ORIGINAL RESEARCH ARTICLE

Drug Safety 2004; 27 (10): 745-756
0114-5916/04/0010-0745/$31.00/0

Haematological Effects of Interferon-β-1a (Rebif®) Therapy in Multiple Sclerosis

Peter Rieckmann,[1] *Paul O'Connor*,[2] *Gordon S. Francis*,[3] *Graham Wetherill*[4,5] and *Enrica Alteri*[6]

1 Department of Neurology, Würzburg, Germany
2 St Michaels Hospital, University of Toronto, Toronto, Ontario, Canada
3 Neurology Clinical Development, Serono Inc., Rockland, Massachussetts, USA
4 Biostatistics, Serono International, Geneva, Switzerland
5 Biometric Solutions Ltd, St Ives, Cambridgeshire, UK
6 Worldwide Clinical Safety and Pharmacovigilance, Serono International, Geneva, Switzerland

Abstract

Introduction: Interferon-β-1a (Rebif®) is an established treatment for relapsing-remitting multiple sclerosis (MS) and haematological changes are commonly reported in clinical trials of this agent. The combined clinical trial and postmarketing safety database for subcutaneous interferon-β-1a (Rebif®) allows a comprehensive, retrospective assessment of both common and infrequent haematological effects associated with interferon-β therapy.

Methods: Haematological laboratory abnormalities were analysed from six randomised, controlled clinical trials of subcutaneous interferon-β-1a in MS, five of which were placebo-controlled. Treatment data were collected from 2482 patients for up to 6 months, 1178 patients for up to 2 years and 786 patients for up to 6 years. Total interferon-β-1a doses ranged from 22μg once weekly to 44μg three times weekly. Postmarketing surveillance data were also analysed.

Results: Treatment with interferon-β-1a led to asymptomatic dose-related reductions in all cell lineages under investigation, predominantly white blood cells. The greatest differences between interferon-β-1a therapy and placebo were seen for total leucocyte and neutrophil counts. At least two-thirds of patients affected by cytopenia experienced the onset of cytopenia within the first 6 months of therapy. The majority of events were mild and generally resolved within 3–4 months, while continuing therapy. Dose reductions were uncommon and only a small proportion (6 of 727; 0.8%) of patients stopped treatment over 2 years because of haematological abnormalities when receiving the highest dose of interferon-β-1a, 44μg three times weekly. Postmarketing safety reports were similarly related to asymptomatic cytopenias, although one case of potentially related autoimmune haemolytic anaemia was reported.

Conclusion: Although haematological abnormalities are common and dose-related in patients with MS receiving interferon-β-1a, the events are mainly mild and transient, with little impact on adherence to therapy. Haematological events are rarely of clinical significance and do not adversely affect the benefit-to-risk ratio that favours high-dose interferon-β-1a therapy.

Introduction

Interferon-β is one of a family of naturally occurring proteins that have immunomodulatory, antiviral and antiproliferative effects. It is widely used as a long-term immunomodulatory therapy for multiple sclerosis (MS).[1,2] Interferon-β-1a is synthesised in mammalian cells, so it is glycosylated and has an amino acid structure that is identical to the natural molecule. Rebif® [1] (Serono, Geneva, Switzerland) is an interferon-β-1a that is administered subcutaneously. There is evidence that the effects of interferon-β in MS are dose-dependent;[1,3,4] therefore, Rebif® is administered at a recommended dose of 44μg three times weekly, although a 22μg three times weekly dose is also available.

Interferon-β has been extensively tested in both relapsing-remitting MS (RRMS) and secondary progressive MS (SPMS). In large clinical trials, the main adverse events are flu-like symptoms, injection-site reactions and laboratory abnormalities,[1-9] including asymptomatic white blood cell (WBC) abnormalities and raised liver aminotransferase levels.[10] The benefits of enhanced efficacy provided by high-dose, high-frequency therapy with interferon-β must be considered alongside any dose-related changes in the safety and tolerability of this drug.

In order to assess the incidence of changes in WBC counts in patients with MS receiving interferon-β-1a and the impact of those changes on patient safety, information was collated from several clinical trials of subcutaneously administered interferon-β-1a and from postmarketing surveillance; these were integrated into a single safety database. The studies utilised doses ranging from 22μg once weekly to 44μg three times weekly in patients with a broad range of ages and degrees of disability. Patients with suspected MS, RRMS and SPMS were included.

Methods

Clinical Studies

Haematological safety data were analysed retrospectively from six controlled, double-blind, randomised, clinical trials of interferon-β-1a in MS, five of which were placebo-controlled and one employed an active control.[1,4,8,9,11,12] For the purposes of this study an adverse haematological event was defined as either a symptomatic event (e.g. bleeding, infection, etc.) felt by the physician to be caused by a haematological abnormality or a laboratory result that was a clinically significant change from baseline. A laboratory abnormality was any event that differed from the laboratory normal values. Given that laboratory abnormalities were almost universally asymptomatic and that similar severity grades of abnormalities can be considered as adverse events by some clinicians and not by others, it was deemed important to focus on all the laboratory abnormalities to eliminate subjectivity in reporting. Adverse event rates and laboratory abnormalities were collected for up to 6 years within the clinical trials, although comparison with placebo continued only to a maximum of 3 years.

Data on treatment discontinuations because of adverse events were obtained from the six controlled studies, including their extension phases up to 6 years, as well as data from another 17 uncontrolled MS studies.[13] Data on serious adverse events (SAEs) were obtained from all 23 studies and from postmarketing surveillance.

The database of the controlled studies included 2482 patients (termed the 'controlled population'), comprising of 1500 patients with RRMS and 982 patients with SPMS. Placebo was administered to 441 patients with RRMS and 383 patients with SPMS. Interferon-β-1a was administered to 1059 patients with RRMS and 599 patients with SPMS. The 6-month data included all patients from the controlled population. The 2-year and 6-year data were from subsets of the controlled population and included 1178 patients from two studies (560 with RRMS[1] and 618 with SPMS[11]), of whom 392 were receiving placebo (years 1–2 or 1–3 only) and 786 were receiving interferon-β-1a. The total pooled safety data, including those from the 17 uncontrolled studies, provided information on a total of 3995 patients (termed the 'total population') with over 7800 patient-years of exposure.

1 The use of trade names is for product identification purposes only and does not imply endorsement.

Table I. Definitions of grades of haematological abnormality (National Cancer Institute Common Toxicity Criteria)

Parameter	Grade 1	Grade 2	Grade 3	Grade 4
Total WBC	3.0×10^9/L to <LLN	2.0 to $<3.0 \times 10^9$/L	1.0 to $<2.0 \times 10^9$/L	$<1.0 \times 10^9$/L
Neutrophils	1.5×10^9/L to <LLN	1.0 to $<1.5 \times 10^9$/L	0.5 to $<1.0 \times 10^9$/L	$<0.5 \times 10^9$/L
Lymphocytes	$0.75–1.0 \times 10^9$/L	0.5 to $<0.75 \times 10^9$/L	0.5 to 0.2×10^9/L	$<0.2 \times 10^9$/L
Platelets	75×10^9/L to <LLN	50 to $<75 \times 10^9$/L	25 to $<50 \times 10^9$/L	$<25 \times 10^9$/L
Haemoglobin	100 g/L to <LLN	80 to <100 g/L	65 to <80 g/L	<65 g/L

LLN = lower limit of normal; **WBC** = white blood cell.

Six-month clinical trial data were used to explore differences in the haematological changes associated with different doses of interferon-β-1a during the initial months of therapy, whereas 2-year and 6-year clinical trial data provided information on the onset and recovery of events following administration of the approved doses (22μg and 44μg three times weekly) for MS.

Routine laboratory tests were performed during the clinical trials, generally at baseline, at weeks 2, 4 and 12, and then every 3 months thereafter. Haematological measures included WBC count, differential, haemoglobin and platelet counts. The National Cancer Institute Common Toxicity Criteria (version 3.0, 2003) were used to grade toxicity (table I) below the lower limit of normal (LLN). Due to variations in scales used to grade lymphopenia, a LLN was set at 1.0×10^9/L for all samples. Data are presented based on laboratory measures. Because all abnormalities were asymptomatic, adverse event reporting underestimates the impact on these variables and may vary between sites for similar grades of laboratory abnormality.

To assess the persistence of haematological changes, shift tables were prepared to document the number of patients who had normal, high or low haematological parameters at baseline, cross-correlated with their status at specific timepoints during the study. Persistent new-onset elevations were calculated based on the number of patients with elevated values at the endpoint divided by the number at risk (those with high or normal values) at baseline.

Postmarketing Data

Rebif® has been on the market from mid-1998. Since then, safety data from postmarketing sources have been recorded in a dedicated database and analysed periodically in compliance with existing regulations worldwide. For the purpose of this publication, all medically confirmed cases in the Serono postmarketing safety database with an initial receipt date up to 30 September 2003 were considered. In the database, the coding dictionary used is the WHO's Adverse Reaction Terminology (WHO-ART) and verbatim terms are coded. The search was conducted either by verbatim terms or by the WHO-ART Preferred Term and Body System.

Events that are labelled, particularly if non-serious, are typically under-reported to the sponsor. Therefore, common non-serious events, such as haematological disorders, are best analysed using clinical study data. However, spontaneous reporting can generate signals, or confirm a signal generated during clinical studies. The definition of 'serious' used is in agreement with the International Conference on Harmonisation (ICH) guidelines (ICH E2A): a serious event is one that is fatal, life-threatening, disabling, incapacitating, results in hospitalisation, prolongs hospitalisation, is a congenital anomaly or birth defect, or is otherwise considered medically significant.

The spontaneously reported postmarketing data provided clinical, but no laboratory, information based on approximately 60 000 patients, with approximately 145 000 patient-years of exposure, as of September 2003.

Statistics

The analysis was performed on all available data from the studies under consideration. The Cochran-Armitage Trend Test on binomial proportions was used to detect any dose-effect relationships. Comparisons of proportions affected were made using Fisher's Exact Test. The 95% CIs were estimated using the binomial distribution. All tests were two-sided with a significance level of 0.05. Values <0.0005 are expressed as $p < 0.001$. All computations were performed using SAS (SAS Institute Inc.,

Cary, North Carolina, USA) version 8.02 for Windows. Descriptive statistics (mean, median) were reported for all the continuous variables assessed. For nominal variables, the distribution tables (numbers and percentages) were displayed for each treatment and each visit.

Results

Patient Demographics

The ratio of male to female patients was approximately 1 : 2 in studies of RRMS and 2 : 3 in those of SPMS. Median (mean) patient age in the total population was 37 (37.1) years: 35 (35.3) years in patients with RRMS and 44 (43.1) years in those with SPMS; 90% of patients were aged between 22 and 52 years. Patients were predominantly Caucasian (97.1%).

Exposure to Interferon-β-1a

All patients who received at least one dose of the study drug during the clinical trials were included in the analyses of safety (table II). In the controlled population, 1658 patients received interferon-β-1a (Rebif®) and 824 patients received placebo. In the Prevention of Relapses and Disability by Interferon-β-1a Subcutaneously in Multiple Sclerosis (PRISMS)[1] and Secondary Progressive Efficacy Clinical Trial of Recombinant Interferon-β-1a in Multiple Sclerosis (SPECTRIMS)[11] studies, safety data were available for patients who started the study receiving interferon-β-1a, either 22μg (n = 398) or 44μg (n = 388) three times weekly, and who continued therapy for up to 6 years, plus 392 patients who received placebo for 2 years. The proportion of patients who completed each interval is also shown in table II.

Incidence of White Blood Cell Toxicity in the Controlled Population

Examination of the results for the controlled population by study demonstrated heterogeneity in the proportions of patients affected by WBC toxicity, with one study, the EVidence of Interferon Dose-response: European North American Comparative Efficacy (EVIDENCE)[4] trial, showing consistently lower rates than the other studies. Differences be-

Table II. Patient numbers contributing data during various time intervals, the proportion completing the interval and the cumulative treatment exposure and time on study for the controlled population

Parameter	Treatment duration (months)	Placebo (treatment-years)	22μg qw (treatment-years)	44μg qw (treatment-years)	22μg tiw (treatment-years)	44μg tiw (treatment-years)
Patient number	6	824 (372)	435 (198)	98 (44)	398 (180)	727 (327)
	24	392 (748)			398 (740)	388 (723)
	72				398 (1870)	388 (1792)
Patients reaching timepoint (%)	6[a]	96	98	96	97	96
	12[b]	96			93	93
	24[b]	89			89	89
	36[b]				85	83
	48[b]				80	76
	60[b]				64	60
	72[c]				30	29

a Six studies.
b Two studies.
c One study.
qw = once weekly; **tiw** = three times weekly.

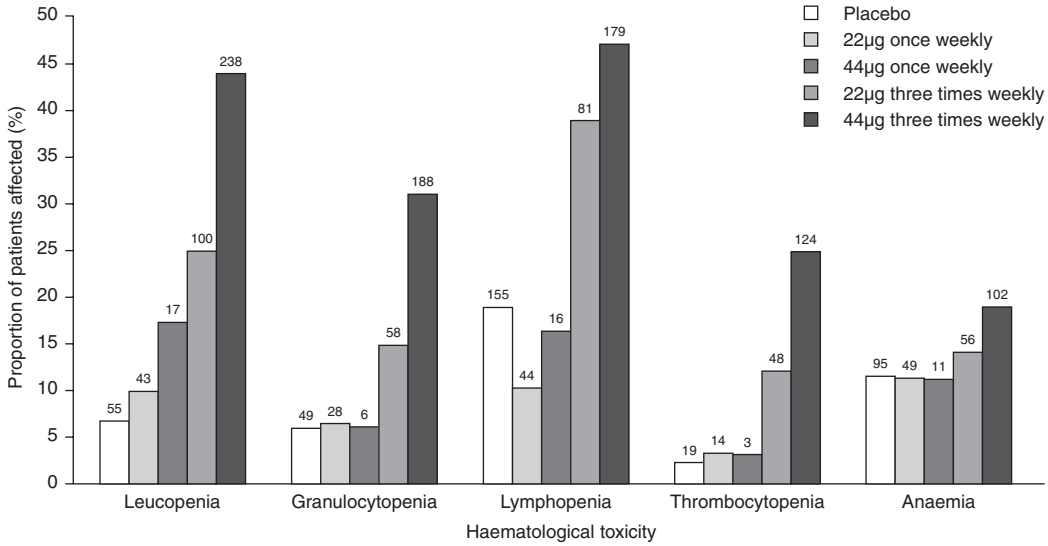

Fig. 1. The number and proportion (%) of patients with haematological abnormalities, based on laboratory testing, during the first 6 months of treatment with interferon-β-1a (Rebif®) in all six studies. The comparisons of 22μg and 44μg three times weekly with placebo were significant for all measures ($p < 0.002$), apart from anaemia. Differences between 22μg and 44μg three times weekly were significant ($p < 0.001$) for leukopenia and granulocytopenia ($p < 0.001$ for both) and thrombocytopenia ($p < 0.05$).

tween EVIDENCE and the other studies include different time (1999–2000 for 6-month data versus 1995–6 for the other studies), geography (predominantly US-based versus Europe, Canada and Australia) and product container (liquid in prefilled syringes versus liquid in vials), although the formulation was unchanged. It is not clear that any of these factors would differentially affect haematological results. For the abnormalities with potentially more clinical relevance (grades 3 or 4), there were no substantial differences between studies. For this reason, dose comparisons may be best assessed using data from PRISMS[1] and SPECTRIMS,[11] as presented below.

During the first 6 months of therapy, asymptomatic reductions were seen in several haematological variables (figure 1). There was evidence of a dose-effect relationship for leucopenia ($p < 0.001$, linear trend analysis), lymphopenia ($p < 0.001$), granulocytopenia ($p < 0.001$) and thrombocytopenia ($p < 0.001$), but not for anaemia ($p = 0.16$). There were no notable changes in the numbers of basophils, eosinophils or monocytes. Most of these laboratory abnormalities were of mild severity. For the doses approved for MS therapy, 22μg and 44μg three times weekly, differences between the proportion of patients with abnormal results for interferon-β-1a and placebo were significant ($p < 0.001$) for all comparisons, except for anaemia. A difference between the two approved doses ($p < 0.05$) was seen for granulocytopenia, leucopenia and thrombocytopenia.

For patients with 2 years of exposure to interferon-β-1a, there were significant differences in the proportion of patients for each haematological variable, for both doses, compared with placebo, except for anaemia in the 22μg three times weekly group (table III); most of these abnormalities were mild (grade 1). In this 2-year cohort study, there is a sufficient duration of treatment to generate information on time to onset and average duration of laboratory abnormalities. The median (mean) time to onset for haematological abnormalities was 1.6 (4.8) months, for thrombocytopenia it was 1.5 (3.6) months and for anaemia it was 3.0 (7.0) months.

Longer-term data (6-year follow-up from the PRISMS[1] and SPECTRIMS[11] studies) demonstrated that the proportion of patients with haematological abnormalities increased somewhat compared with the proportions affected after 2 years (table IV). Interpretation of these long-term rates of haematological abnormalities must take into consideration

Table III. Percentage of patients receiving interferon-β-1a for 2 years in the controlled population experiencing haematological toxicity (by grade and treatment group)[a]

Haematological toxicity	Grade	Placebo (n = 392)	22μg tiw (n = 398)	44μg tiw (n = 388)
Leucopenia	0	90	68[b]	48[bc]
	1	8	25	34
	2	1	7	17
	3	0	0.3	1
	4	0.3	0	0
Granulocytopenia	0	89	78[b]	63[bc]
	1	8	15	19
	2	3	6	14
	3	0.3	0.5	3
	4	0.3	0	0.3
Lymphopenia	0	84	70[b]	55[bc]
	1	10	18	21
	2	5	10	20
	3	1	2	4
	4	0	0	0.3
Thrombocytopenia	0	96	86[b]	73[bc]
	1	4	14	27
	2	0	0	0.3
Anaemia	0	83	79	71[bd]
	1	15	20	27
	2	1	1	2
	3	0.3	0	0

a Percentages are rounded to nearest integer, except for values <0.5%.

b $p \leq 0.001$ compared with placebo.

c $p \leq 0.001$ compared with 22μg tiw.

d $p < 0.05$ compared with 22μg tiw.

tiw = three times weekly.

the fact that comparable placebo values are not available for the 6-year data and that factors other than interferon-β-1a therapy could contribute to these laboratory abnormalities.

Severity of Abnormalities

Most of the haematological abnormalities were mild: only 0.2% of the controlled population (6 out of 2482 patients) experienced grade 4 toxicity during the first 6 months of therapy: 2 out of 824 (0.2%) placebo-treated patients and 4 out of 1658 (0.2%) interferon-treated patients. After 2 years of therapy, 1 of the 392 (0.3%) patients receiving placebo and 3 of the 786 (0.4%) treated patients experienced grade 4 toxicity.

Considering grades 3–4 toxicity as a measure of a clinically significant effect, the proportions of patients experiencing such toxicities when receiving 22μg or 44μg three times weekly (the approved doses) compared with placebo are shown in table IV.

In the total population database, eight patients receiving interferon-β-1a had haematological abnormalities that were classified as SAEs by the treating physician. Seven patients had lymphopenia (six patients receiving 44μg three times weekly and one receiving 22μg three times weekly), and one patient had anaemia (receiving 44μg three times weekly). Five patients taking placebo also experienced such SAEs: four had anaemia and one had lymphopenia. The most common haematological SAE during interferon-β-1a therapy was lymphopenia that had an incidence rate of 0.09 per 100 patient-years, compared with 0.12 per 100 patient-years for placebo. The SAEs related to lymphopenia may partly be an artefact of the rating scales used by the individual sites, particularly as all cases were asymptomatic. When using the Common Toxicity Criteria scale,

Table IV. Cumulative proportion (%) of haematological abnormalities over time (with proportion grades 3 or 4 in parentheses) in patients from the Prevention of Relapses and Disability by Interferon-β-1a Subcutaneously in Multiple Sclerosis (PRISMS) and Secondary Progressive Efficacy Clinical Trial of Recombinant Interferon-β-1a in Multiple Sclerosis (SPECTRIMS) studies, receiving interferon β-1a for up to 6 years

Treatment regimen	Treatment duration (months)	Leucopenia	Neutropenia	Lymphopenia	Thrombocytopenia	Anaemia
22μg tiw (n = 398)	6[a]	25 (0)	15 (0.3)	20 (1.0)	12 (0)	14 (0)
	24[a]	32 (0.3)	22 (0.5)	30 (2.0)	14 (0)	21 (0)
	72	39 (0.3)	28 (0.5)	40 (3.0)	14 (0)	29 (0.3)
44μg tiw (n = 388)	6[bc]	44 (1.0)	31 (3.1)	34 (2.3)	25 (0)	19 (0)
	24[bc]	52 (1.0)	37 (3.6)	45 (3.9)	27 (0)	28 (0)
	72[c]	56 (1.3)	44 (3.6)	52 (5.2)	29 (0)	36 (0.5)
Placebo (n = 392)	6	8 (0.3)	7 (0.3)	12 (1.0)	3 (0)	13 (0.3)
	24	10 (0.3)	11 (0.5)	16 (1.0)	4 (0)	17 (0.3)

a All comparisons (Fisher's exact test) significantly different ($p < 0.002$) from placebo, excluding anaemia.

b All comparisons between 44μg tiw and placebo are significant ($p < 0.05$).

c All comparisons between doses are significant ($p < 0.05$), excluding anaemia at 6 months.

tiw = three times weekly.

there are only two patients with grade 4 toxicity (very severe or life-threatening), one of each receiving 22μg once weekly and 44μg three times weekly. All SAE cases resolved following dose interruption, and no deaths related to haematological abnormalities were reported.

Persistence of Abnormalities

The mean (median) duration of laboratory abnormalities were 3.8 (3.0) months for leucopenia, 4.5 (3.0) months for lymphopenia, 3.0 (3.0) months for neutropenia, 3.6 (2.8) months for thrombocytopenia and 4.2 (3.0) months for anaemia for patients in the controlled population. Table V presents data on the cumulative proportion of patients who had any abnormality during the study and the proportion whose endpoint value was abnormal. The table demonstrates that, although the proportions of patients experiencing haematological abnormalities were high, most of these subsequently resolved. For example, in patients receiving the highest dose of interferon-β-1a, 44μg three times weekly, most of the abnormalities had resolved by 2 years, while patients continued on therapy. The proportions of patients with abnormal values that subsequently resolved were 71% (37 out of 52) for leucopenia, 76% (34 out of 45) for lymphopenia, 81% (30 out of 37) for neutropenia, 81% (21 out of 27) for thrombocytopenia and 68% (19 out of 28) for anaemia. The proportions of patients with persistent abnormalities were not substantially greater than for recipients of placebo. Of the abnormalities that persisted, the proportions rated mild were 87% (13 out of 15) for leucopenia, 55% (6 out of 11) for lymphopenia, 71% (5 out of 7) for neutropenia, 89% (8 out of 9) for anaemia and 100% (5 out of 5) for thrombocytopenia. For more severe events, i.e. grades 2–4, 11 out of 12 (92%) events in the placebo group, 22 out of 27 (81%) events in the 22μg three times weekly group and 52 out of 62 (84%) events in the 44μg three times weekly group resolved by the end of the 2-year interval.

Treatment Discontinuation and Dose Modification

In the total population, 12 of 3995 (0.3%) patients receiving interferon-β-1a experienced 16

Table V. Proportion (%) of patients showing haematological abnormalities at any time during the interval and the proportion with persistent abnormalities at the end of the interval

Treatment duration (mo)	Treatment regimen	Leucopenia		Neutropenia		Lymphopenia		Thrombocytopenia		Anaemia	
		any	last	any	last	any	last	any	last	any	last
6	Placebo (n = 824)	7	2	6	1	7	2	2	1	12	6
	22μg qw (n = 435)	10	6	6	3	8	4	3	2	11	7
	44μg qw (n = 98)	17	7	6	4	14	5	3	4	11	6
	22μg tiw (n = 398)	25	12	15	5	20	10	12	4	14	7
	44μg tiw (n = 727)	33	17	26	9	25	11	17	8	14	7
24	Placebo (n = 392)	10	2	11	2	16	2	4	1	17	4
	22μg tiw (n = 398)	32	8	22	4	30	12	14	2	21	8
	44μg tiw (n = 388)	52	15	37	7	45	11	27	5	28	9

mo = months; **qw** = once weekly; **tiw** = three times weekly.

haematological events leading to treatment withdrawal (eight lymphopenia, five leucopenia, one each of thrombocytopenia, granulocytopenia and leucocytosis). The time to treatment discontinuation varied considerably (2–14 months). In the controlled population, six patients reported nine events leading to treatment withdrawal (four each of lymphopenia and leucopenia and one leucocytosis); two patients were receiving 22μg three times weekly and four were receiving 44μg three times weekly (0.8% of the 44μg three times weekly group). In the uncontrolled population, five patients reported six events leading to treatment withdrawal (four lymphopenia, one leucopenia and one granulocytopenia). In the clinical trial protocols, grade 4 toxicity required treatment discontinuation even if the events were asymptomatic. This is important when considering reports of lymphopenia, as these were graded using the WHO scale for leucocytes because the WHO scale does not grade lymphopenia. This leads to an overestimation of the severity of lymphopenia. The current Common Toxicity Criteria scale does not designate grade 4 toxicity for lymphopenia.

Dose reductions for haematological events occurred in 49 (3.0%) patients on 171 occasions. The reasons for dose reductions, by type of abnormality, were lymphopenia (n = 30), leucopenia (n = 26), granulocytopenia (n = 16), anaemia (n = 5), thrombocytopenia (n = 2) and bone marrow depression (n = 1).

Drug Interactions

To examine whether the use of concomitant medications was associated with any specific adverse events, data from the PRISMS study[1] were investigated and rates of adverse events noted when patients were receiving medications commonly used in MS. These concomitant medications were corticosteroids (frequently used to manage relapses), antidepressants (frequently used both for depression and pain management in MS) and hormonal therapy (for women). There was an increase in both leucocytosis and lymphopenia in association with corticosteroid use that was similar in placebo- and interferon-β-1a-treated patients. The leucocytosis is consistent with the demargination associated with corticosteroid use, but the explanation for lymphopenia associated with corticosteroid use is

less clear. Concomitant use of antidepressants produced a small increase in lymphopenia in patients receiving interferon-β-1a, 44μg three times weekly. However, this difference may be a chance finding, because no such change was found in patients receiving the 22μg three times weekly dose. Concomitant hormonal therapy in women was not associated with any specific changes in adverse events.

Clinically Apparent Events

As stated above, symptomatic events directly related to haematological abnormalities were rare. However, haematological abnormalities may be causally linked with adverse events in other areas, particularly infections and haemorrhagic events. Contrasting adverse events between groups with the largest dose difference, placebo compared with interferon-β-1a 44μg three times weekly, infectious events such as viral respiratory tract infections and urosepsis were comparable between groups (22.5% and 19.9%, respectively) after 6 months; the difference becoming slightly greater after 2 years. Less common infections (fungal, parasitic, herpes zoster) occurred in 1.6% of patients on placebo and 2.8% receiving interferon-β-1a 44μg three times weekly at 6 months and in 6.6% and 8.0% at 24 months. Haemorrhagic events were reported in 1.8% and 3.8% of patients on placebo and interferon-β-1a 44μg three times weekly, respectively, at 6 months but in 16.3% and 15.0% at 24 months. Other pulmonary, cardiac and allergic adverse events were very uncommon and found to be similar between groups.

Postmarketing Surveillance Data

Since the introduction of Rebif® to the market, a total of 117 events related to abnormalities of WBCs, red blood cells (RBCs) or platelets have been reported to the manufacturers. This figure represents 8.3% of all adverse events reported in association with the use of Rebif®. The proportion of events that were considered serious (15 out of 117, 12.8%) is less than the 17.2% of all spontaneously reported events that were considered serious.

The cases recorded in the database essentially confirm the findings from the clinical studies. The majority (87.2%) of the reports are non-serious, predominantly isolated WBC reductions (43 reports, 36.8%), most commonly neutrophils, with infrequent reports of reduced platelet counts (12%) or RBCs (7%). A decrease in counts of two cell lineages (WBC and RBC, WBC and platelets) has been reported in 15 (12.8%) cases, while four cases involved all three lineages. Detailed information was provided by reporters in only approximately 50% of cases, but from such information it appears that in about two-thirds of instances the event onset is within 3 months of the start of treatment. Additionally, from available information, the events respond favourably to dose reduction or discontinuation.

It is very difficult to evaluate the severity of the events reported, as outside the study setting no common scale for severity is used, and the judgements of the reporters, if provided, cannot be compared. When severity was mentioned, clinicians indicated in their reports that the event was 'mild' in the majority of the 29 cases with these data provided, or they simply specified absolute WBC counts, which were mainly grades 1 and 2, with three reports of grade 3 abnormalities. All of the non-serious cases of RBC or platelet decreases were grade 1.

Considering the limitations of spontaneous reporting and the uncertainty regarding the denominator, little can be said about a dose relationship. When the dose was provided in the reports, the cases occurred more frequently when the patients were taking interferon-β-1a 44μg three times weekly. This may reflect the fact that the majority of patients are currently using this dosage regimen, although controlled clinical data support a dose effect.

Serious events affecting WBCs, RBCs and platelets have been reported rarely with Rebif®. The majority of the 15 serious case reports are confounded by the concomitant use of other potentially haematotoxic drugs, such as mitoxantrone, or by the presence of underlying illnesses that provide a more plausible explanation for the event. Other reports are so poorly documented that a complete evaluation cannot be performed.

There has been one case that represents a possible safety signal. A 40-year-old woman with MS had taken Rebif®, 22μg three times weekly, for 2.5 years when she was admitted to hospital with anaemia and jaundice. Blood analysis was consistent with an autoimmune haemolytic anaemia (increased bilirubin and lactic dehydrogenase levels, positive

Coomb's test, presence of spherocytes, polychromasia and increased numbers of reticulocytes). The patient was treated with prednisolone 40mg daily and folic acid 5mg twice daily. Rebif® and mefenamic acid, a drug known to be associated with haemolytic anaemia, were both stopped, following which the patient progressively recovered. When the haemoglobin had reached normal levels, the patient was restarted on Rebif® with close monitoring, but the blood abnormalities began to recur within a few months, at which point Rebif® therapy was discontinued permanently.

Discussion

Up to 35% of patients in clinical trials were reported to have an adverse event involving haematological parameters – defined as a change from baseline values that was considered clinically significant.[1] Similar abnormalities have been reported in patients treated with interferon-α in other indications,[14-16] suggesting that this effect is typical of type-1 interferons. The mechanism by which haematological effects occur is believed to be related to bone marrow suppression.

As the haematological abnormalities are almost universally asymptomatic, adverse event reporting in clinical trials underestimates the full effect of therapy and is subject to variation in interpretation by clinicians. A more precise measure of the haematological impact of interferon-β therapy is based on laboratory values. The proportions of patients with various haematological laboratory abnormalities seen in the Rebif® safety database were dose-related and, for the highest dose, 44μg three times weekly, ranged from 19–44% after 6 months of therapy (compared with 3–13% for placebo) and ranged from 2–52% after 24 months of therapy (compared with 4–17% for placebo). The greatest difference compared with placebo involved leucocytes and neutrophils, with the least difference seen for haemoglobin. For the interferon-β-1a 44μg three times weekly dose, all comparisons with placebo were highly significant.

The laboratory abnormalities observed in the clinical trials were asymptomatic, and most were graded as mild. Routine infections were slightly more common in placebo-treated patients compared with high-dose interferon. However, during the first 6 months of therapy, there was a trend to an increase of atypical infections with high-dose interferon therapy, a trend that was less apparent after 2 years of therapy. Likewise, haemorrhagic events, while infrequent, appeared increased with therapy during the first 6 months, but this trend reversed by 2 years. There may thus be a slight increase in such events during the early months of therapy, although the absolute difference between groups is small (1–2%). Observations made during the 2 years demonstrates that comparable proportions of placebo and interferon-treated patients were affected.

Cytopenia generally reversed, either spontaneously or with dose reduction, and rarely led to the discontinuation of therapy. Most of the abnormalities occurred during the first 6 months of therapy and long-term trials demonstrated that the prevalence decreased substantially over time, with only small numbers of new incident cases reported after 6 months. Serious or life-threatening events were very uncommon.

These data are supported by the postmarketing surveillance observations, even bearing in mind the limitations of spontaneous reporting. In 5 years of postmarketing experience, only one severe reaction appears to have been associated with Rebif®; other cases were either confounded by another disease or by the use of concomitant medication. The single case has a possible autoimmune aetiology and probably differs in aetiopathogenesis from the common, non-serious and generally mild haematological disturbances observed both in clinical studies and reported in the postmarketing setting. The autoimmune hypothesis is consistent with findings in the above cases and with an association of other autoimmune events reported rarely with interferon therapy, including thyroid[17-19] and hepatic,[10,20] though more commonly reported with interferon-α than interferon-β use.[7,21] Cases of severe haematological events are more common with interferon-α,[21-25] although events reported with interferon-β include autoimmune haemolytic anaemia after a 2-month course of an unspecified interferon-β administered twice weekly,[26] and aplastic anaemia after 1 year of therapy with intramuscular interferon-β-1a once weekly.[27] It is also important to consider that interferon-α has been advocated as a therapy for various forms of haemolytic anaemia and thrombo-

cytopenia.[28-34] The paucity of published case reports of serious haematological toxicity related to interferon-β treatment of MS patients provides further evidence against a major risk of toxicity.

There are three interferon-β products approved for use in MS. Interferon-β-1b (Betaseron®, Schering AG, Berlin, Germany) is administered subcutaneously at a dose of 825μg (8 MIU) every other day. This higher dose is required because of lower bioactivity. No data exist directly comparing Betaseron® and Rebif®, although the product information for Betaseron®[35] indicates that the haematological profiles are comparable. A direct comparative study of 677 RRMS patients on Rebif® or the interferon-β-1a, Avonex® (Biogen, Cambridge, Massachusetts, USA) 30μg administered intramuscularly once weekly, demonstrated significantly enhanced efficacy of the Rebif® regimen on relapse and magnetic resonance imaging outcomes for up to an average of 64 weeks.[4,36] More patients on Rebif® than Avonex® had haematological events considered as adverse events (13.6% vs 5.3%; $p < 0.001$) compared with the total number of asymptomatic reductions in WBC counts (27% vs 9%; $p < 0.001$).[37] Clearly, not all WBC reductions are considered as adverse events by clinicians, yet all are asymptomatic. These proportions represent any occurrence during the average 64-week exposure. The proportions with abnormal counts at the final assessment were 9% and 2% for Rebif® and Avonex®, respectively (pre-treatment values were abnormal in 2% of both groups). Furthermore, for each regimen, the proportion of patients with clinically more relevant, severe events was low, both for changes considered as adverse events (0.9% vs 0.6%) and for asymptomatic decreased WBC counts (1.5% vs 0.6%). Two (0.6%) patients receiving Rebif®, but no patient receiving Avonex®, stopped therapy for haematological toxicity. Thus, although there is a substantial dose difference, there is relatively little impact on treatment adherence and most toxicity is of mild grade. When considering therapy, an assessment of relative benefit-to-risk is important. In terms of efficacy, the number needed to treat (NNT) to have one extra patient remain free of relapses was 12,[36] while the NNT to induce one additional adverse event-related dropout, including haematological adverse events, was 91,[37] a benefit-to-risk ratio that favours high-dose, high-frequency interferon-β-1a therapy.

Conclusion: Therapeutic Guidelines

Monitoring of patients for haematological abnormalities is recommended in the US prescribing information for Rebif®[38] at baseline, regular intervals (1, 3 and 6 months) following initiation of treatment and periodically thereafter in the absence of clinical symptoms. Nevertheless, the data available from this extensive pooled database indicate that, although these abnormalities are common during treatment with interferon-β-1a, they are rarely considered clinically significant and are not associated with infections or other complications. Dose modifications for haematological toxicity were made in 3% of patients in the controlled population and cessation of therapy occurred in 0.4% of all patients and 0.8% of those on the highest dose, i.e. 44μg three times weekly. Despite the limited impact of interferon on blood lines, prudence would suggest that patients with grade 3 WBC abnormalities should probably have their dose reduced or interrupted until values fall to grade 2 or less. Grade 4 events are so unusual that other potential aetiologies should be explored. For platelets and haemoglobin, any toxicity of grade 2 or higher is very uncommon and not substantially different from changes seen in placebo-treated patients. Grade 2 toxicity or greater should prompt an investigation for other causes while considering interferon dose modification. Overall, haematological changes related to interferon-β therapy, while common, do not appear to constitute a safety concern at the doses used to treat MS.

Acknowledgements

The authors wish to express thanks to the patients who have participated in the studies, contributing data to this analysis and to the physicians and other study site personnel responsible for their care. Studies from which data have been utilised include PRISMS, SPECTRIMS, ETOMS, OWIMS, Nordic SPMS and EVIDENCE. Sarah-Jane Blake, Caudex Medical Ltd, provided valuable manuscript assistance.

Drs Rieckmann and O'Connor have received funding for research activities and honoraria for lectures and advisory panels from the manufacturer of Rebif®; Drs Francis and Alteri are employees of Serono; Dr Wetherill provided consultant statistical input to Serono.

References

1. PRISMS Study Group, University of British Columbia MS/MRI Analysis Group. PRISMS-4: long-term efficacy of interferon-β-1a in relapsing MS. Neurology 2001; 56: 1628-36
2. Jacobs LD, Cookfair DL, Rudick RA, et al. Intramuscular interferon beta-1a for disease progression in relapsing multiple sclerosis. Ann Neurol 1996; 39: 285-94
3. The IFNβ Multiple Sclerosis Study Group. Interferon beta-1b is effective in relapsing-remitting multiple sclerosis: I. Clinical results of a multicenter, randomized, double-blind, placebo-controlled trial. Neurology 1993; 43: 655-61
4. Panitch H, Goodin DS, Francis G, et al. Randomized, comparative study of interferon β-1a treatment regimens in MS: the EVIDENCE trial. Neurology 2002; 59: 1496-506
5. European Study Group on Interferon β-1b in Secondary Progressive MS. Placebo-controlled multicentre randomised trial of interferon beta-1b in treatment of secondary progressive multiple sclerosis. Lancet 1998; 352: 1491-7
6. Bayas A, Rieckmann P. Managing the adverse effects of interferon-β therapy in multiple sclerosis. Drug Saf 2000; 22 (2): 149-59
7. Walther EU, Hohfeld R. Multiple sclerosis: side effects of interferon beta therapy and their management. Neurology 1999; 53: 1622-7
8. Once Weekly Interferon for MS Study Group. Evidence of interferon B-1a dose response in relapsing-remitting MS. Neurology 1999; 53: 679-86
9. Comi G, Filippi M, Barkhof F, et al. Effect of early interferon treatment on conversion to definite multiple sclerosis: a randomised study. Lancet 2001; 357: 1576-82
10. Francis G, Grumser Y, Alteri E. Hepatic reactions during treatment of multiple sclerosis with interferon-beta-1a: incidence and clinical significance. Drug Saf 2003; 26 (11): 815-27
11. SPECTRIMS Study Group, Hughes RAC. Randomized controlled trial of interferon-beta-1a in secondary progressive MS: clinical results. Neurology 2001; 56: 1496-504
12. Sorensen PS, Andersen O, Elovaara I, et al. Double-blind placebo-controlled study of once weekly, low dose interferon beta-1a in secondary progressive multiple sclerosis (SPMS): Nordic SPMS study [abstract]. Mult Scler 2001; 7 Suppl. 1: S94
13. Rebif integrated summary of safety. Serono (Data on file)
14. Toccaceli F, Rosati S, Scuderi M, et al. Leukocyte and platelet lowering by some interferon types during viral hepatitis treatment. Hepato Gastroenterology 1998; 45: 1748-52
15. Ernstoff MS, Lembersky BC, Kirkwood JM. Fluorouracil, interferon-alpha, and colon cancer: rational pursuit of synergism between antimetabolites and biologicals. J Clin Oncol 1989; 7: 1764-5
16. Gugliotta L, Bagnara GP, Catani L, et al. In vivo and in vitro inhibitory effect of α-interferon on megakaryocyte colony growth in essential thrombocythaemia. Br J Haematol 1989; 71: 177-81
17. Rotondi M, Mazziotti G, Biondi B, et al. Long-term treatment with interferon-β therapy for multiple sclerosis and occurrence of Graves' disease. J Endocrinol Invest 2000; 23: 321-4
18. McDonald MD, Pender MP. Autoimmune hypothyroidism associated with interferon beta-1b treatment in two patients with multiple sclerosis. Aust N Z J Med 2000; 30: 278-9
19. Durelli L, Ferrero B, Oggero A, et al. Autoimmune events during interferon beta-1b treatment of multiple sclerosis. J Neurol Sci 1999; 162: 74-83
20. Duchini A. Autoimmune hepatitis and interferon beta-1a for multiple sclerosis. Am J Gastroenterol 2002; 97: 767-8
21. Vial T, Descotes J. Clinical toxicity of the interferons. Drug Saf 1994; 10: 115-50
22. Sevastianos VA, Deutsch M, Dourakis SP, et al. Pegylated interferon α-2b-associated autoimmune thrombocytopenia in a patient with chronic hepatitis C. Am J Gastroenterol 2003; 98: 406-707
23. Sagir A, Wettstein M, Heintges T, et al. Autoimmune thrombocytopenia induced by PEG-IFN-α 2b plus ribavirin in hepatitis C. Dig Dis Sci 2002; 47: 562-3
24. Tomita N, Motomura S, Ishigatsubo Y. Interferon-alpha-induced pure red cell aplasia following chronic myelogenous leukemia. Anticancer Drugs 2001; 12: 7-8
25. Landau A, Castera L, Buffet C, et al. Acute autoimmune hemolytic anemia during interferon alpha therapy for chronic hepatitis C. Dig Dis Sci 1999; 44: 1366-7
26. Kazuta Y, Watanabe N, Sagawa K, et al. A case of autoimmune hemolytic anemia induced by IFN-β therapy for type-C chronic hepatitis. Fukushima J Med Sci 1995; 41: 43-9
27. Aslam AK, Singh T. Aplastic anemia associated with interferon β-1a. Am J Ther 2002; 9: 522-3
28. Hrstkova H, Bajer M, Michalek J. Recombinant human interferon α-2a therapy in children with chronic immune thrombocytopenic purpura. J Pediatr Hematol Oncol 2002; 24: 299-303
29. Cesana C, Brando B, Boiani E, et al. Effective treatment of autoimmune hemolytic anemia and hairy cell leukemia with interferon-α. Eur J Haematol 2002; 68: 120-1
30. Barel C, Bilger K, Ninet J, et al. Treatment of hepatitis C virus-associated thrombocytopenic purpura with a combination of interferon alpha-2b and ribavirin. J Clin Gastroenterol 2002; 35: 200-1
31. Rajan S, Liebman HA. Treatment of hepatitis C related thrombocytopenia with interferon alpha. Am J Hematol 2001; 68: 202-9
32. Dikici B, Bosnak M, Kara IH, et al. Interferon-alpha therapy in idiopathic thrombocytopenic purpura. Pediatr Int 2001; 43: 577-80
33. Donato H, Kohan R, Picon A, et al. α-Interferon therapy induces improvement of platelet counts in children with chronic idiopathic thrombocytopenic purpura. J Pediatr Hematol Oncol 2001; 23: 598-603
34. Vianelli N, Gugliotta L, Tura S, et al. Interferon-α2a treatment in a pregnant woman with essential thrombocythemia. Blood 1994; 83: 874-5
35. Berlex Laboratories. Betaseron® (interferon beta-1b) [package insert]. Berlex Laboratories, 2003
36. Panitch H. Differences between IFN beta-1A 44μg tiw and 30μg qw sustained to 16 months: final evidence results [abstract]. Int J MS Care 2003; 5: 80
37. Sandberg M. Comparative risk-benefit analysis in EVIDENCE [abstract]. Mult Scler 2003; 9: S139
38. Serono International. Rebif® (interferon beta-1a) [package insert]. Serono International, 2003

Correspondence and offprints: *Peter Rieckmann*, Department of Neurology, Würzburg, Josef Schneider STR 11, 97080, Germany.
E-mail: p.rieckmann@mail.uni-wuerzburg.de