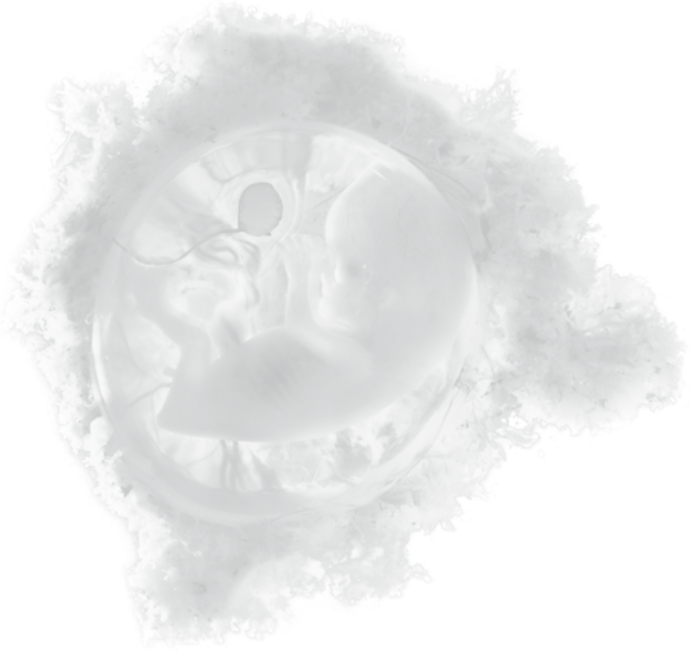
TABLE OF CONTENTS

CHAPTER 1

GENERAL INTRODUCTION



Special situations in MS

Multiple sclerosis (MS) is a chronic, incurable disease. Currently, treatments offered to patients with MS do not substantially prevent progressive disability. The Holy Grail in MS research is to develop a definitive cure for MS or at least a method for halting disease progression. Greater knowledge about the etiology and pathogenesis is required to be able to move forward towards a better treatment.

Central to this thesis is the concept that through study of natural disease course and natural modifiers, crucial lessons can be learned about etiology, pathogenesis, prognosis and perhaps ultimately new treatment options in MS. This thesis will focus on two special and different situations in MS: pregnancy and MS, and childhood onset of MS.

Pregnancy strongly influences disease course in MS. The number of relapses in MS is decreased during pregnancy. However, during the first three months after delivery the number of relapses increases. Alterations in the immune system during pregnancy, essential for a successful pregnancy, likely cause this disease amelioration. Studying clinical features and immune functions during and after pregnancy in MS can provide insight into the pathogenesis of MS in general. It can also prove to be of clinical benefit to women with MS that are pregnant or have a childwish, in supplying both patients and doctors with more detailed information about disease course around pregnancy. Furthermore it may reveal possible biomarkers for predicting postpartum relapse.

In a small minority of patients clinical debut of MS occurs in childhood. Diagnosis of MS in children can be difficult, because of the many diseases that can mimic MS in childhood. More information about natural disease course could therefore help to improve diagnosis and management of MS in these children. Furthermore, MS in children allows better study of potential risk factors involved in pathogenesis, that are present early in life, than is possible in patients with an adult onset.

The subsequent parts of this introduction will discuss respectively in more detail: the general aspects of MS, pregnancy in MS, and childhood onset of MS. This chapter will conclude with the scope and outline of this thesis.

I. GENERAL ASPECTS OF MULTIPLE SCLEROSIS

MS is a chronic inflammatory disease affecting the central nervous system (CNS).[1] It is the most common chronic neurological disease in young adults. MS affects all regions of the CNS, including the optic nerve. Patients can have a broad spectrum of signs and symptoms, including problems with ambulation, visual and sensory disturbances, fatigue and cognitive disorders.

Epidemiology

MS has a life time prevalence of 1 per 1000 individuals in the western world.[1] Women are more often affected than men with a male to female ratio of 1:3.[2-3] This gender difference in incidence becomes apparent after puberty. Before puberty the male to female ratio is 1:1.[4] Age of onset follows a unimodal distribution with a peak between the ages of 20 to 40 years.[5] For women the onset of MS thereby falls in the childbearing years. MS can also have its onset in childhood. In up to 5.0% of the total MS population, onset of MS is before the age of 16 years and in less than 0.6% onset is before the age of ten years.[3,6-9]

Disease course

Most patients (80-90%) experience a relapsing remitting (RR) disease course (Figure 1). A minority (10-20%) of the adult patients exhibit a primary progressive (PP) disease course. PP-MS in children is very rare.[1,9-11] RR-MS is characterized by exacerbations followed by a variable extent of improvement. A majority (65%) of these patients with RR-MS eventually develop a secondary progressive disease course, in which the disease progresses slowly and exacerbations tend to be less frequent.[1] Patients with a childhood onset take years longer to develop irreversible disabilities, but do so at a younger age than adult onset MS (Figure 2).[9-11]

Diagnosis of MS

A golden standard for the diagnosis of MS is lacking. Instead, the diagnosis of MS relies on a combination of clinical and/or paraclinical parameters. Widely accepted criteria for the diagnosis are the revised McDonald criteria.[12] The McDonald criteria allow for an early and reliable diagnosis. Essential for the diagnosis MS is dissemination in time and in space. When clinical features fail to provide evidence of dissemination in time and space, paraclinical features can be used to fulfil diagnostic criteria, such as magnetic resonance imaging (MRI), visual evoked potentials or presence of intrathecal (oligoclonal) antibody production.

When clinical criteria for dissemination in space or in time cannot be fulfilled, the Barkhof MRI criteria for dissemination in space or the Barkhof MRI criteria for dissemination in time can be used (Table 1). The specific criteria for the diagnosis PP-MS are depicted in Table 2.[12]

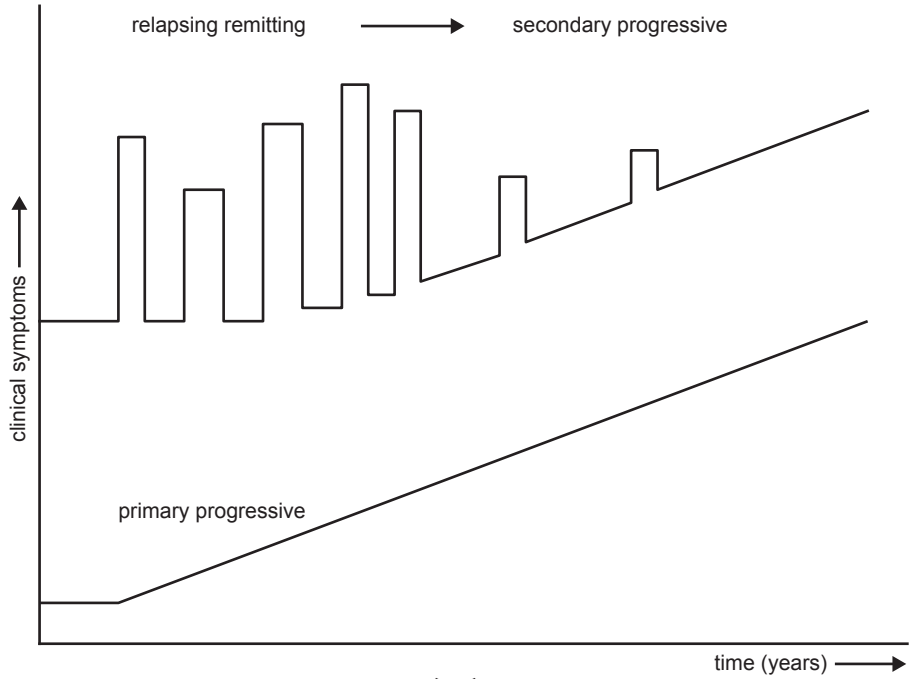


Figure 1. Disease course in multiple sclerosis (MS)
The majority of MS patients (80-90%) start with a relapsing-remitting (RR) disease course, during which they suffer alternating periods of clinical symptoms followed by (partial) recovery. Around 60-70% of these RR patients eventually develop a secondary progressive disease course, characterized by a slow and gradual progression of clinical symptoms. A minority (10-20%) of the adult patients exhibit a primary progressive (PP), characterized by a slow and gradual progression of clinical symptoms without an RR phase.

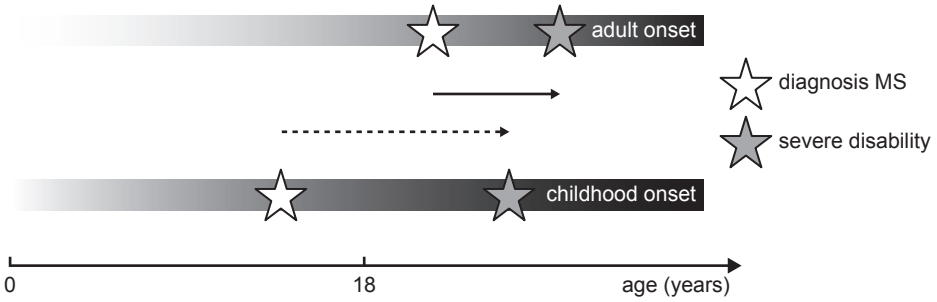


Figure 2. Clinical course differs in childhood and adult onset in multiple sclerosis (MS)
Time from diagnosis to severe disability is longer in patients with childhood onset MS (dotted line) than in adult onset MS (fixed line). Nevertheless, patients with childhood onset MS reach severe disability at a younger age.

Table 1. Barkhof MRI criteria for diagnosing MS[12]

Barkhof criteria for dissemination in place (at least 3 out of 4)	Barkhof criteria for dissemination in time (at least 1 out of 2)
≥1 gadolinium-enhancing lesion or ≥9 T2 lesions *	Gadolinium enhancement at ≥3 months after onset of the initial clinical event, if not at the site corresponding to the initial event.
≥1 infratentorial lesion **	Detection of a new T2 lesion if it appears at any time compared with a reference scan done at least 30 days after the onset of the initial clinical event.
≥1 juxtacortical lesion	
≥1periventricular lesion	

* An enhancing spinal cord lesion is considered to be equivalent to an enhancing brain lesion, and individual spinal cord lesions can contribute together with individual brain lesions to reach the required number of T2 lesions.
** A spinal cord lesion can be considered equivalent to a brain infratentorial lesion.
MS: Multiple sclerosis

Table 2. McDonald criteria for the diagnosis primary progressive multiple sclerosis[12]

1	One year of disease progression (prospectively or retrospectively determined)	
2	Plus two out of these three criteria	
	a	Positive brain MRI (≥9 T2 lesions or ≥4 T2 lesions with positive VEP)
	b	Positive myelum MRI (≥2 focal T2 lesions)
	c	Positive CSF (increased IgG-index or isoelectric focussing evidence of oligoclonal IgG bands (or both)

VEP: visual evoked potential, CSF: cerebrospinal fluid

Pathogenesis of MS: focus on immunology

The exact cause of MS is unknown. MS is presumed to be the effect of an unfortunate interplay between environmental exposure and genetic susceptibility.[1]

MS is considered to be an inflammatory disease of the central nervous system (CNS).[1,13] Active lesions in MS are characterized by disruption of the blood brain barrier (BBB), local edema and demyelination.[14] Macrophages containing myelin associated proteins and T cells and are found surrounding damaged myelin sheaths.[14] Perivascular radial demyelination with sharp borders and axonal injury is observed .[14-15] In the chronic phase of the lesion no active demyelination is observed, but instead a process of gliosis and reduced axonal density is found.[14]

Natural killer (NK) cells and antigen presenting cells (APC), like monocytes, macrophages, and dendritic cells of the innate arm of the immune system present autoantigens to T cells of the adaptive arm of the immune system (Figure 3)**.** Auto reactive lymphocytes migrate through the disrupted BBB. Disease activity is mediated by interferon γ (IFN-γ) secreting CD4+ Thelper 1 (Th1) cells. Recently, an important pro-inflammatory T cell subset of

interleukin (IL)-17 producing T helper cells (Th17) has been implicated in MS disease activity, controlled by IL-1, IL-6, IL-18, IL-23 and possibly IL-21.[13,116-17] This Th17 subset is involved in BBB disruption and brain inflammation.[18] The immunosuppressive regulatory T cells (Treg) form another important T cell subset in MS. Treg play an important role in the maintenance of self-tolerance in autoimmune animal models and are controlled by transforming growth factor β (TGF-β).[13,19] Defects in regulatory T cell function have been described in MS.[20-22]

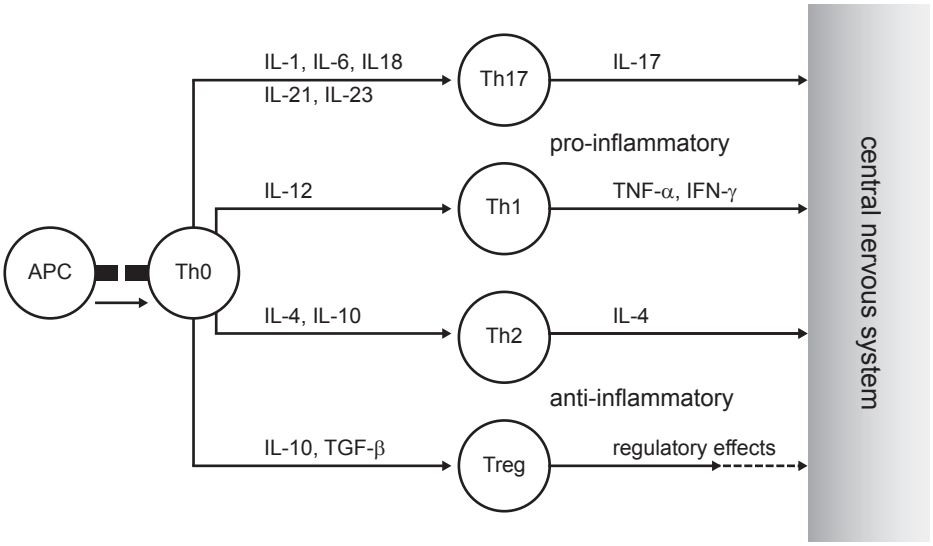


Figure 3. T cell differentiation in multiple sclerosis (MS)
Antigen presenting cells (APC) present autoantigens to naïve CD4+ T cells (Th0). The most relevant cytokines are depicted for differentiation towards T helper (Th)1, Th2, Th17 cells and regulatory T cells (Treg). Disease activity in multiple sclerosis (MS) is probably driven by pro-inflammatory Th1 and Th17 cells. Th2 cells and Treg likely protect against MS.

II. PREGNANCY AS A NATURAL MODIFIER OF MS

Pregnancy is the only known natural modifier of disease course in MS.[1,23] During the last trimester a striking reduction of 70% in relapse frequency is reported, whereas interferon beta therapy results only in a 30-35% reduction of the relapse rate.[1] Understanding the underlying biological mechanisms of this powerful natural modifier of MS will give better understanding of pathogenesis and possibly treatment of MS.

A. Clinical features of MS and pregnancy

Influence of pregnancy on MS disease course

MS presentation in women happens often in the childbearing years.[1] Onset risk of MS is reduced during pregnancy.[24] Pregnancy has an ameliorating effect on clinical disease activity, which is schematically shown in Figure 4. During the third trimester, at a group level, the

annualized relapse rate decreases with 70%, compared to the year preceding pregnancy. During this third trimester only one in every twenty women will have a relapse. However, in the first three months after delivery disease activity is known to rebound, resulting in a relapse in one in every three women with MS.[23,25-27] A year after delivery the relapse rate is normalized and comparable with the year preceding pregnancy.[23,25]

Illustrative of decreased disease activity is the reduced number of lesions on MRI during pregnancy as was reported by a case report showing MRI data of two pregnant patients that were included in a sequential MRI study.[28] After delivery an increase in the number of new or enlarging MRI lesions was observed.[29]

The numbers of relapses in the year preceding pregnancy and during pregnancy in addition to the duration of disease were unfavourable predictors of postpartum relapse, although with poor sensitivity and specificity.[25] A recent study showed that exclusive breastfeeding was protective against postpartum relapse.[30] Yet, many other studies failed to find an association between breastfeeding and postpartum relapse.[23,25-26,31-32] We can conclude that good clinical predictors of postpartum relapse are lacking. Instead of clinical predictors, biomarkers could be an alternative for predicting postpartum relapse. Currently, no biomarkers have been identified that are able to reliably predict postpartum relapse.

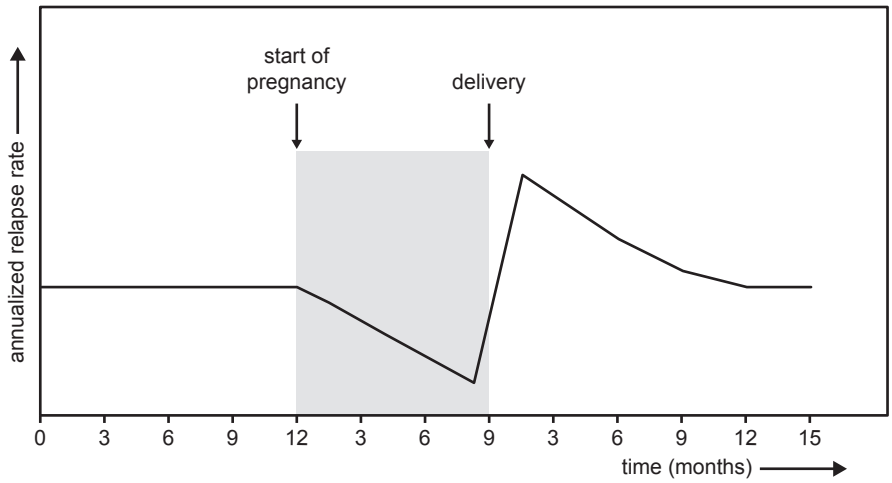


Figure 4. Relapse rate before, during and after pregnancy
During pregnancy, an evident drop in relapse rate is observed during the last three months of pregnancy, whereas in the first three months after delivery the relapse rate rebounds and overshoots. One year after delivery, the relapse rate is comparable with the year preceding pregnancy.

Influence of MS on pregnancy and pregnancy outcome

No major adverse effects in birth outcome have been reported in mothers with MS.[27,33-34] Neonates being born to mothers with MS were more frequently small for their gestational age (13%), in comparison to neonates being born to healthy mothers (11%).[33] This was not

confirmed in another cohort.[27] Women with MS have a slightly higher percentage (26-32%) of medically assisted deliveries (forceps or vacuum-assisted delivery or caesarean section), compared to healthy mothers (20-23%).[27,33] These data may be partially biased because the MS mothers in these studies were three years (mean) older than the healthy mothers.

Pregnancy in systemic lupus erythematosus and rheumatoid arthritis.
In other autoimmune disorders such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA), disease course is also altered during pregnancy. SLE is a systemic autoimmune disease affecting mostly women in their childbearing years. Multiple studies on disease activity have been published and were reviewed recently.[35-36] Large differences between studies on disease activity during pregnancy were observed. These conflicting results could mostly be explained by different disease definitions and definitions of a SLE flare. An increase of SLE activity during pregnancy was found in most studies. In RA, a majority of patients (65-90%) experience an improvement during pregnancy.[35-36] After delivery, an increase of disease activity was observed in approximately 80-90% of RA patients.[36]

B. Pregnancy-induced tolerance: a link to MS amelioration during pregnancy?
Because the fetus expresses paternal antigens, it is considered to be semi-allogenic. Rejection of these paternal antigens, expressed by trophoblast cells in early implantation and pregnancy, is inhibited.[37-38] A well known proposition is that the adaptive arm of the immune system is skewed during pregnancy from a pro-inflammatory phenotype towards an anti-inflammatory phenotype.[37-40] Compensating for the decreased adaptive pro-inflammatory immune functions, activity of innate immune functions increases, allowing both a successful pregnancy and defence against infections during pregnancy.[38,41-43]

As pregnancy induces altered immunological activity and MS and other autoimmune diseases display an altered disease course during pregnancy, it is logical to study changes in the innate and adaptive arms of immune system during pregnancy in MS.

C. Biology of normal pregnancy
In this part I will respectively discuss the immunological changes in the uterus, the changes of the adaptive arm of the immune system and finally the changes in the innate arm of the immune system.

Normal pregnancy: The uterus as an immune privileged site
The semi-allogenic fetus is allowed to grow inside the uterus. This tolerance against paternal antigens is not systemic, but limited to the uterus. Therefore, this pregnancy-induced tolerance is not a case of central immunological tolerance.[44] In human pregnancy, fetal trophoblast cells invade the decidua and replace the endothelium of the uterine spiral arteries, thus ensuring adequate blood supply. The interface between the fetal placenta and the maternal decidua is one of the supposed sites of this regulation of tolerance and is shown in Figure 5. This implantation side is infiltrated with maternal leucocytes. Most of

these cells are NK cells, next to monocytes and T cells.[38] A second interface between the fetal semi-allograft and the maternal immune system is the syncytiotrophoblast, that forms the villous surface of the haemochorial placenta.[45-46] Syncytiotrophoblast cells do not express detectable levels of major histocompatiblity complex (MHC) class I and class II molecules. Trophoblast cells do not express MHC class II molecules, which can be recognized by CD4+ T cells. Also the classical MHC class I molecules human leucocyte antigens (HLA)-A, HLA-B and HLA-D are not expressed by trophoblast cells. This lack of classical MHC class molecules helps limiting maternal T cell awareness of the semi-allogenic fetus. Trophoblast cells do express the MHC class I molecules HLA-C, HLA-E and HLA-G, which interact with the uterine NK cells.[45,47] HLA-E has a role in inhibition of NK cytotoxicity.[48] Interestingly, the killer cell immunoglobulin-like receptors (KIRs), produced by NK cells, show an expression pattern that is biased towards recognition of HLA-C. HLA-C is the only polymorphic classical MHC I molecule expressed by placental trophoblasts that is in direct contact with uterine tissue.[46,49] These KIR receptors also interact with HLA-E and HLA-G.[38]

Another mechanism for maternal immune suppression is placental production of indoleamine-2,3 dioxygenase (IDO).[50] IDO can inhibit proliferation and function of T cells by depletion of tryptophan in T cells. Catabolites of trypotophan are also able to inhibit T cell proliferation.[51]

Galectin-1 recently gained much attention. This glycan binding protein is synthesized by a wide variety of cells including immune cells. Galectin-1 can modulate T cell homeostasis by interfering with T cell activation, promoting T cell apoptosis, T cell migration and regulating Th1/Th2 cytokine balance towards Th2 predominance.[50,52-53] Galectin-1 has an important role at the feto-maternal interface and is secreted by decidual tissue.[52]

Normal pregnancy: adaptive immunity

Human CD4+ T helper lymphocytes can be classified into functional subsets on the basis of their pattern of cytokine production.[54] Type 1 CD4+ T cells (Th1 cells) produce interleukin-2 (IL-2), tumor necrosis factor alpha (TNF-α) and interferon-gamma (IFN-γ). Th1 cells are the main effectors of phagocyte-induced host defence. On the other hand, type 2 CD4+ T cells (Th2 cells) produce IL-4, IL-5, IL-13 and IL-10. Th2 cells are mainly responsible for phagocyte-independent host defence. The Th1/Th2 paradigm has long dominated the discussion around pregnancy related tolerance. The ratio between the pro-inflammatory Th1 and anti-inflammatory Th2 cells is changed towards a more Th2 phenotype during pregnancy.[37,55] In recurrent spontaneous abortions and in pre-eclampsia increased Th1 immunity was observed.[39,55-56] However, Th2 type immunity was also observed in recurrent abortions.[41,57] Knock out mice for the Th2 type cytokines IL-4, IL-5, IL-9 and IL-13 are able to reproduce, demonstrating that Th2 type immunity is not essential for successful pregnancy.[58] These findings suggest that the Th1/Th2 balance shifting theory as an explanation of pregnancy related tolerance is too simple.[59] More key players in immunology were identified at the feto-maternal interface like monocytes and NK cells, as were two functional subsets of T cells: Treg and Th17 cells. Treg have been found to be highly important for a successful

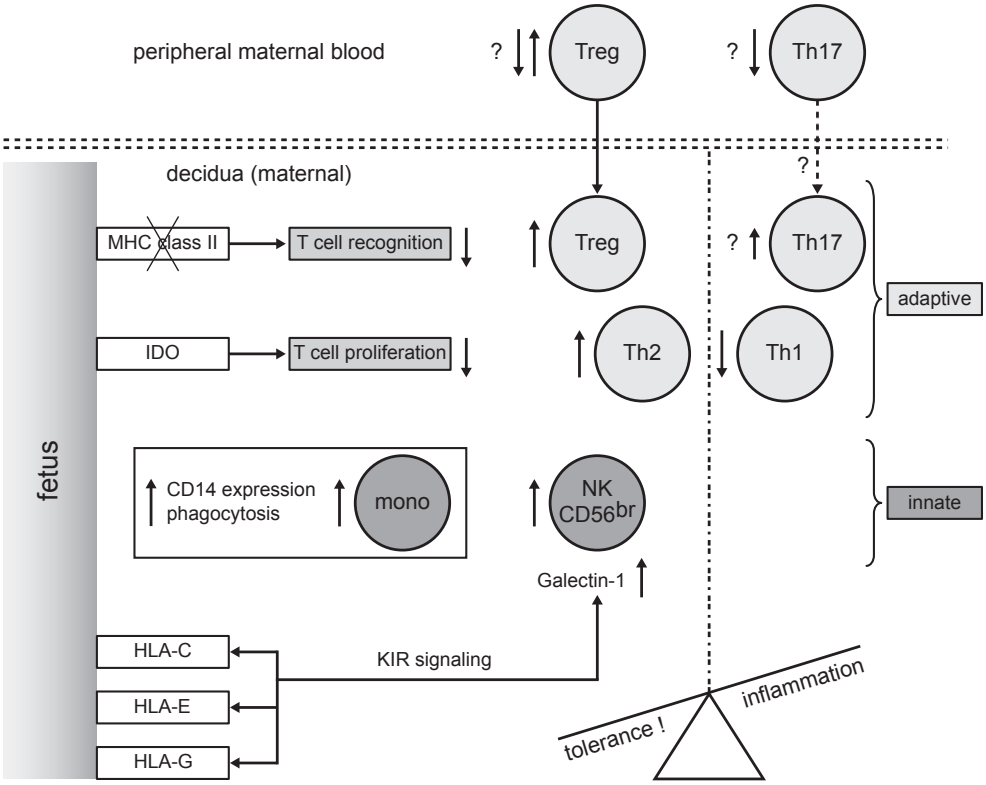


Figure 5. Mechanisms of pregnancy-induced tolerance in normal pregnancy
At the feto-maternal interface several mechanisms are shown that help to inhibit rejection of paternal antigens by T cells. The absence of major histocompatiblity complex (MHC) class II molecules limits T cell awareness of paternal antigens. Also classical MHC class I molecules human leucocyte antigen (HLA)-A and HLA-B are not expressed at the feto-maternal interface. Instead the HLA-C and the non-classical MHC class I molecules HLA-E and HLA-G are expressed. HLA-C, HLA-E and HLA-G interact with CD56^{bright} Natural Killer (NK) cells by killer cell immunoglobulin-like receptor (KIR) signalling. Numbers of CD56^{bright} NK cells are increased in the decidual tissue. Increased expression of indoleamine-2,3 dioxygenase (IDO) limits T cell proliferation by depletion of tryptophan in T cells. Catabolites of trypotophan are also able to inhibit T cell proliferation. Galectin-1 can modulate T cell function by interfering with T cell activation, promoting T cell apoptosis, T cell migration and regulating Th1/Th2 cytokine balance towards Th2 predominance. The number of monocytes is increased and they display increased expression of CD14 and increased phagocytosis. Normal pregnancy is characterized by an increase of anti-inflammatory Th2 cells and a decrease of pro-inflammatory Th1 cells. Regulatory T cells (Treg) migrate into the decidual tissue and their numbers in the decidual tissue are increased. There is some evidence that pro-inflammatory Th17 cells numbers are increased in the decidual tissue. There is no available data on Th17 migration into the decidua from the peripheral maternal blood. Data on alterations in numbers of circulating Treg and Th17 in peripheral maternal blood are conflicting.

pregnancy.[60] Several studies have examined the frequency of circulating Treg in peripheral blood during pregnancy. Both increases and decreases in frequencies of circulating Treg were reported.[61-67] These differences in frequencies of circulating T cell frequencies are likely based on the different flow cytometry gating strategies used to identify Treg populations in peripheral blood. Early in pregnancy, increased numbers of Treg are found in the decidua.[68-69] These decidual Treg are able to inhibit CD4+ T cell proliferation[68] and are decreased in recurrent abortions, implying a role in the maintenance of successful pregnancy.[67-70] The frequencies of Th17 cells during pregnancy have not been studied extensively. One study showed no change in the frequency of Th17 cells during pregnancy[71] while another study found decreased numbers of Th17 cells during normal pregnancy.[67] Unexpectedly, numbers of these pro-inflammatory Th17 cells are increased in the decidua compared to peripheral blood.[71]

Normal pregnancy: innate immunity

As discussed before, in human pregnancy the fetal implantation side is infiltrated by monocytes, granulocytes, NK cells and to a lesser extent with T cells. The majority of immune cells are derived from the innate arm of the immune system. During normal pregnancy the numbers of circulating monocytes and granuloctyes are increased.[43] Not only the numbers of these cells are increased, the phagocytic function of these cells and the expression of the endotoxin receptor CD14 and CD64, the high affinity receptor for the Fc fragment of IgG, is increased.[43,72-75] Another important cell type at the feto-maternal interface are NK cells. In humans, NK cells are divided into two distinct types of cells based on their expression of CD16 and CD56.[76] $CD16^{neg}CD56^{bright}$ cells are poorly cytotoxic, whereas $CD16^{pos}CD56^{dim}$ cells are highly cytotoxic but poor at cytokine production.[53] During pregnancy the numbers of $CD56^{dim}$ cells are decreased and numbers of $CD56^{bright}$ cells are increased, both in blood and in the decidua.[46,53] $CD56^{bright}$ NK cells are able to regulate T cell function by limiting T cell survival.[77] Trophoblast cells express HLA-C which is the ligand for KIR expressed by uterine NK cells. Complex interactions between activating and inhibiting KIR types and HLA-C subtypes codetermine the success of pregnancy.[78]

Overall, it is clear that the maternal immune system is altered to allow for a successful pregnancy, but the precise mechanisms behind these changes and the possible interactions of these mechanisms remain to be elucidated.

D. Biology of pregnancy in MS

Immunological changes during pregnancy in MS. What is known?

MS is considered to be a T cell driven autoimmune disease. Hence most studies on immunological changes during pregnancy in MS focussed on T cell subsets, yielding conflicting results on Treg frequency in peripheral blood. One study showed an increase of the frequency of circulating Treg in peripheral blood during the first two trimesters followed by a decrease in the third trimester.[79] Another study showed a decrease in Treg during the

last two trimesters.[80] A study from Finland showed no differences in Treg frequencies during pregnancy.[81] This last study also found decreased production of the Th1 cytokine IFN-γ by peripheral blood cells during pregnancy.[81] This was also found before in another small study.[82] IFN-γ production increased during pregnancy.[79] Surprisingly, a recent study showed that MS patients with a postpartum relapse have lower numbers of IFN-γ producing $CD4^{pos}$ cells during the third trimester.[83] No studies have been done on frequency of the pro-inflammatory Th17 cells during pregnancy in MS.

As soluble CD95 can block CD95 mediated T cell apoptosis, it has been hypothesized that pregnancy may induce an alteration in soluble CD95 levels. No evidence of altered levels of soluble CD95 in pregnant MS patients was found.[84]

Innate immune alterations during pregnancy in MS patients were investigated in a single study. This study described an increased number of circulating anti-inflammatory $CD56^{bright}$ NK cells.[80]

Soluble HLA-G is increased in the CSF of MS patients. In MS patients high levels of soluble HLA-G in serum and CSF are associated with fewer MRI abnormalities.[85-86] After delivery in MS patients serum level of soluble HLA-G is decreased. This drop in serum level of soluble HLA-G was associated with an increased number of postpartum relapses.[87]

It is clear that many questions on the biology of pregnancy in MS remain. Some changes that have been reported were unexpected and have not been successfully reproduced in other cohorts.

Pregnancy in the animal model of MS: of mice and women

Experimental autoimmune encephalomyelitis (EAE) is the animal model of MS. In EAE myelin components are injected into susceptible animals, emulsified in very strong adjuvants. This leads to a CD4+ T cell mediated autoimmune disease with similarities to MS.[13,88] The induced EAE in animals does not equal MS in humans, but important lessons can still be learned from this animal model.

Several studies have examined the effect of pregnancy and/or estrogens on EAE. Mice immunized during pregnancy showed a decreased incidence of EAE.[89] Mice with pre-existing EAE that became pregnant displayed an ameliorated disease course.[89] In the brain of these mice no decrease in inflammatory cell infiltration was observed, compared to non-pregnant mice. In murine EAE, immunisation during pregnancy resulted in a decreased incidence and clinical severity.[90] TNF-α and IL-7 production were decreased and IL-10 production was increased. These data were confirmed by a recent study.[91] This study also found the presence of microparticles, exosomes derived from the serum of pregnant mice, with an immune suppressive function. In EAE mice treated with estrogens disease severity was reduced.[92-94] In these estrogen-treated mice IL-10 production was increased.[92]

Estriol treatment in MS

A cross-over study attempted to mimic pregnancy in women with MS by giving them the pregnancy hormone estriol in a dosage (8 mg/day) comparable with levels of estriol

found during pregnancy during two periods of six months.[95] Six patients with RR MS and six patients with SP MS were entered in the study. Ten patients finished the study protocol (two SP MS patients were excluded during the study because of protocol violation). Estriol treatment did not result in a significant decrease in relapse rate, likely because of the small numbers. The patients with RR-MS showed a decrease in MRI disease activity and a decrease in IFN-γ production by peripheral blood mononuclear cells (PBMC). Also an increased production of the Th2 cytokines IL-5 and IL-10 by PBMC was observed. This increase of IL-10 was possibly due to an increased number of CD64+ cells (monocytes/macrophages).[96] The use of oral contraceptives in the general population is not related with a decreased risk of MS.[97]

Other pregnancy related hormones and MS

During pregnancy cortisol levels increase and they decline after delivery.[98] The rise in cortisol during pregnancy is driven by placental production of corticotrophin releasing hormone (CRH). Hypothalamic production of CRH is decreased during pregnancy and is restored around three months after delivery. High levels of cortisol are likely to be beneficial to MS, as high dose corticosteroids are used in the treatment of acute relapses in MS.[99] No studies on cortisol levels during pregnancy in MS have been performed.

The exact role of prolactin in MS is still to be elucidated. Prolactin is implicated in white matter regeneration.[100] In EAE inhibition of prolactin production by bromocriptin is able to reduce severity of EAE.[101-102] In MS patients acute relapses are accompanied by a surge in prolactin.[103-104] A decrease in prolactin accompanied reduced MRI disease activity in MS patients treated with monthly pulses of corticosteroids.[105] Prolactin levels during MS pregnancy have not been studied yet.

III. WHEN MS STARTS IN CHILDHOOD

In recent years, the awareness is increased that MS can already start during childhood.[106-109] Establishing a definite diagnosis of MS after a first demyelinating event in children can be difficult. Differential diagnosis is more complex than in adults as other diseases may mimic MS during childhood. Neurological deficits are sometimes subtle and neurological examination of children is more challenging. These factors often lead to both patient and doctors delay in the diagnosis of MS.

Studying MS in children can augment understanding of MS in general. Children with MS uniquely allow the study of potential environmental influences. Migration studies show that early (childhood) changes in geographical location influence the risk of MS, suggesting a role of environmental influences in the development of MS.[110] The chances of identifying an unknown environmental factor X are higher in children as they are closer in time to the moment of exposure to this environmental influence (Figure 6).

Before we can advance in studying childhood onset in MS, a first necessity is a reliable diagnosis of MS in children.

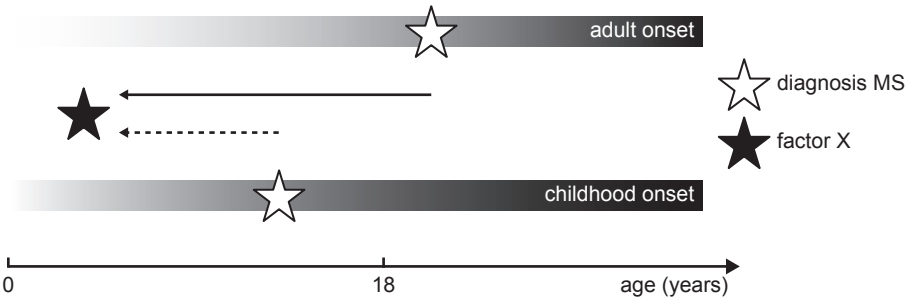


Figure 6. Putative environmental factor X and age at onset
It is hypothesized that a possible environmental factor X exist early in life, which is capable of influencing the risk of multiple sclerosis (MS) later in life. The chances of identifying such an environmental factor X are better in patients with childhood onset (dotted line) than in patients with adult onset (fixed line), because they are closer to the moment of exposure to this environmental influence.

Adult MRI criteria for diagnosis of pediatric MS

In both adults and children the diagnosis of MS relies on dissemination in time and space (McDonald criteria).[12] A definition of pediatric MS has been proposed in a consensus statement by the international pediatric MS study group (IPMSSG), depicted in Table 3.

A cross-sectional study in children with MS showed that only 50% of the children with MS fulfilled the Barkhof MRI criteria for dissemination in space.[111] The Barkhof MRI criteria have been developed in adults to discriminate between MS lesions and atherosclerotic ischemic lesions. Children rarely have atherosclerosis, but they can have typical childhood diseases MRI abnormalities similar to MS. Because of a lack of clinical studies, implementing MRI criteria in children is a problem and especially in very young children, aged under ten years. This was also stressed in a review on MRI abnormalities in children with multiple sclerosis.[107] It might be that the Barkhof MRI criteria for dissemination in space therefore are not suitable for the diagnosis of MS in children.

Table 3. Proposed criteria for pediatric MS[109]

Multiple demyelinating episodes of the CNS fulfilling dissemination in time and space criteria
Dissemination in space criteria • Two clinical locations separated in space (as in McDonald criteria) • Barkhof MRI criteria can be used to fulfil dissemination in space criteria (see Table 1) • Abnormal CSF (elevated IgG index and/ or oligoclonal IgG bands) in combination with ≥2 lesions on MRI
Dissemination in time criteria • Two clinical episodes separated in time (as in McDonald criteria) • New T2 or gadolinium enhanced lesions 3 months after the first demyelinating event (age ≥ 10 years)
A first episode compatible with ADEM cannot be considered as the first event of MS

CNS: central nervous system, CSF: cerebrospinal fluid, ADEM: acute disseminated encephalomyelitis, MS: multiple sclerosis

Distinguishing between MS and ADEM

In children, more frequently than in adults, a first demyelinating event cannot be distinguished from acute disseminated encephalomyelitis (ADEM).[112-115] Several definitions for ADEM have been proposed.[109,116] The IPMSSG introduced a definition of ADEM, shown in Table 4.[109] When these typical ADEM cases have relapses, it becomes difficult to distinguish between ADEM and MS, because these cases then fulfil the McDonald criteria for dissemination in time and in space. For this purpose, the international pediatric MS study group introduced the terms recurrent ADEM and multiphasic ADEM (criteria are depicted in Table 5), thus separating these entities from MS.[109] Because a first event compatible with ADEM cannot count as a first event in MS, problems arise when a first event compatible with ADEM is followed by an event not compatible with ADEM. Then neither the diagnosis MS nor the diagnosis recurrent/multiphasic ADEM can be made.

Clinical data on long term follow-up supporting these proposed criteria is lacking. This leads to uncertainty in patients, parents and physicians about prognosis and possible future therapeutic options after a first demyelinating event. It is crucial to determine which parameters reliably predict outcome after a first demyelinating event in children.

Table 4. Proposed criteria for pediatric ADEM[109]

All criteria must be fulfilled
A first clinical event with a presumed inflammatory or demyelinating cause
Acute or subacute onset
Affecting multifocal areas of the CNS
Clinical: polysymptomatic
Encephalopathy • Behavioral change (confusion, excessive irritability) • Alteration in consciousness (lethargy, coma)
No other etiologies can explain the event
Event should be followed by (partial) improvement (clinical or radiological)
No history of a prior demyelinating event
New or fluctuating symptoms, signs or MRI findings occurring within 3 months of the inciting ADEM event are considered part of the acute event
Neuro-imaging shows focal or multifocal lesion(s), predominantly involving white matter, without radiological evidence of previous destructive white matter changes: • Brain MRI (FLAIR, T2) shows large >1-2 cm lesions, that are multifocal, hyperintense and located supratentorial and infratentorial in the white matter or grey matter (basal ganglia/thalamus) • In rare cases, brain MRI shows a large single lesion (>1-2 cm), predominantly in the white matter • Spinal cord MRI may show confluent intramedullary lesions with variable enhancement

ADEM: acute disseminated encephalomyelitis, CNS: central nervous system, FLAIR: fluid attenuated inversion recovery

Table 5. Proposed criteria for recurrent ADEM and multiphasic ADEM[109]

Recurrent ADEM	Multiphasic ADEM
New event of ADEM with a recurrence of the initial symptoms and signs, 3 or more months after the first ADEM event, without involvement of new clinical areas by history, neurologic examination or neuroimaging	ADEM followed by a new clinical event also meeting criteria for ADEM, but involving new anatomic areas of the CNS as confirmed by history, neurologic examination or neuroimaging
Event does not occur while on steroids, and occurs at least 1 month after completing steroid therapy	The subsequent event must occur 1) at least 3 months after the onset of the initial ADEM event and 2) at least 1 month after completing steroid therapy
MRI shows no new lesions; original lesions may have enlarged	The subsequent event must include a polysymptomatic presentation including encephalopathy, with neurologic symptoms or signs that differ from the initial event (mental status changes may not differ from the initial event)
No better explanation exists	The brain MRI must show new areas of involvement but also demonstrate complete or partial resolution of those lesions associated with the first ADEM event

ADEM: acute disseminated encephalomyelitis, CNS: central nervous system

Towards pediatric MRI criteria

Many studies on pediatric MS retrospectively investigated pediatric cases in adult cohorts.[4,6,11] Monophasic variants like ADEM or monophasic optic neuritis were not included in these studies. There is only one follow-up study in children with a broad clinical spectrum of first demyelinating events, like optic neuritis, transverse myelitis and ADEM. This French study showed that next to the Barkhof criteria the presence of lesions perpendicular to the corpus callosum and only well defined lesions on MRI (KIDMUS criteria) were of prognostic value for MS development (Table 6).[114,117] The sensitivity of these KIDMUS criteria was very low (5%) in children aged under ten years.

Recently, two new sets of MRI criteria have been proposed (Table 6). A first set of MRI criteria was developed to distinguish MS patients experiencing their first event from monophasic ADEM patients.[118] A second set of criteria proposed modifications to the Barkhof MRI criteria in order to improve the diagnostic accuracy of these MRI criteria for MS in children. These criteria were initially designed to distinguish between MS (MRI at second MS defining attack) and other, non-demyelinating diseases (SLE and migraine).[119] These criteria have yet to be validated in an independent cohort.

Table 6. Pediatric MRI criteria for diagnosing MS

KIDMUS[113-114] **(1 out of 2) ***	**Callen: MS vs ADEM criteria**[118] **(at least 2 out of 3)**	**Callen: Diagnostic MS criteria**[119] **(at least 2 out of 3)**
Lesions perpendicular to long axis of corpus callosum	Absence of a diffuse bilateral lesion pattern	≥ 5 lesions on T2-weighted images
The sole presence of well-defined lesions	Presence of black holes	≥ 2 periventricular lesions
	≥ 2 periventricular lesions	≥ 1 brainstem lesion

* In the first publication both KIDMUS criteria were required[114]
MS: mulitiple sclerosis, ADEM: acute disseminated encephalomyelitis

Prediction of severity in pediatric MS

Apart from identifying prognostic factors for the diagnosis MS at onset of the disease, prognostic factors for early severity are necessary. Knowledge of such prognostic parameters may help selecting those children with a poor prognosis for future treatment trials. A childhood-onset MS potential index for early severity has been proposed.[120] This index is based on the presence of one or two of the KIDMUS MRI criteria, female gender, a short interval between the first and the second (MS-defining) attack, absence of encephalopathy and progressive disease course. This severity score has yet to be validated in another independent pediatric cohort.

IV. SCOPE AND OUTLINE OF THIS THESIS

This thesis focuses on two topics in MS: pregnancy and young age at onset. The main objective of our studies on MS and pregnancy was to identify possible underlying biological mechanisms of MS disease amelioration during pregnancy. We also aimed to asses in more detail the disease course including quality of life, and to find and confirm risk factors for postpartum relapse. Our main objective in the studies presented in this thesis on onset of MS in children was to improve the difficult diagnostic process in children.

To study pregnancy in MS patients we performed a longitudinal, prospective study in which ambulant relapsing remitting MS patients with a childwish or that were pregnant, were recruited at the Rotterdam MS Center, ErasMS. The study was designed to include patients preconceptionally, but inclusion was also allowed during pregnancy. All MS patients were seen at 10–12 weeks and 28–30 weeks of pregnancy, and at 4–8 weeks after delivery. MS patients were also invited at a time point at least 9 months after delivery, without clinical infection or recent disease activity.

To study onset of MS in children we performed a nationwide study on early prognostic factors after a first demyelinating event. Children under the age of 16 were included with an attack compatible with a demyelinating disease of the CNS in the period 1990-2007. Patients were identified by members of the Dutch Study Group on childhood MS and ADEM.

All major pediatric neurology centres in hospitals in the Netherlands participated with complete geographical coverage of the Netherlands.

The studies concerning MS and pregnancy are described in **chapter 2.** The studies concerning onset of MS in children are described in **chapter 3**.

In **chapter 2.1** we report on clinical and self report scales in women with MS, before, during and after delivery. Quality of life during pregnancy was compared with healthy pregnant women. Possible risk factors for post partum relapse are discussed. **Chapter 2.2** shows data on the effect of breastfeeding in women with MS. **Chapter 2.3** provides data on the possible use of the chemokine interleukin 8 as a biomarker for postpartum relapse.
In **chapter 2.4** we describe a genome wide RNA expression study in monocytes during pregnancy. We set out to identify activation or depression of immunological pathways in monocytes that might explain the amelioration of MS during pregnancy. In **chapter 2.5** we focus on highly relevant T cell subsets. We hypothesized that frequency of Treg and Th17 cells in peripheral blood were altered during pregnancy, explaining the clinical improvement during pregnancy. In **chapter 2.6** we investigate serum levels of leptin during pregnancy and the possible use of leptin as a biomarker for postpartum relapse.

In **chapter 3.1** we report on a large national multicentre study on prognostic factors after a first demyelinating attack. We aimed to identify clinical, radiological and CSF factors predicting development of MS after a first inflammatory demyelinating attack in children. In **chapter 3.2** we set out to find clinical and radiological factors able to predict an early relapse after the diagnosis in children. In **chapter 3.3** we compare four current sets of MRI criteria in our pediatric cohort and determined which are the most useful in clinical practice for distinguishing ADEM from MS.

In the discussion the observations from the studies in chapter 2 and 3 are summarized and discussed in relation to current literature. Recommendations for further research are described.

REFERENCES

1. Compston A, Coles A. Multiple sclerosis. Lancet 2008;372:1502-1517.
2. Alonso A, Hernán MA. Temporal trends in the incidence of multiple sclerosis: a systematic review. Neurology 2008;71:129-135.
3. Orton SM, Herrera BM, Yee IM, Valdar W, Ramagopalan SV, Sadovnick AD, Ebers GC; Canadian Collaborative Study Group. Sex ratio of multiple sclerosis in Canada: a longitudinal study. Lancet Neurol. 2006;5:932-936.
4. Boiko A, Vorobeychik G, Patsy D, Devonshire V Sadovnick D, and the UBC MS Clinic Neurologists. Early onset multiple sclerosis. A longitudinal study. Neurology 2002;59:1006-1010.
5. Mayr WT, Pittock SJ, McClelland RL, Jorgensen NW, Noseworthy JH, Rodriguez M. Incidence and prevalence of multiple sclerosis in Olmsted County, Minnesota, 1985-2000. Neurology 2003;61:1373-1377.
6. Duquette P, Murray TJ, Pleines J, Ebers GC, Sadovnick D, Weldon P, Warren S, Paty DW, Upton A, Hader W, et al. Multiple sclerosis in childhood: clinical profile in 125 patients. J Pediatr. 1987;111:359-363.
7. Ghezzi A, Deplano V, Faroni J, Grasso MG, Liguori M, Marrosu G, Pozzilli C, Simone IL, Zaffaroni M. Multiple sclerosis in childhood: clinical features of 149 cases. Mult Scler. 1997;3:43-46.
8. Sindern E, Haas J, Stark E, Wurster U. Early onset MS under the age of 16: clinical and paraclinical features. Acta Neurol Scand. 1992;86:280-284.
9. Renoux C, Vukusic S, Mikaeloff Y, Edan G, Clanet M, Dubois B, Debouverie M, Brochet B, Lebrun-Frenay C, Pelletier J, Moreau T, Lubetzki C, Vermersch P, Roullet E, Magy L, Tardieu M, Suissa S, Confavreux C; Adult Neurology Departments KIDMUS Study Group. Natural history of multiple sclerosis with childhood onset. N Engl J Med. 2007;356:2603-2613.
10. Confavreux C, Vukusic S. Age at disability milestones in multiple sclerosis. Brain. 2006;129:595-605.
11. Simone IL, Carrara D, Tortorella C, Liguori M, Lepore V, Pellegrini F, Bellacosa A, Ceccarelli A, Pavone I, Livrea P. Course and prognosis in early-onset MS: comparison with adult-onset forms. Neurology 2002;59:1922-1928.
12. Polman CH, Reingold SC, Edan G, Filippi M, Hartung HP, Kappos L, Lublin FD, Metz LM, McFarland HF, O'Connor PW, Sandberg-Wollheim M, Thompson AJ, Weinshenker BG, Wolinsky JS. Diagnostic criteria for multiple sclerosis:2005 revisions to the "McDonald Criteria". Ann Neurol. 2005;58:840-846.
13. McFarland HF, Martin R. Multiple sclerosis: a complicated picture of autoimmunity. Nat Immunol. 2007;8:913-919.
14. Pittock SJ, Lucchinetti CF. The pathology of MS: new insights and potential clinical applications. Neurologist 2007;13:45-56.
15. Trapp BD, Peterson J, Ransohoff RM, Rudick R, Mörk S, Bö L. Axonal transection in the lesions of multiple sclerosis. N Engl J Med. 1998;338:278-285.
16. Tzartos JS, Friese MA, Craner MJ, Palace J, Newcombe J, Esiri MM, Fugger L. Interleukin-17 production in central nervous system-infiltrating T cells and glial cells is associated with active disease in multiple sclerosis. Am J Pathol. 2008;172:146-155.
17. Mills KH. TLR-dependent T cell activation in autoimmunity. Nat Rev Immunol. 2011;11:807-22.
18. Kebir H, Kreymborg K, Ifergan I, Dodelet-Devillers A, Cayrol R, Bernard M, Giuliani F, Arbour N, Becher B, Prat A. Human TH17 lymphocytes promote blood-brain barrier disruption and central nervous system inflammation. Nat Med. 2007;13:1173-1175.
19. Sakaguchi S. Naturally arising Foxp3-expressing CD25+CD4+ regulatory T cells in immunological tolerance to self and non-self. Nat Immunol. 2005;6:345-352.
20. Viglietta V, Baecher-Allan C, Weiner HL, Hafler DA. Loss of functional suppression by CD4+CD25+ regulatory T cells in patients with multiple sclerosis. J Exp Med. 2004;199:971–979.
21. Martinez-Forero I, Garcia-Munoz R, Martinez-Pasamar S, Inoges S, Lopez-Diaz de Cerio A, Palacios R, Sepulcre J, Moreno B, Gonzalez Z, Fernandez-Diez B, Melero I, Bendandi M, Villoslada P. IL-10 suppressor activity and ex vivo Tr1 cell function are impaired in multiple sclerosis. Eur J Immunol. 2008;38:576 –586.
22. Astier AL, Meiffren G, Freeman S, Hafler DA. Alterations in CD46-mediated Tr1 regulatory T cells in patients with multiple sclerosis. J Clin Invest. 2006;116:3252–3257.

23. Confavreux C, Hutchinson M, Hours MM, Cortinovis-Tourniaire P, Moreau T**.** Rate of pregnancy-related relapse in multiple sclerosis. Pregnancy in multiple sclerosis group. N Engl J Med. 1998;339:285-291.
24. Runmarker B, Andersen O. Pregnancy is associated with a lower risk of onset and a better prognosis in multiple sclerosis. Brain 1995;118:253-261.
25. Vukusic S, Hutchinson M, Hours M, Moreau T, Cortinovis-Tourniaire P, Adeleine P, Confavreux C, The Pregnancy In Multiple Sclerosis Group; Pregnancy In Multiple Sclerosis Group.Pregnancy and multiple sclerosis (the PRIMS study): clinical predictors of post-partum relapse. Brain 2004;127:1353-1360.
26. Fernández Liguori N, Klajn D, Acion L, Cáceres F, Calle A, Carrá A, Cristiano E, Deri N, Garcea O, Jaureguiberry A, Onaha P, Patrucco L, Riccio P, Rotta Escalante R, Saladino M, Sinay V, Tarulla A, Villa A.Epidemiological characteristics of pregnancy, delivery, and birth outcome in women with multiple sclerosis in Argentina (EMEMAR study). Mult Scler. 2009;15:555-562.
27. Jalkanen A, Alanen A, Airas L; Finnish Multiple Sclerosis and Pregnancy Study Group. Pregnancy outcome in women with multiple sclerosis: results from a prospective nationwide study in Finland. Mult Scler. 2010;16:950-955.
28. van Walderveen MA, Tas MW, Barkhof F, Polman CH, Frequin ST, Hommes OR, Valk J. Magnetic resonance evaluation of disease activity during pregnancy in multiple sclerosis. Neurology 1994;44:327-329.
29. Paavilainen T, Kurki T, Parkkola R, Färkkilä M, Salonen O, Dastidar P, ElovaaraI, Airas L. Magnetic resonance imaging of the brain used to detect early post-partum activation of multiple sclerosis. Eur J Neurol. 2007;14:1216-1221.
30. Langer-Gould A, Huang SM, Gupta R, Leimpeter AD, Greenwood E, Albers KB, Van Den Eeden SK, Nelson LM. Exclusive breastfeeding and the risk of postpartum relapses in women with multiple sclerosis. Arch Neurol. 2009;66:958-963.
31. Hellwig K, Gold R. Breastfeeding and multiple sclerosis in a German cohort. Mult Scler. 2008;14:718.
32. Airas L, Jalkanen A, Alanen A, Pirttilä T, Marttila RJ. Breast-feeding, postpartum and prepregnancy disease activity in multiple sclerosis. Neurology 2010;75:474-476.
33. Dahl J, Myhr KM, Daltveit AK, Hoff JM, Gilhus NE. Pregnancy, delivery, and birth outcome in women with multiple sclerosis. Neurology 2005;65:1961-1963.
34. Kelly VM, Nelson LM, Chakravarty EF. Obstetric outcomes in women with multiple sclerosis and epilepsy. Neurology 2009;73:1831-1836.
35. Borchers AT, Naguwa SM, Keen CL, Gershwin ME. The implications of autoimmunity and pregnancy. J Autoimmun. 2010;34:287-299.
36. Doria A, Iaccarino L, Arienti S, Ghirardello A, Zampieri S, Rampudda ME, CutoloM, Tincani A, Todesco S. Th2 immune deviation induced by pregnancy: the two faces of autoimmune rheumatic diseases. Reprod Toxicol. 2006;22:234-241.
37. Wegmann TG, Lin H, Guilbert L, Mosmann TR. Bidirectional cytokine interaction in maternal fetal relationship: is successful pregnancy a Th2 phenomenon? Immunol Today 1993;14:353-356.
38. Trowsdale J, Betz AG. Mother's little helpers: mechanisms of maternal-fetal tolerance. Nat Immunol. 2006;7:241-246.
39. Raghupathy R. Th1-type immunity is incompatible with successful pregnancy. Immunol Today. 1997;18:478-482.
40. Piccinni MP. T cell tolerance towards the fetal allograft. J Reprod Immunol. 2010;85:71-75.
41. Chaouat G. Innately moving away from the Th1/Th2 paradigm in pregnancy. Clin Exp Immunol. 2003;131:393-395.
42. Redman CW, Sargent IL. Latest advances in understanding preeclampsia. Science. 2005;308:1592-1594.
43. Sacks G, Sargent I, Redman C. An innate view of human pregnancy. Immunol Today 1999;20:114-118.
44. Chaouat G, Petitbarat M, Dubanchet S, Rahmati M, Ledée N. Tolerance to the foetal allograft? Am J Reprod Immunol. 2010;63:624-636.
45. Sargent IL, Borzychowski AM, Redman CW. NK cells and human pregnancy--an inflammatory view. Trends Immunol. 2006;27:399-404.
46. Moffett A, Loke C. Immunology of placentation in eutherian mammals. Nat Rev Immunol. 2006;6:584-594.

47. Hiby SE, Walker JJ, O'Shaughnessy KM, Redman CW, Carrington M, Trowsdale J, Moffett A. Combinations of maternal KIR and fetal HLA-C genes influence the risk of preeclampsia and reproductive success. J Exp Med. 2004;200:957-965.
48. Ishitani A, Sageshima N, Hatake K. The involvement of HLA-E and -F in pregnancy. J Reprod Immunol. 2006;69:101-113.
49. Sharkey AM, Gardner L, Hiby S, Farrell L, Apps R, Masters L, Goodridge J, Lathbury L, Stewart CA, Verma S, Moffett A. Killer Ig-like receptor expression in uterine NK cells is biased toward recognition of HLA-C and alters with gestational age. J Immunol. 2008;181:39-46.
50. Terness P, Kallikourdis M, Betz AG, Rabinovich GA, Saito S, Clark DA. Tolerance signaling molecules and pregnancy: IDO, galectins, and the renaissance of regulatory T cells. Am J Reprod Immunol. 2007;58:238-254.
51. Frumento G, Rotondo R, Tonetti M, Damonte G, Benatti U, Ferrara GB. Tryptophan-derived catabolites are responsible for inhibition of T and natural killer cell proliferation induced by indoleamine 2,3-dioxygenase. J Exp Med. 2002;196:459-468.
52. Blois SM, Ilarregui JM, Tometten M, Garcia M, Orsal AS, Cordo-Russo R, Toscano MA, Bianco GA, Kobelt P, Handjiski B, Tirado I, Markert UR, Klapp BF, Poirier F, Szekeres-Bartho J, Rabinovich GA, Arck PC. A pivotal role for galectin-1 in fetomaternal tolerance. Nat Med. 2007;13:1450-1457.
53. Karimi K, Arck PC. Natural Killer cells: keepers of pregnancy in the turnstile of the environment. Brain Behav Immun. 2010;24:339-347.
54. Mosmann TR, Coffman RL. TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. Annu Rev Immunol. 1989;7:145-173.
55. Piccinni MP, Beloni L, Livi C, Maggi E, Scarselli G, Romagnani S. Defective production of both leukemia inhibitory factor and type 2 T-helper cytokines by decidual T cells in unexplained recurrent abortions. Nat Med. 1998;4:1020-1024.
56. Saito S, Sakai M. Th1/Th2 balance in preeclampsia. J Reprod Immunol. 2003;59:161-173.
57. Bates MD, Quenby S, Takakuwa K, Johnson PM, Vince GS. Aberrant cytokine production by peripheral blood mononuclear cells in recurrent pregnancy loss? Hum Reprod. 2002;17:2439-2444.
58. Fallon PG, Jolin HE, Smith P, Emson CL, Townsend MJ, Fallon R, Smith P, McKenzie AN. IL-4 induces characteristic Th2 responses even in the combined absence of IL-5, IL-9, and IL-13. Immunity 2002;17:7-17.
59. Chaouat G, Ledée-Bataille N, Dubanchet S, Zourbas S, Sandra O, Martal J. TH1/TH2 paradigm in pregnancy: paradigm lost? Cytokines in pregnancy/early abortion: reexamining the TH1/TH2 paradigm. Int Arch Allergy Immunol. 2004;134:93-119.
60. Aluvihare VR, Kallikourdis M, Betz AG. Regulatory T cells mediate maternal tolerance to the fetus. Nat Immunol. 2004;5:266-271.
61. Heikkinen J, Möttönen M, Alanen A, Lassila O. Phenotypic characterization of regulatory T cells in the human decidua. Clin Exp Immunol. 2004;136:373-378.
62. Somerset DA, Zheng Y, Kilby MD, Sansom DM, Drayson MT. Normal human pregnancy is associated with an elevation in the immune suppressive CD25+ CD4+ regulatory T cell subset. Immunology 2004;112:38-43.
63. Tilburgs T, Roelen DL, van der Mast BJ, de Groot-Swings GM, Kleijburg C, Scherjon SA, Claas FH. Evidence for a selective migration of fetus-specific CD4+CD25bright regulatory T cells from the peripheral blood to the decidua in human pregnancy. J Immunol. 2008;180:5737-5745.
64. Tilburgs T, Roelen DL, van der Mast BJ, van Schip JJ, Kleijburg C, De Groot-Swings GM, Kanhai HH, Claas FH, Scherjon SA. Differential distribution ofCD4(+)CD25(bright) and CD8(+)CD28(-) T cells in decidua and maternal blood during human pregnancy. Placenta 2006;27 Suppl A:S47-53.
65. Steinborn A, Haensch GM, Mahnke K, Schmitt E, Toermer A, Meuer S, Sohn C. Distinct subsets of regulatory T cells during pregnancy: is the imbalance of these subsets involved in the pathogenesis of preeclampsia? Clin Immunol. 2008;129:401-412.
66. Förger F, Marcoli N, Gadola S, Möller B, Villiger PM, Østensen M. Pregnancy induces numerical and functional changes of CD4+CD25 high regulatory T cells in patients with rheumatoid arthritis. Ann Rheum Dis. 2008;67:984-990.
67. Santner-Nanan B, Peek MJ, Khanam R, Richarts L, Zhu E, Fazekas de St Groth B, Nanan R. Systemic increase in the ratio between Foxp3+ and IL-17-producing CD4+ T cells in healthy pregnancy but not in preeclampsia. J Immunol. 2009;183:7023-7030.

68. Sasaki Y, Sakai M, Miyazaki S, Higuma S, Shiozaki A, Saito S. Decidual and peripheral blood CD4+CD25+ regulatory T cells in early pregnancy subjects and spontaneous abortion cases. Mol Hum Reprod. 2004;10:347-353.
69. Saito S, Nakashima A, Shima T, Ito M. Th1/Th2/Th17 and regulatory T cell paradigm in pregnancy. Am J Reprod Immunol. 2010;63:601-610.
70. Yang H, Qiu L, Chen G, Ye Z, Lü C, Lin Q. Proportional change of CD4+CD25+ regulatory T cells in decidua and peripheral blood in unexplained recurrent spontaneous abortion patients. Fertil Steril. 2008;89:656-661.
71. Nakashima A, Ito M, Yoneda S, Shiozaki A, Hidaka T, Saito S. Circulating and decidual Th17 cell levels in healthy pregnancy. Am J Reprod Immunol. 2010;63:104-109.
72. Sacks GP, Studena K, Sargent K, Redman CW. Normal pregnancy and preeclampsia both produce inflammatory changes in peripheral blood leukocytes akin to those of sepsis. A J Obstet Gynecol. 1998;179:80-86.
73. Luppi P, Haluszczak C, Betters D, Richard CA, Trucco M, DeLoia JA. Monocytes are progressively activated in the circulation of pregnant women. J Leukoc Biol. 2002;72:874-884.
74. Naccasha N, Gervasi MT, Chaiworapongsa T, Berman S, Yoon BH, Maymon E, Romero R. Phenotypic and metabolic characteristics of monocytes and granulocytes in normal pregnancy and maternal infection. Am J Obstet Gynecol. 2001;185:1118-1123.
75. Davis D, Kaufmann R, Moticka EJ. Nonspecific immunity in pregnancy: monocyte surface Fcgamma receptor expression and function. J Reprod Immunol. 1998;40:119-128.
76. Cooper MA, Fehniger TA, Caligiuri MA. The biology of human natural killer-cell subsets. Trends Immunol. 2001;22:633-640.
77. Bielekova B, Catalfamo M, Reichert-Scrivner S, Packer A, Cerna M, Waldmann TA,McFarland H, Henkart PA, Martin R. Regulatory CD56(bright) natural killer cells mediate immunomodulatory effects of IL-2Ralpha-targeted therapy (daclizumab) in multiple sclerosis. Proc Natl Acad Sci. U S A. 2006;103:5941-5946.
78. Hiby SE, Apps R, Sharkey AM, Farrell LE, Gardner L, Mulder A, Claas FH, Walker JJ, Redman CC, Morgan L, Tower C, Regan L, Moore GE, Carrington M, Moffett A. Maternal activating KIRs protect against human reproductive failure mediated by fetal HLA-C2. J Clin Invest. 2010;120:4102-4110.
79. Iorio R, Frisullo G, Nociti V, Patanella KA, Bianco A, Marti A, Mirabella M, Tonali PA, Batocchi AP. T-bet, pSTAT1 and pSTAT3 expression in peripheral blood mononuclear cells during pregnancy correlates with post-partum activation of multiple sclerosis. Clin Immunol. 2009;131:70-83.
80. Sánchez-Ramón S, Navarro A J, Aristimuño C, Rodríguez-Mahou M, Bellón JM, Fernández-Cruz E, de Andrés C. Pregnancy-induced expansion of regulatory T-lymphocytes may mediate protection to multiple sclerosis activity. Immunol Lett. 2005;96:195-201.
81. Airas L, Saraste M, Rinta S, Elovaara I, Huang YH, Wiendl H; Finnish Multiple Sclerosis and Pregnancy Study Group. Immunoregulatory factors in multiple sclerosis patients during and after pregnancy: relevance of natural killer cells. Clin Exp Immunol. 2008;151:235-43.
82. Gilmore W, Arias M, Stroud N, Stek A, McCarthy KA, Correale J. Preliminary studies of cytokine secretion patterns associated with pregnancy in MS patients. J Neurol Sci. 2004;224:69-76.
83. Langer-Gould A, Gupta R, Huang S, Hagan A, Atkuri K, Leimpeter AD, Albers KB, Greenwood E, Van Den Eeden SK, Steinman L, Nelson LM. Interferon-gamma-producing T cells, pregnancy, and postpartum relapses of multiple sclerosis. Arch Neurol. 2010;67:51-57.
84. Ehrlich S, Haas J, Zipp F, Infante-Duarte C. Serum levels of soluble CD95 are not associated with amelioration of multiple sclerosis during pregnancy. J Neurol Sci. 2007;252:83-87.
85. Huang YH, Airas L, Schwab N, Wiendl H. Janus head: the dual role of HLA-G in CNS immunity. Cell Mol Life Sci. 2011;68:407-416.
86. Fainardi E, Rizzo R, Melchiorri L, Stignani M, Castellazzi M, Tamborino C, Paolino E, Tola MR, Granieri E, Baricordi OR. CSF levels of soluble HLA-G and Fas molecules are inversely associated to MRI evidence of disease activity in patients with relapsing-remitting multiple sclerosis. Mult Scler. 2008;14:446-454.
87. Airas L, Nikula T, Huang YH, Lahesmaa R, Wiendl H. Postpartum-activation of multiple sclerosis is associated with down-regulation of tolerogenic HLA-G. J Neuroimmunol. 2007;187:205-211.
88. Tsunoda I, Fujinami RS. Two models for multiple sclerosis: experimental allergic encephalomyelitis and Theiler's murine encephalomyelitis virus. J Neuropathol Exp Neurol. 1996;55:673-686.

89. Langer-Gould A, Garren H, Slansky A, Ruiz PJ, Steinman L. Late pregnancy suppresses relapses in experimental autoimmune encephalomyelitis: evidence for a suppressive pregnancy-related serum factor. J Immunol. 2002;169:1084-1091.
90. McClain MA, Gatson NN, Powell ND, Papenfuss TL, Gienapp IE, Song F, Shawler TM,Kithcart A, Whitacre CC. Pregnancy suppresses experimental autoimmune encephalomyelitis throughimmunoregulatory cytokine production. J Immunol. 2007;179:8146-8152.
91. Gatson NN, Williams JL, Powell ND, McClain MA, Hennon TR, Robbins PD, Whitacre CC. Induction of pregnancy during established EAE halts progression of CNS autoimmune injury via pregnancy-specific serum factors. J Neuroimmunol. 2011;230:105-113.
92. Kim S, Liva SM, Dalal MA, Verity MA, Voskuhl RR. Estriol ameliorates autoimmune demyelinating disease: implications for multiple sclerosis. Neurology. 1999;52:1230-1238.
93. Bebo BF Jr, Fyfe-Johnson A, Adlard K, Beam AG, Vandenbark AA, Offner H. Low-dose estrogen therapy ameliorates experimental autoimmune encephalomyelitis in two different inbred mouse strains. J Immunol. 2001;166:2080-2089.
94. Offner H. Neuroimmunoprotective effects of estrogen and derivatives in experimentalutoimmune encephalomyelitis: therapeutic implications for multiple sclerosis. J Neurosci Res. 2004;78:603-624.
95. Sicotte NL, Liva SM, Klutch R, Pfeiffer P, Bouvier S, Odesa S, Wu TC, Voskuhl RR. Treatment of multiple sclerosis with the pregnancy hormone estriol. Ann Neurol. 2002;52:421-428.
96. Soldan SS, Alvarez Retuerto AI, Sicotte NL, Voskuhl RR. Immune modulation in multiple sclerosis patients treated with the pregnancy hormone estriol. J Immunol. 2003;171:6267-6274.
97. Alonso A, Clark CJ. Oral contraceptives and the risk of multiple sclerosis: a review of the epidemiologic evidence. J Neurol Sci. 2009;286:73-75.
98. Mastorakos G, Ilias I. Maternal hypothalamic-pituitary-adrenal axis in pregnancy and the postpartum period. Postpartum-related disorders. Ann N Y Acad Sci. 2000;900:95-106.
99. Miller DM, Weinstock-Guttman B, Béthoux F, Lee JC, Beck G, Block V, Durelli L, LaMantia L, Barnes D, Sellebjerg F, Rudick RA. A meta-analysis of methylprednisolone in recovery from multiple sclerosis exacerbations. Mult Scler. 2000;6:267-273.
100. Gregg C. Pregnancy, prolactin and white matter regeneration. J Neurol Sci. 2009;285:22-27.
101. Riskind PN, Massacesi L, Doolittle TH, Hauser SL The role of prolactin in autoimmune demyelination: suppression of experimental allergic encephalomyelitis by bromocriptine. Ann Neurol. 1991;29:542-547.
102. Dijkstra CD, van der Voort ER, De Groot CJ, Huitinga I, Uitdehaag BM, Polman CH, Berkenbosch F. Therapeutic effect of the D2-dopamine agonist bromocriptine on acute and relapsing experimental allergic encephalomyelitis. Psychoneuroendocrinology 1994;19:135-142.
103. Nociti V, Frisullo G, Tartaglione T, Patanella AK, Iorio R, Tonali PA, Batocchi AP. Multiple sclerosis attacks triggered by hyperprolactinemia. J Neurooncol. 2010;98:407-409.
104. Kira J, Harada M, Yamaguchi Y, Shida N, Goto I. Hyperprolactinemia in multiple sclerosis. J Neurol Sci. 1991;102:61-66.
105. Then Bergh F, Kümpfel T, Schumann E, Held U, Schwan M, Blazevic M, Wismüller A,Holsboer F, Yassouridis A, Uhr M, Weber F, Daumer M, Trenkwalder C, Auer DP. Monthly intravenous methylprednisolone in relapsing-remitting multiple sclerosis - reduction of enhancing lesions, T2 lesion volume and plasma prolactin concentrations. BMC Neurol. 2006 May 23;6:19.
106. Ness JM, Chabas D, Sadovnick AD, Pohl D, Banwell B, Weinstock-Guttman B; International Paediatric MS Study Group. Clinical features of children and adolescents with multiple sclerosis. Neurology 2007;68:S37-45.
107. Banwell B, Shroff M, Ness JM, Jeffery D, Schwid S, Weinstock-Guttman B; International Paediatric MS Study Group. MRI features of paediatric multiple sclerosis. Neurology 2007;68:S46-53.
108. Pohl D, Waubant E, Banwell B, Chabas D, Chitnis T, Weinstock-Guttman B, Tenembaum S; International Paediatric MS Study Group. Treatment of paediatric multiple sclerosis and variants. Neurology 2007;68:S54-65.
109. Krupp LB, Banwell B, Tenembaum S; for the international paediatric MS study group. Consensus definitions proposed for paediatric multiple sclerosis and related disorders. Neurology 2007;68:S7-S12.
110. Ebers GC. Environmental factors and multiple sclerosis. Lancet Neurol. 2008;7:268-277.

111. Hahn CD, Manohar M, Shroff M, Blaser S, Banwell B. MRI criteria for multiple sclerosis. Evaluation in a paediatric cohort. Neurology 2004;62:806-808.
112. Menge T, Hemmer B, Nessler S, Wiendl H, Neuhaus O, Hartung HP, Kieseier BC, Stüve O.Acute disseminated encephalomyelitis: an update. Arch Neurol. 2005;62:1673-1680.
113. Mikaeloff Y, Caridade G, Husson B, Suissa S, Tardieu M; Neuropediatric KIDSEP Study Group of the French Neuropediatric Society.Acute disseminated encephalomyelitis cohort study: Prognostic factors for relapse. Eur J Paediatr Neurol. 2007;11:90-95.
114. Mikaeloff Y, Suissa S, Vallée L, Lubetzki C, Ponsot G, Confavreux C, Tardieu M; KIDMUS Study Group.First episode of acute CNS inflammatory demyelination in childhood: Prognostic factors for multiple sclerosis and disability. J Pediatr. 2004;144:246-252.
115. Tenenbaum S, Chamoles N, Fejerman N. Acute disseminated encephalomyelitis. Along-term follow-up study of 84 paediatric patients. Neurology 2002;59:1224-1231.
116. Tardieu M, Mikaeloff Y. What is acute disseminated encephalomyelitis (ADEM)? Eur J Peadiatr Neurol. 2004;8:239-242.
117. Mikaeloff Y, Adamsbaum C, Husson B, Vallée L, Ponsot G, Confavreux C, Tardieu M, Suissa S; KIDMUS Study Group on Radiology. MRI prognostic factors for relapse after acute CNS inflammatory demyelination in childhood. Brain 2004;127:1942-1947.
118. Callen DJ, Shroff MM, Branson HM, Li DK, Lotze T, Stephens D, Banwell BL. Role of MRI in the differentiation of ADEM from MS in children. Neurology 2009;72:968-973.
119. Callen DJ, Shroff MM, Branson HM, Lotze T, Li DK, Stephens D, Banwell BL. MRI in the diagnosis of paediatric multiple sclerosis. Neurology 2009;72:961-967.
120. Mikaeloff Y, Caridade G, Assi S, Suissa S, Tardieu M. Prognostic factors for early severity in a childhood multiple sclerosis cohort. Paediatrics 2006;118:1133-1139.

CHAPTER 2

PREGNANCY AND MULTIPLE SCLEROSIS

CHAPTER 2.1

PREGNANCY IN MULTIPLE SCLEROSIS: CLINICAL AND SELF-REPORT SCALES

R.F. Neuteboom, A.C.J.W. Janssens, T.A.M. Siepman, I.A. Hoppenbrouwers, I.A. Ketelslegers, N. Jafari, E.A.P. Steegers, C.J.M. De Groot and R.Q. Hintzen

ABSTRACT

Relapse rate is decreased during pregnancy in multiple sclerosis (MS). Risk for postpartum relapse is increased in the first 3 months after delivery. We aimed to study clinical course of MS around pregnancy, using clinical as well as self-report scales, including data on quality of life (QoL), and to identify clinical factors predisposing for postpartum relapse. We performed a prospective, longitudinal study among 35 MS patients and 20 controls. In patients we assessed expanded disability status scale (EDSS), the Guy's neurological disability scale (GNDS) and the multiple sclerosis impact scale 29 (MSIS-29). In patients and controls we assessed the MOS 36 item short form health survey questionnaire (SF36), consisting of eight domains. The previously described surge in relapses after delivery was also obvious in this study ($p = 0.005$). At group level EDSS and MSIS-29 did not show overt fluctuations over time. The GNDS, however, improved during the third trimester, compared to the first trimester ($p = 0.003$). A concomitant improvement in the SF36 domains vitality ($p < 0.001$) and general health ($p = 0.001$) was found in patients. At the final visit, at least 9 months after delivery, no worsening of EDSS, GNDS, MSIS-29 or SF36 was observed compared with the (for MS, beneficial) third trimester. Duration of disease, relapses in the year preceding pregnancy or relapses during pregnancy were not associated with postpartum relapse. QoL is improved during pregnancy. Although relapse rate was increased directly after delivery, in the mid long term after delivery no adverse effects of pregnancy on MS were found.

INTRODUCTION

The clinical debut of multiple sclerosis (MS) in women is often during the childbearing years.[1] Factors associated with family planning are a major concern when having a chronic disease like MS. Pregnancy has a known ameliorating effect on the disease activity.[2] In the third trimester the relapse rate is decreased by around 70% compared to the year preceding pregnancy. Yet in the first 3 months after delivery, one in every three women has a relapse.[2-3]

Several questions arise when counselling women with MS who want to become pregnant. First, the timing of a postpartum relapse, early after delivery, may unfavourably influence daily care for the newborn infant. Prediction of a postpartum relapse would be most helpful in order to anticipate on this postpartum relapse. A previous study showed that the following three clinical factors were found to be predictive of a postpartum relapse: (1) the number of relapses in the year preceding pregnancy, (2) the number of relapses during pregnancy, and (3) the duration of disease.[3] These findings have not been validated in an independent prospective cohort.

Second, the clinical data in the few available studies on pregnancy and MS are limited to the expanded disability status scale (EDSS) and relapse rate. No quality of life (QoL) issues or self report scales were addressed in these studies. Next to insight in disease course in terms of disability and attack frequency, also better insight in QoL during and after pregnancy is necessary to inform women with MS who want to become pregnant.

Third, it has been shown that the relapse rate declines after a peak in the first 3 months after delivery. Nine months after delivery the relapse rate is comparable with the relapse rate in the year preceding pregnancy. Whether QoL at that time has improved or worsened is unknown.

The aims of this prospective and longitudinal study were not only to confirm data on EDSS and relapse rate, but also to address clinical course and outcome by using clinical scales as well as self-report scales including QoL parameters. Our second goal was to identify or confirm clinical risk factors predisposing for postpartum relapse.

MATERIALS AND METHODS

Participants and procedures

The Rotterdam Study on Pregnancy in MS was performed at ErasMS, the MS Centre at Erasmus MC. Ambulant MS patients, with a relapsing remitting disease course, were invited to participate. Control patients were recruited from the out-patient clinic of Obstetrics at the Erasmus MC. Exclusion criteria for MS patients and controls were recurrent abortion, hypertension, diabetes mellitus or other systemic diseases. The study was designed to include patients preconceptionally, but inclusion was also allowed during pregnancy. Inclusion of all controls occurred during the first trimester. All MS patients and controls were seen at 10–12 weeks and 28–30 weeks of pregnancy, and at 4–8 weeks after delivery. MS patients were also invited at a time point at least 9 months after delivery, without clinical infection or recent disease activity.

Data on maternal age, disease duration, and parity were collected at the first visit. Data on gestational age at delivery, multiple pregnancy, (pre)eclampsia, delivery, birth weight, and breastfeeding were collected at the last two visits. Data on relapse rate before pregnancy, during the three trimesters of pregnancy, and every 3 months in the first year after delivery were collected by interview at all these time points. The Guy's neurological disability scale (GNDS), multiple sclerosis impact scale 29 (MSIS-29), and EDSS were assessed in patients at every visit. Both patients and healthy controls were asked to evaluate their health related QoL the month prior to evaluation using the MOS 36 item short form health survey questionnaire (SF36) at each visit.

This study was approved by the ethics committee of the Erasmus MC and all participants gave written informed consent.

Measurements

A relapse was defined as an episode of neurological disturbance for which causative lesions are likely to be inflammatory and demyelinating in nature and lasting more than 24 h.[4] Pseudo-relapse, meaning recurrence of previous signs and symptoms or fever-related worsening, was not considered as a relapse. Disease duration was measured from time of diagnosis.

The EDSS is the most widely used scoring system in MS and consists of findings of the neurological examination.[5] The EDSS is an ordinal score that ranges from 0.0 (no disability) to 10.0 (death due to MS). The EDSS was scored at every visit by trained neurologists (RFN, TAMS, IAH, IAK, NJ, and RQH).

The GNDS consists of 12 items, all generating a score ranging from 0 (optimal health) to 5 (poor health) with a summed maximum total score of 60.[6] Items are cognition, mood, vision, speech, swallowing, lower and upper extremity function, bladder and bowel function, sexual function, fatigue, and one free item addressing another problem not yet contained in other items.

The MSIS-29 is a validated self-report scale consisting of two domains: physical health (20 items) and mental health (nine items).[7-9] Both domains generate a score ranging from 0 (optimal health) to 100 (poor health). The questions reflected on the 2 weeks preceding evaluation.

The SF36 is an instrument comprising four physical health domains and four mental health domains and has been used before in MS patients.[10-12] The physical health domains are physical functioning, role physical functioning, bodily pain, and general health. The four mental health domains are social functioning, vitality, role emotional functioning, and mental health. For each domain a score is generated ranging from 0 (poor health) to 100 (optimal health).

Statistical analysis

Differences in demographic and pregnancy characteristics and the SF36 between patients and healthy women were compared using the Student's t test or Mann–Whitney U test

for continuous variables and chi-square for categorical variables. The same statistics were used for comparing characteristics of MS patients with and without a postpartum relapse. Relapse rate, clinical scales and self-report scales at the different time points were compared to the third trimester using a multiple measurements linear mixed model analysis. The third trimester was used as reference because maximal disease amelioration has been reported before at that time point.[2] A p-value below 0.01 was considered significant. Data were analyzed using SPSS version 16.0.

RESULTS

Characteristics of the study population

Thirty-five women with MS and 20 controls participated in this study. Eighteen MS patients were included before pregnancy, 16 during the first trimester, and one during the third trimester. The mean time from inclusion to pregnancy in the 18 MS patients was 6 [standard deviation (SD): ±3.9, range 1–20] months. The mean time from delivery to the final visit in the MS group was 10 (SD: ±3.5, range 8–24) months. All controls were enrolled in the first trimester of pregnancy.

The controls were recruited from the outpatient clinic of Obstetrics in our hospital. The reasons for their referral were: previous cesarean section (six), history of cardiac problems (three, of which one also had twin pregnancy), medically assisted pregnancy (two), prematurity in previous pregnancy (two), previous post-natal depression (one), uterus duplex (one), epilepsy (one), twin pregnancy (one), metabolic disorder (one). Two women had no specific medical indication.

In the MS patients median disease duration was 4.0 (SD: ±4.0, range 0–13) years. Median EDSS was 1.5 (SD: ±0.8, range 0–4.0) in the first trimester (n = 34). Three MS patients received intravenous immunoglobulins directly after delivery with the aim to prevent possible relapses.[13] One woman had already received corticosteroids as treatment for a postpartum relapse at the time of her first postpartum visit. At time of the final visit four patients used immune modulating drugs.

Data on pregnancy and birth outcome are presented in Table 1. A smaller proportion of women were nulliparous in the control group, compared to the MS patients. Breastfeeding at time of the first postpartum visit was more frequently observed in the MS group, compared to the control group.

Table 1: Maternal age and data on pregnancy and birth outcome

	Patients N=35	Controls N=20
maternal age (±SD) (years)	31.2 (±3.8)	31.3 (±4.6)
nulliparous	71%	35%
cesarean section	14%	25%
assisted vaginal delivery (forceps/vacuum)	11%	5%
multiple (twin) pregnancy	0%	10%
gestational age at delivery (±SD) (weeks)	39.2 (±1.5)	38.1 (±2.7)
prematurity (<37 weeks of gestation)*	6%	5%
birth weight (± SD) (grams) *	3329 (±354)	3496 (±686)
small for gestational age **	0%	0%
breast feeding	69%	40%
(pre)eclampsia	0%	0%

MS = Multiple Sclerosis, SD = standard deviation
* not including twin pregnancy
** small for gestational age is defined as birth weight under -2SD, using standardized intrauterine growth charts

Annualized relapse rate

The annualized relapse rate is depicted in Fig. 1. After delivery a significantly increased relapse rate was observed during the first 3 months after delivery compared to the third trimester (p = 0.005). There was no significant difference in the annualized relapse rate in the year preceding pregnancy compared to the period 9-12 months after delivery.

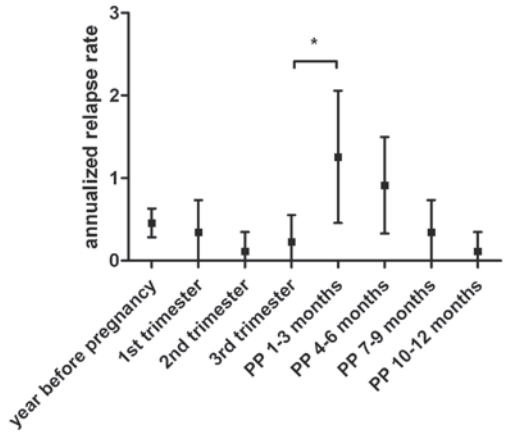


Figure 1. Relapse rate: before, during and after pregnancy in MS patients
Values represent the mean (±95%CI) annualized relapse rate. The third trimester was used as reference.
* P-value<0.01
PP = postpartum

MS specific clinical and self report scales

The MS specific clinical and self report scales are shown in Fig. 2. The GNDS showed a significant drop during the third trimester compared to the first trimester (p = 0.003) and the first postpartum visit (p = 0.001). We observed that about 85% of the mean drop in GNDS was mainly explained by three of the 12 items. These items concerned sexuality (31%), fatigue (28%), and a free item in which a complaint could be issued that was not addressed before in the other eleven items of the GNDS (26%). No differences were observed in the two domains of the MSIS-29 or the EDSS during the study.

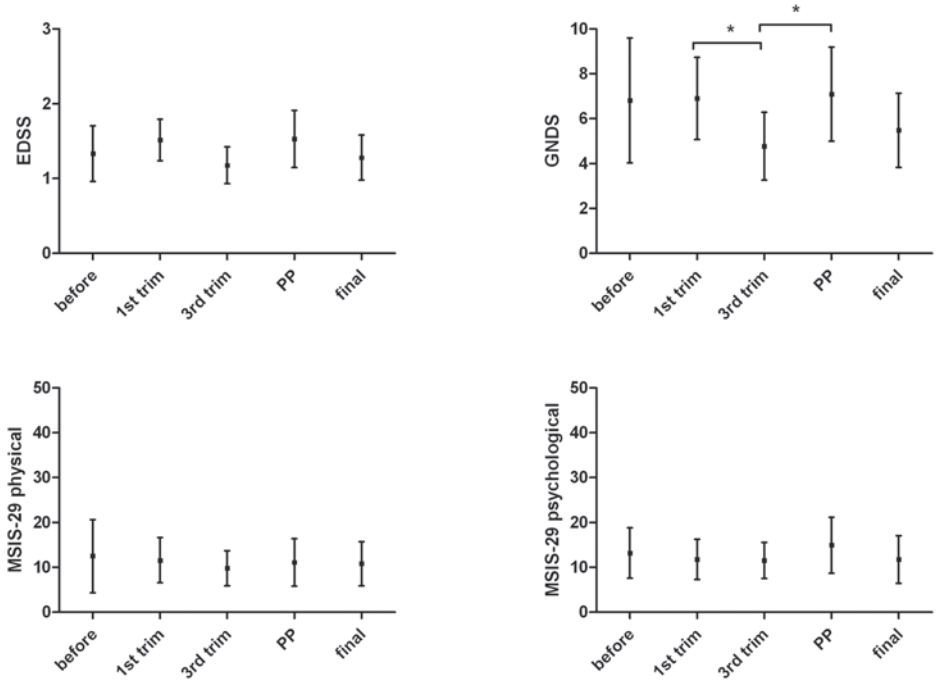


Figure 2. EDSS, GNDS and MSIS29 scores: before, during and after pregnancy in MS

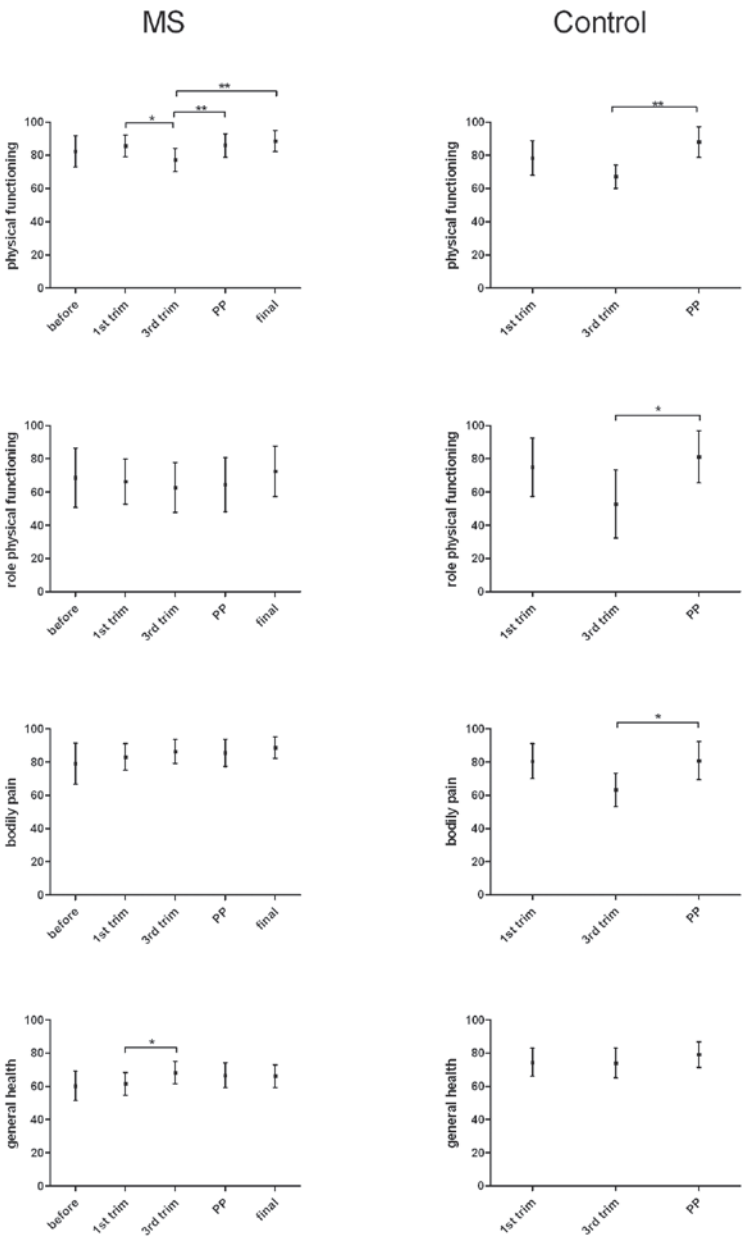
Values represent mean (±95%CI) scores of the EDSS, GNDS, MSIS-29 (physical and psychological subscale). The third trimester was used as reference.

* P<0.01

PP = postpartum, EDSS = expanded disability status scale, GNDS = Guy's neurological disability scale, MSIS-29 = multiple sclerosis impact scale 29

Quality of life

The SF36 was measured in both healthy controls and MS patients. The eight domains of the SF36 are shown in Fig. 3. MS patients reported an improvement in general health and in vitality during the third trimester compared to the first trimester (respectively, p = 0.001 and p < 0.001). Healthy women reported an evident and significant increase in bodily pain


MS
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physical functioning
role physical functioning
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general health
before
1st trim
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PP
final
*
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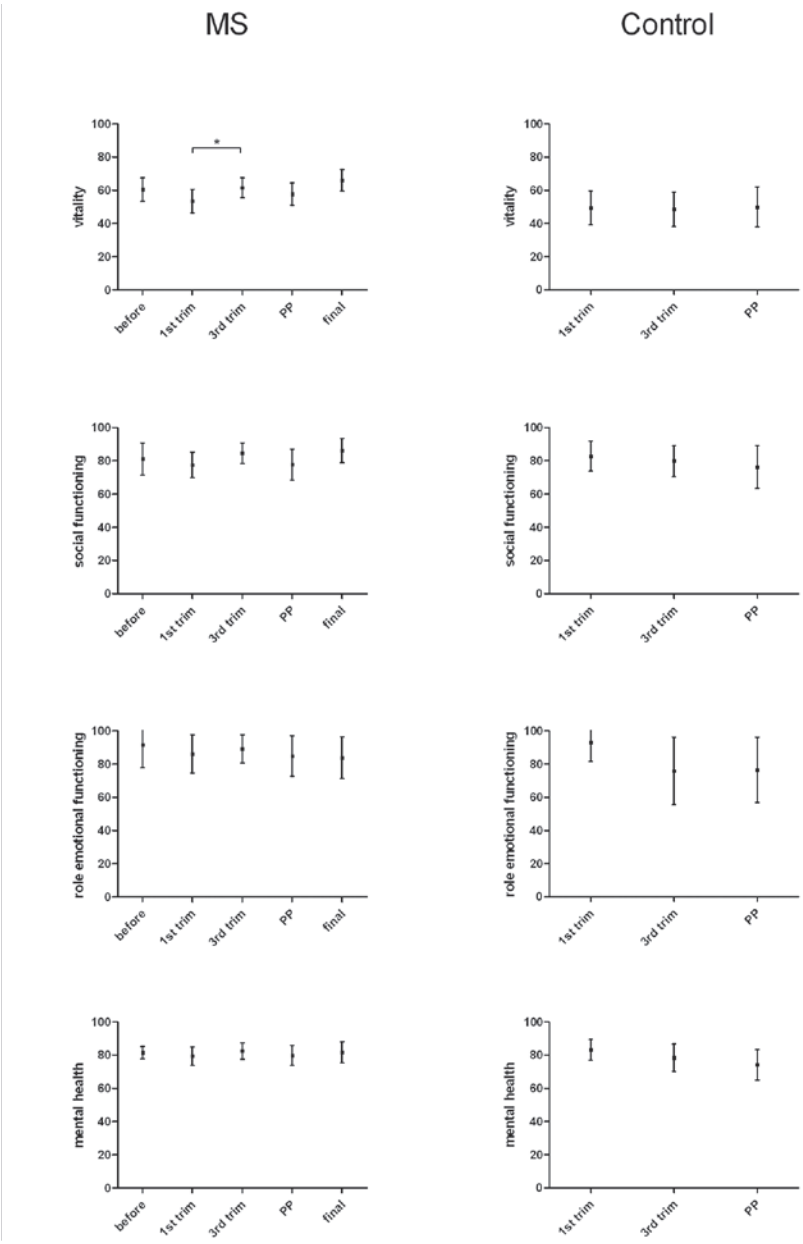


Figure 3. SF36 subscale scores: before, during and after pregnancy in MS and healthy women
Values represent mean (±95%CI) scores on the 8 subscales of the SF36: physical functioning, role physical functioning, bodily pain, general health, vitality, social functioning, role emotional functioning and mental health. The third trimester was used as reference. Scores range from 0 = poor health to 100 = optimal health.
* P-value <0.01, ** P-value <0.001
PP = postpartum, SF36 = MOS 36 item short form health survey questionnaire

at time of the first postpartum visit (p = 0.002) compared to the first trimester. The controls reported significantly more bodily pain than MS patients during the third trimester (p < 0.001). A significant decrease in physical functioning in MS patients was found during the third trimester, compared to the first trimester (p = 0.002). Physical functioning improved significantly after delivery in both MS patients (p < 0.001) and healthy women (p < 0.001). Role physical functioning was decreased in the controls during the third trimester compared the first postpartum visit (p = 0.009). No differences were found in the domains social functioning, role emotional functioning and mental health.

Postpartum relapse

Postpartum relapse occurred in 10 out of 35 MS patient (29%). One out of the three women treated with intravenous immunoglobulins after delivery had a postpartum relapse. The differences between the patients with and without a postpartum relapse are shown in Table 2. Women with a relapse during pregnancy were more likely to also experience a relapse after delivery. This difference was not significant. At the final visit, we found no differences in all assessment instruments, between patients with and without a postpartum relapse. Both groups also showed no differences in all assessment instruments comparing their third trimester visit with their final visit.

Table 2: Characteristics of patients with and without postpartum relapse

	PP relapse N=10	No PP relapse N=25	P-value
maternal age (±SD) (years)	31.1 (±4.5)	31.3 (±3.6)	0.76
disease duration (±SD) (years)	2.9 (±2.0)	5.0 (±4.5)	0.36
relapse in year before pregnancy (%)	50%	44%	1.0
relapse during pregnancy (%)	30%	8%	0.13
nulliparous (%)	70%	72%	1.0
median EDSS first trimester (IQR)	2.0 (1.5-2.13)	1.25 (1.0-1.88)	0.01
assisted vaginal delivery or cesarean section (%)	30%	24%	1.0

SD = standard deviation, PP = postpartum, IQR = interquartile range, EDSS = expanded disability status scale

DISCUSSION

We here present prospective and longitudinal data on clinical and QoL parameters during pregnancy in MS. We found an increase in relapse rate after delivery, comparable with previous studies.[2,3,14] We observed that this increase in relapse rate directly after delivery did not cause sustained adverse effects of pregnancy on MS in all used instruments at time of the final visit, 9 months or more after delivery.

During pregnancy and after delivery we observed no difference in the MS specific assessment instruments EDSS and the self report scale MSIS-29. A small improvement in

the GNDS was observed during the third trimester. Remarkably, this improvement in the GNDS was mainly explained by the domains concerning sexuality, fatigue, and the free item. Much in line with the decrease in fatigue is the improvement in the SF36 domains vitality and general health in MS patients during the third trimester. Contradictory to the amelioration of MS in the third trimester is the decreased health in the SF36 domain physical functioning in MS patients during the third trimester, compared with the first trimester and the first postpartum visit. On the other hand, decreased health in the SF36 domain physical functioning was also observed in controls during the third trimester compared to the postpartum visit. Therefore, this may be explained as a pregnancy effect and not MS related.

As described before[15,16], we recorded no major adverse effects on birth outcome. A higher proportion of neonates small for gestational age in mothers with MS has been reported.[15] We also found a lower birth weight in the MS group, but this did not reach statistical significance.

We also set out to validate several clinical risk factors for postpartum relapse that were previously identified.[3] These were disease duration, number of relapses in the year preceding pregnancy and number of relapses during pregnancy. We were not able to validate these findings in our cohort. We did observe that women with a relapse during pregnancy more likely also had a postpartum relapse, although this was not significant. It is possible that our numbers were too small to demonstrate such effects. Interestingly, a recent study showed that exclusive breastfeeding was associated with a smaller chance of postpartum relapse.[17] We and others could not reproduce these intriguing findings.[2,3,14,18,19] Taken together, we can conclude that, until now, there are no good clinical parameters predicting postpartum relapse. We, therefore, are in need of reliable biomarkers, able to predict a postpartum relapse. Interleukin 8 may be a candidate.[20]

Three points should be taken into account in respect to our data. First, the small numbers of patients and controls limited the conclusions of our study. We were not able to test for interaction effects and related measurements. In order not to make an over-interpretation we considered p-values under 0.01 to be significant and we used one visit as main reference. We chose the third trimester as main reference because maximal disease amelioration was reported at that time point.[2,3]

Second, the controls were referred to the outpatient clinic of Obstetrics mainly because of complications in previous pregnancies. Therefore, the chance of complications in the controls during these studied pregnancies and deliveries was increased. This may have influenced pregnancy outcome and QoL of the controls during and after pregnancy. It may likely explain why the controls reported more bodily pain than the women with MS during the third trimester.

Third, the participating patients in our study had a low EDSS, likely because we selected patients with a child wish. This low disability may have caused floor or ceiling effects limiting the ability of our measurement instruments to detect change. Especially the SF36 is sensitive to floor and ceiling effects.[21] It is possible that subtle differences in QoL were not

detected by the SF36.

We can conclude that in the mid-long term after pregnancy in MS patients EDSS, GNDS, MSIS-29, relapse rate and QoL are not unfavourably altered. We also showed that amelioration of disease course during pregnancy is not only defined by a decreasing relapse rate, but can also be regarded in terms of QoL, mostly appreciated in the SF36 domains vitality and general health. This underlines the necessity for including assessments of QoL when determining MS disease severity. The results from this study will help neurologists and MS-nurses counselling pregnant MS patients or MS patients with a child wish.

REFERENCES

1. Compston A, Coles. Multiple sclerosis. Lancet. 2008;372:1502-1517.
2. Confavreux C, Hutchinson M, Hours MM, Cortinovis-Tourniaire P, Moreau T. Rate of pregnancy-related relapse in multiple sclerosis. Pregnancy in multiple sclerosis group. N Engl J Med. 1998;339:285-291.
3. Vukusic S, Hutchinson M, Hours M, Moreau T, Cortinovis-Tourniaire P, Adeleine P, Confavreux C, The Pregnancy In Multiple Sclerosis Group; Pregnancy In Multiple Sclerosis Group. Pregnancy and multiple sclerosis (the PRIMS study): clinical predictors of post-partum relapse. Brain. 2004;127:1353-1360.
4. Polman CH, Reingold SC, Edan G, Filippi M, Hartung HP, Kappos L, Lublin FD, Metz LM, McFarland HF, O'Connor PW, Sandberg-Wollheim M, Thompson AJ, Weinshenker BG, Wolinsky JS. Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria". Ann Neurol. 2005;58:840-846.
5. Kurtzke JF. Rating neurological impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology. 1983;33:1444-1452.
6. Sharrack B, Hughes RAC. The Guy's neurological disability scale (GNDS): a new disability measure for multiple sclerosis. Mult Scler. 1999;5:223-233.
7. Hobart J, Lamping D, Fitzpatrick R, Riazi A, Thompson A. The multiple sclerosis impact scale (MSIS-29) a new patient-based outcome measure. Brain. 2001;124:962-973.
8. Riazi A, Hobart JC, Lamping DL, Fitzpatrick R, Thompson AJ. Multiple sclerosis impact scale (MSIS-29): reliability and validity in hospital based samples. J Neurol Neurosurg Psychiatry. 2002;73:701-704.
9. Gray O, McDonnell G, Hawkins S. Tried and tested: the psychometric properties of the multiple sclerosis impact scale (MSIS-29) in a population-based study. Mult Scler. 2009;15:75-80.
10. Ware JE, Sherbourne CD. The MOS 36-item short-form health survey (SF36). I. Conceptual framework and item selection. Med Care. 1992;30:473-483.
11. Janssens AC, van Doorn PA, de Boer JB, Kalkers NF, van der Meche FG, Passchier J, Hintzen RQ. Anxiety and depression influence the relation between disability status and quality of life in multiple sclerosis. Mult Scler. 2003;9:397-403.
12. Janssens AC, van Doorn PA, de Boer JB, van der Meché FG, Passchier J, Hintzen RQ. Impact of recently diagnosed multiple sclerosis on quality of life, anxiety, depression and distress of patients and partners. Acta Neurol Scand. 2003;108:389-395.
13. Haas J, Hommes OR. A dose comparison study of IVIG in postpartum relapsing-remitting multiple sclerosis. Mult Scler. 2007;13:900-908.
14. Fernández Liguori N, Klajn D, Acion L, Cáceres F, Calle A, Carrá A, Cristiano E, Deri N, Garcea O, Jaureguiberry A, Onaha P, Patrucco L, Riccio P, Rotta Escalante R, Saladino M, Sinay V, Tarulla A, Villa A. Epidemiological characteristics of pregnancy, delivery, and birth outcome in women with multiple sclerosis in Argentina (EMEMAR study). Mult Scler. 2009;15:555-562.
15. Dahl J, Myhr KM, Daltveit AK, Hoff JM, Gilhus NE. Pregnancy, delivery, and birth outcome in women with multiple sclerosis. Neurology. 2005;65:1961-1963 .
16. Kelly VM, Nelson LM, Chakravarty EF. Obstetric outcomes in women with multiple sclerosis and epilepsy. Neurology. 2009;73:1831-1836.
17. Langer-Gould A, Huang SM, Gupta R, Leimpeter AD, Greenwood E, Albers KB, Van Den Eeden SK, Nelson LM. Exclusive breastfeeding and the risk of postpartum relapses in women with multiple sclerosis. Arch Neurol. 2009;66:958-963.
18. Neuteboom RF, Hintzen RQ. Breast-feeding, postpartum and prepregnancy disease activity in multiple sclerosis. Neurology. 2011;76:1532-1533.
19. Airas L, Jalkanen A, Alanen A, Pirttilä T, Marttila RJ. Breast-feeding, postpartum and prepregnancy disease activity in multiple sclerosis. Neurology. 2010;75:474-476.
20. Neuteboom RF, Verbraak E, Voerman JS, van Meurs M, Steegers EA, de Groot CJ, Laman JD, Hintzen RQ. First trimester interleukin 8 levels are associated with postpartum relapse in multiple sclerosis. Mult Scler. 2009;15:1356-1358.
21. Freeman JA, Hobart JC, Langdon DW, Thompson AJ. Clinical appropriateness: a key factor in outcome measure selection: the 36 item short form health survey in multiple sclerosis. J Neurol Neurosurg Psychiatry. 2000;68:150-156.

CHAPTER 2.2

BREAST-FEEDING, POSTPARTUM AND PREPREGNANCY DISEASE ACTIVITY IN MULTIPLE SCLEROSIS

R.F. Neuteboom and R.Q. Hintzen

Breast-feeding and multiple sclerosis (MS) disease activity is controversial.[1] Previous studies have not shown the effects of breastfeeding on risk of a postpartum relapse.[2-3] In contrast, Langer-Gould et al.[4] suggested that exclusive breast-feeding for at least the first 2 months after delivery protected against postpartum relapse. This finding is not supported by Airas et al.[1], who found no protective effect of breast-feeding on the risk of postpartum relapse. We prospectively studied 35 women with relapsing-remitting MS, as described before.[5] Twenty-four (69%) patients breast-fed their babies for more than 2 months after delivery. Six out of these 24 (25%) who breast-fed their babies had a postpartum relapse in the first trimester after delivery, whereas 4 out of 11 (36%) who did not breastfeed their babies had a postpartum relapse (Fisher exact test: $p = 0.68$). Sixteen patients (46%) had a more severe disease course before pregnancy, defined as an annualized relapse rate greater than or equal to 1. Ten of these 16 (63%) patients breast-fed their babies, vs 14 out of 19 (74%) patients who had a benign disease course before pregnancy (Fisher exact test: $p = 0.71$). Total time of breast-feeding was shorter in the patients with a severe disease course (mean ± SD: 14.8 ± 11.9 weeks) when compared with the patients with a benign disease course (mean ± SD: 21.7 ± 18.9 weeks), although this difference was not significant (Mann-Whitney *U* test, $p = 0.38$). Only 3 patients did not start or discontinued breast-feeding because of a necessity to commence disease-modifying drugs (DMD). In our cohort, disease activity preceding pregnancy was not associated with the decision to start and continue breast-feeding. Therefore, this was not a confounder. Our results, although based on a relatively small cohort, confirm those of Airas et al. and others.[1-3] They all show no protective effect of breast-feeding. This may dampen the promising findings by Langer-Gould et al.[4] In the absence of clinical predictors of postpartum relapse[2], predictive markers are needed. Serum level of Interleukin 8 may be a candidate.[5]

REFERENCES

1. Airas L, Jalkanen A, Alanen A, Pirttilä T, Marttila RJ. Breast-feeding, postpartum and prepregnancy disease activity in multiple sclerosis. Neurology. 2010;75:474-476.
2. Vukusic S, Hutchinson M, Hours M, Moreau T, Cortinovis-Tourniaire P, Adeleine P, Confavreux C, The Pregnancy In Multiple Sclerosis Group; Pregnancy In Multiple Sclerosis Group. Pregnancy and multiple sclerosis (the PRIMS study): clinical predictors of post-partum relapse. Brain. 2004;127:1353-1360.
3. Confavreux C, Hutchinson M, Hours MM, Cortinovis-Tourniaire P, Moreau T. Rate of pregnancy-related relapse in multiple sclerosis. Pregnancy in multiple sclerosis group. N Engl J Med. 1998;339:285-291.
4. Langer-Gould A, Huang SM, Gupta R, Leimpeter AD, Greenwood E, Albers KB, Van Den Eeden SK, Nelson LM. Exclusive breastfeeding and the risk of postpartum relapses in women with multiple sclerosis. Arch Neurol. 2009;66:958-963.
5. Neuteboom RF, Verbraak E, Voerman JS, van Meurs M, Steegers EA, de Groot CJ, Laman JD, Hintzen RQ. First trimester interleukin 8 levels are associated with postpartum relapse in multiple sclerosis. Mult Scler. 2009;15:1356-1358.

CHAPTER 2.3

FIRST TRIMESTER INTERLEUKIN 8 LEVELS ARE ASSOCIATED WITH POSTPARTUM RELAPSE IN MULTIPLE SCLEROSIS

R.F. Neuteboom, E. Verbraak, J.S.A. Voerman, M. Van Meurs, E.A.P. Steegers, C.J.M. De Groot, J. D. Laman and R.Q. Hintzen

ABSTRACT

Pregnancy has an ameliorating effect on multiple sclerosis (MS), but directly after delivery the risk of a relapse is increased. The pro-inflammatory chemokine interleukin 8 is associated with disease activity. We aimed to investigate whether pregnancy-induced fluctuations of interleukin 8 correlate with periods of enhanced and diminished disease activity. Thirty-six women with MS were prospectively studied before, during and after pregnancy. Serum levels of interleukin 8 were significantly decreased during the third trimester ($p = 0.03$). High first trimester serum levels of interleukin 8 were associated with a high risk of postpartum relapse ($p = 0.007$). These results help us to further understand the altered disease course during pregnancy.

INTRODUCTION

Pregnancy ameliorates the disease course of multiple sclerosis (MS) reducing disease activity by 70%.[1] However, 30% of women with MS experience a postpartum relapse in the first 3 months after delivery.[1] It is postulated that shifts from a pro-inflammatory to an anti-inflammatory immune environment and vice versa may be responsible for these fluctuations.[2-3] A candidate mediator of these processes is the pro-inflammatory chemokine interleukin 8 (IL-8, CXCL8). IL-8 is a potent chemo-attractant involved in both attraction and infiltration of leucocytes (mainly neutrophils) at the site of inflammation.[4] In MS patients, IL-8 expression is increased and is associated with increased disease activity.[4-5] Chemokines play an important role for the maintenance of (early) pregnancy.[6] No longitudinal studies on IL-8 levels in maternal serum in women with MS have been provided yet. Our aim was to assess whether pregnancy induces alterations in serum IL-8 levels and whether such alterations are associated with the risk of a postpartum relapse.

PATIENTS AND METHODS

Patients

MS patients with a relapsing-remitting disease course and who were ambulant were recruited at the Rotterdam MS Center, ErasMS. Exclusion criteria were recurrent abortion, history of hypertension and diabetes mellitus. The study was designed to include patients pre-conceptionally though inclusion was also allowed during pregnancy. All MS patients were seen at 10–12 weeks and 28–30 weeks of pregnancy and at 4–8 weeks after delivery. Clinical follow-up data were available for more than 3 months after delivery in order to determine whether a postpartum relapse occurred. A relapse was defined as an episode of neurological disturbance for which causative lesions are likely to be inflammatory and demyelinating in nature and lasting more than 24 hours.[7] MS severity was determined using the Expanded Disability Status Scale (EDSS).[8] This study was approved by the ethical committee of Erasmus University and all patients gave written informed consent.

Serum IL-8 levels

Blood samples were taken at each visit between 10 and 12 am. Serum was stored at -80°C. Serum IL-8 levels were measured by enzyme-linked immunosorbent assay (ELISA), according to the manufacturer's protocol (Sanquin, Amsterdam, The Netherlands). The detection range of the assay was between 1 and 240 pg/ml.

Data analysis

The Mann–Whitney U test was used for a comparison of continuous variables. The Fisher's exact test was used for a comparison of dichotomized data. Serum levels of IL-8 at the different time points were compared using a multiple measurements linear mixed model analysis. P-values below 0.05 were considered significant. Data were analyzed using SPSS version 16.0.

RESULTS

Patients and pregnancy outcome

Serum IL-8 levels were assessed in 36 pregnant women with MS of which 17 were included before pregnancy (mean 6 months (SD: ±3.8) before pregnancy), 17 during the first trimester and 2 during the third trimester. In one MS patient the third trimester visit was lacking. Mean MS disease duration before the start of the studied pregnancy was 4.5 (SD: ±3.8) years. Mean maternal age was 30.9 (SD: ±3.7) years. Median EDSS measured at first trimester visit was 1.5 (range 0–4.0). None of the patients received medication except for three patients who received intravenous immunoglobulins directly after delivery, with the aim of protecting against possible exacerbations.[9] Mean duration of gestation was 39.1 (SD: ±1.6) weeks. Mean birth weight was 3,369 (SD: ±413) grams. None of the children were small for gestational age (defined as less -2 SD using standardized intra-uterine growth charts). Eleven women with MS (31%) had a relapse in the first 3 months after delivery.

Serum IL-8 levels

During the third trimester of pregnancy a significant decrease was observed, compared with the first trimester (Figure 1A). First trimester serum levels were higher in the group with an eventual postpartum relapse compared with the group that did not ($p = 0.015$; Figure 1B). The same held true for the serum levels of IL-8 before pregnancy, although these numbers were small for statistical comparison (data not shown). In order to compare women with high and low IL-8 levels in the first trimester we digitomized the group using the median first trimester levels of IL-8 (=6.8 pg/ml). We observed that serum IL-8 levels during the first trimester below 6.8 pg/ml were highly predictive of being relapse free in the first 3 months after delivery; 1 out of 17 (6%) patients with serum IL-8 levels below 6.8 pg/ml had a postpartum relapse versus 9 out of 17 (53%) with serum IL-8 levels above 6.8 pg/ml ($p = 0.007$). Using this cut-off value results in a sensitivity of 90% (9/10) and specificity of 67% (16/24).

DISCUSSION AND CONCLUSION

Here we have presented a longitudinal and prospective study on serum levels of IL-8 during and around pregnancy in MS patients. We found that during pregnancy IL-8 levels were decreased in the third trimester when compared with the first trimester. This drop in the pro-inflammatory chemokine IL 8 is compatible with the lower disease activity during the third trimester. Moreover, we found that serum levels of IL-8 at the time of the first trimester were associated with postpartum relapse in the first 3 months after delivery. From a clinical point of view the possibility to predict post partum relapses would be welcome, at least for practical reasons such as the ability to identify those patients who would or would not benefit from prophylactic treatment such as intravenous immunoglobulins.[9] Clinical parameters studied before proved to be insufficient predictors of postpartum relapse at

the individual level.[10] Using our, arbitrarily chosen, cut-off level of first trimester IL-8 levels resulted in a, possibly clinically useful, negative predictive value of 94%. Yet the low positive predictive value of 53% and our relative small numbers limit the clinical use of IL-8 as a predictive parameter in this setting for now.

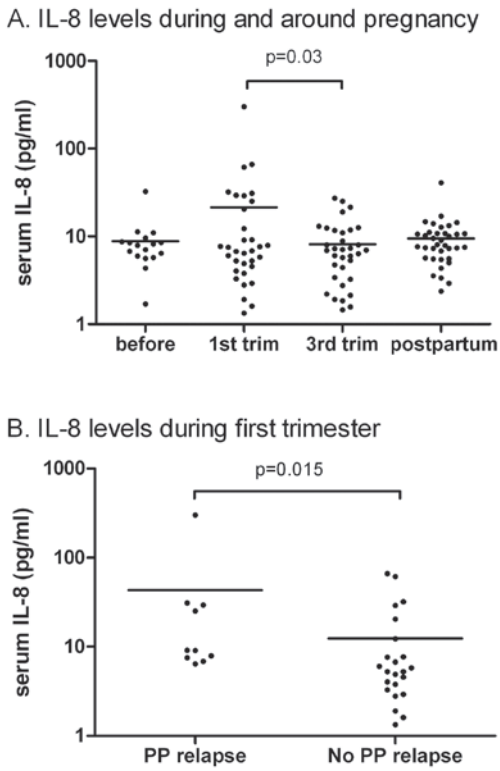


Figure 1. (A) Serum levels of IL-8 in MS patients before pregnancy, at time of the first and third trimester and 4-8 weeks postpartum. Horizontal lines represent means. (B) Serum levels of IL-8 in MS patients with and without a postpartum (PP) relapse and without at time of first trimester. Horizontal lines represent means.

REFERENCES

1. Confavreux C, Hutchinson M, Hours MM, Cortinovis-Tourniaire P, Moreau T and the pregnancy in multiple sclerosis group. Rate of pregnancy-related relapse in multiple sclerosis. N Engl J Med. 1998;339:285-291.
2. Wegmann TG, Lin H, Guilbert L and Mosmann TR. Bidirectional cytokine interaction in maternal fetal relationship: is successful pregnancy a Th2 phenomenon? Immunol Today. 1993;14:353-356
3. Sargent IL, Borzychowski AM and Redman CW. NK cells and human pregnancy - an inflammatory view. Trends Immunol. 2006;27:399-404.
4. Lund BT, Ashikian N, Ta HQ, Chakryan Y, Manoukian K, Groshen S, Gilmore W, Cheema GS, Stohl W, Burnett ME, Ko D, Kachuck NJ, Weiner LP. Increased CXCL8 (IL-8) expression in Multiple Sclerosis. J Neuroimmunol. 2004;155:161-71.
5. Bartosik-Psujek H and Stelmasiak Z. The levels of chemokines CXCL8, CCL2 and CCL5 in multiple sclerosis patients are linked to the activity of the disease. Eur J Neurol. 2005;12:49-54.
6. Hannan NJ and Salamonsen LA. Role of chemokines in the endometrium and in embryo implantation. Curr Opin Obstet Gynecol. 2007;19:266-272.
7. Polman CH, Reingold SC, Edan G, Filippi M, Hartung HP, Kappos L, Lublin FD, Metz LM, McFarland HF, O'Connor PW, Sandberg-Wollheim M, Thompson AJ, Weinshenker BG, Wolinsky JS. Diagnostic criteria for multiple sclerosis:2005 revisions to the "McDonald Criteria". Ann Neurol. 2005;58:840-846.
8. Kurtzke JF. Rating neurological impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology. 1983;33:1444-1452.
9. Haas J and Hommes OR. A dose comparison study of IVIG in postpartum relapsing-remitting multiple sclerosis. Multiple Sclerosis. 2007;13:900-908.
10. Vukusic S, Hutchinson M, Hours M, Moreau T, Cortinovis-Tourniaire P, Adeleine P, Confavreux C, The Pregnancy In Multiple Sclerosis Group; Pregnancy In Multiple Sclerosis Group. Pregnancy and multiple sclerosis (the PRIMS study): clinical predictors of post-partum relapse. Brain. 2004;127:1353-1360.

CHAPTER 2.4

THE MONOCYTE TRANSCRIPTOME DURING PREGNANCY IN MULTIPLE SCLEROSIS: PROMINENT EXPRESSION OF THE FC-RECEPTOR CD64

R.F. Neuteboom, E. Verbraak, A.F. Wierenga-Wolf, J.S.A. Voerman, M. Van Meurs, S.M. Swagemakers, P.J. Van Der Spek, E.A.P. Steegers, C.J.M. De Groot, J.D. Laman and R.Q. Hintzen

ABSTRACT

Background: During the third trimester of pregnancy multiple sclerosis (MS) disease activity is reduced. It is not fully understood which factors mediate this disease amelioration.
Objective: To study alterations of the monocyte transcriptome during pregnancy in MS patients, using a genomewide approach to identify differentially regulated genes.
Methods: Women with MS and healthy controls were longitudinally studied, including a visit before pregnancy.
Results: RNA-microarray analysis was performed in six patients. We found a significant increase of CD64 (Fc gamma receptor 1a, FcgR1a) during the third trimester compared with baseline, confirmed by RT-PCR in a group of ten patients. Analysis with Ingenuity software was performed using all genes expression of which was altered at least 1.5-fold in at least five out of six patients. Major networks that were altered during MS pregnancy were: cell-to-cell signalling and interaction, immune response, and cell signalling. From the genes selected for Ingenuity analysis, seven additional candidate genes, selected for their biological interest, were tested using RT-PCR in ten patients with MS and nine controls. We found an increased expression of JAK2 and STAT1 directly postpartum in patients with MS and in controls.
Conclusion: The increased CD64 expression during pregnancy is indicative of enhanced innate immune functions.

INTRODUCTION

In women, the debut of multiple sclerosis (MS) often occurs during childbearing years.[1] MS is considered to be a T-cell driven autoimmune disease.[2] Disease activity is thought to be mediated by CD4+ Thelper (Th) cells producing pro-inflammatory cytokines, such as IFN-γ and IL-17. T-cell subset differentiation and T-cell reactivity in the central nervous system (CNS) is driven by antigen presenting cells (APC) like dendritic cells and monocytes/macrophages.[2]

Pregnancy has an ameliorating effect on disease activity, especially during the third trimester with a 70% decrease of the annualized relapse rate. However, in the first 3 months after delivery disease activity rebounds and one in every three women with MS experiences a relapse.[3] The underlying biological mechanisms of pregnancy as an important biological modifier of MS disease course remain unclear. Multiple mechanisms have been suggested. Placental factors like trophoblast cells expressing HLA-G class and production of IL-10 and indoleamine-2,3-dioxygenase (IDO) have been reported.[4] Furthermore, a well-known proposition is that adaptive immunity during pregnancy skews from a pro-inflammatory phenotype towards an anti inflammatory phenotype.[4-5] Although one could expect pregnant women to have an increased number of infections, because of the immune-suppressive environment, there is no significant increase in the number of infections, nor evidence for an altered pattern of pathogens. It has been hypothesized that innate immunity is activated to compensate for the presumed decrease in adaptive immunity.[4,6-7] This activation includes expanded numbers and activity of monocytes/macrophages, key players in innate immunity.[8] The activation of monocytes/macrophages has not been explored during MS pregnancy thus far. Hence, the aim of our current study was to identify genes differentially expressed over the course of pregnancy and postpartum.

MATERIALS AND METHODS

Patients and healthy controls

Patients with MS and a relapsing–remitting disease course and who were ambulant were recruited at the Rotterdam MS Centre, ErasMS. Healthy control donors were recruited from our outpatient clinic of obstetrics. Exclusion criteria were recurrent abortion, history of hypertension and diabetes mellitus. The study was designed to include patients preconceptionally and inclusion was also allowed during pregnancy. Inclusion of all healthy controls occurred during the first trimester. All MS patients and controls were seen at 10–12 weeks and 28–30 weeks of pregnancy and at 4–8 weeks postpartum. The occurrence of a clinical infection was determined by anamnesis at the study visit, next to questionnaires.

A relapse was defined as an episode of neurological disturbance for which causative lesions are likely to be inflammatory and demyelinating in nature and lasting more than 24 h.[9] MS severity was determined using the Expanded Disability Status Scale (EDSS).[10] This study was approved by the ethics committee of the Erasmus University Medical Center and all patients gave written informed consent.

Blood samples
Blood samples were taken at each visit between 10 am and 12 noon. Peripheral blood mononuclear cells (PBMC) were isolated using CPT cell preparation tubes (Becton Dickinson, Breda, The Netherlands) and stored in liquid nitrogen until use.

Microarray analysis
CD14+ APC were selected using CD14 microbeads (Miltenyi, Utrecht, The Netherlands). Total RNA isolation from CD14 positive and negative cell fractions was performed using the RNeasy Mini Kit (Qiagen, Venlo, The Netherlands) following the manufacturer's procedures. Subsequently RNA was quantified using the Nanodrop spectrophotometer (Isogen Life Science, IJsselstein, The Netherlands). Integrity of the RNA was assessed by bioanalyser (Agilent, Amstelveen, The Netherlands) analysis. Next, 1mg RNA was amplified and biotin labelled using the IVT labelling kit (Affymetrix, Santa Clara, CA, USA) following the manufacturer's protocol. Human gene chip probe array (Affymetrix) analysis was performed according to the procedures provided by the manufacturer. Genechip probe arrays (Human genome U133 plus 2.0) were hybridized with 15mg cRNA at 45°C for 16 h. Scanning of the genechip arrays was performed using the Genechip Scanner 3000G7 (Affymetrix) at 570 nm. Results were analyzed by using the Genechip Operating Software (Affymetrix).

RNA and RT-PCR analysis
RNA isolation from CD14+ cells was performed by using the GenElute Mammalian Total RNA Miniprep Kit (Sigma, Zwijndrecht, The Netherlands). RNA samples were DNAse I treated to remove contaminating DNA (Invitrogen, Breda, The Netherlands). Using 0.5 mg RNA as template, copy DNA (cDNA) was reverse transcribed by using Superscript II (Invitrogen). Primers and probes were selected by using the Universal ProbeLibrary Assay Design Center (Roche, Almere, The Netherlands). To determine target gene mRNA expression, real-time quantitative reverse transcription PCR was performed using TaqMan technology as described previously. GAPDH mRNA levels were measured as a control to normalize for RNA input. An Applied Biosystems 7900 Sequence Detector was programmed for the initial step of 2 min at 50°C and 10 min at 95°C, followed by 40 thermal cycles of 15 s at 95°C and 1 min at 60°C. For calculation of mRNA expression levels Ct values per gene were applied to standard curves, generated for each specific gene of interest.

Data analysis
The percentage of present calls, noise, background, and on-chip housekeeping gene controls all indicated a high quality of samples and an overall comparability. Probe sets that were not present (according to Affymetrix MAS5.0 software) in any of the Genechips were omitted from further analysis. Raw intensities of the remaining probe sets of each chip were log2 transformed and normalized using quantile normalization. After normalization, the data were back-transformed to normal intensity values. Data analysis was carried out using excel and OmniViz software, version 3.6.0. Differentially expressed genes were identified

using statistical analysis of microarrays (SAM) or by calculating fold changes (at least 1.5-fold up or down in at least five out of six patients). Functional annotation of the results was done using Ingenuity Pathway Analysis (Ingenuity, Mountain View, CA) (www.ingenuity.com). RNA levels at the various time points were compared using a multiple measurements linear mixed model analysis. Bonferroni correction for multiple testing was applied. For comparison of continuous variables the Mann–Whitney U-test was used. For comparison of dichotomized data the Fisher's exact test was used. P values below 0.05 were considered significant. Data were analysed using SPSS version 16.0.

RESULTS

Patients and healthy women

RNA microarray analysis was performed on six women with MS, comparing the baseline (before pregnancy) visit with the third trimester visit. Validation of the microarray data by RT-PCR was performed for 10 women with MS and nine healthy women. In four out of these 10 women with MS both RNA microarray and RT-PCR were performed. None of the women with MS had a relapse, a clinical infection or additional medication during the study course. Demographic and clinical data are depicted in Table 1. No significant differences between the women with MS and healthy women were observed, except that women with MS were more often nullipara. None of the women with MS or healthy women had (pre-)eclampsia.

Table 1. Patients and healthy women, demographic and clinical data

	MS patients n=6 RNA microarray	MS patients n=10 RT-PCR	Healthy n=9 RT-PCR	P Value*
mean maternal age (years ±SD)	33.0 (± 3.0)	32.3 (± 3.7)	32.6 (± 2.0)	n.s.
median EDSS (range)	1.5 (0-2.0)	1.5 (0-2.0)	n.a.	n.s.
nullipara	5/6	10/10	5/9	0.033
caesarean section	1/6	3/10	4/9	n.s.
mean duration of gestation (weeks ±SD)	39.0 (±1.6)	39.4 (±1.4)	38.1 (±2.6)	n.s.
breastfeeding at last visit	5/6	6/10	4/9	n.s.

* MS patients n= 10 (RT-PCR) compared to healthy women n=9 (RT-PCR). Mann-Whitney U test and Fisher's exact test were used.

RNA microarray comparison of baseline and third trimester

Using a very stringent statistical analysis for microarray (SAM), we found a significant increase in expression of CD64 (Fc gamma receptor 1a) during the third trimester compared to the baseline (before pregnancy) visit. Also an increase in ankyrin repeat domain 22 (ANKRD22) expression was observed.

RT-PCR validation of increased CD64 expression

The significant increase in CD64 expression during the third trimester of pregnancy detected by microarray analysis was confirmed by RT-PCR. The primer set used is listed in Table 2. An increase in mRNA expression was observed during the first trimester, although not significant, and during the third trimester ($p<0.05$) when compared to the baseline (before pregnancy) visit ($p<0.05$) (Figure 1). For healthy women no baseline sample was available, so comparison of the baseline with third trimester was not possible. A significant increase of CD64 expression was observed directly post partum ($p<0.05$) in both MS patients and healthy women.

Table 2. Primer sets and function of genes used for RT-PCR

Gene name	Accession number	Primer sequelnces (5' to 3')	Exiqon probe nr	Gene function
CD64	(NM_000566)	F: tgggaaagcatcgctacac R: gcactggagctggaaatagc	18	high affinity receptor to the Fc fragment of IgG3> IgG1>IgG4> IgG2
Stat 1	(AY865620)	F: cagtgggtagacacaaaatgga R: cagaacaagaagagtatggcagaa	2	Cytokine receptor signalling
JAK2	(NM_004972)	F: ggtgaaagtcccatattctggt R: aggccacagaaaacttgctc	50	Cytokine receptor signalling
CD38	(NM_001775)	F: cagcaacaaccctgtttcagt R: ttgagcatcacatggaccac	27	NAD glycohydrolase, cell signalling
IL-8	(NM_000584)	F: agacagcagagcacacaagc R: atggttccttccggtggt	72	Neutrophil chemo-attraction
PTX3	(NM_002852)	F: gcggtgctagaggagctg R: ggaataaaatagctgtttcacaacct	23	Attenuation of neutrophil recruitment
CXCL2	(NM_002089)	F: catcgaaaagatgctgaaaaatg R: ttcaggaacagccaccaata	69	Neutrophil chemo-attraction

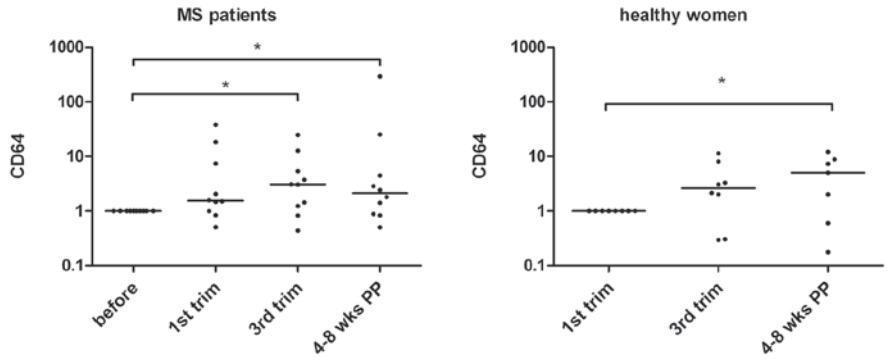


Figure 1. CD64 expression in MS patients and healthy women using quantitative RT-PCR
RNA expression compared to GAPDH (ratio: compared to first sample). Horizontal lines represent medians. Multiple measurements linear mixed model analysis. * P< 0.05.

Pathway analysis of microarray data

To further unravel potential disease ameliorating mechanisms Ingenuity analysis was applied. Therefore all genes that were increased or decreased at least 1.5-fold in five or six out of the six patients studied during the third trimester were entered into the Ingenuity program. Sixty-nine transcripts were found, of which 42 genes could be used in Ingenuity for network analysis. Twenty-one genes were involved in cell-to-cell signalling and interaction. Other molecular and cellular functions included: immune response (20 genes) and cell signalling (16 genes). The major networks found to be involved using Ingenuity web-based software are described in Table 3.

Table 3. Biological networks identified by Ingenuity analysis

Network	Genes in network	Top functions
1	Akt, Apyrase, BDKRB1, CEACAM6, **CXCL2↓,** EDG2, **EREG↓**, **FCGR1A↑**, G alphai, **GAS6↑, GBP1↑, GPSM1↓**, IFNZ, IL1, **IL8↓**, IL18BP, Il1r, **IL1R1↓**, IRAK4, **JAK2↑**, Mapk, **P2RY12↑**, Pdgf, PI3K, **PML↑**, **PTX3↓**, Rac, Ras, **RGS1↓**, SCYE1, **SERPINB2↓**, **STAT1↑**, STAT5a/b, Tgf beta, TYRO3	Cell signalling; cell-to-cell signalling and interaction; hematological system development and function
2	**BRE↓**, C3, **C7ORF16↓**, **CD38↑,** CD44, **CD1C↓**, CFB, CFH, CR1, **CXCL2↓**, **EPB41L3↑**, FCER2, **FCER1A↓**, FPR1, GM2A, IGKC, **IGL@↓**, IL13, IL25, **IL1R1↓**, IL1R2, IL8RB, INDO, MS4A2, **OAS3↑**, **OLR1 (includes EG:4973)↓**, PECAM1, **PLA2G7↓**, PP2A, SLPI, **SORT1↑**, TNF, TNFAIP6, TPT1, WNT5A	Cellular movement; immune response; hematological system development and function
3	ALOX5, BTG1, CD40LG, CDKN1A, DDB2, **DDEF2↑**, EBI3, **EBI2 (includes EG:1880) ↓** ECGF1,FANCC, **FER1L3↑**, FGF13, **GBP1↑**, HOXA5, IFI6, IFI35, **IFI44↑**, **IFI44L↑**, IFITM1, IFNA5 (includes EG:3442), IFNE1, **IL1R1↓**, INDO, **KLF6↓**, **LGALS3BP↑**, MST1, OAS1, PARP9, **PDE4B↓**, PKD1, **PLAC8↑**, SRC, **STAT1↑**, TNFAIP6, TP53	Cell-to-cell signalling and interaction; cellular function and maintenance; immunological disease

Genes in bold are differentially expressed in the microarray analysis (at least 1.5 fold). Arrows indicate upregulation (↑) or downregulation (↓) during pregnancy in MS.

Candidate gene approach RT-PCR data

In addition to CD64, RT-PCR was performed on additional candidate genes. These candidate genes were selected from the top ten upregulated and top 10 downregulated genes found in the major networks identified by Ingenuity analysis. Based on their immunological function we selected IL-8 (CXCL8), CXCL2, CD38, signal transducer and activator of transcription 1 (STAT1), Janus kinase 2 (JAK2) and pentraxin 3 (PTX3). Gene functions and primer sets used are presented in Table 2. None of the genes studied were significantly altered in the third trimester, compared to the first visit. However, in patients with MS we observed a significant increase directly postpartum in JAK2 and STAT1 expression ($p<0.05$) compared to before pregnancy or to the third trimester, respectively. In healthy women we observed a comparable significant increase directly postpartum of both STAT1 and JAK2 expression ($p<0.01$) compared to the first trimester (Figure 2). Expression levels did not

differ significantly between patients with MS and healthy women. Breast feeding was not associated with altered expression levels of the selected candidate genes.

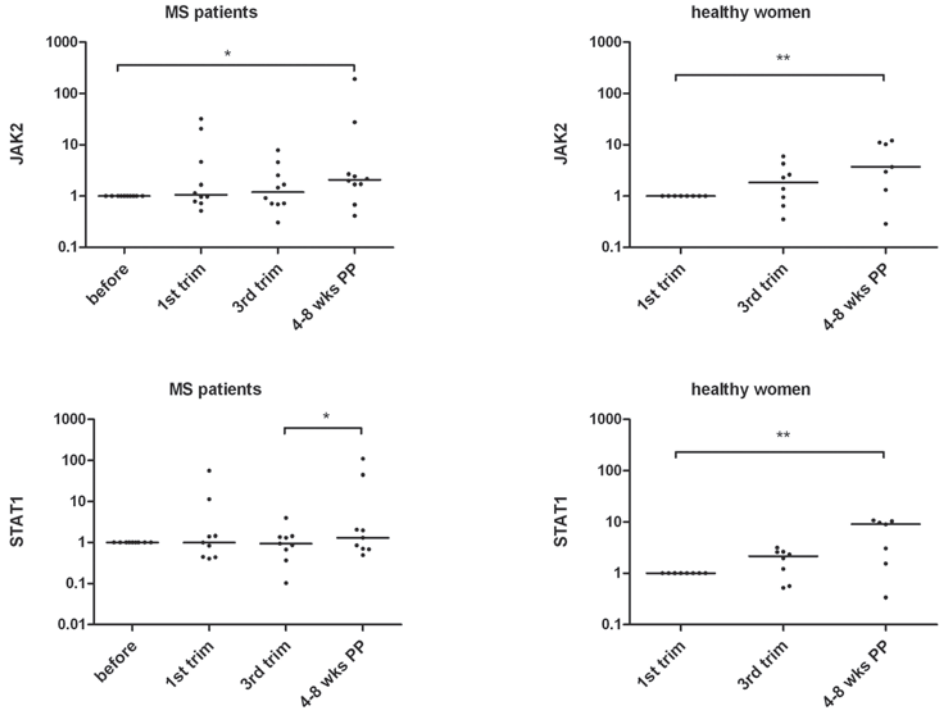


Figure 2. JAK2 and STAT1 expression in MS patients and healthy women using quantitative RT-PCR

RNA expression compared to GAPDH (ratio: compared to first sample). Horizontal lines represent medians. Multiple measurements linear mixed model analysis. * P< 0.05, ** P<0.01.

DISCUSSION

Here we present a longitudinal study on differentially expressed genes during pregnancy in MS patients. Using a genomewide approach on a relatively small number of patients could have limited our results. Still, despite the small number of patients we were able to demonstrate up regulation of CD64 and ANKRD22 during the third trimester of pregnancy in MS patients. Since very little is known in the literature on the potential functions of ANKRD22, we did not further explore this gene in the current study. Increased expression of CD64 during the third trimester was confirmed by quantitative RT-PCR in women with MS. Enhanced CD64 expression in monocytes has been observed before in healthy pregnant women.[11-14] Therefore there is little reason to suggest that our findings are MS specific, and instead pregnancy regulation of CD64 appears to be a general phenomenon. CD64 belongs

to the family of receptors for the Fc portion of immunoglobulins. CD64 is the high affinity receptor to the Fc fragment of IgG. It binds best to IgG3 and IgG1, followed by, with decreasing efficacy, IgG4 and IgG2 .[15] CD64 is constitutively expressed at substantial levels by monocytes and macrophages.[15-16] Ligation of Fc fragment of IgG to CD64 results in phagocytosis, respiratory burst and secretion of proinflammatory cytokines including IL-1 and IL-6.16 CD64 knockout mice show impaired protection against bacterial infection.[17] Monocyte activation, as found in sepsis, is associated with high CD64 expression.[18] Also in systemic lupus erythematosus nephritis increased expression of CD64 on monocytes is a marker of systemic inflammation.[19] Our results therefore support the hypothesis that innate immunity is more activated during pregnancy, likely as a means to prevent infectious complications.[4,6–8] To further explore changes in monocyte RNA expression during pregnancy in MS we searched for genes expression that went up or down at least 1.5-fold in nearly all patients. The gene set derived from this approach was used in Ingenuity pathway analysis software. Assessment by Ingenuity identified the following major networks to be modulated in monocytes during the course of pregnancy: cell-to-cell signalling and interaction, immune response and cell signalling. These results further substantiate the hypothesis that monocyte activation is altered during MS pregnancy. In addition to CD64, we selected several more candidate genes (IL8, CXCL2, STAT1, JAK2, CD38, PTX3) to be studied in a larger patient group and in healthy pregnant women using quantitative RT-PCR. We found no statistically significant differences in expression of these genes of interest comparing the baseline sample with the third trimester sample in MS patients. There were no statistical differences between MS patients and healthy women. As baseline samples were not available in the healthy control group, full comparison with MS patients was limited. We observed a significant increase in expression of STAT1 and JAK2 in both MS patients and healthy women after delivery. The JAK/STAT pathway is crucial in cytokine signalling. Cytokine binding to its receptor causes activation of the intracellular JAK complex and downstream phosphorylation of STAT hereby regulating gene expression. Both JAK2 and STAT1 are crucial in IFN-γ signalling.[20] Increase in STAT1 expression in monocytes after delivery in MS has been observed before.[21] STAT1 is also essential for expression of T box transcription factor (T-bet) the latter being important for Th1 development and IFN-γ production.[22] In monocytes T-Bet is expressed after stimulation with IFN-γ.[23] In MS patients it has already been shown that STAT1 and T-bet expression in monocytes is increased at time of relapse[24] while it is decreased after treatment with glucocorticoids.[25] Also, in rheumatoid arthritis STAT1 expression is increased in synovial tissue compared to healthy controls indicating a pro-inflammatory role.[26] A possible explanation of this rise in STAT1 activation is the postpartum increase of IFN-γ.[27] The postpartum increased expression of JAK2 and STAT1 can therefore, at least in part, be responsible for the increased disease activity after delivery in MS patients. A potential cause of this alteration in monocyte RNA expression during pregnancy could very well be oestrogens which reach peak levels during the third trimester. Oestriol in a dose of 8 mg/day given in two periods of 6 months reduces magnetic resonance imaging (MRI) disease activity in MS[28] and causes an upregulation of the anti-inflammatory cytokine IL-10 in PBMC

mainly because of increased numbers of CD64+ cells [29].[29] In experimental autoimmune encephalomyelitis (EAE) mice treated with oestriol this amelioration of disease severity and increase in T-cell production of IL-10 was also observed.[30] In guinea pigs treatment with oestriol increased expression of Fc gamma receptors in macrophages.[31] In conclusion, we found that the monocyte transcriptome is regulated during pregnancy in patients with MS. These genes are related to: cell-to-cell signalling and interaction, immune response and cell signalling. This is most appreciated through CD64 expression which is strongly upregulated during the third trimester, indicating monocyte activation at this stage.

REFERENCES

1. Compston A, Coles A. Multiple sclerosis Lancet. 2008;372:1502-1517.
2. McFarland HF, Martin R. Multiple sclerosis: a complicated picture of autoimmunity. Nat Immunol. 2007;8:913-919.
3. Confavreux C, Hutchinson M, Hours MM, Cortinovis-Tourniaire P, Moreau T. Rate of pregnancy-related relapse in multiple sclerosis. Pregnancy in multiple sclerosis group. N Engl J Med. 1998;339:285-291.
4. Trowsdale J, Betz AG. Mother's little helpers: mechanisms of maternal-fetal tolerance. Nat Immunol. 2006;7:241-246.
5. Wegmann TG, Lin H, Guilbert L, Mosmann TR. Bidirectional cytokine interaction in maternal fetal relationship: is successful pregnancy a Th2 phenomenon? Immunol Today. 1993;14:353-356.
6. Chaouat G. Innately moving away from the Th1/Th2 paradigm in pregnancy. Clin Exp Immunol. 2003;131:393-95.
7. Redman CW, Sargent IL. Latest advances in understanding preeclampsia. Science. 2005;308:1592-1594.
8. Sacks G, Sargent I, Redman C. An innate view of human pregnancy. Immunol Today. 1999;20:114-118.
9. Polman CH, Reingold SC, Edan G, Filippi M, Hartung HP, Kappos L, Lublin FD, Metz LM, McFarland HF, O'Connor PW, Sandberg-Wollheim M, Thompson AJ, Weinshenker BG, Wolinsky JS. Diagnostic criteria for multiple sclerosis:2005 revisions to the "McDonald Criteria". Ann Neurol. 2005;58:840-846.
10. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology. 1983;33:1444-52.
11. Sacks GP, Studena K, Sargent K, Redman CW. Normal pregnancy and preeclampsia both produce inflammatory changes in peripheral blood leukocytes akin to those of sepsis. A J Obstet Gynecol. 1998;179:80-86.
12. Davis D, Kaufmann R, Moticka EJ. Nonspecific immunity in pregnancy: monocyte surface Fcgamma receptor expression and function. J Reprod Immunol. 1998;40:119-128.
13. Naccasha N, Gervasi MT, Chaiworapongsa T, Berman S, Yoon BH, Maymon E, Romero R. Phenotypic and metabolic characteristics of monocytes and granulocytes in normal pregnancy and maternal infection. Am J Obstet Gynecol. 2001;185:1118-1123.
14. Luppi P, Haluszczak C, Betters D, Richard CA, Trucco M, DeLoia JA. Monocytes are progressively activated in the circulation of pregnant women. J Leukoc Biol. 2002;72:874-884.
15. van de Winkel JG, Anderson CL. Biology of human immunoglobulin G Fc receptors. J Leukoc Biol. 1991;49:511-524.
16. Salmon JE, Pricop L. Human receptors for immunoglobulin G: key elements in the pathogenesis of rheumatic disease. Arthritis Rheum. 2001;44:739-750.
17. Ioan-Facsinay A, de Kimpe SJ, Hellwig SM, van Lent PL, Hofhuis FM, van Ojik HH, Sedlik C, da Silveira SA, Gerber J, de Jong YF, Roozendaal R, Aarden LA, van den Berg WB, Saito T, Mosser D, Amigorena S, Izui S, van Ommen GJ, van Vugt M, van de Winkel JG, Verbeek JS. FcgammaRI (CD64) contributes substantially to severity of arthritis, hypersensitivity responses, and protection from bacterial infection. Immunity. 2002;16:391-402.
18. Danikas DD, Karakantza M, Theodorou GL, Sakellaropoulos GC, Gogos CA. Prognostic value of phagocytic activity of neutrophils and monocytes in sepsis. Correlation to CD64 and CD14 antigen expression. Clin Exp Immunol. 2008;154:87-97.
19. Li Y, Lee PY, Sobel ES, Narain S, Satoh M, Segal MS, Reeves WH, Richards HB. Increased expression of FcgammaRI/CD64 on circulating monocytes parallels ongoing inflammation and nephritis in lupus. Arthritis Res Ther. 2009; DOI: 10.1186/ar2590.
20. Murray PJ. The JAK-STAT signalling pathway: input and output integration. J Immunol. 2007; 178:2623-2629.
21. Iorio R, Frisullo G, Nociti V, Patanella KA, Bianco A, Marti A, Mirabella M, Tonali PA, Batocchi AP. T-bet, pSTAT1 and pSTAT3 expression in peripheral blood mononuclear cells during pregnancy correlates with post-partum activation of multiple sclerosis. Clin Immunol. 2009;131:70-83.
22. Szabo SJ, Kim ST, Costa GL, Zhang X, Fathman CG, Glimcher LH. A novel transcription factor, T-bet, directs Th1 lineage commitment. Cell. 2000;100:655-669.

23. Lighvani AA, Frucht DM, Jankovic D, Yamane H, Aliberti J, Hissong BD, Nguyen BV, Gadina M, Sher A, Paul WE, O'Shea JJ. T-bet is rapidly induced by interferon-gamma in lymphoid and myeloid cells. Proc Natl Acad Sci USA. 2001;98:15137-15142.
24. Frisullo G, Angelucci F, Caggiula M, Nociti V, Iorio R, Patanella AK, Sancricca C, Mirabella M, Tonali PA, Batocchi AP. pSTAT1, pSTAT3, and T-bet expression in peripheral blood mononuclear cells from relapsing-remitting multiple sclerosis patients correlates with disease activity. J Neurosci Res. 2006; 84:1027-1036.
25. Frisullo G, Nociti V, Iorio R, Katia Patanella A, Bianco A, Caggiula M, Sancricca C, Tonali PA, Mirabella M, Batocchi AP. Glucocorticoid treatment reduces T-bet and pSTAT1 expression in mononuclear cells from relapsing remitting multiple sclerosis patients. Clin Immunol. 2007;124:284-293.
26. Kasperkovitz PV, Verbeet NL, Smeets TJ, van Rietschoten JG, Kraan MC, van der Pouw Kraan TC, Tak PP, Verweij CL.Activation of the STAT1 pathway in rheumatoid arthritis. Ann Rheum Dis. 2004;63:233-239.
27. Keski-Nisula L, Hirvonen MR, Roponen M, Heinonen S, Pekkanen J. Maternal and neonatal IL-4 and IFN-gamma production at delivery and 3 months after birth. J Reprod Immunol. 2003;60:25-33.
28. Sicotte NL, Liva SM, Klutch R, Pfeiffer P, Bouvier S, Odesa S, Wu TC, Voskuhl RR. Treatment of multiple sclerosis with the pregnancy hormone estriol. Ann Neurol. 2002;52:421-428.
29. Soldan SS, Alvarez Retuerto AI, Sicotte NL, Voskuhl RR. Immune modulation in multiple sclerosis patients treated with the pregnancy hormone estriol. J Immunol. 2003;171:6267-6274.
30. Kim S, Liva SM, Dalal MA, Verity MA, Voskuhl RR. Estriol ameliorates autoimmune demyelinating disease: implications for multiple sclerosis. Neurology. 1999;52:1230-1238.
31. Gomez F, Ruiz P, Bernal JA, Escobar M, Garcia-Egido A, Lopez-Saez JJ. Enhancement of splenic-macrophage Fcgamma receptor expression by treatment with estrogens. Clin Diagn Lab Immunol. 2001;8:806-810.

CHAPTER 2.5

PREGNANCY-INDUCED FLUCTUATIONS IN FUNCTIONAL T-CELL SUBSETS IN MULTIPLE SCLEROSIS PATIENTS

R.F. Neuteboom, E. Verbraak, A.F. Wierenga-Wolf, M. Van Meurs, E.A.P. Steegers, C.J.M. De Groot, J.D. Laman and R.Q. Hintzen

ABSTRACT

Background: During pregnancy, especially during the third trimester, multiple sclerosis (MS) disease activity is reduced. It is not known which factors mediate this disease amelioration.
Objective: To study whether the frequency of two important T-cell subsets, T-helper 17 (Th17) and regulatory T-cells (Treg), is altered in relation to pregnancy-induced MS disease amelioration.
Methods: Each individual was tested longitudinally, after sampling of blood at timepoints before pregnancy, during the first and third trimester, and in the early post-partum period. Frequencies of Th17 cells were assessed after short (4 hours) re-stimulation of peripheral blood lymphocytes with PMA and ionomycin, followed by flow cytometry using CD4, CD45RO and IL-17A antibodies. To assess peripheral blood Treg frequencies, we used six-colour flow cytometry with antibodies against CD3, CD4, CD25, CD127, FoxP3 and HLA-DR, to specifically identify Treg.
Results: Both MS patients (n=9) and controls (n=8) displayed unaltered Th17 frequencies during pregnancy. In contrast, circulating Treg frequency significantly decreased in MS patients (n=15) during the first and third ($p<0.001$) trimesters compared with the period before pregnancy. In the post-partum period, the frequency of circulating Treg again resurged back to near pre-pregnancy levels. In controls (n=15) comparable frequency kinetics were observed in that post-partum a significant increase in circulating Treg frequency was detected compared with the first ($p < 0.001$) and third ($p = 0.012$) trimester.
Conclusions: Third trimester amelioration is not related to the fluctuation of circulating Th17 cells. Furthermore, a paradoxical decrease of immunosuppressive circulating Tregs can be observed during this phase, both in MS patients and controls.

INTRODUCTION

Pregnancy has an ameliorating effect on the multiple sclerosis (MS) annualized relapse rate. In particular, during the third trimester a 70% decrease in disease activity is observed. However, after delivery disease activity rebounds and one in every three women experience a relapse within the first 3 months.[1] MS is generally considered a CD4+ T-helper (Th) cell driven autoimmune disease.[2] Recently an important proinflammatory T-cell subset, named Th17, has been implicated in MS disease activity.[3] The Th17 subset is involved in the promotion of blood–brain barrier disruption and brain inflammation.[4] Another important T-cell subset in MS are the immunosuppressive regulatory T cells (Treg), the central role of which has been demonstrated in the maintenance of self-tolerance in autoimmune animal models.[5] The mechanisms underlying MS disease amelioration during pregnancy still remain unclear. Alterations in the frequency of Th17 cells and Treg could be involved in disease amelioration. Recently, the role of Treg during pregnancy has gained much attention[6-7] because of their role in immunological tolerance to the fetus.[8] Up to now thorough studies of Treg in MS pregnancy have been scarce and the available information has led to inconsistent conclusions.[9-11] This may at least partly be explained by different flow-cytometric approaches. Treg identification has been much improved by detection of the forkhead box transcription factor p3 (FoxP3)[12] and lack of expression of the IL7Ra chain (CD127).[13] In view of their presumed critical roles in MS, alterations in systemic Th17 and Treg frequencies are potentially crucial to amelioration of the MS disease course. Hence, in a longitudinal study we analysed for the first time whether the frequency of Th17 cells is affected in MS patients and controls during pregnancy. Furthermore, we analysed the frequency of Treg during the course of pregnancy in MS using the recently established stringent Treg markers.

MATERIALS AND METHODS

Patients and controls

Ambulant MS patients with a relapsing–remitting disease course, fulfilling diagnostic criteria,[14] were recruited at the Rotterdam MS Centre, ErasMS. Exclusion criteria were recurrent abortion, history of hypertension and diabetes mellitus. The study was designed to include MS patients pre-conceptionally. All MS patients were seen before pregnancy, at 10–12 and 28–30 weeks of pregnancy and at 4–8 weeks after delivery. MS severity was determined using the Expanded Disability Status Scale (EDSS).[15] Healthy pregnant women were recruited from the outpatient obstetrics clinic at the Erasmus MC. They were also seen at 10–12 and 28–30 weeks of pregnancy and at 4–8 weeks postpartum. This study was approved by the ethics committee of the Erasmus University and all participants gave written informed consent.

Blood samples and cell isolation

Blood samples were taken at each visit between 10 and 12 hours in the morning. Peripheral blood mononuclear cells (PBMCs) were isolated using CPT cell preparation tubes (Becton and Dickinson (BD), Breda, The Netherlands). After isolation PBMCs were stored in liquid nitrogen until use for flow cytometry.

Flow-cytometric analysis

For detection of Th17, PBMCs were re-stimulated with 50 ng/ml PMA, 500 mg/ml ionomycin and Golgi Stop (BD) for 4 h. Subsequently cells were stained using CD45RO-FITC (Dako, Heverlee, Belgium) and CD4-PerCP-Cy5 (BD). Next cells were fixed in 2% paraformaldehyde and permeabilized using 0.5% saponin (Sigma, Zwijndrecht, The Netherlands). Cells were subsequently incubated with anti-IL17A-PE (eBioscience, San Diego, CA). Flow-cytometric analysis was performed using a FACS Calibur (BD). Analysis of Treg was performed using: CD3-PerCP, CD4-AmCyan, CD25-PeCy7 (BD) and CD127-pacific blue (eBioscience). Intracellular staining for FoxP3 was performed using the FoxP3 Staining Buffer Set (eBioscience) in combination with anti-FoxP3-PE (BD). Analysis was performed using an LSRII flow cytometer (BD). Data were analysed using CellQuest Pro and FACS Diva software (BD) for Th17 and Treg subsets, respectively. Analysis of Th17 cells was performed by gating on CD4 followed by selection of the CD45RO+ (memory) cells. Within the CD4/ CD45RO+ gate IL-17+ cells were selected. For Treg analysis CD3+/CD4+ cells were selected followed by gating on FoxP3+/CD127low cells. To confirm Treg identity final selection was performed by gating back on CD25+/CD127low cells.

Statistical analysis

Comparison of continuous variables was performed using the Student's t-test and Mann–Whitney U test. Dichotomized data was analysed using the chi-squared or Fisher's exact test. Data at the various timepoints were compared using a multiple measurements linear mixed model analysis. Data were analysed using SPSS version 16.0.

RESULTS

Clinical data

Treg frequency was studied in 15 women with MS and 15 healthy controls (Table 1). Median EDSS at baseline was 1.0 (range 0–2.5). Th17 frequency was analysed in a subset of nine women with MS and eight controls. Median EDSS was 1.0 (range 0–1.5). None of the patients had a relapse during the 4–8 week follow-up. Patients did not receive immune modifying medication at least 3 months before the first visit and during the study. Demographics and pregnancy outcome are depicted in Table 1.

Table 1. Demographics and pregnancy outcome in MS patients and healthy controls

	MS patients		Healthy women		
	Treg (n=15)	Th17 (n=9)	Treg (n=15)	Th17 (n=8)	P Value*
mean maternal age (years ±SD)	31.5 0 (± 3.7)	30.7 (± 3.9)	31.8 (± 4.6)	30.6 (± 4.6)	n.s.
median EDSS (range)	1.0 (0-2.5)	1.0 (0-1.5)	n.a.	n.a.	n.a.
nullipara	12/15	6/9	7/15	2/8	n.s.
caesarean section	3/15	2/9	3/15	2/8	n.s.
(pre)eclampsia	0/15	0/6	0/10	0/8	n.s.
mean duration of gestation (weeks ±SD)	39.3 (±1.2)	39.3 (±0.8)	37.9 (±2.3)	37.3 (±2.7)	n.s.
breastfeeding at last visit	8/15	4/9	10/15	6/8	n.s.

* P value when comparing MS patients to healthy controls in both Treg and Th17 flowcytometric analysis (Mann-Whitney U test). n.s. = non significant; n.a. = not applicable; EDSS = expanded disability status scale.

Th17 frequency is not altered during the course of pregnancy

In order to analyse whether the pro-inflammatory Th17 cell subset in MS patients or controls is affected by pregnancy, the frequency of CD4+CD45RO+IL17A+ cells (Figure 1A) was assessed. No significant regulation of Th17 frequency was observed during pregnancy either in controls or MS patients (Figure 1B and C). Frequency of Th17 cells was similar for pregnant MS patients and controls.

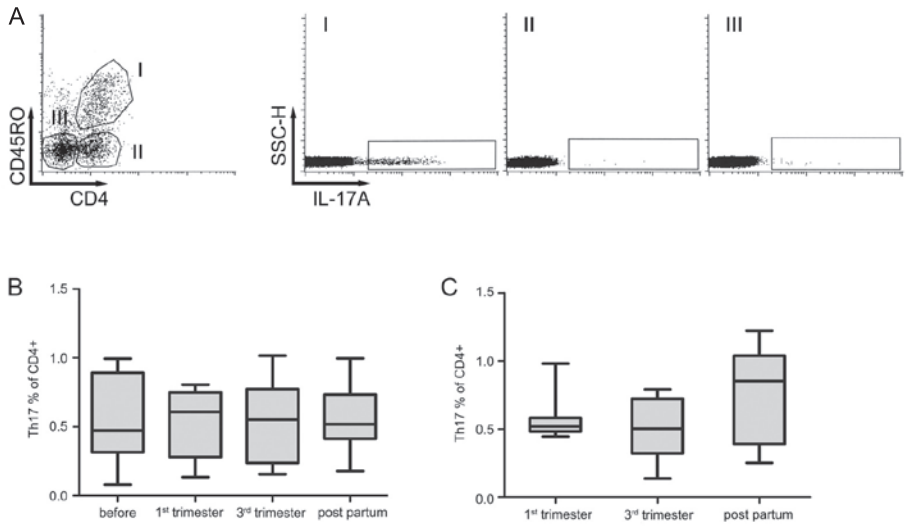


Figure 1. Th17 frequency is stable during pregnancy in MS patients and healthy controls. A) Number of IL-17A (I) producing cells was determined by gating of CD4 and CD45RO double positive cells. In cells positive for CD4 alone (II) or negative for CD4 (III) very low or no IL-17A production was detected. B) Percentage of Th17 cells in MS patients before pregnancy, at first and second trimester and 4-8 weeks postpartum and C) in healthy controls during the 1st and 3rd trimester and after pregnancy. Horizontal lines represent means.

Frequency of Treg is decreased during early and late pregnancy

To assess the effect of pregnancy on the frequency of Treg, cells were gated as shown in Figure 2A. Treg frequency in MS patients (Figure 2B) showed a significant decrease in frequency from before pregnancy compared with the first ($p < 0.001$) and third trimester ($p < 0.001$). Shortly post-partum a significant increase was observed in comparison with the first ($p = 0.005$) and third trimester ($p = 0.003$) and Treg frequency approached pre-pregnancy level. For controls we found frequency kinetics (Figure 2C) comparable to MS patients, in that post-partum a significant increase in Treg frequency was observed in comparison with the first ($p < 0.001$) and third trimester ($p = 0.012$). When HLA-DR was used as a marker of recent Treg activation,[16] similarly significant results were obtained (data not shown). Both in MS patients and controls FoxP3 expression level, as reflected by mean fluorescent intensity (MFI), significantly decreased from the first trimester towards the third trimester (MS: 3941 ± 1440 to 3295 ± 881, $p = 0.001$; and controls: 3577 ± 649 to 3235 ± 658, $p = 0.033$). In MS patients FoxP3 MFI levels significantly increased again to 3810±1176 ($p = 0.003$) compared with the third trimester. Similar to the Th17 subset highly comparable Treg frequencies and MFI levels were found in MS patients and controls.

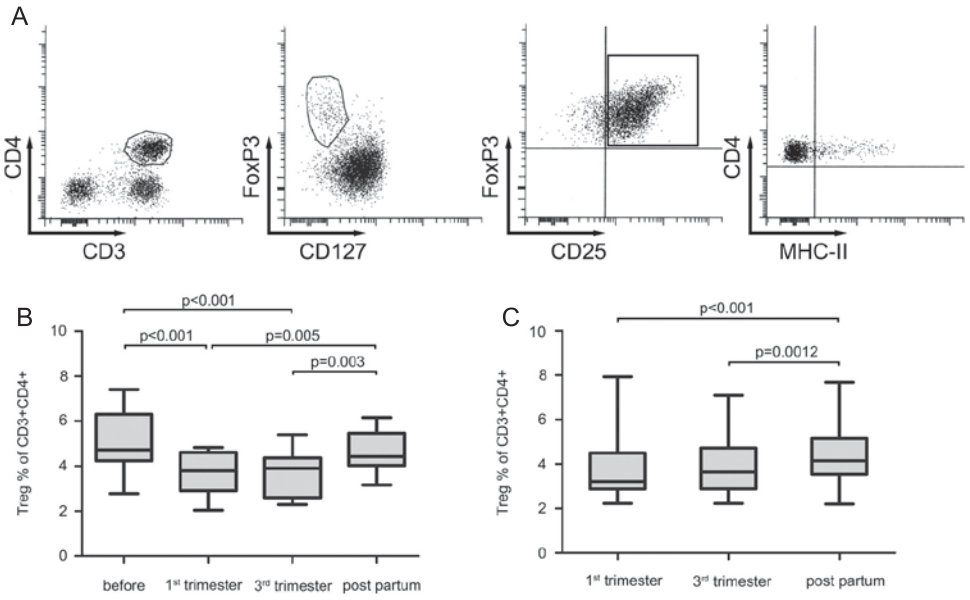


Figure 2. Frequency of peripheral Treg is altered during pregnancy in MS patients and controls. A) Gating strategy for the identification regulatory T cells. Final number of cells in CD25/FoxP3 positive gate were used for further analysis incuding identification of MHCII positive Treg. B) Percentage of CD25+FoxP3+CD127^{low} cells in MS patients within CD3+CD4+ gate before pregnancy, at first and second trimester and 4-8 weeks postpartum. C) Percentage of CD25+FoxP3+CD127^{low} cells in healthy women within CD3+CD4+ gate, at time of first and second trimester and 4-8 weeks postpartum. Horizontal lines represent means.

DISCUSSION

We here present a longitudinal study analysing the frequency of Th17 and Treg in peripheral blood of MS patients versus controls during pregnancy. We hypothesized that alteration in the frequency of Th17 or Treg is involved in MS disease amelioration during pregnancy. We did not find any regulation in the frequency of Th17 cells in the peripheral blood of both MS patients and controls around and during pregnancy. This indicates that peripheral Th17 frequency is not influenced in MS during the course of pregnancy. Recently the Th17 regulating microRNA miR-326 was suggested to be linked to disease severity.[17] It would be attractive for future research to study how expression of miR-326, next to cytokines such as IL-17F or IL-22, would behave over time during pregnancy in MS. Another potential ameliorative mechanism is alteration in Treg frequency. In contrast to what we anticipated in women with MS the percentage of Treg declines during the first and third trimester in comparison with the sample taken before pregnancy. Postpartum (4–8 weeks) a significant increase in Treg frequency was observed compared with the first and third trimester. Although we lacked the before pregnancy time point for healthy controls it appears likely that the kinetics of peripheral Treg frequency is fully comparable to MS patients during pregnancy since frequencies during pregnancy as well as the postpartum increase were highly similar. In addition we also observed a significant decrease in FoxP3 MFI levels in both MS patients and controls from the first towards the third trimester. Previous studies have suggested quite disparate functions for Treg in pregnancy, since they have been described to either increase or decrease in frequency.[9,18-20] These conflicting conclusions emphasize the need for additional studies analysing Treg kinetics during pregnancy using the recently refined Treg cell markers and stringent gating strategies, as were used previously.[21-,22] Here we have shown that the Treg frequency is significantly decreased during the first and third trimester of pregnancy. Since both MS patients and controls demonstrate this reduction, the fluctuation in Treg frequency during pregnancy in MS patients appears to be part of normal pregnancy physiology. One potential disease-modifying mechanism could be the recent notice that Treg can also produce IL-17 under inflammatory conditions.[23-24] As these Treg bear cell markers comparable to Th17 cells, we would have expected to find at least a minimal change in Th17 frequency during pregnancy. The systemic drop in Treg number may be explained by the importance of Treg in pregnancy outcome[25] and their migration to the foetal–maternal interface.[7] Furthermore, our finding of decreased numbers of Treg during the third trimester, the period associated with decreased disease activity, is of special interest as the use of depleting anti-CD25 antibodies decreases Treg number and nevertheless results in an amelioration of the MS disease course.[22] Collectively, the data of the current study do not support the idea that peripheral blood Th17 and Treg cells are involved directly in MS disease course alteration during pregnancy.

REFERENCES

1. Confavreux C, Hutchinson M, Hours MM, Cortinovis-Tourniaire P, Moreau T. Rate of pregnancy-related relapse in multiple sclerosis. Pregnancy in Multiple Sclerosis Group. N Engl J Med. 1998;339:285-291.
2. McFarland HF, Martin R. Multiple sclerosis: a complicated picture of autoimmunity. Nat Immunol. 2007;8:913-919.
3. Tzartos JS, Friese MA, Craner MJ, Palace J, Newcombe J, Esiri MM, Fugger L. Interleukin-17 production in central nervous system-infiltrating T cells and glial cells is associated with active disease in multiple sclerosis. Am J Pathol. 2008;172:146-155.
4. Kebir H, Kreymborg K, Ifergan I, Dodelet-Devillers A, Cayrol R, Bernard M, Giuliani F, Arbour N, Becher B, Prat A. Human TH17 lymphocytes promote blood-brain barrier disruption and central nervous system inflammation. Nat Med. 2007;13:1173-1175.
5. Sakaguchi S. Naturally arising Foxp3-expressing CD25+CD4+ regulatory T cells in immunological tolerance to self and non-self. Nat Immunol. 2005;6:345-352.
6. Somerset DA, Zheng Y, Kilby MD, Sansom DM, Drayson MT. Normal human pregnancy is associated with an elevation in the immune suppressive CD25+ CD4+ regulatory T-cell subset. Immunology. 2004;112:38-43.
7. Tilburgs T, Roelen DL, van der Mast BJ, de Groot-Swings GM, Kleijburg C, Scherjon SA, Claas FH. Evidence for a selective migration of fetus-specific CD4+CD25bright regulatory T cells from the peripheral blood to the decidua in human pregnancy. J Immunol. 2008;180:5737-5745.
8. Aluvihare VR, Kallikourdis M, Betz AG. Regulatory T cells mediate maternal tolerance to the fetus. Nat Immunol. 2004;5:266-271.
9. Iorio R, Frisullo G, Nociti V, Patanella KA, Bianco A, Marti A, Mirabella M, Tonali PA, Batocchi AP. T-bet, pSTAT1 and pSTAT3 expression in peripheral blood mononuclear cells during pregnancy correlates with post-partum activation of multiple sclerosis. Clin Immunol. 2009;131:70-83.
10. Airas L, Saraste M, Rinta S, Elovaara I, Huang YH, Wiendl H. Immunoregulatory factors in multiple sclerosis patients during and after pregnancy: relevance of natural killer cells. Clin Exp Immunol. 2008;151:235-243.
11. Sánchez-Ramón S, Navarro A J, Aristimuño C, Rodríguez-Mahou M, Bellón JM, Fernández-Cruz E, de Andrés C. Pregnancy-induced expansion of regulatory T-lymphocytes may mediate protection to multiple sclerosis activity. Immunol Lett. 2005;96:195-201.
12. Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. Science. 2003;299:1057-1061.
13. Michel L, Berthelot L, Pettré S, Wiertlewski S, Lefrère F, Braudeau C, Brouard S, Soulillou JP, Laplaud DA.Patients with relapsing-remitting multiple sclerosis have normal Treg function when cells expressing IL-7 receptor alpha-chain are excluded from the analysis. J Clin Invest. 2008;118:3411-3419.
14. Polman CH, Reingold SC, Edan G, Filippi M, Hartung HP, Kappos L, Lublin FD, Metz LM, McFarland HF, O'Connor PW, Sandberg-Wollheim M, Thompson AJ, Weinshenker BG, Wolinsky JS. Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria". Ann Neurol. 2005;58:840-846.
15. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology. 1983;33:1444-1452.
16. Baecher-Allan C, Wolf E, Hafler DA. MHC class II expression identifies functionally distinct human regulatory T cells. J Immunol. 2006;176:4622-4631.
17. Du C, Liu C, Kang J, Zhao G, Ye Z, Huang S, Li Z, Wu Z, Pei G. MicroRNA miR-326 regulates TH-17 differentiation and is associated with the pathogenesis of multiple sclerosis. Nat Immunol. 2009;10:1252-1259.
18. Forger F, Marcoli N, Gadola S, Moller B, Villiger PM, Ostensen M. Pregnancy induces numerical and functional changes of CD4+CD25 high regulatory T cells in patients with rheumatoid arthritis. Ann Rheum Dis. 2008;67:984-990.
19. Heikkinen J, Mottonen M, Alanen A, Lassila O. Phenotypic characterization of regulatory T cells in the human decidua. Clin Exp Immunol. 2004;136:373-378.

20. Mjösberg J, Svensson J, Johansson E, Hellström L, Casas R, Jenmalm MC, Boij R, Matthiesen L, Jönsson JI, Berg G, Ernerudh J. Systemic reduction of functionally suppressive CD4dimCD25highFoxp3+ Tregs in human second trimester pregnancy is induced by progesterone and 17beta-estradiol. J Immunol. 2009;183:759-769.
21. Santner-Nanan B, Peek MJ, Khanam R, Richarts L, Zhu E, Fazekas de St Groth B, Nanan R.Systemic increase in the ratio between Foxp3+ and IL-17-producing CD4+ T cells in healthy pregnancy but not in preeclampsia. J Immunol. 2009;183:7023-30.
22. Oh U, Blevins G, Griffith C, Richert N, Maric D, Lee CR, McFarland H, Jacobson S. Regulatory T cells are reduced during anti-CD25 antibody treatment of multiple sclerosis. Arch Neurol. 2009;66:471-479.
23. Beriou G, Costantino CM, Ashley CW, Yang L, Kuchroo VK, Baecher-Allan C, Hafler DA. IL-17-producing human peripheral regulatory T cells retain suppressive function. Blood. 2009;113:4240-4249.
24. Yang XO, Nurieva R, Martinez GJ, Kang HS, Chung Y, Pappu BP, et al. Yang XO, Nurieva R, Martinez GJ, Kang HS, Chung Y, Pappu BP, Shah B, Chang SH, Schluns KS, Watowich SS, Feng XH, Jetten AM, Dong C. Molecular antagonism and plasticity of regulatory and inflammatory T cell programs. Immunity. 2008;29:44-56.
25. Guerin LR, Prins JR, Robertson SA. Regulatory T-cells and immune tolerance in pregnancy: a new target for infertility treatment? Hum Reprod Update. 2009;15:517-535.

CHAPTER 2.6

SERUM LEPTIN LEVELS DURING PREGNANCY IN MULTIPLE SCLEROSIS

R.F. Neuteboom, E. Verbraak, J.S.A. Voerman, M. Van Meurs, E.A.P. Steegers, C.J.M. De Groot, J.D. Laman and R.Q. Hintzen

ABSTRACT

Background: Disease activity in patients with multiple sclerosis (MS) is suppressed during pregnancy, whereas attack frequency increases after delivery. It is yet unclear, which immuno – endocrinological processes mediate these disease fluctuations. Leptin has been identified as a hormone that can influence inflammatory activity.

Objective: The aim of this study was to investigate whether pregnancy-induced fluctuations of serum leptin levels differed between patients with MS and controls and whether serum leptin levels correlate with periods of enhanced and diminished disease activity.

Methods: 36 Women with MS and 17 healthy women were prospectively followed during and after pregnancy. The MS group could be studied already at a timepoint before pregnancy. Serum leptin and soluble leptin receptor (SLR) levels were measured using enzyme-linked immunosorbent assay.

Results: Pre-pregnancy serum leptin levels were (mean ± SD) 22.9 ± 12.8 ng/ml in the MS group. These levels increased in the third trimester to 28.5 ± 15.0 ng/ml ($P = 0.007$). The third trimester serum leptin levels in healthy women were comparable, 29.4 ± 19.0 ng/ml. Serum leptin levels after delivery dropped to 18.5 ± 12.8 ng/ml in women with MS ($P < 0.001$) and to a lesser extend (22.0 ± 17.5 ng/ml) in healthy women ($P = 0.04$). SLR levels showed the same pattern. Remarkably, women with the highest relative decrease in serum leptin levels after delivery had more often a postpartum relapse ($P = 0.008$).

Conclusion: In women with MS, leptin increased during late pregnancy. A postdelivery drop in leptin levels was observed in both the MS and control group. The postdelivery drop was associated with the occurrence of postpartum relapse.

INTRODUCTION

Multiple sclerosis (MS) is the most common neurological disease in young adults, affecting women more than men.[1] Disease presentation is often during childbearing years.[1]

Pregnancy has a known ameliorating effect on the disease activity, especially in the third trimester with a 70% decrease of the annualized relapse rate.[2] However, in the first 3 months after delivery, disease activity rebounds and about one in every three women with MS has a relapse.[2] The underlying biological mechanisms of this natural modifier of MS disease course remain unclear. It is proposed that adaptive immunity during pregnancy skews from proinflammatory toward anti-inflammatory immunity.[3] Regulatory T-cells (Tregs), which are reported to have a protective role in autoimmune diseases such as MS, are altered during pregnancy.[4] Recent findings also suggest an important role for natural killer (NK) cells.[5]

Hormonal factors are likely candidates for explaining this altered disease course during pregnancy. Estriol in a dose of 8 mg/day given in two periods of 6 months reduces magnetic resonance imaging disease activity in patients with MS.[6] It causes an up-regulation of the anti inflammatory interleukin-10 (IL-10) in peripheral blood mononuclear cells mainly because of increased number of CD64+ cells (monocytes/macrophages).[7] In experimental autoimmune encephalomyelitis (EAE), both estrogen and progesterone ameliorate disease severity and alter the profile of cytokine secretion toward a more antiinflammatory immune response.[8-9]

Next to sex steroids, leptin is another hormonal candidate. Leptin, first identified as an adipocyteproduced modulator of energy homeostasis, also has major effects on both innate and adaptive immune responses.[10-13] Elevated serum leptins levels cause a proinflammatory immune response. In monocytes and macrophages, leptin promotes phagocytosis and proliferation together with production of proinflammatory cytokines such as IL-6, IL-12, and tumor necrosis factor-α (TNF-α).[10-11] It induces chemotaxis of neutrophils and increases cytotoxicity of NK cells. Also adaptive immunity is shifted to a proinflammatory response.[10] There is evidence that leptin decreases the numbers of Tregs.[12-13] In EAE, leptin is involved in both induction and progression of EAE.[14] Administration of anti-leptin antibodies or soluble leptin receptors (SLRs) decreases disease severity in EAE.[15] In patients with relapsing remitting MS, serum levels of leptin are increased.[12]

Leptin levels are elevated during pregnancy.[16-18] So far no studies have addressed the relationship between leptin, the SLR, and altered disease course of MS during pregnancy. The aim of this prospective longitudinal study was to investigate whether pregnancy-induced fluctuation of serum levels of leptin and SLR differed between patients with MS and controls and whether serum leptin levels correlate with periods of enhanced and diminished disease activity. Our secondary goal was to study if these natural leptin fluctuations are associated with the occurrence of a postpartum relapse.

PATIENTS AND METHODS

Patients and healthy controls

MS patients with a relapsing remitting disease course and who were ambulant were recruited at the Rotterdam MS center, ErasMS. Healthy control patients were recruited from the outpatient clinic of obstetrics at the Erasmus MC. Exclusion criteria were recurrent abortion, history of hypertension, diabetes mellitus, or systemic diseases such as rheumatoid arthritis or systemic lupus erythematosus. The study was designed to include patients preconceptionally and inclusion was also allowed during pregnancy. Inclusion of all healthy controls occurred during the first trimester. All patients with MS and controls were seen at 10–12 weeks and 28–30 weeks of pregnancy and at 4–8 weeks after delivery. Data on age, ethnicity, parity, gestational age, twin pregnancy, (pre)eclampsia, birth weight, and breastfeeding were collected.

A relapse was defined as an episode of neurological disturbance for which causative lesions are likely to be inflammatory and demyelinating in nature and lasting more than 24 hours.[19] MS severity was determined using the expanded disability status scale (EDSS).[20] This study was approved by the ethics committee of the Erasmus university, and all patients gave written informed consent.

Serum leptin and SLR levels

At each visit, blood samples were taken in the morning between 10 a.m. and 12 a.m. Serum was stored at -80 °C. Serum leptin levels were measured by enzyme-linked immunosorbent assay (ELISA), according to the manufacturer's procedure (R&D systems, Abingdon, UK). The detection range of the assay was between 1.2 and 80 ng/ml. Serum LR levels were measured by ELISA according to the manufacturer's procedure (R&D systems). The detection range of the assay was 0.3 to 20 ng/ml.

Data analysis

For comparison of continuous variables, the Student's t-test and Mann–Whitney U test were used. For comparison of dichotomized data, the chi square or Fisher's exact test was used. Linear regression as used for evaluating the relation between birth weight and leptin levels. Leptin levels at the different timepoints were compared using a multiple measurement linear mixed model analysis. Data were analyzed using SPSS version 16.0.

RESULTS

Patients and healthy controls

Thirty-six pregnant women with MS were studied, of which 17 were included before pregnancy (mean 6 months (SD ± 3.8) before pregnancy), 17 during the first trimester, and two during the third trimester. In one patient with MS, the third trimester visit was lacking. As a control group 17 healthy pregnant women were included, all first seen at time of the first trimester.

Mean MS disease duration before the studied pregnancy was 4.5 years (SD ± 3.8). Median EDSS measured at first trimester visit was 1.5 (range 0–4.0). None of the patients received medication except for three patients who received intravenous immunoglobulins directly after delivery, with the aim to protect for possible exacerbations .[21] Eleven women with MS (31%) had a relapse in the first 3 months after delivery.

Age at time of pregnancy was equally distributed in women with MS and the healthy women (Table 1). No statistical significant differences in women with MS and healthy in demographical and pregnancy outcome parameters were found, except that the women in the control group less often were primipara (Table 1).

Table 1. Data on pregnancy and outcome of women with MS and healthy women

	women with MS n=36	healthy women n=17	P value
mean maternal age (years) (±SD)	30.9 (±3.7)	31.4 (±4.4)	n.s.
- maternal age 20-25	6 %	6 %	n.s.
- maternal age 25-30	36%	35 %	n.s.
- maternal age 30-35	33%	35 %	n.s.
- maternal age 35+	11%	18 %	n.s.
primipari	69 %	35%	0.019
non caucasian ethnicity	6%	6 %	n.s.
twin pregnancy	0 %	12 %	n.s.
(pre)eclampsia	0 %	0 %	n.s.
cesarean section	14 %	24%	n.s
mean duration of gestation (weeks) (±SD)	39.1 (±1.6)	37.9 (±2.8)	n.s.
preterm (<37 weeks gestation)	6%	18%	n.s.
mean birth weight (grams) (±SD)*	3369 (±413)	3476 (±690)	n.s.
small for gestational age **	0 %	0 %	n.s.
breast feeding at last visit	61 %	47%	n.s.

MS = multiple sclerosis, n.s. = not statistically significant, SD = standard deviation
* not including twin pregnancy
** small for gestational age is defined as birth weight –2SD, using standardized intra-uterine growth chart.

Serum leptin and SLR levels before, during, and after pregnancy

Serum levels of leptin during pregnancy in women with MS and healthy women are shown in Figure 1. Serum leptin levels (mean ± SD) in the women with MS before pregnancy were 22.9 ± 12.8 ng/ml. First trimester serum leptin levels were 22.8 ± 13.6 ng/ml in women with MS and 28.4 ± 19.0 ng/ml in healthy women. In women with MS, serum leptin levels increased significantly during the third trimester of pregnancy to 28.5 ± 15.0 ng/ml (P = 0.007, compared to pre-pregnancy visit and P = 0.012, compared to first trimester). In healthy women, serum leptin levels remained constant during the third trimester at 29.4 ± 19.0 ng/ml. Serum levels of leptin after delivery significantly dropped to 18.5 ± 12.8 ng/ml in women

with MS (P < 0.001) and to 22.0 ± 17.5 ng/ml in healthy women (P = 0.036). Serum levels of leptin did not differ between women with MS and healthy women during pregnancy and after delivery.

SLR levels showed the same pattern as the leptin levels in serum in both patients with MS and healthy women. In women with MS, SLR levels (mean ± SD) were 15.3 ± 4.4 ng/ml before pregnancy. During pregnancy, these levels significantly rose to 21.1 ± 9.3 ng/ml (P < 0.001) during the first trimester and to 26.9 ± 8.9 ng/ml (P < 0.001) at time of the third trimester. After delivery, SLR levels significantly dropped to 19.1 ± 5.2 ng/ml (P < 0.001). In healthy women, we observed during the first trimester SLR levels of 20.7 ± 8.2 ng/ml, which significantly increased to 27.9 ± 10.9 ng/ml (P < 0.001) during the third trimester and after delivery significantly decreased to 19.4 ± 7.5 ng/ml (P < 0.001).

Comparison of primipara to multipara did not reveal significant differences in serum leptin or SLR levels before, during, and after pregnancy. Breastfeeding or a cesarean section was not associated with a change in serum levels of leptin. Serum leptin levels in the first or third trimester did not correlate with birth weight.

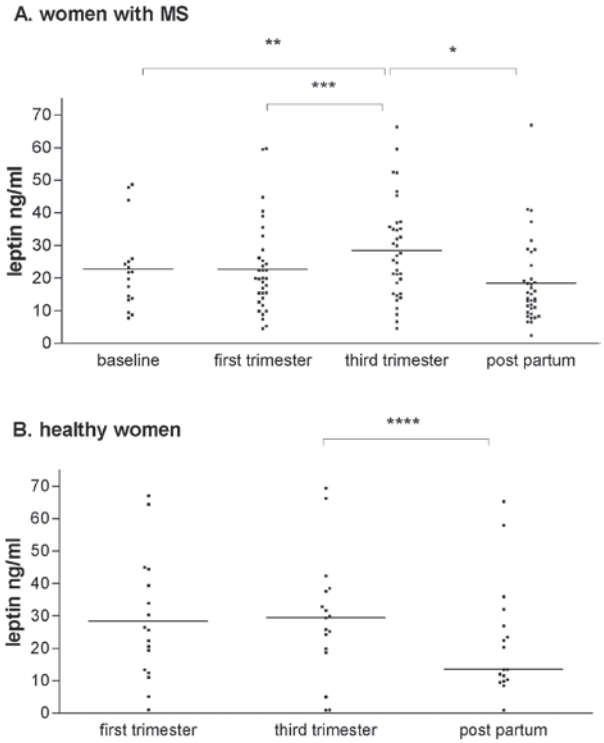


Figure 1. (A) Serum levels of leptin of MS patients before pregnancy, in the first and third trimester of pregnancy and 4-8 weeks post partum. (B) Serum leptin levels of healthy women in the first and third trimester of pregnancy and 4-8 weeks post partum. Horizontal lines represent means.
* p<0.001, ** p=0.007, *** p=0.012, ***** p=0.036

Serum leptin levels: predicting postpartum relapse?

Absolute serum leptin levels during and after pregnancy showed no association with the occurrence of a postpartum relapses. However, there were remarkably large inter-individual differences in the drop of serum leptin levels after delivery compared with the third trimester, both in the MS and healthy group. To assess a possible association of these relative changes with postpartum attack frequency, we split the MS group into two subgroups, using the median percentual postpartum change of leptin (-40%) as a cutoff value (Figure 2). We observed that out of the 17 women with the most relative postpartum decrease in serum levels of leptin, nine (53%) had a postpartum relapse (Figure 2). However, out of the 18 women with the least relative decrease or even an increase, only two (11%) had a postpartum relapse (Figure 2). This indicates that a more substantial relative drop in serum leptin levels after delivery is associated with postpartum relapse (P = 0.008).

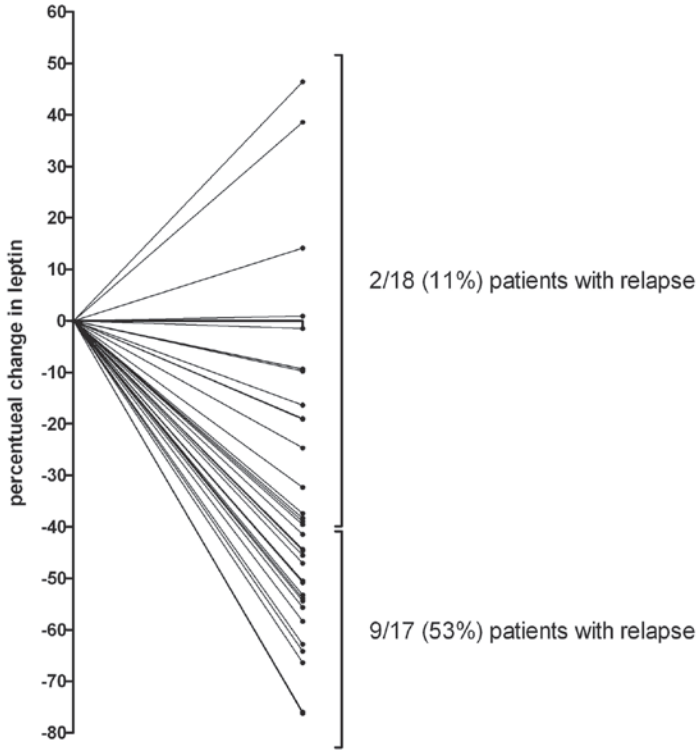


Figure 2. Relative changes in serum leptin levels in MS patients comparing the third trimester (set at 0) with the post partum period. The group was digotomized using the median relative change (= −40%) as a cut-off. Difference in risk of postpartum relapse in these two groups was significant (p=0.008)

DISCUSSION

This prospective study is the first to report longitudinal measurements of leptin before, during, and after pregnancy in patients with MS. We observed a significant increase in serum levels of leptin in women with MS during the third trimester when compared to baseline and first trimester samples. The decrease in leptin after delivery in the women with MS and in healthy women was comparable with earlier studies in healthy women.[16-17]

In contrast with the patients with MS, we observed no leptin increase during pregnancy in healthy women. As we had no sample before pregnancy in this group, we cannot rule out that leptin was already at its peak level at the first trimester measurement. On the exact timepoint of leptin peak level during pregnancy conflicting results have been reported, suggesting high variability.[16-18] Possibly, in healthy women, serum levels were already increased before our measurement in the first trimester and, therefore, an increase during pregnancy would be missed.

It was noted that the healthy group contained less primipara women than the MS group. However, no significant difference between leptin serum levels between primipara and multipara was found, so it seems not likely that this difference influenced our results.

It has been demonstrated that children of mothers with MS have a lower birth weight.[22] We also observed a nonsignificant trend in the same direction. We found no relation between maternal serum leptin levels and birth weight in women with MS or healthy women, which was in line with an earlier study in healthy pregnant women.[23] In addition, we confirm the absence of an association between maternal serum leptin levels and breastfeeding as is reported in healthy women.[24]

As an adipose tissue-derived energy homeostasis regulator, high serum levels are associated with a high body mass index (BMI) also during pregnancy.[25] A shortcoming of our study is that we were not able to match women with MS and healthy women for BMI, thus limiting direct comparison between the both groups. However, the strength of our study is that we longitudinally followed both the women with MS and the healthy women, allowing us to study intra-individual changes in serum leptin levels, irrespective of BMI. Furthermore, BMI alone cannot account for the increase of leptin during pregnancy. The rise of leptin during pregnancy precedes the increase in BMI and abruptly decreases after delivery[16,18] because, next to the adipose tissue, the placenta becomes the most important source of leptin during pregnancy. [26-27]

Leptin, as discussed before, has important effects on both the innate and adaptive immune response. Elevated serum leptin levels cause a proinflammatory immune response. The ameliorating effect of the third trimester of pregnancy on disease activity, therefore, seems in contrast with the elevated leptin levels during pregnancy. First, it should be noted that not all effects of leptin are proinflammatory. In patients with relapsing remitting MS, serum leptin enhances the release of the anti-inflammatory cytokine IL-10 in peripheral blood mononuclear cells, next to proinflammatory cytokines (IL-6, TNF-α).[28-29] Furthermore, we observed that SLR is equally increased during pregnancy. The leptin levels, as we

measured, represent total leptin levels, including both bound and free leptin. SLR modulates leptin activity by competing with the binding to the membrane bound receptor.[30] Leptin binds to SLR with the same affinity as to its membrane bound receptor in a molar ratio of 1:1.[31] Considering the molecular weight ratio of leptin to SLR (=7.7:1)[31], during and around pregnancy a constant molar serum leptin to SLR ratio of around 10 is observed (data are not shown). The elevated SLR levels could be a counterbalance for leptin availability during pregnancy. However, the constant tenfold molar excess of leptin over its soluble receptor indicates that the absolute levels of free leptin are still elevated during pregnancy and decrease after delivery, despite the change in SLR levels.

Notably, we found that women with MS with the largest relative decrease in serum leptin levels after delivery more often had a postpartum relapse. It could be hypothesized that the increase of leptin in the third trimester has a delayed effect, priming the immune system to a more proinflammatory immune response. Supportive of this hypothesis, earlier studies show that an increase in serum leptin levels precedes a relapse in humans and the clinical signs in EAE[32–34], but still future research on this item would be needed.

A large relative drop after the delivery could well be a marker for increased risk of a postpartum relapse. However in our study, the postpartum visit took place 4–8 weeks after delivery, and some of the postpartum relapses would have already taken place. So before serum leptin levels can be clinically useful as a marker for postpartum relapse, our result have to be affirmed in a new study with additional samples taken within the first week after delivery, to make a prediction at a more clinically useful timepoint.

We conclude that our data support the hypothesis that leptin is a factor associated with MS disease activity. The exact role of leptin in the innate and adaptive immunological pathways still remains to be elucidated.[35]

REFERENCES

1. Compston A, Coles A. Multiple Sclerosis. Lancet. 2002;359:1221-1231.
2. Confavreux C, Hutchinson M, Hours MM, Cortinovis-Tourniaire P, Moreau T. Rate of pregnancy-related relapse in multiple sclerosis. Pregnancy in multiple sclerosis group. N Engl J Med. 1998;339:285-291.
3. Wegmann TG, Lin H, Guilbert L, Mosmann TR. Bidirectional cytokine interaction in maternal fetal relationship: is successful pregnancy a Th2 phenomenon? Immunol Today. 1993;14:353-356.
4. Mellor AL, Munn D. Policing pregnancy: Tregs help keep the peace. Trends Immunol. 2004;25:563-565.
5. Sargent I, Borzychowski AM, Redman CWG. NK cells and human pregnancy – an inflammatory view. Trends Immunol. 2006;27:399-404.
6. Sicotte NL, Liva SM, Klutch R, Pfeiffer P, Bouvier S, Odesa S, Wu TC, Voskuhl RR.Treatment of multiple sclerosis with the pregnancy hormone estriol. Ann Neurol. 2002;52:421-428.
7. Soldan SS, Alvarez Retuerto AI, Sicotte NL, Voskuhl RR. Immune modulation in multiple sclerosis patients treated with the pregnancy hormone estriol. J Immunol. 2003;171:6267-6274.
8. El-Etr M, Vukusic S, Gignoux L, Durand-Dubief F, Achiti I, Baulieu EE, Confavreux C. Steroid hormones in multiple sclerosis. J Neurol Sci. 2005;233:49–54.
9. R. Druckmann and M.A. Druckmann. Progesterone and the immunology of pregnancy, J Steroid Biochem Mol Biol 2005;97:389–396.
10. Lago F, Dieguez C, Gomez Reino J, Gualillo O. The emerging role of adipokines as mediators of inflammation and immune responses. Cytokine growth factor rev. 2007;18:313-325.
11. Loffreda S, Yang SQ, Lin HZ, Karp CL, Brengman ML, Wang DJ, Klein AS, Bulkley GB, Bao C, Noble PW, Lane MD, Diehl AM.Leptin regulates proinflammatroy immune responses. FASEB J. 1998;12:57-65.
12. Matarese G, Carrieri PB, La Cava A, Perna F, Sanna V, De Rosa V, Aufiero D, Fontana S, Zappacosta S.Leptin increase in multiple sclerosis associates with reduced number of CD4+CD25+ regulatory T cells. Proc Natl Acad Sci USA. 2005;102:5150-5155.
13. De Rosa V, Procaccini C, Calì G, Pirozzi G, Fontana S, Zappacosta S, La Cava A, Matarese G. A key role of leptin in the control of regulatory T Cell proliferation. Immunity. 2007;26:241-255.
14. Matarese G, Di Giacomo A, Sanna V, Lord GM, Howard JK, Di Tuoro A, Bloom SR, Lechler RI, Zappacosta S, Fontana S. Requirement for leptin in the induction and progression of autoimmune encephalomyelitis. J Immunol. 2001;166:5909-5916.
15. De Rosa V, Procaccini C, La Cava A, Chieffi P, Nicoletti GF, Fontana S, Zappacosta S, Matarese G. Leptin neutralization interferes with pathogenic T cell autoreactivity in autoimmune encephalomyelitis. J Clin Invest. 2006;116:447-455.
16. Hardie L, Trayhurn P, Abramovich D, Fowler P. Circulating leptin in women: a longitudinal study in the menstrual cycle and during pregnancy. Clin Endocrinol. 1997;47:101-106.
17. Schubring C, Englaro P, Siebler T, Blum WF, Demirakca T, Kratzsch J, Kiess W. Longitudinal analysis of maternal serum leptin levels during pregnancy, at birth and up to six weeks after birth: relation to body mass index, skinfolds, sex steroids and umbilical cord blood leptin levels. Horm Res. 1998;50:276-283.
18. Highman T, Friedman J, Huston L, Wong W, Catelano P. Longitudinal changes in maternal leptin serum concentrations, body composition an resting metabolic rate in pregnancy. Am J Obstet Gynecol. 1998;178:1010-1015.
19. Polman CH, Reingold SC, Edan G, Filippi M, Hartung HP, Kappos L, Lublin FD, Metz LM, McFarland HF, O'Connor PW, Sandberg-Wollheim M, Thompson AJ, Weinshenker BG, Wolinsky JS.Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria". Ann Neurol. 2005;58:840-846.
20. Kurtzke JF. Rating neurological impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology. 1983;33:1444-1452.
21. Haas J, Hommes OR. A dose comparison study of IVIG in postpartum relapsing-remitting multiple sclerosis. Mult Scler. 2207;13:900-908.
22. Dahl J, Myhr KM, Daltveit AK, Hoff JM, Gilhus NE. Pregnancy, delivery, and birth outcome in women with multiple sclerosis. Neurology. 2005;65:1961-1963.

23. Grisaru-Granovsky S, Eitan R, Algur N, Schimmel MS, Diamant YZ, Samueloff A. Maternal and umbilical cord serum leptin concentrations in small-for-gestational-age and in appropriate-for-gestational-age neonates: a maternal, fetal, or placental contribution? Biol Neonate. 2003;84:67-72.
24. Butte NF, Hopkinson JM, Nicolson MS. Leptin in human reproduction: serum leptin levels in pregnant and lactating women. J Clin Endocrinol Metab. 1997;82:585-589.
25. Hendler I, Black well SC, Mehta SH, Whitty JE, Russell E, Sorokin Y, Cotton DB.The levels of leptin, adiponectin, and resistin in normal weight, overweight, and obese pregnant women with and without preeclampsia. Am J Obstet Gynecol. 2005;193:979-983.
26. Hauguel-de Mouzon S, Lepercq J, Catalano P. The known and unknown of leptin in pregnancy. Am J Obstet Gynaecol. 2006;194:1537-1545.
27. Henson MC, Castracane VD. Leptin in pregnancy: an update. Biol Reprod. 2006;74:218-29.
28. Chatzantoni K, Papathanassopoulos P, Gourzoulidou E, Mouzaki A. Leptin and its soluble receptor in plasma of patients suffering from remitting-relapsing multiple sclerosis (MS). In vitro effects of leptin on type-1 and type-2 cytokine secretion by peripheral blood mononuclear cells, T-cells and monocytes of MS patients. J Autoimmun. 2004;23:169-177.
29. Frisullo G, Angelucci F, Mirabella M, Caggiula M, Patanella K, Nociti V, Tonali PA, Batocchi AP. Leptin enhances the release of cytokines by peripheral blood mononuclear cells from relapsing multiple sclerosis patients. J clin immunol. 2004;24:287-293.
30. Yang G, Ge H, Boucher A, Yu X, Li C. Modulation of direct leptin signaling by soluble leptin receptor. Mol Endocrinol. 2004;18:1354-1362.
31. Stein K, Vasquez-Garibay E, Kratzsch J, Romero-Velarde E, Jahreis G. Influence of nutritional recovery on the leptin axis in severely malnourished children. J Clin Endocrinol Metab. 2006;91:1021-1026.
32. Batocchi AP, Rotondi M, Caggiula M, Frisullo G, Odoardi F, Nociti V, Carella C, Tonali PA, Mirabella M. Leptin as a marker of multiple sclerosis activity in patients treated with interferon-beta. J Neuroimmunol. 2003;139:150-154.
33. Frisullo G, Mirabella M, Angelucci F, Caggiula M, Morosetti R, Sancricca C,Patanella AK, Nociti V, Iorio R, Bianco A, Tomassini V, Pozzilli C, Tonali PA,Matarese G, Batocchi AP.. The effect of disease activity on leptin, leptin receptor and suppressor of cytokine signallling-3 in relapsing-remitting multiple sclerosis. J Neuroimmunol. 2007;192:174-183.
34. Sanna V, Di Giacomo A, La Cava A, Lechler RI, Fontana S, Zappacosta S et al. Leptin surge precedes onset of autoimmune encephalomyelitis and correlates with development of pathogenic T cell responses. J Clin Invest. 2003;111:241-250.
35. Matarese G, Leiter EH, La Cava A. Leptin in autoimmunity: many questions, some answers. Tissue Antigens. 2007;70:87-95.

CHAPTER 3

CHILDHOOD ONSET IN MULTIPLE SCLEROSIS

CHAPTER 3.1

PROGNOSTIC FACTORS AFTER A FIRST ATTACK OF INFLAMMATORY CNS DEMYELINATION IN CHILDREN

R.F. Neuteboom, M. Boon, C.E. Catsman-Berrevoets, J.S. Vles, R.H. Gooskens, H. Stroink, R.J. Vermeulen, J.J. Rotteveel, I.A. Ketelslegers, E. Peeters, B.T. Poll-The, J.F. De Rijk-Van Andel, A. Verrips and R.Q. Hintzen

ABSTRACT

Objective: To identify clinical, radiologic, or CSF factors that predict conversion to multiple sclerosis (MS) after a first attack of inflammatory demyelination in children.

Methods: In this nationwide retrospective multicenter study in the Netherlands, 117 children below age 16 were included. Fifty-four children presented with a monofocal clinically isolated syndrome (CIS) and 63 children with a polyfocal CIS (PCIS).

Results: A second MS-defining attack occurred in 43% of the CIS cases, compared to 21% of the patients with PCIS onset ($p < 0.006$). Basal ganglia and thalamic lesions and lesions larger than 2 cm on MRI (considered typical of ADEM) were observed during PCIS, irrespective of the presence of encephalopathy. No significant difference in developing MS was found in children with PCIS with or without encephalopathy. Elevated IgG index and presence of oligoclonal CSF bands were more often observed in children who developed MS. Both Barkhof and KIDMUS MRI criteria shared a high specificity and had a high positive predictive value for conversion to MS. In children under the age of 10, the Barkhof criteria had a higher sensitivity than the KIDMUS criteria, but still lower than in older children.

Conclusions: Barkhof and KIDMUS MRI criteria share a high specificity and positive prognostic value for conversion to multiple sclerosis (MS). Sensitivity of these criteria is poor, especially in children below 10 years of age. Basal ganglia lesions can occur in patients who later develop MS. A substantial number of patients presenting with polyfocal onset and no encephalopathy remained monophasic.

INTRODUCTION

Onset of multiple sclerosis (MS) before the age of 16 years is reported in 2.2–5.0% of the total MS population and before the age of 10 years is observed in 0.2– 0.6%.[1-5] Diagnosis of MS in children can be more difficult than in adults. More frequently than in adults, the first attack of inflammation cannot be distinguished from acute disseminated encephalomyelitis (ADEM).[6-9]

Predicting development of MS after a first demyelinating attack is useful because of uncertainty of caregivers about prognosis and possible future therapeutic options. A cross-sectional study in children with MS showed that half of the children with MS fulfilled the Barkhof MRI criteria, which are widely accepted as prognostic for developing MS after a first clinical attack in adults.[10-11] A French study in children with a broad clinical spectrum of first demyelinating attacks showed that next to the Barkhof criteria the presence of lesions perpendicular to the corpus callosum and only well-defined lesions on MRI (KIDMUS criteria) were prognostic for developing MS.[8,12] A recent review on MRI abnormalities in children with MS stressed the problem of implementing the adult criteria to children and especially to the very young, aged under 10 years.[13] The aim of this nationwide study was to identify clinical, radiologic, or CSF factors predicting development of MS after a first inflammatory demyelinating attack in children.

METHODS

Patients

Patients were identified by members of the Dutch study group on childhood MS and ADEM. Eleven major neuropediatric centers in hospitals in nine large cities participated with complete geographic covering of the Netherlands. Children under the age of 16 with an attack, in the period 1990–2007, compatible with a demyelinating disease of the CNS, based on clinical features, were included. No other diagnosis than demyelinating disease of the CNS, like bacterial or viral infection of the CNS or a vasculitis, was allowed. Neuromyelitis optica (Devic disease) was also excluded. Length of follow-up time was determined by the last visit or telephone contact with a neurologist or pediatrician.

Definitions

A clinically isolated syndrome (CIS) was defined as a monofocal attack of CNS demyelination (in optic nerve, brainstem, hemisphere, cerebellum, or spinal cord). A polyfocal clinically isolated syndrome (PCIS) was defined as a polyregional attack, implicating multiple CNS lesions. The term ADEM was avoided because of the incomplete consensus on diagnosis, especially considering the diagnostic weight of presentation with encephalopathy and findings on MRI, which are studied here as independent variables.[14-15] This enabled us to dissect patients with PCIS into subgroups with and without encephalopathy at onset, without discussion whether they should be labeled as ADEM. Encephalopathy was defined as altered consciousness or evident change of behavior at time of debut of the attack (3

days before or after the first contact with the treating physician) not related to seizures or antiepileptic treatment. We considered a second attack within 1 month after the first attack or a second attack while on steroids or a second attack within 1 month after steroid discontinuation to be part of the initial first attack. Thus such cases were still labeled as monophasic.

A multiphasic disease course with at least 1 month between two attacks was considered MS when there was also dissemination in space, according to the revised McDonald criteria.[11]

Demographic data.
Demographic, clinical, and laboratory data were collected from chart records by a trained researcher using standardized recording sheets and kept in a database. Collected patient characteristics were age at onset and gender. Furthermore we scored the presence of a preceding infection or vaccination in the 4 weeks before onset, meningism, seizures, fever, headache, and the occurrence of a second and third attack.

MRI data
Available scans were done at 1.0 or 1.5 Tesla and typically consisted of transverse T1, T2, and proton density 3–5 mm images. In most cases T2-weighted fast-fluid-attenuated inversion recovery scans were available. Gadolinium was not administered routinely. To avoid reader bias, available MRI scans at baseline were centrally re-evaluated using a standardized MRI record form in consensus by two experienced MRI assessors (R.F.N. and R.Q.H.), blinded to clinical symptoms, disease evolution, and initial MRI analysis of the local neuroradiologist. Lesions were scored on transverse proton density and T2-weighted images. Periventricular lesions were defined as lesions in direct contact with the ventricular system. Juxtacortical lesions were defined as lesions in direct contact with the cortical gray matter with no intervening white matter. Infratentorial locations consisted of brainstem and cerebellar lesions. We assessed the fulfillment (at least three out of four) of the Barkhof criteria: 1) at least nine lesions on the T2-weighted images; 2) presence of at least three periventricular lesions; 3) presence of at least one juxtacortical lesion; 4) presence of at least one infratentorial lesion.[16] Additional variables tested for prediction of MS conversion were white matter lesions perpendicular to the corpus callosum, gray matter lesions in the thalamus or basal ganglia, and T2 lesion size (large lesions were defined as lesions with a maximal diameter over 2 cm, small lesions had a maximal diameter below 2 cm). We also scored the sole presence of well-defined lesions, which were defined by clear lesions borders; an abrupt decrease in intensity of T2-weighted signal at the borderline between lesion and surrounding brain tissue.[12]

CSF data
Data on CSF were collected from the period during or soon after the first clinical attack. IgG index upper cutoff value of 0.68 was selected.[17] CSF oligoclonal bands (OCB) detected with immunoelectric focusing were positive in case of two or more bands present in CSF, but not in serum.

Statistical analysis

Descriptive data were compared by means of the x^2 test and Fisher exact test for proportions and the *t* test and Mann-Whitney test for continuous measures. Survival curves were estimated using the Kaplan-Meier method. Time zero for the survival analysis was taken as the date of the first attack. The primary endpoint was conversion to MS. Time to conversion to MS was defined by the time between the first and second attack. For event-free subjects the follow-up period ended on the date of the last known visit, at which point the time was censored. *p* Value below 0.05 was considered significant. Statistical analysis was performed using SPSS version 11.0.

RESULTS

A total of 117 children were included in the study with a mean follow-up of 54 months (median 43 months, range 5–201 months). Clinical characteristics are depicted in table 1. Sixty-five (56%) children had a first presentation below the age of 10 years and 40 (34%) below the age of 6. In total, 37 of the 117 patients were diagnosed with MS, fulfilling the clinical McDonald criteria.

PCIS was seen in 63 children. Thirteen of them (21%) had a second MS defining attack. The mean time to this second attack was 24.7 months (median: 10 months, range: 2–79 months). In 5 (38%) children this second attack was again a polyfocal attack. Children with PCIS and encephalopathy, fulfilling the proposed international criteria for ADEM,[14] had a lower age at onset and a higher prevalence of seizures than children with PCIS without encephalopathy, but showed no significant difference in the other clinical features. Children with PCIS without encephalopathy progressed to MS more frequently then children with PCIS with encephalopathy, but this difference was not significant (table 1). Basal ganglia or thalamic lesions and large lesions on MRI were both seen in children with PCIS with and without encephalopathy (table 1). Using the definitions from the international pediatric MS study group,[14] we found only two cases of multiphasic ADEM and no cases of recurrent ADEM.

Fifty-four children presented with CIS. Of these 54 children, 12 had optic neuritis, 17 isolated transverse myelitis, 18 brainstem symptoms, 4 hemispheric symptoms, and 3 had cerebellar symptoms. Twenty-four (44%) children with a CIS had a second, MS defining, attack. The mean time to conversion to MS after a CIS was 17.7 months (median: 12 months, range: 2–75 months).

Children with a final diagnosis of PCIS were younger, had significantly more often encephalopathy, headache, fever, and seizures, as well as a preceding infectious episode within the 4 weeks preceding the onset of initial attack (table 2). Mean time to MS diagnosis in the total group was 18.6 months (median: 11 months, range: 2–79). This was significantly shorter than the duration of follow-up of both the groups with a final diagnosis of CIS (mean: 52.7 months, median: 42.5 months, range: 12–162 months) and PCIS (mean: 44.8 months, median: 32 months, range: 5–201 months).

Table 1. Clinical and MRI features at baseline in patients with a diagnosis of CIS and PCIS at onset

	type of onset CIS	PCIS				
		all	with encepha-lopathy	no encepha-lopathy	p-value*	p-value**
clinical features	n=54	n=63	n=36	n=27		
mean age at onset (years)	10.5	6.8	5.6	8.4	<0.001	0.011
male gender (%)	44	65	72	55	0.025	n.s.
meningism (%)	2	22	31	11	0.001	n.s.
headache (%)	2	32	31	33	<0.001	n.s.
fever (%)	4	38	42	33	<0.001	n.s.
seizures (%)	0	24	36	7	<0.001	0.008
infections (%)	20	49	55	41	0.002	n.s.
vaccination(%)	2	3	6	0	n.s.	n.s
cinical outcome at end of follow-up						
progression to MS(%)	44	21	17	26	0.006	n.s.
MRI features	n=49	n=61	n=35	n=26		
thalamus / basal ganglia lesions (%)	4	56	51	62	<0.001	n.s.
large lesions (%)	10	67	80	50	<0.001	0.014

* CIS compared with PCIS (all)
** PCIS with encephalopathy compared to PCIS without encephalopathy
CIS = clinically isolated syndrome, PCIS = polyfocal clinically isolated syndrome, MS = multiple sclerosis, n.s. = not significant

Table 2. Clinical and CSF features and at baseline of children with a final diagnosis of CIS, PCIS and MS

	final diagnosis at end of follow-up monophasic			
	CIS	PCIS	MS	P-value*
clinical data	n=30	n=50	n=37	
mean age at onset (years) range (years)	9.6 1-15	6.4 1-14	10.4 1-15	<0.001
follow up mean (months)	52.7	44.8	66	n.a.
follow up median (months)	42.5	32	60	n.a.
male gender (%)	43	66	51	n.s.
encephalopathy (%)	0	60	16	<0.001
meningism (%)	3	20	11	n.s.
headache (%)	3	34	8	0.005
fever (%)	7	42	8	<0.001

seizures (%)	0	28	3	0.02
preceding infection (%)	27	50	24	0.015
CSF data				P-value**
IgG- index	n=17	n=26	n=29	
>0.68 (%)	17	27	66	<0.001
oligoclonal banding	n=16	n=19	n=26	
present (%)	0	16	46	0.001

* MS compared to PCIS
** MS compared to CIS and PCIS
CIS = clinically isolated syndrome, PCIS = polyfocal clinically isolated syndrome, MS = multiple sclerosis, n.a. = not applicable, n.s. = not significant

Table 3. MRI features of children with MS and monophasic disease (CIS and PCIS) younger and older than ten years at onset of symptoms

	MS			monophasic disease			p-value		
	all n=35	<10 yr n=11	≥10yr n=24	all n=75	<10 yr n=48	≥10 yr n=27	all	<10yr	>10yr
Barkhof									
≥ 9 lesions T2 (%)	66	64	67	17	21	11	<0.001	0.005	<0.001
infratentorial lesion (%)	88	91	88	43	46	37	<0.001	0.007	<0.001
juxtacortical lesions (%)	74	64	79	31	33	26	<0.001	n.s.	<0.001
≥ 3 periventricular lesions (%)	60	55	63	5	4	7	<0.001	<0.001	<0.001
3-4 Barkhof criteria (%)	60	45	67	7	6	7	<0.001	0.008	<0.001
supplemental									
lesions perpendicular to corpus callosum (%)	60	27	75	5	6	4	<0.001	0.037	<0.001
thalamus / basal ganglia lesions (%)	20	36	13	40	48	26	0.039	n.s.	n.s.
large lesions (%)	20	55	4	52	58	41	0.002	n.s.	0.002
small lesions (%)	74	64	79	25	25	26	<0.001	0.013	<0.001
only well-defined lesions (%)	40	36	67	8	6	13	<0.001	0.018	<0.001
KIDMUS (%)	49	18	63	4	4	4	<0.001	n.s.	<0.001

CIS = clinically isolated syndrome, PCIS = polyfocal clinically isolated syndrome, MS = multiple sclerosis, KIDMUS criteria = presence of both lesions perpendicular to the corpus callosum and only well-defined lesions, <10 yr = age at onset under 10 years, ≥10yr = age at onset above 10 years, n.s. = not significant

CSF was obtained in all but one patient. IgG index was tested in 72 children within 1 month and in one child within 5 months after onset. An elevated IgG index was significantly more frequently seen in children with MS. OCB were tested in 61 children within 1 month and in two children within 5 months after onset. OCB was significantly more often seen in children with MS (table 2).

A total of 110 brain MRIs at baseline were available. Five children had only a spinal cord MRI and two baseline MRI scans were not available. MRI data are shown in table 3. Each of the four individual Barkhof criteria was significantly more often observed with children with MS. This was not the case for juxtacortical lesions in children younger than 10 years. Presence of at least three Barkhof criteria was significantly more frequently found in children with diagnosed MS in both age groups.

Survival analysis of the children scoring at least three Barkhof criteria showed a shorter mean time to the second attack of 45 months (95% CI: 18–73) vs 123 months (95% CI: 103–142) (log-rank test p value < 0.0001) in the other children (figure A). When stratified for age below and above 10 years, difference in time to second attack remained significant.

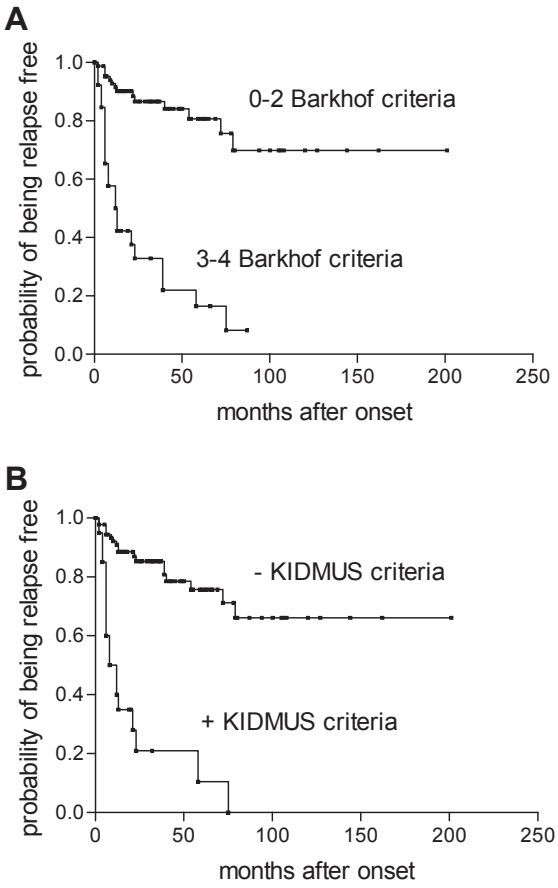


Figure 1. (A) Survival curves of the time preceding the second attack according to the presence of 0-2 and 3-4 Barkhof criteria on baseline MRI. Log-rank test p<0.0001. (B) Survival curves of the time preceding the second attack according to the presence of both lesions perpendicular to the corpus callosum and the presence of only well-defined lesions (KIDMUS criteria) on baseline MRI. Log-rank test p<0.0001.

Lesions perpendicular to the corpus callosum and only well-defined lesions (the KIDMUS criteria) and small lesions were significantly more often found in children with MS. Large lesions and basal ganglia lesions were significantly less frequently observed in children with MS. This difference was not seen in children aged under 10. Positivity of both KIDMUS criteria was observed significantly more often in children with MS in the total group but not in the group younger than 10 years.

Survival analysis of the children with the KIDMUS criteria showed a shorter mean time to the second attack of 22 months (95% CI: 10–33) vs 143 months (95% CI: 119–168) (log-rank test *p* value: < 0.0001) in the children without (figure B). When stratified for age below and over 10 years, difference in time to second attack remained significant. The test properties of the Barkhof and KIDMUS criteria are described in table 4.

Table 4. Test properties of the Barkhof and KIDMUS criteria

	Barkhof			KIDMUS		
	all	<10 years	≥10 years	all	<10 years	≥10 years
sensitivity (%)	60	45	67	49	18	63
specificity (%)	92	94	93	96	96	96
positive predictive value (%)	81	63	89	83	50	85
negative predictive value (%)	83	88	75	81	84	80

KIDMUS criteria = presence of both lesions perpendicular to the corpus callosum and only well-defined lesions, Barkhof criteria = (1) ≥ 9 T2 lesions, (2) infratentorial lesions, (3) juxtacortical lesions, (4) ≥ 3 periventricular lesions, <10 years = age at onset under 10 years, ≥10years = age at onset above 10 years

DISCUSSION

This nationwide study in the Netherlands focused solely on patients from neuropediatric clinics. Due to the retrospective nature of this study, follow-up time of children with a monophasic course was shorter than those with MS (table 2). Nevertheless, the time to conversion to MS in the MS group was significantly shorter than the duration of follow-up of the children who remained monophasic. This indicates that follow-up even in the monophasic patients was likely sufficient to detect the occurrence of a second attack. It is not excluded that children who are now monophasic during follow-up still can have a second MS defining attack in the future.

A diagnosis of ADEM frequently incorporates ADEM- like MRI lesions. These typically large lesions are often related to encephalopathy, making it difficult to independently assess the contribution of such MRI abnormalities and encephalopathy to further conversion into MS. Therefore we avoided the term ADEM, but split the polyfocal onset group into subgroups with and without encephalopathy, regardless of MRI findings. The obligatory presence of encephalopathy for ADEM diagnosis, as proposed by the international pediatric MS study group,14 may carry the risk of underestimation of ADEM cases. In a recent study, not all children with a brain MRI typical of ADEM had encephalopathy.[8] The definition of

encephalopathy deserves further specification in future studies. It has been proposed to include in the definition of encephalopathy the presence of irritability and lethargy.[14] These two features were not part of our definition of encephalopathy, because they may also be observed in any generally sick child.

We also found the presence of large lesions and basal ganglia or thalamic lesions, considered typical of ADEM, in children who had a second MS defining attack and even in children without encephalopathy. Furthermore, we found no statistical difference in progression to MS in the children with PCIS with or without encephalopathy, although there was a trend that children with encephalopathy remained more often monophasic. The 21% of children developing MS in the whole PCIS group was in line with results reported before.[8] Also time to a third attack during follow-up of children with MS was not related to either CIS or PCIS onset type (not shown). An interesting finding was that seizures almost exclusively occurred in the group with a monophasic PCIS, which makes seizures a possible relevant prognostic factor. Using the definitions from the international pediatric MS study group,[14] we found only two cases of multiphasic ADEM and no cases of recurrent ADEM, indicating that these, at least in our study group, constitute a small subgroup of total numbers of children with multiphasic demyelinating diseases.

In adults it is suggested that an elevated CSF IgG index is predictive of MS.[18] In children this has been studied in less detail. We found elevated IgG indices in the majority of cases tested. This sign of intrathecal IgG production was significantly more often found in children with MS. OCB in adults is more sensitive and specific than IgG index in the CSF.[18] OCB, when tested, was found significantly more frequently in the group diagnosed with MS than in the group with a final diagnosis of monophasic CIS or PCIS, as has been reported before.[19]

Three points should be taken into account in respect to the CSF data. First, we did not have information on CSF IgG index and OCB in all patients. Second, some samples were not taken within 1 month after onset of symptoms, although this only occurred in less than 5% of the children. Finally, data were collected in multiple laboratories in a period of more than 10 years, so OCB were not tested uniformly. However, the main technical variance of OCB testing lies in sensitivity. Perhaps future testing in a more optimized and protocolized fashion may even improve the predictive power of this test.

A prognostic factor for progression to MS was the presence of at least three positive Barkhof criteria in children aged above and below 10 years at onset. In the French study it was observed that lesions perpendicular to the corpus callosum as well as only welldefined lesions were prognostic factors for developing MS.[10] We confirm this finding, although not in the group with age at onset below 10 years.

Comparison of test qualities of the Barkhof and KIDMUS criteria showed a low sensitivity, but high specificity for both sets of criteria. Especially in children below 10 years, sensitivity was low. In adults the sensitivity of the Barkhof criteria is 49–74%.[16] The low sensitivity of the KIDMUS criteria in children under age 10 (18%) has been reported earlier and is confirmed here.[10] It should be noted that both our and the other available retrospective studies did not use standardized MRI protocols.

Better tools to predict development of MS are needed. The recent worldwide collaborative efforts will provide longer follow-up data on disease course and disability.[20] Such studies will include the assessment of the possible extra value of scoring dissemination in time by MRI, the validation of novel MRI variables, and novel biomarkers. The possibility of a subgroup remaining monophasic after presentation with polyfocal CIS, reminiscent of ADEM but without encephalopathy, deserves further study.

REFERENCES

1. Duquette P, Murray TJ, Pleines J, Ebers GC, Sadovnick D, Weldon P, Warren S, Paty DW, Upton A, Hader W, et al. Multiple sclerosis in childhood: clinical profile in 125 patients. J Pediatr. 1987;111:359-363.
2. Ghezzi A, Deplano V, Faroni J, Grasso MG, Liguori M, Marrosu G, Pozzilli C, Simone IL, Zaffaroni M. Multiple sclerosis in childhood: clinical features of 149 cases. Mult Scler. 1997;3:43-46.
3. Sindern E, Haas J, Stark E, Wurster U. Early onset MS under the age of 16: clinical and paraclinical features. Acta Neurol Scand. 1992;86:280-284.
4. Renoux C, Vukusic S, Mikaeloff Y, Edan G, Clanet M, Dubois B, Debouverie M, Brochet B, Lebrun-Frenay C, Pelletier J, Moreau T, Lubetzki C, Vermersch P, Roullet E, Magy L, Tardieu M, Suissa S, Confavreux C; Adult Neurology Departments KIDMUS Study Group. Natural history of multiple sclerosis with childhood onset. N Engl J Med. 2007;356:2603-2613.
5. Boiko A, Vorobeychik G, Patsy D, Devonshire V Sadovnick D, and the UBC MS Clinic Neurologists. Early onset multiple sclerosis. A longitudinal study. Neurology. 2002;59:1006-1010.
6. Menge T, Hemmer B, Nessler S, Wiendl H, Neuhaus O, Hartung HP, Kieseier BC, Stüve O. Acute disseminated encephalomyelitis: an update. Arch Neurol. 2005;62:1673-1680.
7. Mikaeloff Y, Caridade G, Husson B, Suissa S, Tardieu M; Neuropediatric KIDSEP Study Group of the French Neuropediatric Society. Acute disseminated encephalomyelitis cohort study: prognostic factors for relapse. Eur J Paediatr Neurol. 2007;11:90-95.
8. Mikaeloff Y, Suissa S, Vallée L, Lubetzki C, Ponsot G, Confavreux C, Tardieu M;KIDMUS Study Group. First episode of acute CNS inflammatory demyelination in childhood: prognostic factors for multiple sclerosis and disability. J Pediatr. 2004;144:246-252.
9. Tenenbaum S, Chamoles N, Fejerman N. Acute disseminated encephalomyelitis. Along-term follow-up study of 84 pediatric patients. Neurology. 2002;59:1224-1231.
10. Hahn CD, Manohar M, Shroff M, Blaser S, Banwell B. MRI criteria for multiple sclerosis. Evaluation in a pediatric cohort. Neurology. 2004;62:806-808.
11. Polman CH, Reingold SC, Edan G, Filippi M, Hartung HP, Kappos L, Lublin FD, Metz LM, McFarland HF, O'Connor PW, Sandberg-Wollheim M, Thompson AJ, Weinshenker BG, Wolinsky JS. Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria". Ann Neurol. 2005;58:840-846.
12. Mikaeloff Y, Adamsbaum C, Husson B, Vallée L, Ponsot G, Confavreux C, Tardieu M, Suissa S; KIDMUS Study Group on Radiology. MRI prognostic factors for relapse after acute CNS inflammatory demyelination in childhood. Brain. 2004;127:1942-1947.
13. Banwell B, Shroff M, Ness JM, Jeffery, D Schwid S, Weinstock-Guttman B; for the international pediatric MS study group. MRI features of pediatric multiple sclerosis. Neurology. 2007;68:S46-S53.
14. Krupp LB, Banwell B, Tenembaum S; for the international pediatric MS study group. Consensus definitions proposed for pediatric multiple sclerosis and related disorders. Neurology. 2007;68:S7-S12.
15. Tardieu M, Mikaeloff Y. What is acute disseminated encephalomyelitis (ADEM)? Eur J Peadiatr Neurol. 2004;8:239-242.
16. Korteweg T, Tintoré M, Uitdehaag B, Rovira A, Frederiksen J, Miller D, Fernando K, Filippi M, Agosta F, Rocca M, Fazekas F, Enzinger C, Matthews P, Parry A, Polman C, Montalban X, Barkhof F.MRI criteria for dissemination in space in patients with clinically isolated syndromes: a multicentre follow-up study. Lancet Neurol. 2006;5:221-227.
17. Eeg-Olofsson O, Link H, Wigertz A. concentrations of CSF proteins as a measure of blood brain barrier function and synthesis of IgG within the CNS in "normal" subjects from the age of 6 months to 30 years. Acta Paediatr Scand. 1981;70:167-170.
18. Freedman MS, Thompson EJ, Deisenhammer F, Giovannoni G, Grimsley G, Keir G, Ohman S, Racke MK, Sharief M, Sindic CJ, Sellebjerg F, Tourtellotte WW.Recommended standard of cerebrospinal fluid analysis in the diagnosis of multiple sclerosis. Arch neurol. 2005;62:865-870.
19. Pohl D, Rostasy K, Reiber H, Hanefeld F. CSF characteristics in early-onset multiple sclerosis. Neurology. 2004;63:1966-1967.
20. Belman AL, Chitnis T, Renoux C, Waubant E; for the international pediatric MS study group. Challenges in the classification of pediatric multiple sclerosis and future directions. Neurology. 2007;68:S70-S74.

CHAPTER 3.2

BARKHOF MAGNETIC RESONANCE IMAGING CRITERIA PREDICT EARLY RELAPSE IN PEDIATRIC MULTIPLE SCLEROSIS

R.F. Neuteboom, I.A. Ketelslegers, M. Boon, C.E. Catsman-Berrevoets and R.Q. Hintzen

On behalf of the Dutch childhood MS and ADEM study group

ABSTRACT

We sought to identify clinical and radiologic features predicting early relapse after a diagnosis of multiple sclerosis in children. In this nationwide retrospective multicenter study in The Netherlands, we included 28 children with multiple sclerosis with onset before age 16 years. Magnetic resonance mages and clinical features at the onset of disease were evaluated. The mean follow-up time was 55 months. Twenty children (71%) had a relapse during follow-up. We found that the presence of at least three of four Barkhof magnetic resonance imaging criteria at the onset of multiple sclerosis signs is predictive of early relapse after a diagnosis of multiple sclerosis in children ($P < 0.05$).

INTRODUCTION

The onset of multiple sclerosis before age 16 years is very rare.[1-5] Multiple studies on the prognostic value of demographic and clinical features of the course of this disease were reported in adult cohorts, not including magnetic resonance imaging data.[2-6] Several recent studies reported on the prognostic value of multiple clinical features, including magnetic resonance imaging and cerebrospinal fluid data, after a first demyelinating attack in pediatric cohorts.[7-9] These studies shed more light on the sometimes difficult process of diagnosing multiple sclerosis in children. Still, little is known about identifying the subgroup of children who will undergo early relapse after a diagnosis of multiple sclerosis. This knowledge would be useful when counselling children and their parents. Furthermore, it would enable us to select children eligible for immunomodulatory treatment in future trials. Recently, a childhood-onset multiple sclerosis potential index for early severity was proposed.[10] This nationwide study sought to identify clinical and radiologic factors predicting early relapse after a diagnosis of multiple sclerosis, and to determine the childhood-onset multiple sclerosis potential index for early severity in another cohort.

METHODS

Patients and definitions

Patients were identified by members of the Dutch study group on childhood multiple sclerosis and acute disseminated encephalomyelitis. We included children with multiple sclerosis who exhibited their first attack before age 16 years, with brain magnetic resonance imaging scans available for evaluation, during the period 1990-2007.

A multiphasic disease course with at least 1 month between two clinical attacks was considered indicative of multiple sclerosis when there was also dissemination in space, according to the revised McDonald criteria.[11] We considered a second attack within 1 month after the first attack, or a second attack during the administration of steroids, or a second attack within 1 month after steroid discontinuation, to be part of the initial first attack. Thus, such cases were still labeled as monophasic. No diagnosis other than demyelinating disease of the central nervous system, such as bacterial or viral infection of the central nervous system or a vasculitis, was allowed. Neuromyelitis optica (Devic's disease) was also excluded. The length of follow-up time was determined by the most recent visit or telephone contact with a neurologist or pediatrician.

Demographic and clinical data

Demographic and clinical data were collected from chart records by a trained researcher, using standardized recording sheets. Patient characteristics included age at onset and sex. We also scored the presence of polyfocal onset, encephalopathy, the use of disease-modifying drugs, the occurrence of a relapse after the diagnosis of multiple sclerosis, and time between the first attack and the diagnosis of multiple sclerosis. Encephalopathy was defined as altered consciousness or evident change of behaviour at the time of debut of the attack, not related to seizures or antiepileptic treatment.

Magnetic resonance imaging data

All magnetic resonance imaging scans of the brain were evaluated as previously described.[7] We assessed the fulfilment of at least three out of four Barkhof criteria: (1) at least nine lesions on the T2-weighted images; (2) the presence of at least three periventricular lesions; (3) the presence of at least one juxtacortical lesion; and (4) the presence of at least one infratentorial lesion.[11] We also scored the presence of lesions in the thalamus and basal ganglia. Furthermore, we scored the presence of white-matter lesions perpendicular to the corpus callosum and the sole presence of well defined lesions. The last two variables are known as the KIDMUS criteria.[9]

Childhood-onset multiple sclerosis potential index for early severity

The childhood-onset multiple sclerosis potential index for early severity was calculated according to the methods of Mikaeloff et al.[10] This index is based on the presence of one or two of the KIDMUS magnetic resonance imaging criteria, female sex, a short interval between the first and second (multiple sclerosis-defining) attack, absence of encephalopathy, and a progressive disease course.

Statistical Analysis

Descriptive data were compared by means of the Fisher's exact test for proportions and the Mann Whitney U test for continuous measures. Survival curves were estimated using the Kaplan-Meier method. Time zero for the survival analysis was taken as the date of the first attack. The primary endpoint was the first relapse after the diagnosis of multiple sclerosis. Time to conversion to multiple sclerosis was defined as the time between the first and second attacks. For event-free patients, the follow-up period ended on the date of the last known visit, at which point the time was censored. We considered $P < 0.05$ significant. Statistical analysis was performed using SPSS, version 16.0 (SPSS, Inc., Chicago, IL).

RESULTS

Twenty-eight children with childhood multiple sclerosis were included. All children exhibited relapsing, remitting multiple sclerosis. The mean age at onset was 10.7 years (S.D., ±3.9 years; median, 12.0 years). Ten (36%) children were less than age 10 years, and four (14%) children were aged less than 6 years, at onset. Twenty children (71%) manifested a relapse after the diagnosis of multiple sclerosis during follow-up. Three of these 20 children underwent interferon-β treatment at that time. The mean time from the diagnosis of multiple sclerosis to this relapse was 26 months (range, 2-96 months; median, 16 months). The mean follow-up time in the group with no relapse after a diagnosis of multiple sclerosis was 51 months (range, 18-95 months; median, 42 months). None of the children exhibited a progressive course during follow-up. The clinical and magnetic resonance imaging features at time of the first attack are listed in Table 1. The presence of three or more periventricular lesions, lesions perpendicular to the corpus callosum, and at least three of four Barkhof

magnetic resonance imaging criteria were associated with a relapse during follow-up. Moreover, the absence of polyfocal onset with encephalopathy, and the absence of thalamic and basal-ganglia lesions, were associated with relapse during follow-up.

Table 1. Clinical and MRI features at time of the first attack

	all n=28	relapse after diagnosis MS present n=20	absent n=8	p-value
clinical features at first attack				
age at onset MS >10 years	19	15	4	n.s.
female gender	14	11	3	n.s.
time between first attack and diagnosis MS <12 months	15	13	2	n.s.
PCIS with encephalopathy	5	1	4	0.015
PCIS	9	4	5	n.s.
MRI features at first attack				
≥9 lesions on T2	21	17	4	n.s.
infratentorial lesions	25	19	6	n.s.
juxtacortical lesions	21	16	5	n.s.
≥3 periventricular lesions	17	15	2	0.030
thalamic / basal ganglia lesions	6	2	4	0.038
lesions perpendicular to corpus callosum	16	14	2	0.044
only well defined lesions	17	13	4	n.s.
3 or 4 Barkhof criteria	18	16	2	0.011
1 or 2 KIDMUS criteria	21	15	6	n.s.
2 KIDMUS criteria	15	13	2	n.s.

MS = multiple sclerosis, PCIS = polyfocal clinically isolated syndrome,
Barkhof criteria: 1) at least nine lesions on the T2-weighted images; 2) presence of at least three periventricular lesions; 3) presence of at least one juxtacortical lesion; 4) presence of at least one infratentorial lesion
KIDMUS criteria: 1) white matter lesions perpendicular to the corpus callosum; 2) only well defined lesions

The mean childhood-onset multiple sclerosis potential index for early severity was 7.4 (S.D. ±3.1) in the group with relapse after follow-up, and this index was lower in the group without relapse, at 4.9 (S.D. ±3.8). This difference was not statistically significant.

A survival analysis of the children meeting at least three Barkhof criteria indicated a shorter mean time to first relapse of 31 months after a diagnosis of multiple sclerosis (95% confidence interval, 16-45 months), versus 62 months (95% confidence interval, 39-84 months) (log-rank test P value, 0.049) in the other children (Fig 1).

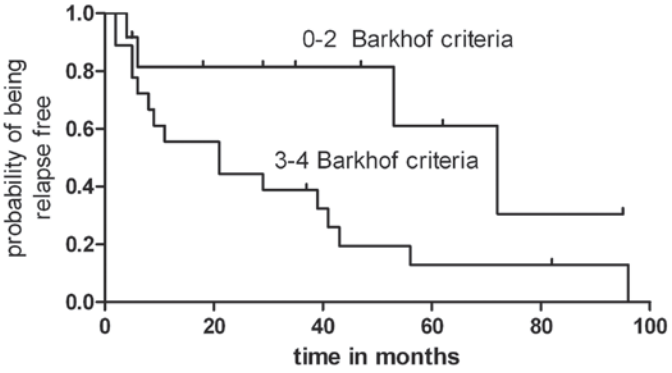


Figure 1. Survival curves of the time preceding the next relapse after diagnosis MS according to the presence of 0-2 and 3-4 Barkhof criteria on baseline MRI (Log-rank test p=0.049). (Barkhof MRI criteria: 1. at least nine lesions on the T2-weighted images; 2. presence of at least three periventricular lesions; 3. presence of at least one juxtacortical lesion; 4. presence of at least one infratentorial lesion)

DISCUSSION

In this nationwide multicenter study, we evaluated several clinical and radiologic parameters in children with a diagnosis of multiple sclerosis. Using survival analysis, we found that the presence of at least three positive Barkhof criteria in children was a prognostic factor for early relapse after a diagnosis of multiple sclerosis.

The retrospective nature of our study and the relatively small number of patients limited our analysis. The minimal follow-up time in the group with no relapse after the diagnosis of multiple sclerosis was 18 months. Naturally, the children with no relapse may exhibit relapse in the future.

We know of only one other study to report on prognostic factors for early relapse in a pediatric cohort .[10] This French study, investigating prognostic factors for early severity in children with multiple sclerosis, indicated that the presence of one or two of the KIDMUS magnetic resonance imaging criteria, female sex, a short interval between the first and second (multiple sclerosis-defining) attacks, the absence of encephalopathy, and a progressive course of disease were independent prognostic factors for early severity. Except in terms of progressive course of the disease, we observed the same trend for all of these features, but these differences did not achieve statistical significance. The childhood-onset multiple sclerosis potential index for early severity was higher in children who manifested a relapse after the diagnosis of multiple sclerosis, although this difference was not significant.

A difference between our study and this French study was that their endpoint definition was the occurrence of a relapse after the diagnosis of multiple sclerosis or development of an expanded disability status score[12] of 4.Nevertheless, a majority (>95%) of those children who reached the endpoint manifested a relapse after the diagnosis of multiple sclerosis before ever developing an expanded disability status score of 4, making our study comparable with

the French study.

We agree with the French study on the importance of using magnetic resonance imaging criteria, to identify those children with a high risk of early relapse. Still larger collaborative prospective studies are required to address this question fully. These efforts should also include an international consensus on the definition of disease severity.

REFERENCES

1. Sindern E, Haas J, Stark E, Wurster U. Early onset MS under the age of 16: clinical and paraclinical features. Acta Neurol Scand. 1992;86:280-284.
2. Duquette P, Murray TJ, Pleines J, Ebers GC, Sadovnick D, Weldon P, Warren S, Paty DW, Upton A, Hader W, Nelson R, Auty A, Neufeld B, Meitzer C. Multiple sclerosis in childhood: clinical profile in 125 patients. J Pediatr. 1987; 111:359-363.
3. Ghezzi A, Deplano V, Faroni J, Grasso MG, Liguori M, Marrosu G, Pozzilli C, Simone IL, Zaffaroni M. Multiple sclerosis in childhood: clinical features of 149 cases. Mult Scler. 1997;3:43-46.
4. Renoux C, Vukusic S, Mikaeloff Y, Edan G, Clanet M, Dubois B, Debouverie M,Brochet B, Lebrun-Frenay C, Pelletier J, Moreau T, Lubetzki C, Vermersch P, Roullet E, Magy L, Tardieu M, Suissa S, Confavreux C; Adult Neurology Departments KIDMUS Study Group. Natural history of multiple sclerosis with childhood onset. N Engl J Med. 2007;356:2603-2613.
5. Boiko A, Vorobeychik G, Patsy D, Devonshire V Sadovnick D, and the UBC MS Clinic Neurologists. Early onset multiple sclerosis. A longitudinal study. Neurology. 2002;59:1006-1010.
6. Simone IL, Carrara D, Tortorella C, Liguori M, Lepore V, Pellegrini F, Bellacosa A, Ceccarelli A, Pavone I, Livrea P. Course and prognosis in early-onset MS: comparison with adult-onset forms. Neurology. 2002;59:1922-1928.
7. Neuteboom RF, Boon M, Catsman Berrevoets CE, Vles JS, Gooskens RH, Stroink H, Vermeulen RJ, Rotteveel JJ, Ketelslegers IA, Peeters E, Poll-The BT, De Rijk-Van Andel JF, Verrips A, Hintzen RQ; Prognostic factors after a first attack of inflammatory CNS demyelination in children. Neurology. 2008;71:967-973.
8. Mikaeloff Y, Suissa S, Vallée L, Lubetzki C, Ponsot G, Confavreux C, Tardieu M;KIDMUS Study Group. First episode of acute CNS inflammatory demyelination in childhood: prognostic factors for multiple sclerosis and disability. J Pediatr 2004;144:246-252.
9. Mikaeloff Y, Adamsbaum C, Husson B, Vallée L, Ponsot G, Confavreux C, Tardieu M, Suissa S; KIDMUS Study Group on Radiology. MRI prognostic factors for relapse after acute CNS inflammatory demyelination in childhood. Brain. 2004;127:1942-1947.
10. Mikaeloff Y, Caridade G, Assi S, Suissa S, Tardieu M. Prognostic factors for early severity in a childhood multiple sclerosis cohort. Pediatrics. 2006;118:1133-1139.
11. Polman CH, Reingold SC, Edan G, Filippi M, Hartung HP, Kappos L, Lublin FD, Metz LM, McFarland HF, O'Connor PW, Sandberg-Wollheim M, Thompson AJ, Weinshenker BG, Wolinsky JS. Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria". Ann Neurol. 2005;58:840-846.
12. Kurtzke JF. Rating neurological impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology. 1983;33:1444-1452.

CHAPTER 3.3

A COMPARISON OF MRI CRITERIA FOR DIAGNOSING PEDIATRIC ADEM AND MS

I.A. Ketelslegers, R.F. Neuteboom, M. Boon, C.E. Catsman-Berrevoets and R.Q. Hintzen

On behalf of the Dutch childhood MS and ADEM study group

ABSTRACT

Background: Brain MRI is a useful tool for diagnosing inflammatory demyelinating disorders in children. However, it remains unclear which are the most reliable criteria for distinguishing multiple sclerosis (MS) from monophasic disorders such as acute disseminated encephalomyelitis (ADEM). We therefore compared the 4 current sets of MRI criteria in our Dutch pediatric cohort and determined which are the most useful in clinical practice for distinguishing ADEM from MS.

Methods: We included 49 children who had had a demyelinating event and an MRI scan within 2 months of their first clinical attack. Twenty-one patients had ADEM and remained relapse-free after at least 2 years of follow-up. Twenty-eight patients had a definitive diagnosis of MS. We assessed the sensitivity and specificity of the following MRI criteria: Barkhof criteria, KIDMUS criteria, Callen MS-ADEM criteria, and Callen diagnostic MS criteria.

Results: The Callen MS-ADEM criteria had the best combination of sensitivity (75%) and specificity (95%). The KIDMUS criteria had higher specificity (100%), but much lower sensitivity (11%). The Barkhof criteria had a sensitivity of 61% and a specificity of 91%. The Callen diagnostic MS criteria were the most sensitive (82%), but were only 52% specific for distinguishing a first attack of MS from ADEM.

Conclusions: The results in our cohort demonstrate that the new Callen criteria for multiple sclerosis-acute disseminated encephalomyelitis (MS-ADEM) are the most useful for differentiating a first attack of MS from monophasic ADEM. Although the Callen diagnostic MS criteria are more sensitive, they lack the specificity necessary to differentiate MS from ADEM.

INTRODUCTION

In children who have a first attack of immune-mediated demyelination, it is usually difficult to distinguish multiple sclerosis (MS) from acute disseminated encephalomyelitis (ADEM). This distinction is important, because ADEM is considered a monophasic disease with a more benign course, while MS is a lifelong chronic illness. Multiple subsequent attacks leading to a diagnosis of MS occur in approximately 20% of children initially diagnosed with ADEM.[1-5]

MRI is a useful tool in confirming the diagnosis of acute immune-mediated demyelination and may become more useful for recognizing patients who are at risk of future attacks of demyelination or MS. Until recently, 2 sets of criteria were available for predicting conversion to MS after a first demyelinating event: the Barkhof MRI criteria were developed for adult patients[6] and the MRI KIDMUS criteria were the first criteria developed for children.[7] In our Dutch cohort of pediatric MS patients, we have already confirmed that both sets of criteria have a high specificity and positive predictive value.[4] However, their sensitivity is poor, especially in children younger than 10 years.[4-5,7-8]

Two recent studies by one group emphasize the need to develop MRI criteria in children that can reliably distinguish the first attack of MS from ADEM and other neurologic diseases.[8-9] The first study developed criteria that can help distinguish patients with MS at first attack from patients with monophasic ADEM.[8] The second study proposed modifications to the Barkhof criteria that would enhance the diagnostic accuracy of these criteria for MS in children. These criteria were initially designed to distinguish between MS (at second attack) and other, nondemyelinating, diseases (SLE and migraine). As the authors state, it is necessary to evaluate the usefulness of these criteria in predicting conversion to MS at the first demyelinating attack.[9]

Although all these criteria were developed for a slightly different purpose, it is of interest to assess to what extent these criteria are useful to distinguish MS at first attack from monophasic ADEM, and in that way can predict conversion to MS. Therefore we compared the test properties of the existing MRI criteria for MS in distinguishing MS at first attack from ADEM and validated the recently proposed criteria in our Dutch pediatric cohort.

METHODS

Patients and definitions

We included consecutive patients under 17 years old whose first demyelinating event of the CNS had occurred between 1995 and 2008. All children were identified by the Dutch Study Group for Pediatric MS, which consists of 15 major pediatric neurology centers in the Netherlands. Uniform definitions were used across sites. Clinical data were retrieved from a central database.[4]

Children were only eligible for this study when a cerebral MRI scan had been obtained within 2 months of the first clinical event and when a follow-up time of at least 2 years was reached. We classified children as either monophasic ADEM or MS (based on clinical

features).[10] The only difference with the International Pediatric MS Study Group consensus definition for monophasic ADEM outcome[10] was that we did not strictly require the presence of encephalopathy at onset. In all MS cases the diagnosis was based on the clinical course (exacerbations), and not solely on new MRI activity. All MS cases in this study had more than 2 additional exacerbations after the initial clinical attack. We excluded patients with multiphasic or recurrent variants of ADEM as well as patients with neuromyelitis optica.[10]

Standard protocol approvals, registrations, and patient consents
This study was approved by the Medical Ethical Committees of the Erasmus University Medical Center in Rotterdam and of the other participating centers. Written informed consent was obtained from all patients or their parents.

MRI analysis
All MRI scans were performed on a 1.5-TeslaMRI scanner with slice thicknesses of 3–5 mm. The lesions was determined on T2-weighted and fluid-attenuated inversion recovery sequences. T1-weighted images were used to determine the presence of black holes. All scans were scored blinded to diagnosis by 2 experienced raters (I.A.K. and R.F.N.). All lesions were located and measured in accordance with the technique described previously.[9] We defined small lesions as being less than 2 centimeters in diameter in the axial dimension, and large lesions as having a maximum diameter of more than 2 centimeters.

MRI scans were classified as meeting the published Barkhof,[6] KIDMUS,[7] or Callen criteria (see table 2).[8-9]

Statistical analysis
We counted the total number of patients in the MS group and in the ADEM group who fulfilled the 4 sets of MRI criteria. Subsequently we calculated the sensitivity, specificity, positive predictive value, and negative predictive value of the different sets of criteria. We also assessed the interrater reliability (Cohen κ). Analyses were performed using SPSS version 14.0.

RESULTS

Twenty-eight patients with MS and 21 patients with ADEM met our inclusion criteria (figure). Their clinical characteristics are shown in table 1. All patients in the ADEM group were polysymptomatic at presentation. Encephalopathy occurred more frequently in the ADEM group (52% vs 7% in the MS group). All patients with MS experienced subsequent clinical attacks. Two of the patients with MS had an initial demyelinating event consistent with the diagnosis of ADEM (with encephalopathy), but later went on to have multiple relapses typical for MS and demonstrated new lesion accrual on MRI.

Table 2 shows the test properties of the 4 sets of MRI criteria. The application of the newly proposed Callen MS-ADEM criteria had the best sensitivity (75%) and specificity

(95%) for distinguishing MS at first attack from ADEM. Their proposed diagnostic MS criteria were 82% sensitive and 52% specific (table 2).

The Cohen κ value for interrater reliability was 0.84 for the MS-ADEM criteria and 1.0 for the other criteria.

Table 1. Patient characteristics

	MS patients N=28	ADEM patients N=21
female / male	14 / 14	5 / 16
age at onset disease, y, mean ± SD	12.41 ± 3.8	6.76 ± 4.3
follow-up time, y, mean ± SD	3.6 ± 2.9	3.8 ± 2.6
clinical presentation at first attack:		
monofocal	13 (46%)	0 (0%)
polyfocal without encephalopathy	13 (46%)	10 (48%)
polyfocal with encephalopathy	2 (7%)	11 (52%)

ADEM = acute disseminated encephalomyelitis; MS = multiple sclerosis.

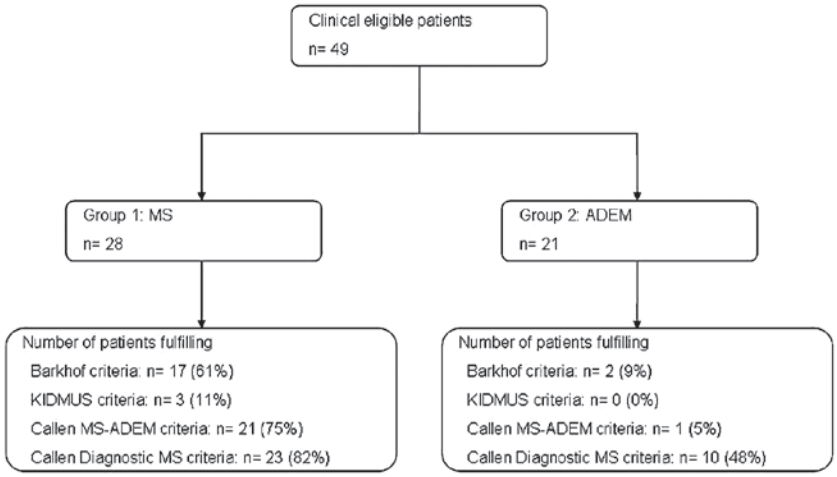


Figure 1. STARD flow diagram
ADEM = acute disseminated encephalomyelitis; MS = multiple sclerosis.

Table 2. Test properties of the available sets of MRI criteria when applied to distinguish between ADEM and MS at first attack*

	Barkhof (at least 3 out of 4) - ≥ 9 lesions on T2-weighted images or 1 gadolinium enhancing lesion - ≥ 3 periventricular lesions - ≥ 1 juxtacortical lesion - ≥ 1 infratentorial lesion	KIDMUS (1 out of 2) - lesions perpendicular to long axis of corpus callosum - the sole presence of well-defined lesions	KIDMUS (both) - lesions perpendicular to long axis of corpus callosum - the sole presence of well-defined lesions	Callen MS vs ADEM criteria (at least 2 out of 3) - absence of a diffuse bilateral lesion pattern - presence of black holes - ≥ 2 periventricular lesions	Callen Diagnostic MS criteria (at least 2 out of 3) - ≥ 5 lesions on T2-weighted images - ≥ 2 periventricular lesions - ≥ 1 brainstem lesion
Sensitivity (%)	61	57	11	75	82
Specificity (%)	91	95	100	95	52
Positive predictive value (%)	90	94	100	96	70
Negative predictive value (%)	63	63	46	74	69

* Fulfilling the criteria at first MRI correlates with conversion to MS
ADEM = acute disseminated encephalomyelitis; MS = multiple sclerosis.

DISCUSSION

In our cohort, we confirmed the high sensitivity and specificity of the newly proposed Callen MS-ADEM criteria.[8] The sensitivity of these criteria in distinguishing between ADEM and MS after a first demyelinating event in children is higher than of the previous available Barkhof and KIDMUS MRI criteria.

Unlike the original study, we did not require the presence of encephalopathy for the clinical diagnosis of ADEM in the present study. It has previously been shown that ADEM-like disease also exists without encephalopathy.[1,4] Children with a polysymptomatic disease with encephalopathy are likely to have different MRI lesion characteristics, including larger and more diffuse bilateral cerebral lesions, than children without encephalopathy. Since "the absence of a diffuse bilateral lesion pattern" is included in the criteria to distinguish MS at first attack from ADEM,[8] we wanted to investigate whether these criteria were still applicable in children with monophasic polyfocal disease without encephalopathy at onset. It is interesting that irrespective of monophasic polyfocal disease with or without encephalopathy, the specificity of these criteria remained the same when applied to our pediatric population.

In addition to this, we reclassified our groups according to the international consensus definition for ADEM, thus excluding patients without encephalopathy at onset. This did not significantly change the sensitivity and specificity of the criteria (data not shown).

We found a perfect interrater reliability. This is notable because "diffuse bilateral lesions" remains a subjective criterion, lacking a precise definition in the literature.[8]

Large lesions are more frequently seen in children with a monophasic disease than in children with MS, but this difference is only observed after the age of 10 years.[4] We would therefore recommend studying these criteria in 2 larger groups of children: 1 group below 10 years old and 1 group above this age.

We confirmed that the newly proposed Callen diagnostic MS criteria have a high sensitivity, but the lowest specificity when used to distinguish between ADEM and MS at first attack.[8] In their original study, these criteria were designed to distinguish between MS at the time of second attack and other, nondemyelinating, diseases.[9] However, at the initial presentation, the MRI distinction between a first attack of MS and other demyelinating disease such as ADEM is usually more difficult, which may explain the lower sensitivity and specificity when applied at the time of first attack.

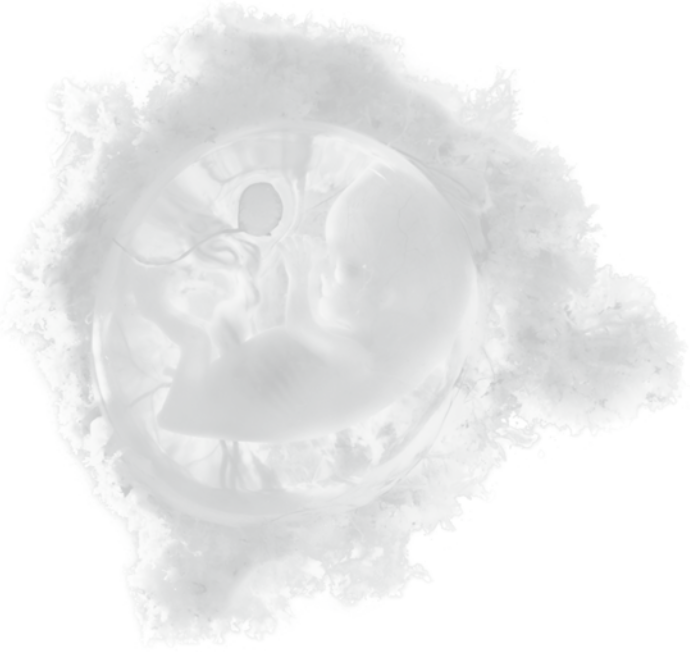
Of all criteria currently available, the Callen MS-ADEM criteria appear to be the most useful in clinical practice for children with a first demyelinating event, specifically for distinguishing between children with a monophasic polysymptomatic demyelinating event (ADEM) and those who are at risk of developing a second demyelinating attack and thus of converting to MS. Still, these findings need to be interpreted with caution because of the limited sample size in our study and in previous studies. Furthermore, although the length of follow-up time is comparable in both groups, the time in the ADEM group is still relatively short to state with complete certainty that they will remain monophasic in the future. It will be important to examine whether these criteria reliably predict future conversion to MS in a prospective cohort of children with a first attack of demyelination. Multinational collaborations would significantly help this endeavor.

REFERENCES

1. Mikaeloff Y, Suissa S, Vallée L, Lubetzki C, Ponsot G, Confavreux C, Tardieu M; KIDMUS Study Group. First episode of acute CNS inflammatory demyelination in childhood: prognostic factors for multiple sclerosis and disability. J Pediatr. 2004;144:246-252.
2. Mikaeloff Y, Caridade G, Husson B, Suissa S, Tardieu M; Neuropediatric KIDSEP Study Group of the French Neuropediatric Society. Acute disseminated encephalomyelitis cohort study: prognostic factors for relapse. Eur J Paediatr Neurol. 2007;11:90-95.
3. Dale RC, Pillai SC. Early relapse risk after a first CNS inflammatory demyelination episode: examining international consensus definitions. Dev Med Child Neurol. 2007;49:887-893.
4. Neuteboom RF, Boon M, Catsman Berrevoets CE, Vles JS, Gooskens RH, Stroink H, Vermeulen RJ, Rotteveel JJ, Ketelslegers IA, Peeters E, Poll-The BT, De Rijk-Van Andel JF, Verrips A, Hintzen RQ; Prognostic factors after a first attack of inflammatory CNS demyelination in children. Neurology. 2008;71:967-973.
5. Banwell B, Ghezzi A, Bar-Or A, Mikaeloff Y, Tardieu M. Multiple sclerosis in children: clinical diagnosis, therapeutic strategies, and future directions. Lancet Neurol. 2007;6:887-902.
6. Barkhof F, Filippi M, Miller DH, Scheltens P, Campi A, Polman CH, Comi G, Adèr HJ, Losseff N, Valk J. Comparison of MRI criteria at first presentation to predict conversion to clinically definite multiple sclerosis. Brain. 1997;120:2059-2069.
7. Mikaeloff Y, Adamsbaum C, Husson B, Vallée L, Ponsot G, Confavreux C, Tardieu M, Suissa S; KIDMUS Study Group on Radiology. MRI prognostic factors for relapse after acute CNS inflammatory demyelination in childhood. Brain. 2004;127:1942-1947.
8. Callen DJ, Shroff MM, Branson HM, Li DK, Lotze T, Stephens D, Banwell BL. Role of MRI in the differentiation of ADEM from MS in children. Neurology. 2009;72:968-973.
9. Callen DJ, Shroff MM, Branson HM, Lotze T, Li DK, Stephens D, Banwell BL. MRI in the diagnosis of pediatric multiple sclerosis. Neurology. 2009;72:961-967.
10. Krupp LB, Banwell B, Tenembaum S. Consensus definitions proposed for pediatric multiple sclerosis and related disorders. Neurology. 2007;68:S7-12.

CHAPTER 4

GENERAL DISCUSSION AND FUTURE PERSPECTIVES



SPECIAL SITUATIONS IN MS

Through study of natural disease course and natural modifiers, important lessons can be learned about etiology, pathogenesis, prognosis and perhaps ultimately new treatment options in multiple sclerosis (MS). This thesis focusses on two special and different situations in MS: pregnancy and MS and childhood onset of MS. Our studies on MS pregnancy had several aims. We wanted to asses clinical disease course of MS around pregnancy and study possible predictors of postpartum relapse We also aimed to investigate possible underlying biological mechanisms of disease amelioration during pregnancy. The aim of our studies on childhood onset of MS was to improve the diagnosis of MS in children. We also set out to identify parameters to identify children with MS developing an early relapse after the diagnosis of MS.

In this chapter, the findings and lessons learned from our studies will be summarized and discussed. Recommendations for future research will be addressed.

I. PREGNANCY IN MULTIPLE SCLEROSIS: SOME ANSWERS, BUT ALSO NEW QUESTIONS

What did we learn from the clinical studies on MS during pregnancy

In this part the main findings and conclusions of the clinical studies on pregnancy and MS, described in **chapter 2.1, 2.2** and **2.3,** will be discussed.

Clinical course of MS during and after pregnancy

In **chapter 2.1** we describe the clinical course of multiple sclerosis before, during and after pregnancy.[1] The strength of our study was that we not only studied relapse rate, but also clinical MS scales: the expanded disability status scale (EDSS), the Guy's neurological disability scale (GNDS), the multiple sclerosis impact scale 29 (MSIS-29), an MS self-report scale, and a scale addressing health related quality of life (QoL): the MOS 36 item short form health survey questionnaire (SF-36). We found that the relapse rate increased in the first three months after delivery, yet normalized within one year after delivery, thereby confirming previous studies.[2-4] Health-related QoL was improved during pregnancy, most appreciated in the SF-36 domains vitality and general health. A year after delivery we found no adverse effects on MS disease activity at group level, measured by the EDSS, MSIS-29, and the GNDS. A year after delivery QoL, measured by the SF-36, was not unfavorably altered when compared with QoL during pregnancy. This indicates that, although the number of relapses is increased in the short term after delivery, there are no adverse effects of pregnancy on disease course in the mid-long term after delivery. These results will help neurologists and MS nurses in counseling women with MS who want to become pregnant.

Predicting postpartum relapse

MS relapses are never welcome, but certainly not during the first three months after delivery. Predicting such a post-partum relapse would be most helpful. One important implication of

identifying women with a high risk for a postpartum relapse is that those women would be eligible for possible prophylactic treatment (i.e. corticosteroids).

Until now the only known predictors of a postpartum relapse, identified by the French Pregnancy in MS (PRIMS) study, were: number of relapses in the year preceding pregnancy, number of relapses during pregnancy, and duration of disease.[3] We were not able to reproduce these results **(chapter 2.1).** Most likely, the relative small number of patients in our cohort limited our conclusions.

Recently, breastfeeding was shown to have a protective effect on disease activity after delivery.[5] We and others did not confirm this finding **(chapter 2.2)**.[2-4,6-8]

We did find that high serum level of the chemokine interleukin-8 (IL-8) during the first trimester was associated with a high chance of postpartum relapse **(chapter 2.3)**.[9] Although the low positive predictive value will likely limit clinical use of IL-8 as a predictor of postpartum relapse, this study is the first to shed light on possible biomarkers predicting postpartum relapse. Confirmation of our finding in another cohort is required before IL-8 can be used in clinical practise.

What did we learn from the immunological studies in MS pregnancy

The main findings and conclusions of the immunological studies, described in **chapter 2.4, 2.5** and **2.6,** will be discussed in the following part. At the start of our studies we hypothesized that during pregnancy in MS patients the adaptive arm of the immune system is skewed from pro-inflammatory to anti-inflammatory. Compensating for this decrease in pro-inflammatory adaptive immune function, we expected the innate arm of the immune system to be more activated.

Innate immunity: increased monocyte activation during MS pregnancy

In **chapter 2.4** we chose to perform a genome wide approach on alterations of the transcriptome of monocytes of MS patients before and during the third trimester of pregnancy.[10] We hoped to identify activation or depression of immunological pathways in monocytes that might explain the amelioration of MS during pregnancy. We found that during pregnancy expression of the Fc receptor CD64 was increased. This was in line with previous studies in healthy pregnant women.[11-14] CD64 belongs to the family of receptors for the Fc portion of immunoglobulins, and is the high affinity receptor for the Fc fragment of IgG.[15] It binds best to IgG3 and IgG1, followed by, with decreasing efficacy, IgG4 and IgG2.[15] Ligation of the Fc fragment of IgG to CD64 results in phagocytosis, respiratory burst and secretion of pro-inflammatory cytokines.[16] Monocyte activation, such as found in sepsis, is associated with high CD64 expression.[17] In another autoimmune disease, systemic lupus erythematosus nephritis, increased expression of CD64 on monocytes is correlated with systemic inflammation.[18] Our results therefore support our hypothesis that the innate arm of the immune system is more activated during pregnancy,

Adaptive immunity: frequencies of circulating regulatory T cells (Treg) and T helper (Th)17 cells

In **chapter 2.5** we studied both Treg and Th17 cells as both subsets are implicated in both pregnancy homeostasis and MS pathology.[19]

We first focused on the anti-inflammatory Treg. Previous reports showed conflicting results in healthy women.[20-26] This was also the case in the few available studies on Treg frequency in peripheral blood in MS during pregnancy.[27-29] A major problem interpreting these studies in both healthy women and MS patients was the methodological diversity. Different gating strategies identifying Treg and the absence of a pre-pregnancy baseline sample or postpartum sample hindered comparison of these studies. Another issue was that some studies sampled at random moments during pregnancy, instead of sampling at a fixed moment. Therefore we performed a longitudinal study including a baseline pre-pregnancy sample and two samples after delivery, in addition to two samples during pregnancy in a fixed schedule.

Unexpectedly, we found that the numbers of circulating Treg were decreased, and not increased, during the first and third trimester of pregnancy. A possible explanation of this decline in circulating Treg could be that circulating Treg are recruited to the fetus implantation site from the peripheral maternal blood.[25] Intriguingly, treatment with anti-CD25 antibodies (daclizumab) was also characterized by decreased numbers of Treg in peripheral blood in MS patients, comparable with the situation during pregnancy. This was not negatively correlated with the reduction in the observed MRI disease activity during treatment with daclizumab.[30] This indicates that sustained suppressed numbers of Treg in peripheral blood do not necessarily negatively influence MS.

As a next step we focussed on Th17 cells. Th17 cells have been implicated in blood brain barrier disruption and brain inflammation in MS pathology.[31-33] Frequencies of Th17 cells during pregnancy have not been studied extensively. One study showed no differences during pregnancy[34] while another study found decreased numbers of Th17 cells during pregnancy.[26] We found no differences in the frequencies of circulating Th17 cells during pregnancy in MS patients and healthy controls.

Taken together our results do not support the hypothesis that peripheral blood Th17 cells and Treg are directly involved in MS disease course alteration during pregnancy.

Leptin: a bridge between endocrinology and immunology?

In **chapter 2.6** we studied serum levels of leptin before, during and after pregnancy in MS patients and healthy controls.[35] Leptin was first identified as an adipocyte derived modulator of energy homeostasis. Leptin also has major effects on both innate and adaptive immune responses, shifting both the innate and adaptive arm of the immune system to a more pro-inflammatory response.[36-39]

We observed a significant increase in serum levels of leptin in women with MS during the third trimester, compared to baseline and first trimester samples[35] as was observed before in healthy women.[40-42] Serum levels of leptin during pregnancy were not associated

with a postpartum relapse. Therefore serum levels of leptin during pregnancy cannot be used as a biomarker for postpartum relapse.

Notably, we found that women with MS with the largest relative decrease in serum leptin levels after delivery more often had a postpartum relapse. Perhaps the increase of leptin in the third trimester is part of a process, priming the immune system to a more pro-inflammatory immune response. Supportive of this hypothesis is that previous studies show that an increase in serum leptin levels precedes a relapse in humans and the clinical signs in experimental autoimmune encephalomyelitis (EAE).[43-45] The priming of the immune system during pregnancy to a more pro-inflammatory response was also suggested by a recent study that found that a decline in CD4+ IFN-γ producing cells during the end of pregnancy was associated with postpartum relapse in MS patients.[46]

Future perspective on remaining questions in MS pregnancy

The regulation of immune tolerance and reactivity during pregnancy is highly complex. Our data was not supportive for a role of circulating Treg and Th17 cells in disease ameliorating during pregnancy in MS. As hypothesized we found evidence of increased activation of the innate arm of the immune system. A logical next step is to further explore innate immunity during pregnancy in MS. As Natural Killer (NK) cells are abundant at the implantation site this cell type is a likely candidate for further research (figure 1).[47]

It may also be possible that amelioration of MS during pregnancy is not related to immune cell populations in the peripheral maternal blood. Adding pregnancy serum to T cell cultures suppresses T cell proliferation in EAE.[48] It can be hypothesized that pregnancy related serum factors, possibly derived from the fetal/placental compartment, migrate to the maternal peripheral blood compartment. These serum factors may exert effects on the immune system, eventually influencing disease activity in the brain. These putative pregnancy related serum factors are a target for future research. Several candidates will be discussed below. It is possible that these pregnancy related serum factors can also be used as biomarkers to predict postpartum relapse.

The following part of the discussion will first discuss possible other innate immune functions that may influence pregnancy induced disease ameliorations, focussing on NK cells. Hereafter possible serum pregnancy factors will be discussed. Finally, possible candidate biomarkers for postpartum relapse and potential treatment options for postpartum relapse will be discussed.

Focussing on the innate immunity (dark gray circles) we found, as hypothesized, evidence of increased activation of monocytes. Other innate immune cells present in the decidua are uterine natural killer cells (uNK). These uNK cells play an important role in pregnancy maintenance. NK cells are also present in peripheral blood. NK cells are divided into two distinct types based on their expression of CD16 and CD56. CD16-CD56^{bright} (marked CD56+ in figure) cells are poorly cytotoxic, whereas CD16+CD56^{dim} (marked CD56- in figure) cells are highly cytotoxic but poor at cytokine production. CD16-CD56^{bright} cells increase during pregnancy and CD16+CD56^{dim} cells decrease during pregnancy in the maternal peripheral blood.

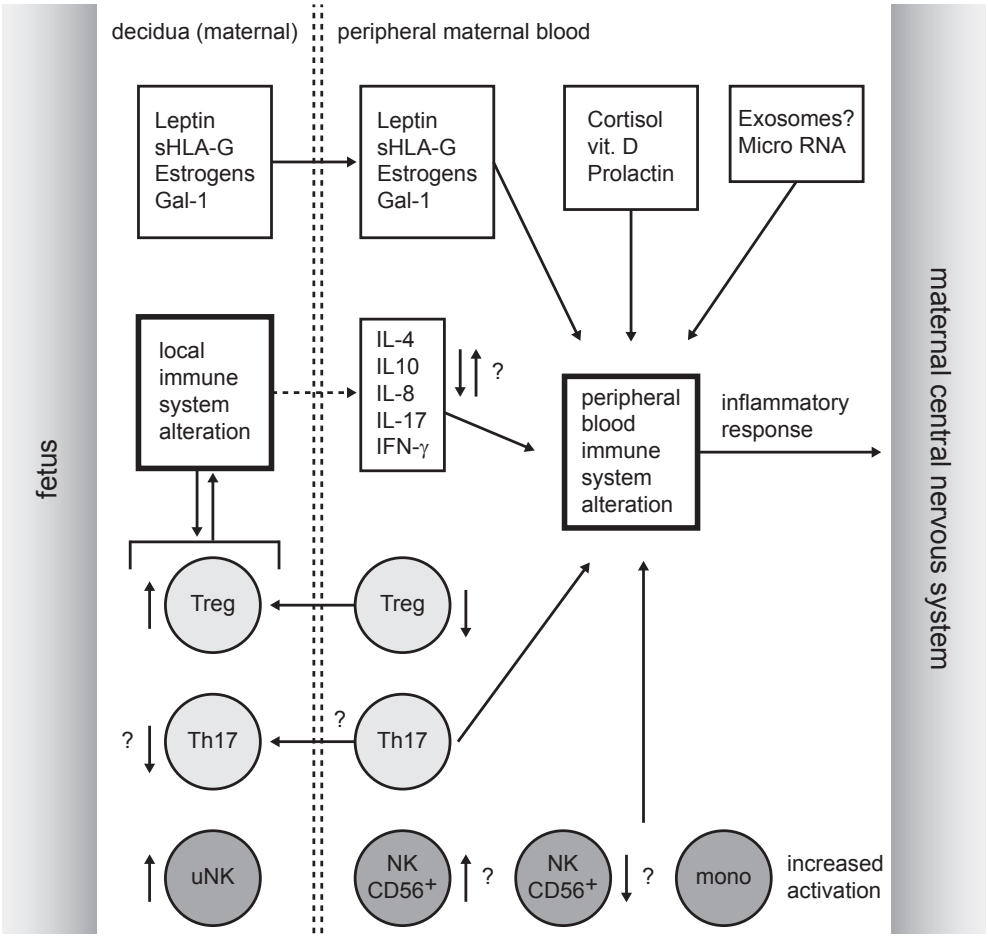


Figure 1. Immune regulation during MS pregnancy: a summary of our results and adding some putative mechanisms

Focussing on the adaptive immunity (light gray circles), we found that the numbers of circulating Treg decreased in the peripheral blood whereas the number of circulating Th17 cells remained the same. The decrease in circulating Treg can possibly be explained by entry of Treg from the peripheral blood into the decidua. It is unknown if Th17 cells enter the decidua from the maternal peripheral blood.

Next to immune cell populations in the peripheral blood, it is also possible that certain pregnancy related serum factors exert effects on the immune system, eventually influencing disease activity in the brain. Possible serum factors include: cytokines/chemokines (i.e. interleukin(IL)-4, IL-10, IL-8, IL-17, IFN-γ), hormonal factors (i.e.: leptin, cortisol, vitamin D, estrogens, prolactin), or other factors (micro RNA, soluble HLA-G (sHLA-G), Galectin-1 (Gal-1), pregnancy related immune suppressive exosomes).

Further study of innate immunity: NK cells

NK cells are not only effector cells of the innate arm of the immune system. NK cells are also able to modify outcome of adaptive immune responses.[49] The NK cell response is based on the several NK subsets and biased to either cytokine expression or cytotoxicity. In humans NK cells are divided into two major subtypes based on their differentiated ability for cytokine expression and cytotoxic capacity. CD16+CD56^{dim} NK cells constitute around 90% of the NK cells in peripheral blood and are highly cytotoxic. Most NK cells in lymph nodes are CD16-CD56^{bright} and poorly cytotoxic, but readily produce cytokines.[50-51]

Uterine NK cells represent a special subset of NK cells.[52] These cells are CD16-CD56^{bright} and produce pro-angiogenic factors like vascular endothelial growth factor (VEGF).[53] These cells are supposed to play an important role in trophoblast invasion and remodelling of decidual arteries. Uterine NK cell function is highly dependent on environmental changes.[51] One of the protective mechanisms of NK cells in pregnancy is secretion of Galectin 1 (Gal-1). Gal-1 expression is increased in uterine NK cells.[52] Gal-1 modulates T cell homeostasis by interfering with T cell activation, promoting T cell apoptosis, T cell migration and regulating the Th1/Th2 cytokine balance towards Th2 predominance.[51,54-55] Uterine NK cells interact with HLA-C molecules using killer immunoglobulin like receptors (KIR). This KIR can be activating (KIR2DS) or inhibiting (KIR2DL). The HLA-C ligands for KIR on trophoblast cells can belong to two groups, HLA-C1 and HLA-C2. This maternal–fetal immunological interaction therefore involves two polymorphic gene systems, maternal KIRs and fetal HLA-C molecules. NK-cell function is therefore likely to vary in each pregnancy. Some KIR/HLA-C combinations might be more favourable to trophoblast-cell invasion than other combinations.

Interestingly, treatment in MS patients with daclizumab or IFN-β is associated with an increase in circulating CD56^{bright} NK cells.[56-57] Expansion of CD56^{bright} NK cells during treatment with daclizumab is positively correlated with the contraction of the number of CD4+ and CD8+ T cells. In vitro these NK cells are able to inhibit T cell survival. In EAE, mice deprived of NK cells, showed earlier disease and more severe disease than controls.[58]

During pregnancy an increase in CD56^{bright} NK cells and decrease in CD56^{dim} NK cells is observed in the peripheral blood of MS patients.[29] Unfortunately, no sample before pregnancy was available in this study. Currently no data on cytokine or Gal-1 profile of these CD56^{bright} NK cells during pregnancy in MS patients are available.

In conclusion, NK cells play an important regulatory role in successful pregnancy. Exploring these subsets of NK cells and their cytokine expression or cytotoxic capacity during pregnancy in MS patients is therefore a logical next step. In the ideal situation both placental tissue and samples of peripheral blood would be available for studying both uterine and peripheral blood populations of NK cells.

Pregnancy related serum factors

In the next part I will discuss possible pregnancy related serum factors, likely to have an influence on MS disease activity during and after pregnancy.

Estrogens

Serum levels of estrogens increase during pregnancy. The three main increased estrogens in pregnancy are: estrone, 17-β estradiol and estriol. In the early phase of pregnancy these estrogens are produced by the ovaries. In the later phase of pregnancy the placenta becomes the major supplier of estrogens. In late pregnancy the most abundant estrogen is estriol. Estriol treatment in relapsing remitting MS patients positively influences MRI disease activity[59] and causes an anti-inflammatory Th2 immune respons.[60] In EAE mice treated with estrogens disease severity is also reduced.[61-63] In these estrogen treated mice IL-10 production is increased.[61] It can be hypothesized that changes in the concentration of estrogens during and after pregnancy may be linked to altered disease activity.

Prolactin

Inhibition of prolactin production by bromocriptin reduces severity of EAE.[64-65] During relapses in MS patients an increase in prolactin is observed.[66-67] These findings indicate that prolactin may negatively influence disease activity in MS. Alternatively, breast-feeding, a situation with increased levels of prolactin, is implicated to protect against postpartum relapse[5], although not confirmed by us.[6] No studies on prolactin levels during and after MS pregnancy have been performed yet.

Cortisol

Pregnancy induced hypercortisolism may be related to decreased disease activity during pregnancy whereas the relative drop in cortisol after delivery may be related to the increased risk of a postpartum relapse. Serum cortisol levels have not been evaluated in MS pregnancy.

Vitamin D

In general, incidence of MS increases as the distance from the equator increases.[68] This has led to the hypothesis that exposure to sunlight and vitamin D are involved in MS pathogenesis and that appropriate levels may protect against MS.[69] Serum vitamin D levels in MS patients during and after pregnancy have been explored.[70] Vitamin D serum levels after delivery are lower in the patients that exclusively breastfed their children compared to patients that did not. Surprisingly, this study found that high serum levels of vitamin D after delivery are associated with a postpartum relapse. Confirmation of these is results is necessary, because this study had some methodological limitations like the lack of a baseline sample before pregnancy and lack of healthy controls.

Cytokines

Our data are not supportive for a role of peripheral blood Treg and Th17 cells in disease amelioration during pregnancy in MS. A possible explanation for the decrease in circulating Treg in the peripheral maternal blood, is that they enter the decidua[25] to locally exert regulatory effects and to favour a local anti-inflammatory response. This local decidual immune response could cause an increase in serum levels of anti-inflammatory cytokines

like IL-4 an IL-10, whereas serum levels of pro-inflammatory cytokines like IFN-γ and the Th17 cell product IL-17 could decrease. It can be postulated that these changes in serum concentrations of pro-inflammatory and anti-inflammatory cytokines regulate not only local decidual immune function, but also systemic immune function, eventually affecting disease activity in the brain.

Soluble human leucocyte antigen (HLA)-G

Expression of non-classical major histocompatibility complex (MHC) class I molecule HLA-G by trophoblast cells is one of the several mechanisms for limiting T cell awareness of the semi-allogenic foetus.[71] In MS patients high levels of soluble HLA-G in serum and CSF are associated with a reduced lesion burden on MRI, suggesting that high soluble HLA-G levels ameliorate MS.[72-73] Low serum levels of soluble HLA-G during pregnancy in MS patients are associated with an increased risk of a postpartum relapse.[74] The possible predictive value of low serum levels of soluble HLA-G as a biomarker has yet to be studied.

Pregnancy related micro RNA (miRNA)

MiRNA are 20-24 nucleotides long and are derived from longer RNA precursors. MiRNA bind to messenger RNA (mRNA), leading to inhibition of translation, or mRNA degradation. Analysis of white matter tissue lesions and blood from patients with MS showed that the presence of a specific miRNA, i.e. miRNA-326 is strongly upregulated in active lesions and in peripheral blood cells in MS patients and promotes Th17 cell differentiation.[75-76]

In pregnancy, placental expression of several miRNA is increased and likely plays an important role in implantation and pregnancy.[77] During pregnancy these miRNA are also detectable in serum and after delivery they disappear.[78-79] It can be hypothesized that, specific pregnancy related, miRNA expression are associated with amelioration of MS during pregnancy.

Immunosuppressive exosomes in pregnancy serum

Recently, it was found that administration of pregnancy-derived serum exosomes to mice with established EAE reduced clinical severity.[80] These exosomes, containing cytosol and exposing extracellular domains of membrane bound proteins, are able to suppress activation T cells in vitro. They suppress activation of myelin-specific T cells, as was measured by a reduction in proliferation and IFN-γ production.[81] The role of these immunosuppressive exosomes in during pregnancy in MS patients has not been investigated yet.

Serum levels of Gal-1

Gal-1 has strong immunomodulatory effects. Gal-1 can modulate T cell homeostasis by interfering with T cell activation, promoting T cell apoptosis, T cell migration and regulating Th1/Th2 cytokine balance towards Th2 predominance.[51,54-55] During pregnancy Gal-1 expression is increased in uterine NK cells. It can be hypothesized that high serum levels of Gal-1 are associated with MS amelioration during pregnancy.

Prediction and possible treatment of postpartum relapse: future perspectives

In the absence of good clinical predictors of postpartum relapse we are in need of biomarkers. We found that high serum levels of the chemokine IL-8 during the first trimester were associated with a high chance of postpartum relapse.[9] Although the negative predictive value was high, the low positive predictive value will limit direct clinical use. Another study shows that low serum levels of soluble HLA-G during pregnancy in MS patients are associated with an increased risk of a postpartum relapse.[82] A possible predictive value of serum levels of soluble HLA-G remains to be investigated. No other studies investigating biomarkers for predicting postpartum relapse have been published. Ideally, such a biomarker should be able to predict postpartum relapse before delivery, in order to allow for patients and physicians to anticipate. Candidate biomarkers are summed in table 1.

The implication of a reliable predictor of a postpartum relapse is that a possible prophylactic treatment could be initiated. Both intravenous immunoglobulins (IVIG) and intravenous methylprednisolone have been studied.[83-87] No prospective double-blind placebo-controlled studies on the use of IVIG or corticosteroids after delivery have been performed, so no definite positive advice on standard treatment with IVIG or corticosteroids after delivery can be given yet. Recently a study started on treatment after delivery with estradiol and progestin aiming to reduce postpartum relapses.[88] The results from this study will be available soon.

Table 1. Potential biomarkers for prediction of postpartum relapse

		Evidence for potential use as biomarker?
Hormonal	Leptin	Studies have shown no evidence
	Vitamine D	No, but needs validation
	Estrogen	No studies performed
	Cortisol	No studies performed
	Prolactin	No studies performed
Cytokines/chemokines	IL-4	No studies performed
	IL-10	No studies performed
	IL-8	Yes, but needs validation in larger cohort
	IL-17	No studies performed
	IFN-γ	No studies performed
Other immunological	Exosomes	No studies performed
	Micro RNA	No studies performed
	Soluble HLA-G	Yes, but predictive value needs to be assessed
	Galectin-1	No studies performed

Another approach might be continuation of interferon-ß, copaxone or natalizumab during pregnancy or an early (re)start after delivery. Although there is some data suggesting that use of interferon-ß and copaxone during the first weeks of pregnancy might be safe[89-96], there is insufficient safety data on continuing these disease modulating drugs (DMD) throughout the entire pregnancy. An early start immediately after delivery is not likely to influence the increased risk of a postpartum relapse, as all of these DMD have a run-in period of several weeks to months, exceeding the acute phase after delivery.

A first step forward would be to perform a double-blind placebo-controlled study on IVIG and/or corticosteroids after delivery.

II. TOWARDS AN EARLY AND RELIABLE DIAGNOSIS OF MS IN CHILDHOOD

What did we learn from our studies on childhood onset in MS

In the following part the main findings and conclusions of our studies on childhood onset of MS described in **chapter 3.1, 3.2** and **3.3** will be discussed.

Prognostic factors after a first demyelinating attack in children

We performed a retrospective nationwide study in all large neuro-pediatric centres in The Netherlands, described in **chapter 3.1**.[97] We included the full spectrum of acquired demyelinating syndromes (ADS) of the central nervous system (monofocal attacks and polyfocal attacks, with and without encephalopathy). 44% of the children with a monofocal attack developed MS, whereas 21% of the children with a polyfocal attack developed MS. We found that both the Barkhof MRI-criteria and the KIDMUS MRI-criteria were able to predict a future diagnosis of MS after a first demyelinating event. However, in very young children, aged under ten, we found that the sensitivity of especially the KIDMUS criteria was very low (18%). CSF analysis showed that an increased IgG index and presence of oligoclonal banding both were able to predict MS. Strikingly, children with and without encephalopathy both displayed MRI abnormalities as seen in typical ADEM cases (large lesions and basal ganglia/thalamic lesions).

Prediction of early relapse after diagnosis of MS in children

When children are to be selected for treatment trials with disease modulating drugs, it would be very useful to predict which children are likely to have a relapse shortly after the diagnosis of MS, indicative for a high disease activity. In the study described in **chapter 3.2** we found that the children with MRI features consistent with three or four out of the four Barkhof criteria for dissemination in space were more likely to have a relapse soon after their second, MS defining, attack.[98] We could not reproduce the predictive value of the childhood-onset MS potential index for early severity as was proposed before.[99]

MRI criteria for discriminating ADEM and MS

Recently, two new sets of MRI criteria were developed with the aim to aid in a reliable diagnosis of MS. One set of MRI criteria was developed to discriminate between MS and other neurological diseases causing white matter lesions (systemic lupus erythematosus (SLE) and migraine).[100] Another set of MRI criteria was developed to discriminate MS from ADEM.[101] In **chapter 3.3** we investigated the capacity of all known diagnostic MRI criteria for children to differentiate MS from acute disseminated encephalomyelitis (ADEM).[102] We found that the new Callen criteria for discriminating MS from ADEM were superior to the other sets of criteria.

Future perspective on improving diagnosis of MS in childhood

Important controversies about defining ADS

To improve the difficult diagnostic process in children, the international pediatric MS study group (IPMSSG) suggested a set of diagnostic criteria.[103] However, several questions remain concerning these definitions and diagnostic criteria for ADS. Multiphasic disseminated demyelinating disease can be MS, recurrent ADEM or multiphasic ADEM, or an ADEM attack followed by a non-ADEM attack. The rationale for these several entities is that not every multiphasic disseminated demyelinating disease is MS, although dissemination in time and space criteria for the diagnosis MS are fulfilled. ADEM followed by a second demyelinating relapse can be a self-limiting disease with only two or three attacks and not a chronic lifelong disease like MS. Whether this approach is correct, will become clear after long-term prospective follow up during the next years. Until then caution is warranted before diagnosing MS in children.

Another problem arises with the definition of ADEM. Currently ADEM is defined as a polyfocal attack with encephalopathy and typical ADEM features on MRI.[103] We showed that children with polyfocal attacks without encephalopathy can also show typical ADEM MRI features.[97] This indicates that encephalopathy may not be obligatory for a diagnosis of ADEM, as was also suggested by others.[104-105] Using the IPMSSG definitions, an ADEM type attack may not be taken into account when making a diagnosis of MS. Using a too strict definition for ADEM may falsely allow children with polyfocal attacks with ADEM-type MRI abnormalities, but without encephalopathy, to be grouped into the group eligible for future MS diagnosis.

When a first ADEM episode is followed by a second ADEM episode the diagnosis of multiphasic or recurrent ADEM can be made, based on the presence of respectively new or only old symptoms. Whether multiphasic ADEM and recurrent ADEM are two distinct entities is disputable. No rationale was provided for separating recurrent ADEM cases and multiphasic cases in the consensus statement by the IPMSSG. Recent studies show that only a minority of relapsing ADEM cases fulfil the criteria for recurrent ADEM.[97,105-108] The number of recurrent ADEM cases, described in literature is small and currently it is unknown if outcome is different in multiphasic or recurrent ADEM.

MRI diagnostic criteria in children

Multiple studies have shown that the Barkhof criteria, used in adult MS, are also valuable in childhood MS [109-110].109-110 The KIDMUS criteria have been shown to differentiate between monophasic and multiphasic ADS.[110] We validated both the Barkhof criteria and KIDMUS criteria in children with ADS, and found a high specificity but mediocre sensitivity for both criteria. Especially the sensitivity of the KIDMUS criteria was low (18%) in the group younger than 10 years. The KIDMUS criteria are not the only MRI criteria for the diagnosis MS in a paediatric population. The Callen diagnostic MS criteria were shown to differentiate between MS and other diseases (migraine and SLE) at the time of the second attack.[100] Differentiating between migraine and MS will not likely be a true diagnostic dilemma. Exploring the capacity of these Callen diagnostic MS MRI criteria to differentiate between MS and other ADS in a full pediatric ADS spectrum cohort at time of the first attack would be more useful.

The Callen ADEM-MS criteria were found to differentiate between MS and ADEM cases in childhood.[101] Unfortunately, only a selection of ADS cases was included in this study, namely children that eventually would develop MS and typical monophasic ADEM cases. No cases of multiphasic or recurrent ADEM were included. We were able to reproduce the findings from this study.[102] Despite the fact that our definition of ADEM did not require the presence of encephalopathy, we still found similar results. This again stresses that encephalopathy may not be obligatory for the diagnosis ADEM.

For the diagnosis of MS in adults the new 2010 revised McDonald diagnostic criteria have been proposed.[111] For fulfilment of dissemination in space (DIS) criteria one or more T2 lesion(s) are required in at least two of the following four areas of the CNS: (1) periventricular, (2) juxtacortical, (3) infratentorial and (4) spinal cord. When a subject has a brainstem or spinal cord syndrome, the symptomatic lesions are excluded from the criteria and do not contribute to lesion count. These DIS MRI criteria should be evaluated in a paediatric ADS cohort.

The clinical relevance of dissemination in time (DIT) MRI criteria has not been studied systematically in children yet. Using MRI for DIT in children may result in an earlier diagnosis. The role of a second MRI may prove especially interesting in children with ADEM and ADEM-like disease, because new, clinically silent lesions are thought to be rare in ADEM and multiphasic ADEM and may be a sign the diagnosis of MS.[112]

International collaboration

MS in children is a rare disease. Therefore, international collaboration is crucial to ensure a large enough number of patients, critical for answering the many unresolved questions. Possible regional differences in incidence and clinical features worldwide also make that an international approach is essential. The IPMSSG is an example of such a worldwide collaboration. It forms a network of clinicians, basic scientists and representatives of MS societies, dedicated to improve healthcare and research in pediatric MS and other forms of ADS.

The first step forwards is the use of international accepted definitions for pediatric MS and other ADS by collaborative international multicentre studies. These definitions should address the controversies mentioned in the sections above. We also need standardized, long term, prospective follow-up data on disease course, including a standardized MRI follow-up protocol in the full spectrum of children with ADS.

REFERENCES:

1. Neuteboom RF, Janssens AC, Siepman TA, Hoppenbrouwers IA, Ketelslegers IA, Jafari N, Steegers EA, de Groot CJ, Hintzen RQ. Pregnancy in multiple sclerosis: clinical and self-report scales. J Neurol. 2012;259:311-317.
2. Confavreux C, Hutchinson M, Hours MM, Cortinovis-Tourniaire P, Moreau T**.** Rate of pregnancy-related relapse in multiple sclerosis. Pregnancy in multiple sclerosis group. N Engl J Med. 1998;339:285-291.
3. Vukusic S, Hutchinson M, Hours M, Moreau T, Cortinovis-Tourniaire P, Adeleine P, Confavreux C, The Pregnancy In Multiple Sclerosis Group; Pregnancy In Multiple Sclerosis Group. Pregnancy and multiple sclerosis (the PRIMS study): clinical predictors of post-partum relapse. Brain 2004;127:1353-1360.
4. Fernández Liguori N, Klajn D, Acion L, Cáceres F, Calle A, Carrá A, Cristiano E, Deri N, Garcea O, Jaureguiberry A, Onaha P, Patrucco L, Riccio P, Rotta Escalante R, Saladino M, Sinay V, Tarulla A, Villa A.Epidemiological characteristics of pregnancy, delivery, and birth outcome in women with multiple sclerosis in Argentina (EMEMAR study). Mult Scler. 2009;15:555-562.
5. Langer-Gould A, Huang SM, Gupta R, Leimpeter AD, Greenwood E, Albers KB, Van Den Eeden SK, Nelson LM. Exclusive breastfeeding and the risk of postpartum relapses in women with multiple sclerosis. Arch Neurol. 2009;66:958-963.
6. Neuteboom RF, Hintzen RQ. Breast-feeding, postpartum and prepregnancy disease activity in multiple sclerosis. Neurology 2011;76:1532.
7. Airas L, Jalkanen A, Alanen A, Pirttilä T, Marttila RJ. Breast-feeding, postpartum and prepregnancy disease activity in multiple sclerosis. Neurology 2010;75:474-476.
8. Portaccio E, Ghezzi A, Hakiki B, Martinelli V, Moiola L, Patti F, La Mantia L, Mancardi GL, Solaro C, Tola MR, Pozzilli C, De Giglio L, Totaro R, Lugaresi A, De Luca G, Paolicelli D, Marrosu MG, Comi G, Trojano M, Amato MP; MS Study Group of the Italian Neurological Society. Breastfeeding is not related to postpartum relapses in multiple sclerosis. Neurology 2011;77:145-150.
9. Neuteboom RF, Verbraak E, Voerman JS, van Meurs M, Steegers EA, de Groot CJ, Laman JD, Hintzen RQ. First trimester interleukin 8 levels are associated with postpartum relapse in multiple sclerosis Mult Scler. 2009;15:1356-1358.
10. Neuteboom RF, Verbraak E, Wierenga-Wolf AF, Voerman JS, Van Meurs M, Swagemakers SM, van de Spek PJ, Steegers EA, de Groot CJ, Laman JD, Hintzen RQ. The monocyte transcriptome during pregnancy in multiple sclerosis: prominent expression of the Fc-receptor CD64. Mult Scler. 2011;17:389-496.
11. Sacks GP, Studena K, Sargent K, Redman CW. Normal pregnancy and preeclampsia both produce inflammatory changes in peripheral blood leukocytes akin to those of sepsis. A J Obstet Gynecol. 1998;179:80-86.
12. Luppi P, Haluszczak C, Betters D, Richard CA, Trucco M, DeLoia JA. Monocytes are progressively activated in the circulation of pregnant women. J Leukoc Biol. 2002;72:874-884.
13. Naccasha N, Gervasi MT, Chaiworapongsa T, Berman S, Yoon BH, Maymon E, Romero R. Phenotypic and metabolic characteristics of monocytes and granulocytes in normal pregnancy and maternal infection. Am J Obstet Gynecol. 2001;185:1118-1123.
14. Davis D, Kaufmann R, Moticka EJ. Nonspecific immunity in pregnancy: monocyte surface Fcgamma receptor expression and function. J Reprod Immunol. 1998;40:119-128.
15. van de Winkel JG, Anderson CL. Biology of human immunoglobulin G Fc receptors. J Leukoc Biol. 1991;49:511-524.
16. Salmon JE, Pricop L. Human receptors for immunoglobulin G: key elements in the pathogenesis of rheumatic disease. Arthritis Rheum. 2001;44:739-750.
17. Danikas DD, Karakantza M, Theodorou GL, Sakellaropoulos GC, Gogos CA. Prognostic value of phagocytic activity of neutrophils and monocytes in sepsis. Correlation to CD64 and CD14 antigen expression. Clin Exp Immunol. 2008;154:87-97.
18. Li Y, Lee PY, Sobel ES, Narain S, Satoh M, Segal MS, Reeves WH, Richards HB. Increased expression of FcgammaRI/CD64 on circulating monocytes parallels ongoing inflammation and nephritis in lupus. Arthritis Res Ther. 2009; DOI: 10.1186/ar2590.

19. Neuteboom RF, Verbraak E, Wierenga-Wolf AF, van Meurs M, Steegers EA, de Groot CJ, Laman JD, Hintzen RQ. Pregnancy-induced fluctuations in functional T-cell subsets in multiple sclerosis patients. Mult Scler. 2010;16:1073-1078.
20. Heikkinen J, Möttönen M, Alanen A, Lassila O. Phenotypic characterization of regulatory T cells in the human decidua. Clin Exp Immunol. 2004;136:373-378.
21. Steinborn A, Haensch GM, Mahnke K, Schmitt E, Toermer A, Meuer S, Sohn C. Distinct subsets of regulatory T cells during pregnancy: is the imbalance of these subsets involved in the pathogenesis of preeclampsia? Clin Immunol. 2008;129:401-412.
22. Förger F, Marcoli N, Gadola S, Möller B, Villiger PM, Østensen M. Pregnancy induces numerical and functional changes of CD4+CD25 high regulatory T cells in patients with rheumatoid arthritis. Ann Rheum Dis. 2008;67:984-990.
23. Somerset DA, Zheng Y, Kilby MD, Sansom DM, Drayson MT. Normal human pregnancy is associated with an elevation in the immune suppressive CD25+ CD4+ regulatory T-cell subset. Immunology 2004;112:38-43.
24. Tilburgs T, Roelen DL, van der Mast BJ, van Schip JJ, Kleijburg C, De Groot-Swings GM, Kanhai HH, Claas FH, Scherjon SA. Differential distribution ofCD4(+)CD25(bright) and CD8(+)CD28(-) T-cells in decidua and maternal blood during human pregnancy. Placenta 2006;27:S47-53.
25. Tilburgs T, Roelen DL, van der Mast BJ, de Groot-Swings GM, Kleijburg C, Scherjon SA, Claas FH. Evidence for a selective migration of fetus-specific CD4+CD25bright regulatory T cells from the peripheral blood to the decidua in human pregnancy. J Immunol. 2008;180:5737-5745.
26. Santner-Nanan B, Peek MJ, Khanam R, Richarts L, Zhu E, Fazekas de St Groth B, Nanan R. Systemic increase in the ratio between Foxp3+ and IL-17-producing CD4+ T cells in healthy pregnancy but not in preeclampsia. J Immunol. 2009;183:7023-730.
27. Iorio R, Frisullo G, Nociti V, Patanella KA, Bianco A, Marti A, Mirabella M, Tonali PA, Batocchi AP. T-bet, pSTAT1 and pSTAT3 expression in peripheral blood mononuclear cells during pregnancy correlates with post-partum activation of multiple sclerosis. Clin Immunol. 2009;131:70-83.
28. Sánchez-Ramón S, Navarro A J, Aristimuño C, Rodríguez-Mahou M, Bellón JM, Fernández-Cruz E, de Andrés C. Pregnancy-induced expansion of regulatory T-lymphocytes may mediate protection to multiple sclerosis activity. Immunol Lett. 2005;96:195-201.
29. Airas L, Saraste M, Rinta S, Elovaara I, Huang YH, Wiendl H; Finnish Multiple Sclerosis and Pregnancy Study Group. Immunoregulatory factors in multiple sclerosis patients during and after pregnancy: relevance of natural killer cells. Clin Exp Immunol. 2008;151:235-243.
30. Oh U, Blevins G, Griffith C, Richert N, Maric D, Lee CR, McFarland H, Jacobson S. Regulatory T cells are reduced during anti-CD25 antibody treatment of multiple sclerosis. Arch Neurol. 2009;66:471-479.
31. Kebir H, Kreymborg K, Ifergan I, Dodelet-Devillers A, Cayrol R, Bernard M, Giuliani F, Arbour N, Becher B, Prat A. Human TH17 lymphocytes promote blood-brain barrier disruption and central nervous system inflammation. Nat Med. 2007;13:1173-1175.
32. Tzartos JS, Friese MA, Craner MJ, Palace J, Newcombe J, Esiri MM, Fugger L. Interleukin-17 production in central nervous system-infiltrating T cells and glial cells is associated with active disease in multiple sclerosis. Am J Pathol. 2008;172:146-155.
33. McFarland HF, Martin R. Multiple sclerosis: a complicated picture of autoimmunity. Nat Immunol. 2007;8:913-919.
34. Nakashima A, Ito M, Yoneda S, Shiozaki A, Hidaka T, Saito S. Circulating and decidual Th17 cell levels in healthy pregnancy. Am J Reprod Immunol. 2010;63:104-109.
35. Neuteboom RF, Verbraak E, Voerman JS, van Meurs M, Steegers EA, de Groot CJ, Laman JD, Hintzen RQ. Serum leptin levels during pregnancy in multiple sclerosis. Mult Scler. 2009;15:907-912.
36. Lago F, Dieguez C, Gomez Reino J, Gualillo O. The emerging role of adipokines as mediators of inflammation and immune responses. Cytokine growth factor rev. 2007;18:313-325.
37. Loffreda S, Yang SQ, Lin HZ, Karp CL, Brengman ML, Wang DJ, Klein AS, Bulkley GB, Bao C, Noble PW, Lane MD, Diehl AM. Leptin regulates proinflammatory immune responses. FASEB J. 1998;12:57-65.
38. Matarese G, Carrieri PB, La Cava A, Perna F, Sanna V, De Rosa V, Aufiero D, Fontana S, Zappacosta S. Leptin increase in multiple sclerosis associates with reduced number of CD4+CD25+ regulatory T cells. Proc Natl Acad Sci USA. 2005;102:5150-5155.
39. De Rosa V, Procaccini C, Calì G, Pirozzi G, Fontana S, Zappacosta S, La Cava A, Matarese G. A key role of leptin in the control of regulatory T cell proliferation. Immunity 2007;26:241-255.

40. Hardie L, Trayhurn P, Abramovich D, Fowler P. Circulating leptin in women: a longitudinal study in the menstrual cycle and during pregnancy. Clin Endocrinol. 1997;47:101-106.
41. Schubring C, Englaro P, Siebler T, Blum WF, Demirakca T, Kratzsch J, Kiess W. Longitudinal analysis of maternal serum leptin levels during pregnancy, at birth and up to six weeks after birth: relation to body mass index, skinfolds, sex steroids and umbilical cord blood leptin levels. Horm Res. 1998;50:276-283.
42. Highman T, Friedman J, Huston L, Wong W, Catelano P. Longitudinal changes in maternal leptin serum concentrations, body composition an resting metabolic rate in pregnancy. Am J Obstet Gynecol. 1998;178:1010-1015.
43. Batocchi AP, Rotondi M, Caggiula, Frisullo G, Odoardi F, Nociti V et al. Leptin as a marker of multiple sclerosis activity in patients treated with interferon-beta. J Neuroimmunol. 2003;139:150-154.
44. Frisullo G, Mirabella M, Angelucci F, Caggiula M, Morosetti R, Sancricca C,Patanella AK, Nociti V, Iorio R, Bianco A, Tomassini V, Pozzilli C, Tonali PA, Matarese G, Batocchi AP. The effect of disease activity on leptin, leptin receptor and suppressor of cytokine signallling-3 in relapsing-remitting multiple sclerosis. J Neuroimmunol. 2007;192:174-183.
45. Sanna V, Di Giacomo A, La Cava A, Lechler RI, Fontana S, Zappacosta S, Matarese G. Leptin surge precedes onset of autoimmune encephalomyelitis and correlates with development of pathogenic T cell responses. J Clin Invest. 2003;111:241-250.
46. Langer-Gould A, Gupta R, Huang S, Hagan A, Atkuri K, Leimpeter AD, Albers KB, Greenwood E, Van Den Eeden SK, Steinman L, Nelson LM. Interferon-gamma-producing T cells, pregnancy, and postpartum relapses of multiple sclerosis. Arch Neurol. 2010;67:51-57.
47. Trowsdale J, Betz AG. Mother's little helpers: mechanisms of maternal-fetal tolerance. Nat Immunol. 2006;7:241-246.
48. Langer-Gould A, Garren H, Slansky A, Ruiz PJ, Steinman L. Late pregnancy suppresses relapses in experimental autoimmune encephalomyelitis: evidence for a suppressive pregnancy-related serum factor. J Immunol. 2002;169:1084-1091.
49. Moretta L, Ferlazzo G, Bottino C, Vitale M, Pende D, Mingari MC, Moretta A. Effector and regulatory events during natural killer-dendritic cell interactions. Immunol Rev. 2006;214:219-228.
50. Cooper MA, Fehniger TA, Caligiuri MA. The biology of human natural killer-cell subsets. Trends Immunol. 2001;22:633-640.
51. Karimi K, Arck PC. Natural Killer cells: keepers of pregnancy in the turnstile of the environment. Brain Behav Immun. 2010;24:339-347.
52. Koopman LA, Kopcow HD, Rybalov B, Boyson JE, Orange JS, Schatz F, Masch R, Lockwood CJ, Schachter AD, Park PJ, Strominger JL. Human decidual natural killer cells are a unique NK cell subset with immunomodulatory potential. J Exp Med. 2003;198:1201-1212.
53. Hanna J, Goldman-Wohl D, Hamani Y, Avraham I, Greenfield C, Natanson-Yaron S, Prus D, Cohen-Daniel L, Arnon TI, Manaster I, Gazit R, Yutkin V, Benharroch D, Porgador A, Keshet E, Yagel S, Mandelboim O. Decidual NK cells regulate key developmental processes at the human fetal-maternal interface. Nat Med. 2006;12:1065-1074.
54. Blois SM, Ilarregui JM, Tometten M, Garcia M, Orsal AS, Cordo-Russo R, Toscano MA, Bianco GA, Kobelt P, Handjiski B, Tirado I, Markert UR, Klapp BF, Poirier F, Szekeres-Bartho J, Rabinovich GA, Arck PC. A pivotal role for galectin-1 in fetomaternal tolerance. Nat Med. 2007;13:1450-1457.
55. Terness P, Kallikourdis M, Betz AG, Rabinovich GA, Saito S, Clark DA. Tolerance signaling molecules and pregnancy: IDO, galectins, and the renaissance of regulatory T cells. Am J Reprod Immunol. 2007;58:238-254.
56. Bielekova B, Catalfamo M, Reichert-Scrivner S, Packer A, Cerna M, Waldmann TA, McFarland H, Henkart PA, Martin R. Regulatory CD56(bright) natural killer cells mediate immunomodulatory effects of IL-2Ralpha-targeted therapy (daclizumab) in multiple sclerosis. Proc Natl Acad Sci USA. 2006;103:5941-5946.
57. Saraste M, Irjala H, Airas L. Expansion of CD56Bright natural killer cells in the peripheral blood of multiple sclerosis patients treated with interferon-beta. Neurol Sci. 2007;28:121-126.
58. Zhang B, Yamamura T, Kondo T, Fujiwara M, Tabira T. Regulation of experimental autoimmune encephalomyelitis by natural killer (NK) cells. J Exp Med. 1997;186:1677-1687.
59. Sicotte NL, Liva SM, Klutch R, Pfeiffer P, Bouvier S, Odesa S, Wu TC, Voskuhl RR. Treatment of multiple sclerosis with the pregnancy hormone estriol. Ann Neurol. 2002;52:421-428.

60. Soldan SS, Alvarez Retuerto AI, Sicotte NL, Voskuhl RR. Immune modulation in multiple sclerosis patients treated with the pregnancy hormone estriol. J Immunol. 2003;171:6267-6274.
61. Kim S, Liva SM, Dalal MA, Verity MA, Voskuhl RR. Estriol ameliorates autoimmune demyelinating disease: implications for multiple sclerosis. Neurology 1999;52:1230-1238.
62. Bebo BF Jr, Fyfe-Johnson A, Adlard K, Beam AG, Vandenbark AA, Offner H. Low-dose estrogen therapy ameliorates experimental autoimmune encephalomyelitis in two different inbred mouse strains. J Immunol. 2001;166:2080-2089.
63. Offner H. Neuroimmunoprotective effects of estrogen and derivatives in experimentalutoimmune encephalomyelitis: therapeutic implications for multiple sclerosis. J Neurosci Res. 2004;78:603-624.
64. Riskind PN, Massacesi L, Doolittle TH, Hauser SL The role of prolactin in autoimmune demyelination: suppression of experimental allergic encephalomyelitis by bromocriptine. Ann Neurol. 1991;29:542-547.
65. Dijkstra CD, van der Voort ER, De Groot CJ, Huitinga I, Uitdehaag BM, Polman CH, Berkenbosch F. Therapeutic effect of the D2-dopamine agonist bromocriptine on acute and relapsing experimental allergic encephalomyelitis. Psychoneuroendocrinology 1994;19:135-142.
66. Kira J, Harada M, Yamaguchi Y, Shida N, Goto I. Hyperprolactinemia in multiple sclerosis. J Neurol Sci. 1991;102:61-66.
67. Nociti V, Frisullo G, Tartaglione T, Patanella AK, Iorio R, Tonali PA, Batocchi AP. Multiple sclerosis attacks triggered by hyperprolactinemia. J Neurooncol. 2010;98:407-409.
68. Compston A, Coles A. Multiple sclerosis. Lancet. 2008;372:1502-1517.
69. Ascherio A, Munger KL, Simon KC. Vitamin D and multiple sclerosis. Lancet Neurol. 2010;9:599-612.
70. Langer-Gould A, Huang S, Van Den Eeden SK, Gupta R, Leimpeter AD, Albers KB, Horst R, Hollis B, Steinman L, Nelson LM. Vitamin D, Pregnancy, Breastfeeding, and Postpartum Multiple Sclerosis Relapses. Arch Neurol. 2011;68:310-313.
71. Sargent IL, Borzychowski AM, Redman CW. NK cells and human pregnancy--an inflammatory view. Trends Immunol. 2006;27:399-404.
72. Huang YH, Airas L, Schwab N, Wiendl H. Janus head: the dual role of HLA-G in CNS immunity. Cell Mol Life Sci. 2011;68:407-416.
73. Fainardi E, Rizzo R, Melchiorri L, Stignani M, Castellazzi M, Tamborino C, Paolino E, Tola MR, Granieri E, Baricordi OR. CSF levels of soluble HLA-G and Fas molecules are inversely associated to MRI evidence of disease activity in patients with relapsing-remitting multiple sclerosis. Mult Scler. 2008;14:446-454.
74. Airas L, Nikula T, Huang YH, Lahesmaa R, Wiendl H. Postpartum-activation of multiple sclerosis is associated with down-regulation of tolerogenic HLA-G. J Neuroimmunol. 2007;187:205-211.
75. Junker A, Krumbholz M, Eisele S, Mohan H, Augstein F, Bittner R, Lassmann H, Wekerle H, Hohlfeld R, Meinl E. MicroRNA profiling of multiple sclerosis lesions identifies modulators of the regulatory protein CD47. Brain 2009;132:3342-3352.
76. Du C, Liu C, Kang J, Zhao G, Ye Z, Huang S, Li Z, Wu Z, Pei G. MicroRNA miR-326 regulates TH-17 differentiation and is associated with the pathogenesis of multiple sclerosis. Nat Immunol. 2009;10:1252-1259.
77. Prieto DM, Markert UR. MicroRNAs in pregnancy. J Reprod Immunol. 2011;88:106-111.
78. Miura K, Miura S, Yamasaki K, Higashijima A, Kinoshita A, Yoshiura K, Masuzaki H. Identification of pregnancy-associated microRNAs in maternal plasma. Clin Chem. 2010;56:1767-1771.
79. Kotlabova K, Doucha J, Hromadnikova I. Placental-specific microRNA in maternal circulation - identification of appropriate pregnancy-associated microRNAs with diagnostic potential. J Reprod Immunol. 2011;89:185-191.
80. Gatson NN, Williams JL, Powell ND, McClain MA, Hennon TR, Robbins PD, Whitacre CC. Induction of pregnancy during established EAE halts progression of CNS autoimmune injury via pregnancy-specific serum factors. J Neuroimmunol. 2011;230:105-113.
81. Williams J, Smith K, Cox G, Shawler T, McTigue D, Whitacre C. Exosomes: Mediators of pregnancy-associated immune modulation and neuroprotection in experimental autoimmune encepahlomyelits (abstract). J Neuroimmunol. 2010;228:59.
82. Airas L, Nikula T, Huang YH, Lahesmaa R, Wiendl H. Postpartum-activation of multiple sclerosis is associated with down-regulation of tolerogenic HLA-G. J Neuroimmunol. 2007;187:205-211.

83. Achiron A, Rotstein Z, Noy S, Mashiach S, Dulitzky M, Achiron R. Intravenous immunoglobulin treatment in the prevention of childbirth-associated acute exacerbations in multiple sclerosis: a pilot study. J Neurol. 1996;243:25-28.
84. Achiron A, Kishner I, Dolev M, Stern Y, Dulitzky M, Schiff E, Achiron R. Effect of intravenous immunoglobulin treatment on pregnancy and postpartum-related relapses in multiple sclerosis. J Neurol. 2004;251:1133-1137.
85. Haas J, Hommes OR. A dose comparison study of IVIG in postpartum relapsing-remitting multiple sclerosis. Mult Scler. 2007;13:900-908.
86. Hellwig K, Beste C, Schimrigk S, Chan A. Immunomodulation and postpartum relapses in patients with multiple sclerosis. Ther Adv Neurol Disord. 2009;2:7-11.
87. de Seze J, Chapelotte M, Delalande S, Ferriby D, Stojkovic T, Vermersch P. Intravenous corticosteroids in the postpartum period for reduction of acute exacerbations in multiple sclerosis. Mult Scler. 2004;10:596-597.
88. Vukusic S, Ionescu I, El-Etr M, Schumacher M, Baulieu EE, Cornu C, Confavreux C; The Prevention of Post-Partum Relapses with Progestin and Estradiol in Multiple Sclerosis (POPART'MUS) trial: rationale, objectives and state of advancement. Prevention of Post-Partum Relapses with Progestin and Estradiol in Multiple Sclerosis Study Group. J Neurol Sci. 2009;286:114-118.
89. Amato MP, Portaccio E, Ghezzi A, Hakiki B, Zipoli V, Martinelli V, Moiola L, Patti F, La Mantia L, Mancardi GL, Solaro C, Tola MR, Pozzilli C, De Giglio L, Totaro R, Lugaresi A, Di Tommaso V, Paolicelli D, Marrosu MG, Comi G, Pellegrini F, Trojano M; MS Study Group of the Italian Neurological Society. Pregnancy and fetal outcomes after interferon-ß exposure in multiple sclerosis. Neurology 2010;75:1794-1802.
90. Hellwig K, Agne H, Gold R. Interferon beta, birth weight and pregnancy in multiple sclerosis. J Neurol. 2009;256:830-831.
91. Salminen HJ, Leggett H, Boggild M. Glatiramer acetate exposure in pregnancy: preliminary safety and birth outcomes. J Neurol. 2010;257:2020-2023.
92. Sandberg-Wollheim M, Frank D, Goodwin TM, Giesser B, Lopez-Bresnahan M, Stam-Moraga M, Chang P, Francis GS. Pregnancy outcomes during treatment with interferon beta-1a in patients with multiple sclerosis. Neurology 2005;65:802-806.
93. Sandberg-Wollheim M, Alteri E, Stam Moraga M, Kornmann G. Pregnancy outcomes in multiple sclerosis following subcutaneous interferon beta-1a Therapy. Mult Scler. 2011;17:423-430.
94. Weber-Schoendorfer C, Schaefer C. Multiple sclerosis, immunomodulators, and pregnancy outcome: a prospective observational study. Mult Scler. 2009;15:1037-1042.
95. Fragoso YD, Finkelsztejn A, Kaimen-Maciel DR, Grzesiuk AK, Gallina AS, Lopes J, Morales NM, Alves-Leon SV, de Almeida SM. Long-term use of glatiramer acetate by 11 pregnant women with multiple sclerosis: a retrospective, multicentre case series. CNS Drugs 2010;24:969-976.
96. Patti F, Cavallaro T, Lo Fermo S, Nicoletti A, Cimino V, Vecchio R, Laisa P, Zarbo R, Zappia M. Is in utero early-exposure to interferon beta a risk factor for pregnancy outcomes in multiple sclerosis? J Neurol. 2008;255:1250-1253.
97. Neuteboom RF, Boon M, Catsman Berrevoets CE, Vles JS, Gooskens RH, Stroink H, Vermeulen RJ, Rotteveel JJ, Ketelslegers IA, Peeters E, Poll-The BT, De Rijk-Van Andel JF, Verrips A, Hintzen RQ. Prognostic factors after a first attack of inflammatory CNS demyelination in children. Neurology 2008;71:967-973.
98. Neuteboom RF, Ketelslegers IA, Boon M, Catsman-Berrevoets CE, Hintzen RQ; Dutch Study Group on Childhood Multiple Sclerosis and Acute Disseminated Encephalomyelitis. Barkhof magnetic resonance imaging criteria predict early relapse in pediatric multiple sclerosis. Pediatr Neurol. 2010;42:53-55.
99. Mikaeloff Y, Caridade G, Assi S, Suissa S, Tardieu M. Prognostic factors for early severity in a childhood multiple sclerosis cohort. Paediatrics 2006;118:1133-1139.
100. Callen DJ, Shroff MM, Branson HM, Lotze T, Li DK, Stephens D, Banwell BL. MRI in the diagnosis of paediatric multiple sclerosis. Neurology 2009;72:961-967.
101. Callen DJ, Shroff MM, Branson HM, Li DK, Lotze T, Stephens D, Banwell BL. Role of MRI in the differentiation of ADEM from MS in children. Neurology 2009;72:968-973.
102. Ketelslegers IA, Neuteboom RF, Boon M, Catsman-Berrevoets CE, Hintzen RQ; Dutch Pediatric MS Study Group. A comparison of MRI criteria for diagnosing pediatric ADEM and MS. Neurology 2010;74:1412-1415.

103. Krupp LB, Banwell B, Tenembaum S; for the international pediatric MS study group. Consensus definitions proposed for pediatric multiple sclerosis and related disorders. Neurology 2007;68:S7-S12.
104. Alper G, Heyman R, Wang L. Multiple sclerosis and acute disseminated encephalomyelitis diagnosed in children after long-term follow-up: comparison of presenting features. Dev Med Child Neurol. 2009;51:480-486.
105. Dundar NO, Anlar B, Guven A, Serdaroglu A, Yarar C. Relapsing acute disseminated encephalomyelitis in children: further evaluation of the diagnosis. J Child Neurol. 2010;25:1491-1497.
106. Suppiej A, Vittorini R, Fontanin M, De Grandis D, Manara R, Atzori M, Gallo P,Battistella PA. Acute disseminated encephalomyelitis in children: focus on relapsing patients. Pediatr Neurol. 2008;39:12-7.
107. Rostásy K, Nagl A, Lütjen S, Roll K, Zotter S, Blaschek A, Korenke GC, Karenfort M, Gotwald T, Holthausen H. Clinical outcome of children presenting with a severe manifestation of acute disseminated encephalomyelitis. Neuropediatrics 2009;40:211-217.
108. Mar S, Lenox J, Benzinger T, Brown S, Noetzel M. Long-term prognosis of pediatric patients with relapsing acute disseminated encephalomyelitis. J Child Neurol. 2010;25:681-688.
109. Hahn CD, Manohar M, Shroff M, Blaser S, Banwell B. MRI criteria for multiple sclerosis. Evaluation in a pediatric cohort. Neurology 2004;62:806-808.
110. Mikaeloff Y, Adamsbaum C, Husson B, Vallée L, Ponsot G, Confavreux C, Tardieu M, Suissa S; KIDMUS Study Group on Radiology. MRI prognostic factors for relapse after acute CNS inflammatory demyelination in childhood. Brain 2004;127:1942-1947.
111. Polman CH, Reingold SC, Banwell B, Clanet M, Cohen JA, Filippi M, Fujihara K, Havrdova E, Hutchinson M, Kappos L, Lublin FD, Montalban X, O'Connor P, Sandberg-Wollheim M, Thompson AJ, Waubant E, Weinshenker B, Wolinsky JS. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. Ann Neurol. 2011;69:292-302.
112. Dale RC, de Sousa C, Chong WK, Cox TC, Harding B, Neville BG. Acute disseminated encephalomyelitis, multiphasic disseminated encephalomyelitis and multiple sclerosis in children. Brain 2000;123:2407-2422.

SUMMARY

SAMENVATTING

LIST OF ABBREVIATIONS

DANKWOORD

CURRICULUM VITAE

LIST OF PUBLICATIONS

PORTFOLIO

SUMMARY

Chapter 1, the introduction, summarizes current knowledge regarding two special and different situations in multiple sclerosis (MS): Childhood onset MS and MS during pregnancy.

Chapter 2 describes the clinical (**chapter 2.1-2.3**) and biological studies (**chapter 2.4-2.6**) on pregnancy and MS. In **chapter 2.1** we studied the clinical course of multiple sclerosis before, during and after pregnancy. We found that the relapse rate increased in the first three months after delivery, yet normalized within one year after delivery. Health-related quality of life (QoL) was improved during pregnancy, most appreciated in the MOS 36 item short form health survey questionnaire (SF-36) domains vitality and general health. Nine months or more after delivery we found no adverse effects on MS disease activity at group level, measured by the expanded disability status scale (EDSS), multiple sclerosis impact scale 29 (MSIS-29), and the Guy's neurological disability scale (GNDS). Nine months or more after delivery QoL, measured by the SF-36, was not unfavorably altered when compared with QoL during pregnancy. This indicates that, although the number of relapses is increased in the short term after delivery, there are no adverse effects of pregnancy on disease course in the mid-long term after delivery. Until now the only known predictors of a postpartum relapse are: number of relapses in the year preceding pregnancy, number of relapses during pregnancy and duration of disease. We were not able to reproduce these findings. In **chapter 2.2** we describe data on breastfeeding and disease activity that does not support the recent claim that breastfeeding protects against postpartum relapse. In **chapter 2.3** we found that high serum levels of the chemokine interleukin-8 (IL-8) during the first trimester were associated with postpartum relapse. The low positive predictive value will likely limit clinical use of IL-8 as a predictor of postpartum relapse. In **chapter 2.4** we performed a genome wide approach on alterations of the transcriptome of monocytes of MS patients before and during the third trimester of pregnancy. We found that during pregnancy expression of the Fc receptor CD64 was increased. Our results therefore support the hypothesis that the innate arm of the immune system is more activated during pregnancy. In **chapter 2.5** we investigated the numbers of circulating regulatory T cells (Treg) and T helper (Th)17 cells. Unexpectedly, we found that the numbers of circulating Treg were decreased, during the first and third trimester of pregnancy in both MS patients and healthy controls. We found no differences in the frequencies of circulating Th17 cells during pregnancy in MS patients and healthy controls. We concluded that our results did not support our hypothesis that peripheral blood Th17 and Treg cells are directly involved in MS disease course alteration during pregnancy. In **chapter 2.6** we studied serum levels of leptin before, during and after pregnancy in MS patients and healthy controls. We observed a significant increase in serum levels of leptin in women with MS during the third trimester, compared to baseline and first trimester samples. Serum levels of leptin during pregnancy were not associated with a postpartum relapse. Therefore, serum levels of leptin during pregnancy cannot be used as a biomarker for postpartum relapse. We found that women with MS with the largest relative decrease in serum leptin levels after delivery more often had a postpartum relapse.

Chapter 3 describes the studies on childhood onset in MS. We performed a retrospective nationwide study in all large neuro-pediatric centers in The Netherlands, described in **chapter 3.1**. We included the full spectrum of acquired demyelinating syndromes (ADS) of the central nervous system. 44% of the children with a monofocal attack developed MS, whereas 21% of the children with a polyfocal attack developed MS. Both the Barkhof MRI-criteria and the KIDMUS MRI-criteria were able to predict a future diagnosis of MS after a first demyelinating event. In the very young, aged under ten, we found that the sensitivity of especially the KIDMUS criteria was very low (18%). Cerebrospinal fluid (CSF) analysis showed that an increased IgG index and presence of oligoclonal banding both were able to predict MS. Strikingly, children with and without encephalopathy both display MRI abnormalities as seen in typical acute disseminated encephalomyelitis (ADEM) cases (large lesions and basal ganglia/thalamic lesions). In **chapter 3.2** we found that children with MS, with MRI features consistent with three or four out of the four Barkhof criteria for dissemination in space, were more likely to have a relapse soon after their second, MS defining, attack. We could not reproduce the predictive value of the childhood-onset MS potential index for early severity. In **chapter 3.3** we investigated the capacity of all known diagnostic MRI criteria for children to differentiate MS from acute disseminated encephalomyelitis (ADEM). We found that the Callen criteria for discriminating MS from ADEM had the best test properties.

In **chapter 4**, the discussion, the observations from the studies in chapter 2 and 3 are summarized and discussed in relation to current literature. Recommendations for further research are described.

SAMENVATTING

Hoofdstuk 1, de introductie, vat de huidige kennis samen over twee bijzondere en verschillende situaties bij multipele sclerose (MS): Debuut van MS op de kinderleeftijd en MS tijdens zwangerschap.

In **hoofdstuk 2** worden de klinische (**hoofdstuk 2.1-2.3**) en biologische studies (**hoofdstuk 2.4-2.6**) over zwangerschap en MS beschreven. **hoofdstuk 2.1** gaat over het klinisch beloop van MS voor, tijdens en na de zwangerschap. Het aantal MS aanvallen nam toe gedurende de eerste drie maanden na de bevalling. Eén jaar na de bevalling normaliseerde het aantal aanvallen weer. Gezondheidgerelateerde kwaliteit van leven (KvL) was verbeterd tijdens de zwangerschap. Dit was het meest duidelijk zichtbaar bij de MOS 36 item short form health survey questionnaire (SF-36) domeinen vitaliteit en algehele gezondheid. Minimaal negen maanden na de bevalling vonden we geen nadelige effecten van zwangerschap op MS ziekteactiviteit, gemeten door de expanded disability status scale (EDSS), multiple sclerosis impact scale 29 (MSIS-29) en Guy's neurological disability scale (GNDS). Minimaal negen maanden na de bevalling was ook KvL, gemeten door de SF-36, niet ten nadele veranderd, vergeleken met KvL tijdens de zwangerschap. Dit betekent dat er geen nadelige effecten zijn van zwangerschap op ziekteactiviteit op de middellange termijn, ondanks de verhoogde ziekteactiviteit direct na de bevalling. De enige bekende voorspellers van een aanval direct na de bevalling zijn: het aantal aanvallen in het jaar voorafgaand aan de zwangerschap, het aantal aanvallen tijdens de zwangerschap en de duur van ziekte. Wij hebben de voorspellende waarde van deze parameters niet kunnen bevestigen. **Hoofdstuk 2.2** gaat over borstvoeding en ziekteactiviteit. Enige tijd geleden werd een artikel gepubliceerd dat melding maakte van de mogelijk beschermende rol van borstvoeding tegen een aanval direct na de bevalling. Wij hebben geen aanwijzing voor een beschermende rol van borstvoeding tegen een aanval direct na de bevalling gevonden. In **hoofdstuk 2.3** vonden we dat een hoge waarde in serum van het chemokine interleukine-8 (IL-8) tijdens het eerste trimester geassocieerd was met een MS aanval direct na de bevalling. De lage positief voorspellende waarde zal waarschijnlijk het gebruik van IL-8 als voorspeller in de praktijk beperken. In **hoofdstuk 2.4** bespreken we een studie naar de veranderingen van het transcriptoom van monocyten van MS patiënten, voor en tijdens de zwangerschap. We vonden dat tijdens de zwangerschap de expressie van de Fc receptor CD64 verhoogd was. Deze resultaten ondersteunen daarmee de hypothese dat aangeboren immuniteit meer geactiveerd is tijdens de zwangerschap. **Hoofdstuk 2.5** gaat over het percentage circulerende regulatoire T cellen (Treg) en T helper (Th)17 cellen. Tegen onze verwachting in vonden we dat het aantal circulerende Treg gedaald was tijdens het eerste en derde trimester van de zwangerschap bij MS patiënten en gezonde controles. We vonden geen verschillen in percentages van circulerende Th17 cellen tijdens en na de zwangerschap bij MS patiënten en gezonde controles. Wij concludeerden dat circulerende Treg en Th17 cellen niet direct betrokken zijn bij de verbetering van MS tijdens de zwangerschap. In **hoofdstuk 2.6** beschrijven we een studie naar serumwaarden

van leptine voor, tijdens en na de zwangerschap bij MS. We observeerden dat leptine significant verhoogd was tijdens de zwangerschap bij MS patiënten, vergeleken met voor de zwangerschap en tijdens het eerste trimester van de zwangerschap. Leptine waarden in serum tijdens de zwangerschap waren niet geassocieerd met een aanval in de eerste drie maanden na de bevalling. Daarom kan leptine niet gebruikt worden om een aanval direct na de bevalling te voorspellen. Wel vonden we dat vrouwen met de grootste relatieve daling in serum waarden van leptine na de bevalling de grootste kans hadden op een aanval in de eerste drie maanden na de bevalling.

In **hoofdstuk 3** worden de studies over debuut van MS op de kinderleeftijd besproken. **Hoofdstuk 3.1** gaat over een retrospectieve studie, waarbij alle grote kinderneurologische centra in Nederland participeerden. Wij includeerden kinderen met aandoeningen binnen het volledige spectrum van verkregen auto-immuun demyeliniserende aandoeningen van het centrale zenuwstelsel. 44% van de kinderen met een monofocale aanval kreeg uiteindelijk de diagnose MS. Van de kinderen met een polyfocale aanval werd bij 21% uiteindelijk de diagnose MS gesteld. Wij vonden dat zowel de Barkhof MRI criteria en de KIDMUS MRI criteria in staat waren om een uiteindelijke diagnose MS te voorspellen ten tijde van de eerste aanval. Echter, bij kinderen jonger dan tien jaar was de sensitiviteit van met name de KIDMUS MRI criteria erg laag (18%). Analyse van de liquor cerebrospinalis toonde dat een verhoogde IgG index en de aanwezigheid van oligoklonale banden in liquor cerebrospinalis een toekomstige diagnose MS konden voorspellen. Opvallend was dat zowel kinderen met als zonder encephalopathie afwijkingen op de MRI hebben, die typisch zijn voor een acute gedisseminеerde encephalomyelitis (ADEM) (grote afwijkingen en afwijkingen in de basale kernen en thalamus). In **hoofdstuk 3.2** vonden we dat kinderen met MS, waarbij de MRI ten tijde van de eerste aanval aan minimaal drie van de vier Barkhof MRI criteria voor disseminatie in plaats voldeed, sneller een aanval krijgen na hun tweede aanval. We konden de voorspellende waarde van de childhood-onset MS potential index for early severity, ontworpen om snelle progressie van MS te voorspellen, niet aantonen. In **hoofdstuk 3.3** beschrijven we de capaciteit van alle bekende diagnostische MRI criteria bij kinderen om MS van ADEM te differentiëren. Wij vonden dat van alle vier onderzochte criteria sets de Callen criteria voor het onderscheid tussen ADEM en MS de beste test eigenschappen hadden.

In **hoofdstuk 4**, de discussie, worden alle bevindingen van de studies uit hoofdstuk 2 en 3 samengevat en besproken in relatie tot de huidige literatuur. Vervolgens worden aanbevelingen voor toekomstig onderzoek gedaan.

LIST OF ABBREVIATIONS

ADEM:	Acute disseminated encephalomyelitis
ADS:	Acquired demyelinating syndromes
ANKRD22:	Ankyrin repeat domain 22
APC:	Antigen presenting cell
BBB:	Blood brain barrier
BMI:	Body mass index
CIS:	Clinically isolated syndrome
CNS:	Central nervous system
CRH:	Corticotrophin releasing hormone
CPT:	Cell preparation tubes
CSF:	Cerebrospinal fluid
DIS:	Dissemination in space
DIT:	Dissemination in time
DMD:	Disease-modifying drugs
EAE:	Experimental autoimmune/allergic encephalomyelitis
EDSS:	Expanded disability status scale
ELISA:	Enzyme-linked immunosorbent assay
FcgR1a:	Fc gamma receptor 1a
FLAIR:	Fluid attenuated inversion recovery
FoxP3:	Forkhead box transcription factor p3
Gal-1:	Galectin 1
GNDS:	Guy's neurological disability scale
HLA:	Human leucocyte antigen
IDO:	Indoleamine-2,3 dioxygenase
IFN-γ:	Interferon γ
IL:	Interleukin
IPMSSG:	International paediatric MS study group
IQR:	Interquartile range
IVIG:	Intravenous immunoglobulins
JAK2:	Janus kinase 2
KIR:	Killer cell immunoglobulin-like receptor
MFI:	Mean fluorescence intensity
MHC:	Major histocompatiblity complex
MOG:	Myelin oligodendrocte glycoprotein
MRI:	Magnetic resonance imaging
mRNA:	Messenger RNA
MS:	Multiple sclerosis
MSIS- 29:	Multiple sclerosis impact scale 29
NK:	Natural killer

OCB:	Oligoclonal bands
PBMC:	Peripheral blood mononuclear cells
PP-MS:	Primary progressive multiple sclerosis
PCIS:	Polyfocal clinically isolated syndrome
PTX3:	Pentraxin 3
QoL:	Quality of life
RA:	Rheumatoid arthritis
RR-MS:	Relapsing remitting multiple sclerosis
RT-PCR:	Real time polymerase chain reaction
SAM:	Statistical analysis of microarrays
SD:	Standard deviation
SF36:	MOS 36 item short form health survey questionnaire
sHLA-G:	Soluble HLA-G
SLE:	Systemic lupus erythematosus
SLR:	Soluble leptin receptor
STAT1:	Signal transducer and activator of transcription 1
T-bet:	T box transcription factor
TGF- ß:	Transforming growth factor ß
Th:	Thelper
TNF- α:	Tumor necrosis factor alpha
Treg:	Regulatory T cells
VEP:	Visual evoked potential
VEGF:	Vascular endothelial growth factor

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CURRICULUM VITAE

Rinze Frederik Neuteboom was born on the 24th of November 1976 in Apeldoorn, The Netherlands. He attended secondary school at the Stedelijk Gymnasium Apeldoorn, from which he graduated in 1995. He started medical school in 1995 at the Erasmus University of Rotterdam. During this period he worked at the Department of Neuro-Anatomy assisting classes of neuro-anatomy. In 2001 he graduated from medical school cum laude and started his residency in Neurology at the Department of Neurology at the Erasmus MC in Rotterdam (head: Prof. Dr. P.J. Koudstaal, later Prof. Dr. P.A.E. Sillevis-Smitt). He finished his Neurology training in 2010. From 2003 he combined his residency with the research underlying this thesis with Prof. Dr. R.Q. Hintzen and Prof. Dr. J.D. Laman. In 2009 he started a two year clinical fellowship in Pediatric Neurology under Prof. Dr. W.F.M. Arts, including a one year residency in Pediatrics under Dr. M. De Hoog. Since 2011 he has been working as a pediatric neurologist in the Sophia Children's Hospital, with special interest in neuro-inflammatory diseases in childhood.

LIST OF PUBLICATIONS

1. ***Neuteboom RF,*** De Groot CJM, Hintzen RQ. Multiple sclerose en zwangerschap. Nederlands tijdschrift voor neurologie. 2003;104:79-83.
2. ***Neuteboom RF***, Garssen MPJ, Hintzen RQ. Neuromyelitis optica (ziekte van Devic): een zware variant van multiple sclerose. Ned Tijdschr Geneeskd. 2004;148:1097-1102.
3. ***Neuteboom RF***, Catsman-Berrevoets CE, Hintzen RQ. Multiple sclerose bij kinderen. Ned Tijdschr Geneeskd. 2007;151:1464-1468.
4. ***Neuteboom RF***, Boon M, Catsman Berrevoets CE, Vles JS, Gooskens RH, Stroink H, Vermeulen RJ, Rotteveel JJ, Ketelslegers IA, Peeters E, Poll-The BT, De Rijk-Van Andel JF, Verrips A, Hintzen RQ. Prognostic factors after a first attack of inflammatory CNS demyelination in children. Neurology 2008;71:967-973.
5. ***Neuteboom RF,*** Verbraak E, Voerman JS, van Meurs M, Steegers EA, de Groot CJ, Laman JD, Hintzen RQ. First trimester interleukin 8 levels are associated with postpartum relapse in multiple sclerosis. Mult Scler. 2009;15:1356-1358.
6. ***Neuteboom RF,*** Verbraak E, Voerman JS, van Meurs M, Steegers EA, de Groot CJ, Laman JD, Hintzen RQ. Serum leptin levels during pregnancy in multiple sclerosis. Mult Scler. 2009; 15:907-912.
7. Ketelslegers IA, Catsman-Berrevoets CE, Boon M, Eikelenboom MJ, Stroink H, ***Neuteboom RF***, Aarsen FK, van de Putte EM, Hintzen RQ. Fatigue and depression in children with multiple sclerosis and monophasic variants. Eur J Paediatr Neurol. 2010;14:320-325.
8. ***Neuteboom RF,*** Ketelslegers IA, Boon M, Catsman-Berrevoets CE, Hintzen RQ; on behalf of the Dutch study group on childhood ADEM and MS. Barkhof MRI criteria predict early relapse in pediatric multiple sclerosis. Pediatr Neurol. 2010;42:53-55.
9. Ketelslegers IA, ***Neuteboom RF***, Boon M, Catsman-Berrevoets CE Hintzen RQ. A comparison of MRI criteria for diagnosing paediatric ADEM and MS. Neurology 2010;74:1412-1415.
10. ***Neuteboom RF,*** Verbraak E, Wierenga-Wolf AF, van Meurs M, Steegers EA, de Groot CJ, Laman JD, Hintzen RQ. Pregnancy-induced fluctuations in functional T-cell subsets in multiple sclerosis patients. Mult Scler. 2010;16:1073-1078.
11. Ghezzi A, Banwell B, Boyko A, Amato MP, Anlar B, Blinkenburg M, Boon M, Filippi M, Jozwiak S, Ketelslegers I, Kornek B, Lim M, Lindstrom E, Nadj C, ***Neuteboom R,*** Rocca MA, Rostasy K, Tardieu M, Wassmer E, Catsman-Berrevoets C, Hintzen R. The management of multiple sclerosis in children: a European view. Mult Scler. 2010;16:1258-1267.
12. ***Neuteboom RF,*** Verbraak E, Wierenga-Wolf AF, Voerman JS, Van Meurs M, Swagemakers SM, van der Spek PJ, Steegers EA, de Groot CJ, Laman JD, Hintzen RQ. The monocyte transcriptome during pregnancy in multiple sclerosis: prominent expression of the Fc-receptor CD64. Mult Scler. 2011;17:389-396.
13. Ketelslegers IA, Visser IE, ***Neuteboom RF***, Boon M, Catsman-Berrevoets CE, Hintzen RQ. Disease course and outcome of acute disseminated encephalomyelitis is more severe in adults than in children. Mult Scler. 2011;17:441-448.

14. ***Neuteboom RF,*** Hintzen RQ. Breast-feeding, postpartum and prepregnancy disease activity in multiple sclerosis. Neurology 2011;76:1532.
15. ***Neuteboom RF,*** Janssens AC, Siepman TA, Hoppenbrouwers IA, Ketelslegers IA, Jafari N, Steegers EA, de Groot CJ, Hintzen RQ. Pregnancy in multiple sclerosis: clinical and self-report scales. J Neurol. 2012;259:311-317.
16. ***Neuteboom RF,*** Lequin MH, de Wit MC, Catsman-Berrevoets CE. Lumbar puncture in paediatric stroke. Lancet 2011;378:848.
17. Ketelslegers IA, Catsman-Berrevoets CE, ***Neuteboom RF,*** Boon M, Van Dijk KGC, Eikelenboom MJ, Gooskens RHJM, Niks EH, Overweg-Plandsoen WCG, Peeters EAJ, Peeters-Scholte CMPCD, Poll-The BT, Rijk- van Andel JF, Samijn JPA, Snoeck IN, Stroink H, Vermeulen RF, Verrips A, Vles JSH, Willemsen MAAP, Rodrigues Pereira R, Hintzen RQ. Incidence of acquired demyelinating syndromes of the CNS in Dutch children: a nationwide study. J Neurol. 2012 accepted.

PHD PORTFOLIO: SUMMARY OF PHD TRAINING AND TEACHING

1. PhD training	Year	Workload (ECTS)
General and specific courses		
Biemond courses 2002, 2003, 2004, 2005, 2006, 2007, 2008, 2009, 2010, 2011		5.7
ESNI neuro-immunology course	2002	1.2
Seminars and workshops		
Voorjaarsvergadering NVKN 2007,2008,2009,2010,2011		1.0
Presentations		
MS research dagen (2 oral presentations)	2006	2.0
ECTRIMS (oral presentation)	2006	1.0
Voorjaarsvergadering NVKN (oral presentation)	2007	1.0
Wetenschappelijke vergadering NVN (oral presentation)	2007	1.0
EPNS (oral presentation)	2007	1.0
ACTRIMS/ECTRIMS/LACTRIMS (2 poster presentations)	2008	2.0
MS research dagen (oral presentation)	2008	1.0
ECTRIMS (poster presentation)	2009	1.0
MS research dagen (oral presentation)	2011	1.0
ECTRIMS (poster presentation)	2011	1.0
(Inter)national conferences		
MS research dagen 2002, 2003, 2004, 2005, 2006, 2008, 2011		4.0
ECTRIMS 2006, 2009, 2011		2.7
EPNS	2007	1.0
ACTRIMS/ECTRIMS/LACTRIMS	2008	1.0
ISPNO	2011	1.0
2. Teaching		
Nascholing regionale neurologen Zuid-West Nederland	2007	0.5
Najaarsvergadering NVKN	2009	0.5
Pediatric Neurology Training for residents in pediatrics	2010	0.5
Training neuro-pediatric nurses 2010, 2011		0.5
MOLMED training 2005, 2008, 2011		1.5
Total		33.1