Chapter 4
General discussion and future perspectives
Summarizing discussion and future perspectives

Introduction

In recent years much has changed in the treatment of relapsing-remitting multiple sclerosis (RRMS). Several new treatments became available and still more are coming up. However, with these new treatments, also “new” side effects were seen. Natalizumab is one of the most effective therapies on the market, but this effective treatment has a down side as well. Progressive multifocal leukoencephalopathy (PML), a severe, potentially life-threatening disease, caused by John Cunningham (JC) virus, is the most important reason not prescribing natalizumab to every patient with RRMS.

The purpose of this thesis was to learn more about clinically relevant issues of natalizumab treatment. Two questions had to be answered:
1. Which factors have a relevant influence on the effectiveness of the treatment?
2. Which are the most important risk factors contributing to the development of PML?

Further we will discuss how this knowledge may lead to a more individualized approach to natalizumab treatment in RRMS patients?

Natalizumab in the treatment of multiple sclerosis

In the first study (chapter 2.1), we investigated the clinical relevance of serum natalizumab concentrations and their relation with anti-natalizumab antibodies. It has already been shown that anti-drug antibody titers are much more difficult to standardize than serum drug concentrations. In a recent paper of van Schouwenburg et al., the difficulties of standardization of these anti-drug antibody measurements are highlighted and the authors conclude that it appears to be difficult to compare the results of different assays. The reason for this is that these results are highly dependent on the characteristics of the monoclonal antibody, most importantly the affinity of the antibody to the drug1.

In the initial phase 3 studies of natalizumab (AFFIRM and SENTINEL), it has been shown that 9-12% of the natalizumab-treated patients developed anti-natalizumab antibodies. In 6% it concerned persistent anti-natalizumab antibodies, which caused a loss of
efficacy as well as an increase in infusion-related reactions and the remaining patients had transient anti-natalizumab antibodies\(^2\). Sorensen et al. found in their study 4.5 % anti-natalizumab antibodies, 3.5% were persistent antibodies\(^3\) and Oliver et al. found in 14.1% of the patients anti-natalizumab antibodies of which 9.4 was persistent\(^4\). In all these studies, the ELISA method was used.

In our study, anti-natalizumab antibodies were present in as many as 58% of the natalizumab-treated patients. At year one after start of the natalizumab, 95.4% of all patients of whom serum was available, was anti-natalizumab antibody negative. So, the total amount of patients with transient anti-natalizumab antibodies we found was much higher than in earlier studies. In our study, we used the RIA method. As was shown in a study of Hart et al., in which they studied the differential effect of drug interference in two common types of assays (RIA and ELISA), which were used to measure anti-adalimumab antibodies\(^5\), the RIA method seems more suitable than the ELISA to detect anti-adalimumab antibodies with free adalimumab circulating in the serum as well.

We showed in one patient an increase in anti-natalizumab antibodies after cessation of the natalizumab therapy. This could suggest that anti-natalizumab antibodies are not measurable in the serum when these antibodies are bound to natalizumab and so called natalizumab-anti-natalizumab immune complexes are formed. The formation of these natalizumab-anti-natalizumab complexes also leads to an increase in clearance of natalizumab, and consequently results in a low serum natalizumab concentration. We could not demonstrate convincing clinical relevance of the measured transient antibodies in our patients. Most likely this is caused by the low antibody-titers, probably in combination with a low affinity, lacking to show a noteworthy effect on the natalizumab concentrations.

We found that the antibody titer was inversely correlated with serum natalizumab concentration (p<0.001) and that only high antibody titers were associated with very low or undetectable serum natalizumab concentrations. Both high antibody titers and low serum natalizumab concentrations were associated with clinical (relapses) and radiological (gadolinium-enhancing lesions on MRI) disease activity, indicating a lack of efficacy of natalizumab. We concluded that measuring natalizumab concentrations is as good as measuring anti-natalizumab antibodies for the evaluation of treatment efficacy. For various practical reasons, however, measurement of natalizumab concentration
could be preferable. Measuring natalizumab concentrations, using a highly specific assay, can also be a guidance in individualizing treatment strategies, which means that it will enable us to lower the dose or frequency of infusions in patients with a high serum natalizumab concentration.

An important consideration in this respect is that the amount of natalizumab infusions may contribute to the risk of PML and an increased interval between two infusions might have a positive effect on the risk of developing PML. This aspect has further been explored in the next chapter.

In the second study (chapter 2.2), we studied the effect of discontinuing the natalizumab treatment in 10 patients. In 7 out of these 10 patients there was clinical and/or radiological activity after discontinuation of treatment during a mean interval of 17 weeks (range 8 – 22 weeks). After discontinuing natalizumab, no other therapy was started. Also in other studies, it has been observed that discontinuing natalizumab treatment resulted in a return of disease activity and that this was independent of starting alternative therapies.\textsuperscript{6,7} These studies did not show a subsequent excessive disease activity, (“rebound”), after cessation of the natalizumab\textsuperscript{6,7}, while in some other studies they suggest it was\textsuperscript{8,9}. More studies have been done recently to further investigate the best strategy after natalizumab discontinuation. This will be further discussed in the future perspectives. As already mentioned before, the mean interval after which we found clinical or radiological disease activity was 17 weeks, with a range of 8-22 weeks. Khatri et al. described that after a single dose of natalizumab, it costs about 3 months to reach a natalizumab level <1 mg/l. A natalizumab level <1 mg/l would result in desaturation of the α4-integrin receptor to <50%\textsuperscript{10}. This was concluded after extrapolating natalizumab levels, which were monitored for 28 days.

To receive more insight in the course of natalizumab levels, which is among other things relevant in how to manage PML, we studied natalizumab levels in 10 RRMS patients who discontinued natalizumab therapy for different reasons (chapter 2.3). The mean treatment durations of these patients, before cessation of the natalizumab, was 23 months (range 12-40 months). We found that the median concentration in patients just before the last natalizumab infusion was 25 (range 18.4-104) mg/l. These concentrations already show that there is a wide range of natalizumab concentrations in the individual patients. Three months after the last natalizumab infusion the median
concentration was 0.28 (range 0.1-1) mg/l. There was still natalizumab detectable in some patients after more than 200 days. As we have shown in chapter 3.3, measuring natalizumab concentration is of major importance for individualized treatment in patients.

**Serological biomarkers and their relevance in natalizumab-associated PML in MS patients**

As already mentioned in the introduction of this chapter, natalizumab is a highly effective treatment for RRMS, but can be complicated by a rare, but potentially life-threatening adverse event, PML. PML is caused by the reactivation and replication of the JCV. JCV seropositivity is a risk factor for the development of PML. It is now known that patients with a high anti-JCV antibody index have a higher risk of developing PML than patients who are anti-JCV antibody negative or have a low anti-JCV antibody index. Nowadays, in the overall population the prevalence of anti-JCV antibodies is 57.1%-58.3% and tends to rise with increasing age\(^\text{11,12}\). The incidence rates based on the estimated natural history are approximately 1%-2\(^\text{13}\). Seroconversion rates in natalizumab-treated MS patients differed from 2%-26.7\(^\text{14,15,16}\). In all these studies there were limited longitudinal data, so the research question of chapter 3.1 was to evaluate the seroconversion rate in the natalizumab-treated RRMS patients of the VU medical center (VUmc). We found in our study population of 179 patients, with a median follow-up of 4.2 years (range 49 days to 12.7 years), an annualized seroconversion rate of 7.1%. This is in the same range as reported earlier by others, but with a much longer follow-up. If we take into account that RRMS patients will use natalizumab for many years, this would lead to a cumulative seroconversion rate of more than 25% in 4 years. This conversion rate is much higher than the estimated natural history conversion rate, and this fact should be taken into account in the risk assessment when considering the start of natalizumab treatment. At this moment, the European Medicines Agency (EMA) recommends retesting of the JCV-serology every 6 months during natalizumab therapy. The EMA decided last year to start an investigation, based on the reported seroconversion rate of 13% in 18 months by Plavina et al.\(^\text{15}\), to reconsider the follow-up strategy of these JCV-seronegative patients. As we mentioned before, natalizumab-treated RRMS patients with a high anti-JCV antibody index have a higher risk than patients with a low anti-JCV antibody index. Plavina et al. provided in their paper a table
with estimated PML risk by anti-JCV antibody index and duration of natalizumab treatment\textsuperscript{15}. For example, a patient with more than 2 years of natalizumab treatment and an index of >1.5 has a risk of 8.1\% to develop PML versus 0.3\% in a patient with the same treatment duration, but with an index of ≤0.9\textsuperscript{15}. In earlier studies it has been suggested that an increase of anti-JCV antibody index could be observed in patients prior to the development of PML\textsuperscript{15,16,17,18}. The major limitation in all these studies was the very limited longitudinal samples that were available. That was the reason for us to further explore this aspect. This has been reported in the next chapter.

In chapter 3.2 the first 4 PML patients from the MS Center of the VUmc of whom extensive longitudinal pre-PML samples were available have been reported. Of 3 out of 4 PML patients, pre-natalizumab samples were also available. All these samples had already a high anti-JCV antibody index. In all samples of these four PML patients, the anti-JCV antibody indices were high, from baseline until the PML diagnosis. The median anti-JCV antibody index over time was 3.04 (range 2.04-3.59). These indices showed no significant increase or decrease over the years (median index baseline 3.05 compared to 3.16 at the time of PML diagnosis, $p=0.72$). The strength of our study was the exceptional high number of pre-PML serum samples in which we tested the anti-JCV antibody index. As mentioned before, all samples had already a high anti-JCV antibody index at baseline, which was also seen in the earlier mentioned studies\textsuperscript{15,16,17,18}. The difference with the patients described by Warnke et al.\textsuperscript{17} was that we did not see a significant increase of anti-JCV antibody index in our four PML patients preceding the PML diagnosis. There have to be taken into account that in patients with a high anti-JCV antibody index (above 3.0), the saturation level of the assay is already technically reached, so no increase of the index can be demonstrated anymore\textsuperscript{15}. The stable high anti-JCV antibody indices correspond to the earlier mentioned findings by Plavina et al.\textsuperscript{15}, that higher anti-JCV antibody indices are associated with a higher risk to develop PML.

One of the most important points of discussion for the future will be if we have to treat all patients the same. As we mentioned before, we think that measuring natalizumab levels could be of more value than measuring anti-natalizumab antibody titers (chapter 2.1), not only because measuring natalizumab levels is easier to standardize, but also because these levels can be used to individualize the treatment. For this purpose, we
studied the effect of plasmapheresis (PLEX) on natalizumab levels in 4 patients who developed PML. The results of this study have been reported in chapter 3.3. The intention of PLEX is to wash out the natalizumab and to restore the immune function. We found that the number of sessions needed to reach natalizumab levels below 1 mg/l (the level where desaturation of the α4-intergrin receptor to <50% is reached\(^\text{10}\)), depends on the natalizumab levels before starting the PLEX. This implicates that patients who have a low serum natalizumab level will not always need five PLEX sessions (standard procedure), whereas on the other hand patients with high serum natalizumab levels may need more than five PLEX sessions. This will lead to a more individualized treatment in natalizumab-associated PML and may prevent unnecessary PLEX sessions / premature terminating PLEX sessions in others.

**Future perspectives**

As mentioned already in the introduction, MS is the most frequent chronic disabling neurological disease in young adults. The frequency and severity of relapses partly determine the prognosis of persisting disability and progression to SPMS, which illustrates the relevance of early and effective treatment with as little as possible adverse effects for these patients.

Natalizumab is a highly effective treatment for RRMS\(^\text{19,20}\). The risk of developing PML is the most important reason to refrain from or to discontinue natalizumab treatment. Plavina et al. developed a risk strategy which takes into account both the anti-JCV antibody index, prior use of immunosuppressive therapy, and the duration of natalizumab treatment\(^\text{15}\).

However, we still would like to predict more precisely which patients using natalizumab are at risk of developing PML. Therefore future strategies to develop better biomarkers or a better combination of multiple biomarkers are still of major importance. Some research has been done with CD62L (L-selectin)\(^\text{21,22}\). To determine CD62L, a laborious procedure with peripheral blood mononuclear cell (PBMC) samples is needed. Furthermore, CD62L has a lower sensitivity, but higher specificity than the anti-JCV antibody index in case of determining the risk of PML. Schwab et al. concluded that there might be an additional value of CD62L next to the anti-JCV antibody index in the future\(^\text{22}\). In contrast, Lieberman et al. recently published a retrospective case-control study that
showed that CD62L was variable in serial sampling and the level was strongly influenced by the viability of the lymphocytes. For these reasons, CD62L seems not a predictive clinical biomarker for the development of PML\textsuperscript{23}. Further research on this and other biomarkers will be needed.

As shown in chapter 2.2, natalizumab discontinuation in RRMS patients can lead to severe clinical and radiological return of disease activity\textsuperscript{24}. More recently, some studies have been performed to reveal the best strategy how to deal with natalizumab discontinuation and how to find the best moment to start an alternative therapy. Iaffaldano et al. studied fingolimod versus interferon-bèta/glatiramer acetate after natalizumab discontinuation and found a superior effect of fingolimod\textsuperscript{25}. Cohen et al. showed that from the patients who switched from natalizumab to fingolimod 20% experienced a relapse in the first 6 months\textsuperscript{26}. The TOFINGO study showed the first evidence that a shorter interval (8-12 weeks) between the switch from natalizumab to fingolimod resulted in less clinical and radiological disease activity than after 16 weeks\textsuperscript{27}. Alping et al. described in an observational study that rituximab had a superior effect and tolerability compared to fingolimod after cessation of natalizumab\textsuperscript{28}.

Starting earlier with a new immunosuppressive drug after cessation of natalizumab might in theory give a greater risk to develop PML, but so far, in the TOFINGO study, no evidence was found that a shorter washout period of fingolimod increases this risk of developing PML. The occurrence of PML has been described in patients discontinuing natalizumab, which was concluded to be still a result of the preceding natalizumab therapy instead of a result of the newly introduced disease-modifying therapy (so called cross-over PML), although it was impossible to rule out any additional effect of steroids or this newly started therapy\textsuperscript{29,30}. The case described by Killestein et al. shows that PML-IRIS may still develop despite the lymphopenia induced by the fingolimod therapy\textsuperscript{30}. The effect of this lymphopenia is reversible in a short time. Considering the fact that some therapies such as alemtuzumab and rituximab result in depletion of circulating B- and T-lymphocytes for over a long period (approximately 6 and 12 months, respectively), this has to be taken into account when considering these therapies after the discontinuation of natalizumab. With the risk of still developing PML within the first months after discontinuation of the natalizumab, this might be a reason to first start, by example, fingolimod before considering a monoclonal antibody as alemtuzumab.
Breakthrough disease after discontinuation of natalizumab is still a problem for which no perfect solution is present, so this will be a major topic for future research.

New therapies are still coming up. Daclizumab high-yield process (HYP), a humanized monoclonal antibody directed against CD25, has been shown to be clinically and radiologically more effective than interferon-bêta in a phase 3 randomized, double-blind trial over 96 weeks (ARR 0.22 and 0.39, respectively and 54% lower amount of lesions with daclizumab HYP)31. Inherent to the better efficacy, more adverse events were observed, so the clinical benefit needs to be shown against the adverse events. Another new monoclonal antibody is ocrelizumab. Ocrelizumab, also a humanized monoclonal antibody that binds to CD20, was compared to interferon-bêta in the OPERA I and II study. The first presented results on the ECTRIMS 2015 showed that the patients treated with ocrelizumab experienced less relapses than the patients treated with interferon-bêta (0.15 and 0.29, respectively). Also less new and especially less gadolinium-enhancing lesions were seen in the patients treated with ocrelizumab32. The most important adverse events were infusion-related events. So, the landscape of treatment for patients with relapsing remitting multiple sclerosis is still changing a lot nowadays. In the future we will learn if these new therapies will replace natalizumab therapy or if they are a good alternative after natalizumab discontinuation in case of high risk of PML.

Until now, natalizumab is one of the most effective therapies and we still have to find a way to treat patients with natalizumab in a way as safe as possible. One possibility, which might also lower the risk to develop PML, is lowering the dose or dose frequency of the natalizumab infusions. In the REFINE study, natalizumab 300 mg i.v. every 4 weeks was compared to 300 mg i.v. every 12 weeks, 300 mg s.c. every 4 and every 12 weeks and 150 mg i.v. and s.c. every 12 weeks. All study arms of every 12 weeks were stopped because of breakthrough disease was observed, which is not unexpected taking into account that (partial) desaturation of the natalizumab receptor will take about 100 days10 and clinical relapses after natalizumab cessation were seen after a mean interval of 17 weeks with a range of 8-22 weeks24. Ryerson et al. retrospectively studied extended interval dosing and found that dosing intervals up to 8 weeks and 5 days did not diminish the clinical and radiological effect of natalizumab. The four PML patients in this study were all in the standard dosing (four weeks) patient group, but because of not
enough patient power of the extended dosing group, no statistically significant PML risk reduction can be achieved.

Foley et al. showed that serum natalizumab concentration and receptor saturation of natalizumab is inversely correlated with lower body weight, so patients with a low bodyweight have a higher natalizumab concentration in their blood as well as higher receptor saturation levels and is suggested to be a risk factor for PML. As already mentioned before, we also found that individual patients have highly variable natalizumab levels. Despite this knowledge, which will be caused by a large variation in pharmacokinetics and patient characteristics, all patients are still being treated with 300 mg natalizumab infusion every four weeks. We believe that almost 80% of the patients have natalizumab levels, which are much higher than needed to have an optimal clinical and radiological efficacy. In line with that, using natalizumab levels for dosing will result in less frequent natalizumab infusions and less hospital visits, which therewith will contribute to improvement in quality of life of RRMS patients. Additionally, there will be enormous financial benefits as well, which is approximately on annual base more than 2.5 million euro’s in the Netherlands only.

Further research to prove that extending dose intervals based on measuring natalizumab levels will result in the same clinical and radiological efficacy and will lead to improvement of quality of life and significant cost-reduction is needed. Whether this personalized regimen will also decrease the risk of PML will be addressed as soon as extended dosing will be applied in large enough cohorts with long enough follow-up.

The landscape of therapeutic drugs available to treat MS continues to expand. As a result clinicians are faced with significant challenges in deciding the optimum treatment strategy for individual patients. A number of factors may influence this decision including JC virus antibody status, efficacy and potential adverse effects of alternate treatment, costs, regulatory and reimbursement issues. Even though quite some work has been done and in general, personalized medicine is practiced more widely now, a number of challenges still exist as mentioned above. Nevertheless, in my opinion a more personalized application of disease modifying therapies is the future, in particular for therapeutic monoclonal antibodies like natalizumab. I hope that this thesis will help to pave the road towards a more individualized therapy.