Treatment response in relation to inflammatory and axonal surrogate marker in multiple sclerosis

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Abstract

Background: This study aimed to investigate if treatment response could retrospectively be related to inflammatory or axonal pathology as measured by plasma surrogate markers.

Methods: In this 1–year observational study 30 MS patients with relapsing–remitting disease were treated with intra–muscular IFNβ–1a or subcutaneous IFNβ–1b. Responders and non–responders were defined according to clinical and MRI criteria. The control group consisted of 14 healthy subjects. Plasma levels of surrogate markers for inflammation (nitric oxide metabolites (NO_x)), astrocytic activation (S100B) and axonal damage (NfH^{SMI35}) were measured using standard assays.

Results: There were 11 non–responders and 19 responders to IFNβ treatment. Median S100B levels were elevated in a higher proportion of treatment responders (63%, 42.9 pg/mL) compared to non–responders (18%, 11.7 pg/mL, p<0.05, Fisher's exact test) and controls (0%, 2 pg/mL, p< 0.001). Levels of NO_x were found to be more frequently elevated in non–responders (72%, 39 μM) compared to healthy controls (0%, 37 μM, p<0.05). Levels of NfH^{SMI35} were more frequently elevated in responders (58%, 300 pg/mL, p<0.001) and non–responders (72%, 500 pg/mL, p<0.001) compared to controls (0%, 4.5 pg/mL).

Conclusion: Patients with relapsing–remitting MS who had surrogate marker supported evidence for astrocytic activation responded more frequently to treatment with IFNβ.

Key words: S100B, neurofilament, NfH^{SMI35}, NO_x, surrogate marker, multiple sclerosis, treatment response

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1 Introduction

Characterising responders and non-responders to treatment in multiple sclerosis (MS) is of relevance for understanding disease progression and selecting effective drug treatment strategies. MS is a heterogeneous disease [1] and it was suggested that the heterogeneity of treatment response could be related to the underlying pathology [2]. Particularly inflammation has been associated with relapses, and relapse frequency early on is related to disease progression. The proven benefit common to all currently recommended treatments is a reduction in the rate of clinical relapse, which has been associated to a transient slowing of disease progression [3]. During the early phase of MS active disease has been related to elevated levels of S100B [4] and the nitric oxide metabolites nitrate and nitrite (NO_x) [5].

The hypothesis underlying this study was that patients who responded to IFNβ treatment would be different from non–responders with regard to their profile of plasma inflammatory (NO_x), astrocytic activation (S100B) and axonal (NfH^{SMI35}) markers.

2 Materials and methods

2.1 Patients

Thirty patients with relapsing remitting multiple sclerosis (RRMS) [6] were included in this cross–sectional study. All patients were treated with IFNβ and recruited from the MS specialist clinic at the Toulouse Purpan Hptial between March 2000 and November 2001. The control group consisted of 14 healthy volunteers. All plasma samples were obtained by antecubital venopuncture at the last clinical assessment. Samples were coded and stored at -70C until analysis. Approval for the study was obtained from the local Ethics Committees. Written informed consent was obtained from each subject.

2.2 Clinical assessment

All patients were assessed by two experienced neurologists (DB, MC). The expanded Disability Status Scale score (EDSS) and the yearly relapse frequency prior to sampling were recorded.

It was decided to declare treatment failure based on clinical and MRI criteria (when available). Patients who suffered from at least two clinical relapses as-

sociated with at least one gadolinium-enhancing lesion on T1 MRI performed at least one month after steroid treatment ("active MRI"), or patients who progressed by over 1 point on the EDSS associated with an active MRI.

2.3 Assays

Plasma levels of S100B, NO_x and NfH^{SMI35} were measured using standard assays) as described previously [7,8,9,10]. The cutoff value for abnormal levels of these biomarkers were defined as the top value of the control group.

2.4 Statistical Analysis

All statistical analyses and graphs were done using SAS software (V8.2, SAS Institute, Inc., Cary, North Carolina, USA). Because of the non–Gaussian distribution all data is presented as the median and interquartile range (IQR). The box (median and 25%-75% cumulative frequency) and whisker (1%-100% cumulative frequency) are shown in the graphs. Because of the small sample size Fisher's exact test was used for comparison of proportions of patients. The linear relationship between continuous variables was evaluated using the non–parametric Spearman correlation coefficient (α=0.05).

3   Results

The baseline characteristic of the MS patients are summarised in Table 1. According to our definition there were 11 responder and 19 non–responder. Patients with MS were significantly younger than the control patients and there were more females. There were no significant differences between responder and non–responder with regard to the EDSS. The choice of the generic drug was not related to treatment response.

The plasma levels for S100B, NO_x and NfH^{SMI35} (median, range) are summarised in Table 1. The cutoff levels were determined as the top value from the control group (S100B = 33 pg/mL, NO_x = 44 μM, NfH^{SMI35} = 250 pg/mL).

In all non–responders MRI was performed. Although a subgroup of responder (n=16) received an MRI brain scan. However the timing of MRI brain scan to relapse and plasma sampling was not rigorous.

A significantly higher proportion of responders (63%) but not of non-responders (18%) had S100B levels above cut–off compared to controls (p=0.021). The

individual values are shown in Figure 1. There were 2 outliers with plasma S100B levels over 300 pg/mL among the responders (Figure 1). Removal of these 2 outliers did not alter statistical significance (p=0.034, Fisher's exact test).

A significant proportion of non–responders (45%) had NO_x levels above cutoff. Also a significant proportion of responders (58%) and non–responders (72%) had NfH^{SMI35} levels above cutoff. There was no significant difference comparing proportions of responders and non–responders for either NO_x or NfH^{SMI35} .

There was no correlation between age, EDSS, relapse frequency and either S100B, NO_x or NfH^{SMI35} for any of the subgroups.

4   Discussion

This study found higher plasma levels S100B, NO_x and NfH^{SMI35} in patients with MS than in healthy controls. In treatment responders the median S100B levels were over 3–fold higher than in non–responders.

We and others previously related elevated CSF S100B to acute disease activity in MS [4]. It is therefore difficult to understand if not paradoxic that treatment responders should have higher plasma S100B levels. A major criticism of this study is the retrospective, the cross–sectional design and the lack of MRI data in some of the patients. However in a longitudinal study on serum S100B in primary progressive MS patients from a clinically negative phase II trial we found similar levels in the non–responders (E Lim, unpublished data).

The current understanding is that IFN–β reduces disease activity by exerting immunomodulatory properties. However *in vivo* and *in vitro* data shows up-regulation of pro–inflammatory factors during IFN–β treatment [11]. On the background that inflammation has been related to protective and destructive mechanisms [12] it can be speculated that these properties might be related in degree to a spectrum of severity. Severe inflammation, blood–brain barrier (BBB) disruption and neutrophil invasion were all related to tissue destruction [13]. Indeed there was a trend for higher NfH^{SMI35} levels in non–responder. Previously we have shown that particular the combination of inflammation with axonal damage indicated poorer prognosis [14].

However low grade inflammation orchestrated by the macrophage and micro–glial network could be neuroprotective. *In vitro* data supports this notion in as nanomolar levels of S100B are neurotrophic [15]. This suggests that moderately elevated S100B levels might be beneficial in the context of IFN–β effects on BBB integrity and inhibition of more severe steps of inflammatory

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activity such as T–cell invasion and subsequent neuro–axonal degeneration.

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Table 1: *Demographic characteristics of patients classified according to clinical criteria of treatment failure. The relapse frequency is given in number of relapses per year. The age and disease duration are given in years. Levels of plasma S100B, NO_x, NfH^{SMI35} median (range) in patients based on clinical criteria of treatment failure. Statistical comparisons are indicated in the following order: control versus non–responder; controls versus responder; non–responder versus responder. NS = not significant.*

|	Control	MS patients		P value
		Non–responder	Responder	
Number	14	11 (37%)	19 (63%)	
Age	36 (24–59)	26 (23–42)	33 (19–40)	<0.05; <0.05; NS
Sex (F:M)	8:6	9:2	16:3	NS
Relapse frequency	N/A	2 (2–8)	1 (0–1)	<0.001
Disease duration	N/A	8 (2–14)	7 (1–18)	NS
EDSS	N/A	3.5 (1.0–6.5)	3.5 (1.5–6.5)	NS
IFN 1a n (%)	N/A	1 (9%)	3 (16%)	
IFN 1b n (%)	N/A	10 (91%)	16 (84%)	
S100B (pg/mL)	2 (0–11)	11.7 (0.1–53.2)	42.9 (16.5–483.0)	NS; <0.001; <0.05
NO_x (μM)	37 (35–39)	42.4 (25.2–74.5)	39 (17.3–81.0)	0.01; NS; NS
NfH^{SMI35} (pg/mL)	4.5 (0–41)	500 (0.1-2,200)	300 (0.1-2,400)	<0.001; <0.001; NS
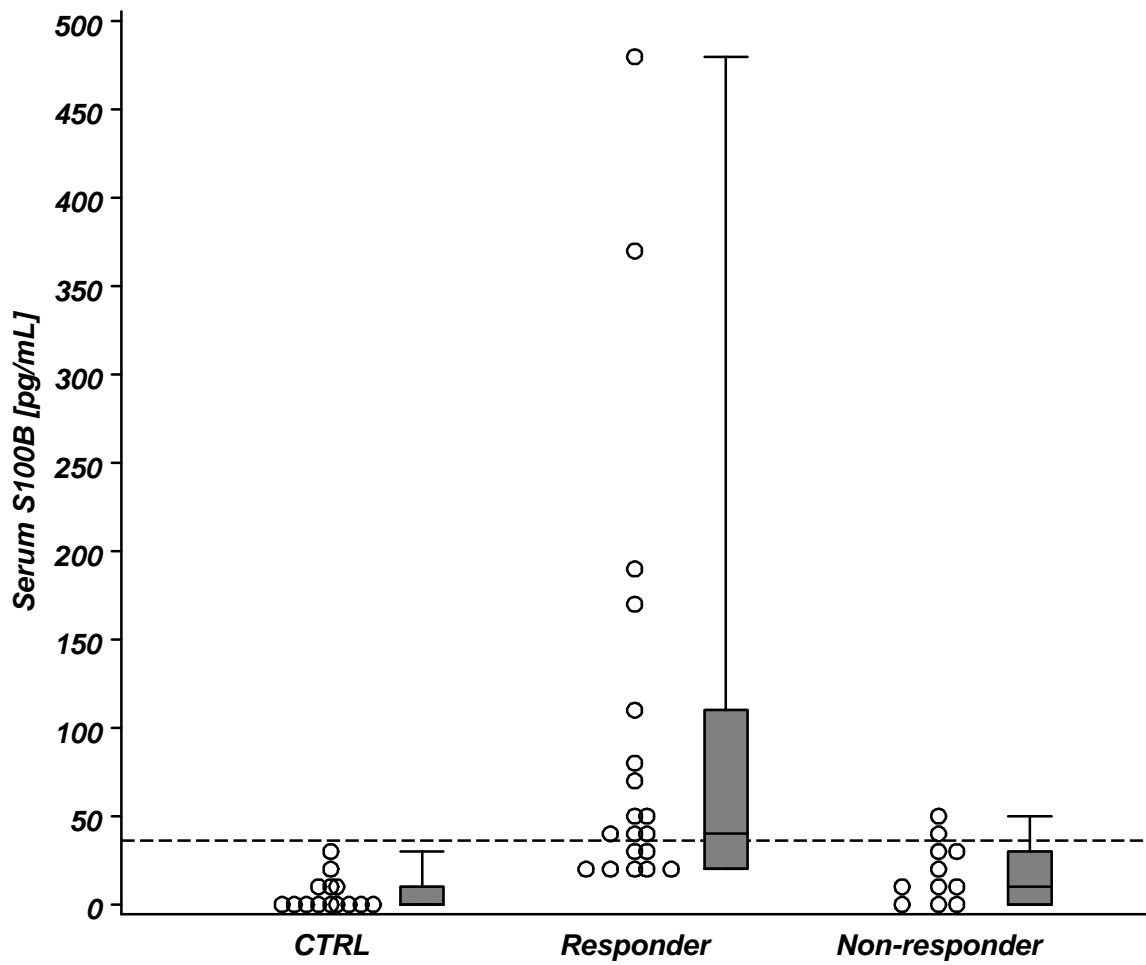
Figure 1. *Plasma S100B (pg/mL) levels in normal controls, treatment non–responders and treatment responders. The dotted horizontal line indicates the cutoff of 33 pg/mL (100% cumulative frequency of the control group).*