

**General Discussion,  
Summary and  
Future Perspectives**

# Chapter 4

## General

The studies presented in this thesis have focused on prediction of the long-term outcome in MS. We set three goals. The main goal was to detect MRI predictors that could be used in multiparametrical models. Secondly, we were also interested whether combining spinal cord and brain MRI predictors would result in stronger predictive models. Thirdly, we tested the added predictive value of MRI predictors over the use of clinical predictors only. The first part of the thesis describes two studies that were performed to enhance knowledge of factors that are related to frequently used MRI predictors (T1LL, chapter 2.1; brain atrophy, chapter 2.2). The second part describes four FU studies of cohorts with different disease durations: ranging from CIS (chapter 3.1) through early MS (chapter 3.2 and 3.3) into advanced MS (chapter 3.4).

In the following part we follow this line and start with discussing on the MRI predictors for measures of focal brain damage (evolution of enhancing lesions, chapter 4.1) and brain atrophy (chapter 4.2). This is followed by a discussion on the predictive value of lesion loads, brain atrophy and spinal cord pathology (chapter 4.3). Chapter 4.4 is focused on answering the question put in the second goal we set: is there added predictive value of spinal cord imaging compared to brain imaging only? Chapter 4.5 evaluates the added value of MRI. Future perspectives are discussed in chapter 4.6.

## Evolution of enhancing lesions

To study the factors that are related to the evolution of enhancing lesions on T1-weighted images we performed a serial study with monthly MRI scans (**Chapter 2.1**). Patients that were selected for the study had active inflammatory disease as evidenced by the presence of at least 5 newly enhancing lesions that could be followed for at least 6 months. Signal intensity of most lesions did not change during FU on T1-weighted images: lesions appeared isointense or hypointense to surrounding white matter on both baseline and 6 month FU MRI images. In the two other patterns signal intensity changed during FU (isointense lesions became hypointense or hypointense lesions became isointense). Most patients showed

evidence of more than one pattern of lesion evolution; some even expressed all four types. The most interesting observation from this serial study is the evidence that evolution of lesions is partially patient-bound. One of the implications of this observation is that some patients are prone to develop destructive hypointense lesions, whereas others tend to develop less destructive lesions. It is tempting to compare our MRI results with data that were produced in the field of histopathology. Histopathological studies describing demyelinating lesions have reported that, superimposed on T-cell and macrophage-mediated inflammation, different types of lesions exist.<sup>1</sup> Specific immunopathological features enable the classification of four distinct patterns. In pattern I, cytotoxicity caused by macrophages and T-cells results in demyelination, whereas in pattern II, there is evidence for antibody-mediated demyelination. Hypoxia-mediated apoptosis of oligodendrocytes is another pattern (III). Pattern IV is seen only in PPMS patients, shows extensive loss of oligodendrocytes, sharply defined lesion edge and no remyelination. Identification of these differing processes that all lead to demyelinating lesions might be important in tailoring therapy. An exciting example is described by Keenan and colleagues who could directly link successful therapeutic plasma exchange to a specific immunopathological lesion pattern: this therapy had success in patients with antibody/complement-associated demyelination (type II), but failed in patients with evidence of type I or III demyelination.<sup>2</sup> These results illustrate that immunopathology might provide clues why therapy fails in one patient and is successful in another. The heterogeneity of these patterns between patients might also explain part of the heterogeneity seen in the clinical course. Importantly, the described heterogeneity of immunopathological patterns in patients with acute fulminate or recently diagnosed MS could not be reproduced in a study that included patients with established MS.<sup>3</sup> This might indicate that, in contrast to early in the disease, later in the disease one common mechanism of demyelination exists. In the described studies patients underwent biopsy (or autopsy) of the brain in order to exclude alternative diagnoses, enabling the authors to determine the immunopathological patterns of demyelination. It is clear that less invasive methods are needed to determine these immunopathological patterns. MRI can be a candidate in this identification process, although so far no sufficiently specific MRI parameters have been identified. Ring enhancement and lesions that show a T2-hypointense rim seem to be strongly associated with types I/II lesions and

to be (virtually) absent in type III lesions.<sup>4</sup> Diffusion weighted imaging, magnetic resonance spectroscopy (e.g. detection of lipid peaks following demyelination and lipid degeneration in macrophages) and other advanced imaging techniques may add more tissue-specific information. Ultra-small superparamagnetic particles of iron oxide (USPIO) may also be of relevance since recent data suggest that USPIO can be used to image entrance of macrophages in the CNS.<sup>5</sup> Lesion type was completely patient-bound in immunopathology, but was only partially patient-bound in our MRI study. This suggests that one immunopathological type of lesion could display several types of evolution on MRI. However, we did of course not have an immunopathological confirmation of our data. Another explanation for the MRI lesion evolution patterns to be more heterogenous than immunopathological is that the evolution patterns on MRI do not only depend on demyelination but also on axonal loss, the amount of edema and remyelination.<sup>6</sup>

### **Determinants of brain atrophy**

In **chapter 2.1** we evaluated associations of MRI parameters with lesion evolution, describing parameters associated with development of destructive T1-hypointense lesions that reflect focal axonal loss. We were also interested in investigating a more global MRI parameter of damage to the brain (brain atrophy). **Chapter 2.2** evaluates determinants of rate of brain atrophy that is assumed to represent total axonal loss and neurodegeneration. Rate of brain atrophy was expressed as annualized percentage of brain volume change (PBVC). Included patients were intercepted directly after the clinical diagnosis of MS according to the Poser criteria.<sup>7</sup> The specific aim was to identify MRI parameters derived from the baseline MRI scan that were associated with PBVC in the first two years after the clinical diagnosis of MS. Data from previous studies indicated the expected atrophy rate to be between 0.6 to 1.4%.<sup>8-10</sup> Our rate of 0.9% is in line with these findings. The main finding from multivariate modeling is that baseline T2LL and normalized brain volume (NBV) explain subsequent rate of atrophy in the first two years after the clinical diagnosis. Accumulated T2LL at 5 years was also found to partially predict atrophy at 14 years FU in a long-term FU of patients with CIS.<sup>11</sup> Since most enhancing lesions in RRMS remain visible on T2-weighted images, T2LL at baseline

MRI represents the total of inflammation. In most studies, T2LL explains only about 10% of concurrent atrophy.<sup>12;13</sup> In univariate analysis of the other lesion loads we found that presence of enhancing lesions and T1LL was associated with PBVC. There is only limited support in the literature for the monitoring of single-dose gadolinium-enhancing lesion volumes to predict subsequent atrophy (rate).<sup>14-16</sup> Indirect evidence also indicates that gadolinium-enhancement is not a strong predictor for subsequent atrophy: immunomodulating therapy has been shown to (almost) completely prevent gadolinium-detectable inflammation but the effect of therapy on the prevention of atrophy is considerably less evident. These results may however be confounded by the effect of anti-inflammatory treatment on reduction of edema. A subset of enhancing lesions that might be more relevant and indicative for tissue destruction are the (incomplete) ring-enhancing lesions. It is thought that the pallor center represents an area of more severe tissue destruction. Several papers have shown the predictive value of these lesions for subsequent atrophy.<sup>15;16</sup> In our study presented in chapter 2.2, we did not specify type of enhancement but our report on the lesion evolution (chapter 2.1) describes the propensity of ring-enhancing lesions to form (destructive) chronic T1 hypointense lesions. This was reported previously by van Waesberghe *et al.*<sup>17</sup> and Ciccarelli *et al.*<sup>18</sup> One would expect that measures of focal brain destruction as represented by chronic T1-hypointense lesions are strongly associated with atrophy as a measure of total destructive changes, and that an overwhelming amount of evidence is to be found in the literature. Although some cross-sectional studies<sup>19;20</sup> report atrophy to be associated with T1LL, this is not confirmed longitudinally by most studies and in our study T1LL did not make it in the final model either. Taken together, this suggests that focal lesion loads have a limited role as a predictor for subsequent atrophy. Cross-sectional lesion loads can be related to atrophy due to direct effects (axonal loss and demyelination cause loss of brain volume) and indirect effects (distant loss of brain volume due to Wallerian degeneration). Kalkers *et al.*<sup>21</sup> confirmed the following hypothesis: overall brain volume is mainly determined by damage of whole brain tissue and to a lesser degree by lesion volume. In their final model that explained 49% of the variance in brain parenchymal fraction, relative peak height of the magnetization transfer ratio histogram was a stronger contributor compared to T1LL. Further research along this line, using newer techniques (e.g. MR spectroscopy) confirmed the importance

of changes in the normal-appearing white matter, both cross-sectionally as longitudinally.<sup>22</sup> These measures could not be included in our study since we focus on broadly applicable techniques that not included MR spectroscopy, T1-mapping and others. Besides T2LL, our final model explaining atrophy rate also included NBV at baseline. Since NBV at baseline represents the total accumulated tissue, and in a way reflects (in a cohort with homogeneous disease duration) atrophy rate up until that time, it seems logically that NBV should predict subsequent atrophy rate. Other longitudinal studies also found that baseline atrophy was predictive for atrophy at the end of FU.<sup>23</sup> It would have been interesting to study atrophy of white matter and gray matter separately, however, due to technical problems with the segmentation we were not able to do this. Several studies have reported (rate of) atrophy to be different for the white and gray matter. Tiberio *et al.* found gray matter atrophy to be progressive in early RRMS, whereas white matter atrophy remained unchanged during FU.<sup>24</sup> In part, this may be explained by the differences in inflammatory activity: in cortical MS lesions, the inflammatory component is less pronounced and in the more inflamed white matter lesions, surrounding edema may be a confounder resulting in volume increases.

## **MRI predictors**

Chapter 3 describes the long-term FU studies we performed to evaluate the predictive value of MRI parameters for disability. To do so, we followed several cohorts, enabling detecting of predictors in the various stages of the disease. The following part will focus on the MRI predictors that were detected in these studies. First we focus on the predictive value of the conventional lesion loads: thereafter we address the value of brain atrophy and spinal cord predictors.

## **T2LL**

Previous studies already identified MRI predictors derived from T2-weighted images in patients that were imaged at presentation with a CIS. Chapter 3.1 describes the long-term FU of patients that were intercepted at the time of initial findings

suggestive of MS. Patients were followed-up for a median of 8.7 years and MRI predictors for clinically relevant disability (EDSS 3 or more) were derived from the baseline T1- and T2-weighted images. Number of T2 lesions and T2LL were already shown to predict disability at FU previously.<sup>25</sup> These previous reports did not take the location of the lesions into account. We aimed at increasing the predictive value of the T2 lesions by using both number and location of the lesions. In our study, infratentorial lesions were found to be the strongest predictor (chapter 3.1). The strong predictive value of infratentorial lesions could be explained firstly by the eloquence of this region. Secondly, one may hypothesize that pathology in the brainstem or cerebellum is an indicator for spinal cord pathology which in turn is strongly correlated to disability.<sup>26</sup> A ten year FU study showed evidence for faster accumulation of lesion load in patients with infratentorial lesions compared to those without. Furthermore EDSS at FU was correlated with infratentorial lesion load.<sup>27</sup> With increasing magnetic field strength lesion detection in the brain is enhanced,<sup>28</sup> therefore caution is needed to translate our findings (MRI scans were performed at 0.6T) to nowadays more common field-strengths of 1.5T and 3.0T. Type of clinical presentation was associated with outcome but was not further evaluated in the multiparametrical regression, this may have confounded our results. Patients with brainstem/cerebellar presentation progressed to clinical relevant disability more often than patients with optic neuritis did. Also the brainstem/cerebellar patients (50%) more often had infratentorial lesions than the optic neuritis patients (39%) did. With longer FU it is expected that patients will develop more severe disability. Future analysis is needed to determine the predictive value of the baseline scan for time to EDSS 4 or 6. Our other long-term FU studies also evaluated the predictive value of lesion loads for disability, providing insight in its relevance in the first years after the clinical diagnosis of MS (chapter 3.2 and 3.3) but also later in the disease (chapter 3.4). As may be expected, T2LLs are low early in the disease (chapter 3.2 and 3.3). None of the lesion loads was statistically significantly correlated with EDSS at baseline but after two years of FU, EDSS correlated moderately with baseline and FU lesion loads. Furthermore, lesion loads at FU are significantly higher in the patients with progression of disability compared to patients without evidence of progressing disability (chapter 3.2). These findings are in line with the most reports from literature that describe poor to moderate predictive value of lesion loads for future (short- and medium

term) disability but also indicate rate of accumulation might be more important than actual lesion load.<sup>29-32</sup> Several explanations have been formulated for this so called clinico-radiological paradox. One of the explanations can be deduced from the threshold hypothesis. In this hypothesis, clinical evidence of the disease (disability) is observed only if the MS related pathology in the brain and spinal cord reach a critical level. In this theory there is a time lag between the development of MS related pathology and MS related disability. This may indicate that long-term FU studies are needed and that previous studies (most of them with short-term FU) have underestimated the predictive value of lesion loads. Rudick *et al.* reported that, in a group of MS patients with median disease duration of six years, T2 lesions occurring between baseline and 2 year FU are correlated more strongly with disability at 13 years FU than they were after 2 years of FU.<sup>33</sup> The long-term predictive value of T2LL can possibly be even stronger if measured earlier in the disease: in patients that were followed 20 years from presentation with a CIS, (accumulation of) T2LL in the first years was a stronger predictor than T2LL later in the disease.<sup>34</sup> In our 12 year FU study of patients with well established MS (median disease duration 5 years) we did not find evidence for T2LL as relevant predictor whereas T1LL was found to be predictive for clinical status.

## **T1LL**

Hypointense T1 lesions are correlated with axonal loss in comparative MRI-histopathology studies.<sup>35</sup> Consequently, chronic hypointense lesions are regarded as a measure of focal axonal loss and thought to represent destructive processes more specifically than T2LL does. In established MS, T1LL predicts disability at FU but no long-term studies on MRI predictors derived from T1-weighted images have been described previously in CIS patients. Therefore, we evaluated the predictive value of gadolinium-enhancement and presence of T1- hypointense lesions in patients with an initial finding suggestive of MS (chapter 3.1) and found that we could not predict outcome at FU. In patients with MS T1-hypointense lesions are frequently seen but in CIS they are still infrequent, this may at least partly explain the lack of predictive power in this phase of the disease. T1LL is also low in newly diagnosed MS patients (chapter 3.2; median 0.2 ml). Although T1LL did not make it into the



final multivariate models for disability at 2- (chapter 3.2) and 5 years (chapter 3.3) after the clinical diagnosis of MS, statistically significant correlations were found with disability. With longer disease duration, the predictive value of T1LL rises (chapter 3.4; median 3.4 ml) and seems to become more important as predictor for disability.<sup>36</sup> The fraction of T2 lesions that are represented as hypointense lesions is unevenly distributed over the various phases of the disease. We showed that newly enhancing lesions in SPMS patients have a propensity to develop into chronic hypointense T1 lesions (chapter 2.1), possibly reflecting a diminished reparative capacity in these patients. Due to the design of this study it was not possible to answer the question that follows directly from these results: does evolution of lesions change in patients that convert from RRMS to SPMS (resulting in more hypointense T1 lesions) or do some patients have inadequate repair mechanisms and are therefore prone to develop destructive lesions resulting in a higher risk to progress to SPMS in a short time. Evidence for the latter comes from a study conducted by Bielekova *et al.*, comparing baseline and FU MRI scans.<sup>37</sup> This study indicated that hypointense T1 lesions are an important marker in segregating phenotypic subgroups that are truly distinct and do not merely represent different stages of the disease. In our 12 year FU study (chapter 3.4), accumulating T1LL was the best predictor for disability as measured by the MSSS. Quantifying T1LL is not without technical problems. For instance, the degree of hypointensity is sequence-dependent, precluding simple study to study comparison and uncomplicated use in multicenter clinical trials.

### **Brain atrophy**

Brain atrophy is regarded as the MRI parameter that most closely reflects neurodegeneration. Robust quantification techniques are available to measure the volume of the brain.<sup>38</sup> Brain atrophy is widely used as MRI predictor and commonly regarded the best MRI predictor for disability, performing better than the lesion loads measures.<sup>20,39</sup> Unfortunately we could not evaluate the predictive value of brain atrophy in our FU study with patients that presented with initial findings suggestive of MS (chapter 3.1). Several reports have described that brain atrophy is present even this early in the disease and discriminates between fast and slow

progression to clinically definite MS.<sup>40</sup> Combining these data with the promising results as a predictor for future disability in patients further in the disease course, it is assumed that brain atrophy can be a good predictor for future disability in patients with a CIS. Gauthier *et al.* evaluated predictors for short-term changes in disability in patients with CIS or low-disability RRMS.<sup>41</sup> Brain parenchymal fraction (BPF) was one of the selected predictors. Odds ratio for disease progression of patients in the lowest BPF quartile was 2.0 indicating a doubled risk at progression. Progression of brain atrophy correlated significantly with change in EDSS during 2-year FU in a cohort of 53 early RRMS patients.<sup>42</sup> In our cohort of patients with a recent diagnosis of MS, rate of brain atrophy was the single best MRI predictor for disease progression in the first 2 years after the diagnosis (chapter 3.2), and was also included in the multiparametrical model predicting disability at 5 years (chapter 3.3). Combined with data already known from the literature, these studies on a homogeneous cohort that was prospectively followed-up provide good evidence of the importance of documenting brain atrophy early in the disease, preferably in the first years following the diagnosis. Fisher *et al.* showed that brain atrophy could also predict disability at longer FU: they studied RRMS patients with low disability (EDSS between 1.0 and 3.5) and found that patients with the highest brain atrophy rates in the first two years of the study were at the highest risk for disease progression at 8 year FU.<sup>23</sup> Our 12-year FU study (chapter 3.4) consisted of patients that had either RRMS or SPMS and disease duration varied widely. In this heterogeneous cohort, ventricular fraction was associated with clinical status at 12-year FU, confirming the value of atrophy measures as predictor for disability at even longer FU. However, this atrophy measure did not survive in the final multiparametrical model. Besides relatively low group size, this may be caused by the used method to determine atrophy: due to the retrospective nature of the study, we had to rely on less accurate measures instead of using more modern and advanced techniques. A report by Sormani *et al.* already found statistical power of one technique can be twice the power of a second technique.<sup>43</sup> Besides measuring the overall rate of brain atrophy it is worthwhile to look at the white-matter, gray-matter and ventricles separately. It is becoming clear that pattern of atrophy differs between these different compartments but predictive value for future disability has not been studied.<sup>12,44;45</sup>

## Spinal cord

The spinal cord is frequently involved in MS but commonly neglected in imaging studies. We studied the value of spinal cord derived predictors for future disability in our cohort with newly diagnosed MS (chapter 3.2 and 3.3) but could not perform this study in other cohorts. In patients that present with a CIS and have an abnormal brain scan silent spinal cord lesions are common though less frequent than in the more advanced stages of disease. Focal T2 spinal cord lesions correlate poorly with disability in cross-sectional studies.<sup>46</sup> Longitudinal studies on the predictive value for disability are sparse and do not provide data to suggest that focal spinal cord lesions should be regarded an important predictor.<sup>47</sup> However reported studies have limited group size and short duration of FU, thereby possibly underestimating the role of focal spinal cord lesions. Our study in chapter 3.3 with more studied patients with longer duration of FU showed that focal spinal cord lesion loads may still play a role as predictor for disability in early RRM. Furthermore, diffuse spinal cord abnormalities were predictive for progression to an EDSS score of 4 or more in our group, adding another predictor derived from conventional spinal cord imaging. Unfortunately we did not perform a 3d volume scan of the spinal cord, thereby precluding the use of another important measure of spinal cord damage: atrophy. Spinal cord atrophy has been described in patients with a CIS and seems to be predictive for the conversion to CDMS.<sup>48</sup> No data exist on the predictive value for future disability. Longitudinal studies reported increased rate spinal cord atrophy in established MS<sup>49</sup> but also in early RRMS<sup>50</sup> with disease duration comparable to our cohort of recently diagnosed patients (chapter 3.2 and 3.3). Although several cross-sectional studies reported correlations between spinal cord area and clinical disability<sup>51;52</sup>, the predictive value of (rate of) spinal cord atrophy is less clear: the limited number of longitudinal studies that have been published report contradictory results. All of these studies have, as is the case for studies on focal pathology, relatively small group sizes and short duration of FU. Up until now, predictive value of spinal cord atrophy has only been shown for patients with advanced RRMS or progressive disease.<sup>47,53</sup> To summarize, incomplete data exists on the value of conventional spinal cord derived predictors for disability, our results however, suggest that besides atrophy also focal and diffuse lesion loads should deserve further attention in future research. More advanced imaging protocols

include diffusion tensor imaging, T1 or T2 relaxation time measurements and other techniques. The first longitudinal diffusion tensor imaging study has already reported on the predictive value of baseline fractional anisotropy for disability at FU after 2 years, indicating the value of these advanced imaging techniques.

### **What disability?**

Clinical manifestations of MS are very heterogeneous. Due to this heterogeneity it is impossible to simply measure disability. Clinical rating systems for disability like the EDSS and the MSFC try to measure the impact of the disease in a standardized fashion. There is, however, no gold-standard for overall disability and different clinical rating scales seem to measure different aspects of the impact of the disease. For example, the EDSS is insensitive for cognitive dysfunction and arm function. Apart from these problems with different rating scales measuring different aspects of disability there are also problems with the psychometric properties of the used rating scales. There is a lack of reliable, validated and responsive clinical rating scales. The EDSS and the MSFC are, despite all discussion, still the most commonly used clinical rating scales in MS. In general, correlations between MRI measures and outcomes on clinical rating scales are weak. This may be partially due to properties of the used clinical rating scales. Another observation is that MRI predictors may vary if different clinical rating scales are used (chapter 3.3).

### **The best MRI predictor(s) for disability: brain or spinal cord?**

Longitudinal studies with long duration of FU are relatively sparse in MS research. Because only well-documented cohorts can be used, most published studies report on small numbers of patients resulting in statistically underpowered studies. Although we did not perform power calculations, these problems probably also occur in our studies. Still, the work presented in this thesis has pointed out several brain and spinal cord MRI measurements that may be used to predict future disability. What is the best MRI predictor for future disability? There is no simple answer to this question. We showed that including spinal cord in the imaging

protocol increases the predictive value of imaging, so, in addition to imaging the brain the spinal cord should also be imaged. At this time there is no evidence of one single best MRI predictor and therefore, risk assessment of patients is best done combining several MRI predictors instead of using just one single predictor. Conceptually, in MS two pathological processes are discerned. The first is focal inflammation, represented by enhancing- and T2-lesions. The second process is neurodegeneration, best represented by hypointense T1-lesions (focal) and atrophy (diffuse). Since representants of inflammation and neurodegeneration are only partly correlated, it seems logical to use representants of both and combine these with spinal cord predictors. Importantly our observations show predictive value of changes in predictors prevails over the use of predictors obtained from only a baseline scan. Therefore, these predictors should be derived from longitudinal observations instead from only one baseline scan.

### **Added value of MRI predictors?**

The predictive value of MRI is sometimes debated due to critics that claim MRI provides no added answers compared to the use of clinical predictors only. It is true that this added predictive value is not systematically investigated. However, we showed in chapter 3.2 that there is added value of MRI predictors above the use of clinical predictors alone. Therefore, when determining the risk for progression of disability, the clinician may use both clinical- and MRI predictors.

Although not within the scope of this thesis and not further discussed here, it is evident that, other paraclinical test can add further predictive value above clinical- and MRI predictors. For example CSF concentrations of neurofilament phosphoforms, peripheral blood chemokine receptors and genetic profiles might provide additional predictive power.

### **Future perspectives**

Our studies have identified several MRI predictors for disability at a group level but in clinical practice at the level of the individual patients there is only a modest

role for MRI predictors. Further research is needed to translate the predictors that were identified in this thesis into more easily interpretable tools that provides the clinician insight in the expected disease course. For example, these requirements could be met by using tables that show evolution of lesion loads or brain/spinal cord volume over time for a given patient compared to the evolution in large cohorts after analogy of the MSSS percentile scores. Ideally this would result in a clinical applicable MRI risk-profile for progression of disability that could be determined for each individual patient. In our studies we used relatively straightforward imaging protocols. Therefore, we could not determine the predictive value of more advanced MRI predictors that can depict abnormalities in the normal-appearing white- and gray matter and will be focus of predictive studies in the future. Also, we did not study cortical lesions separately, and our used sequences are known to underestimate cortical lesion loads. These are all partially consequences of the trade-off between the best possible imaging protocol and a widely applicable imaging protocol, and are also partially caused by the retrospective nature of some of our studies. On the other hand, when looking at the clinical applicability, even our conventional MRI predictors are not used systematically on a large scale in clinical practice today. Maybe this will change in the future with the advanced availability of user-friendly post-processing software.

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