Drug-Induced Progressive Multifocal Leukoencephalopathy: Lessons Learned From Contrasting Natalizumab and Rituximab

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Progressive multifocal leukoencephalopathy (PML) has been identified as a serious adverse drug reaction (ADR) of several immunomodulatory biologicals. In this study, we contrasted the reporting patterns of PML for two biologicals for which the risk was identified at different points in their lifecycle: natalizumab (before reapproval) and rituximab (nine years postapproval). We found that, apart from the differences in clinical characteristics (age, gender, indication, time to event, fatality), which reflect the diversity in context of use, PML reports for natalizumab were more complete and were received sooner after occurrence. This study serves as an important reminder that spontaneous reports should only be used with great caution to quantify and compare safety profiles across products over time. The observed variability in reporting patterns and heterogeneity of PML cases presents challenges to such comparisons. Lumping uncharacterized PML reports together without taking these differences into account may result in biased comparisons and flawed conclusions about differential safety.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC? ☑ PML has recently been identified as a serious ADR of several immunomodulatory biologicals, used under diverse clinical conditions. Although PML has presented a common challenge in the pharmacovigilance of these products, the differences in clinical context in which products are used, as well as the differences in regulatory history of products, may impact on the ADR reporting patterns. • WHAT QUESTION DOES THIS STUDY ADDRESS? ☑ The key question investigated was how spontaneously reported cases of suspected drug-induced PML differ between two drugs (natalizumab and rituximab) with distinct regulatory paths, and used in diverse patient populations. • WHAT THIS STUDY ADDS TO OUR KNOWLEDGE ☑ We found that PML reports are not alike across products and over time: apart from differences in clinical characteristics reflecting the diversity in context of use, PML reports for natalizumab were more complete and were received earlier after occurrence. • HOW THIS MIGHT CHANGE CLINICAL PHARMACOLOGY AND THERAPEUTICS ☑ This study shows that variability in reporting patterns and heterogeneity of cases should be taken into account when comparing the same ADR in different populations.

Progressive multifocal leukoencephalopathy (PML) is a severe viral infection of the human brain associated with poor clinical outcomes, including disability and death.[1] The etiology of PML involves reactivation of the latent John Cunningham virus in the presence of disorders associated with severe cellular immune deficiency. For a long time, PML was a rare condition that was mostly observed among patients with hematological malignancies.[2,3] However, the incidence sharply increased with the onset of the human immunodeficiency virus/AIDS epidemic, and most cases have since then been diagnosed in human immunodeficiency virus-infected patients.[4,5] Over the last decade, however, PML has increasingly been diagnosed in human immunodeficiency virus-negative patients treated with immunomodulatory biologicals, particularly natalizumab and rituximab.[6,7]

In February 2005, only three months after receiving accelerated marketing approval in the United States, the marketing of natalizumab (Tysabri; Biogen Idec/Élan) was suspended after PML had been diagnosed in two patients participating in a clinical trial for multiple sclerosis.[8–10] A third case was subsequently discovered after a reexamination of a patient who had received natalizumab for inflammatory bowel disease, and who was initially falsely diagnosed with drug-induced fatal astrocytoma in 2003.[11] After a reanalysis of previously natalizumab-treated patients confirmed that no additional PML cases had occurred, natalizumab was

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reintroduced in the United States and first approved in Europe in June 2006. It was agreed that specific risk minimization and surveillance measures were to be undertaken to mitigate and further characterize the risk of PML, including a restricted distribution program in the United States and a detailed risk management plan in Europe.[12,13] Since then, the number of adverse drug reaction (ADR) reports for suspected drug-induced PML has vastly increased,[14] and an increasing number of immunomodulatory drugs has been suspected or identified to increase the risk of PML.[15,16] Some of these products had been marketed for multiple years, suggesting that increased awareness may have contributed to the recognition of the risk of drug-induced PML.

Rituximab (Rituxan/Mabthera; Genentech/Hoffmann-La Roche) is an example of a biologic that received increased scrutiny for drug-induced PML relatively late in its lifecycle.[7,17] Rituximab was first approved in 1997 for the treatment of relapsed and refractory follicular lymphoma. Yet, its use has considerably changed throughout its lifecycle, as new indications have been added (including rheumatoid arthritis), and the product became first-line therapy for several lymphoid malignancies. The risk of PML was first included in the United States and European product label in 2007, more than nine years after initial approval, on the basis of data from spontaneous reports of suspected ADRs.[18,19]

The difference in timing with respect to the recognition of PML as a drug-induced condition for natalizumab and rituximab is intriguing, and provides a unique opportunity to contrast the two products in relation to their respective ADR reporting patterns. Although PML has presented a common challenge in the pharmacovigilance of several immunomodulatory biologicals, the differences in clinical context in which products are used, as well as the differences in regulatory history, including indication dynamics of products, may impact on the ADR reporting patterns. This can present challenges to clinical and regulatory decision-making. In this study, we contrasted the PML reporting patterns (e.g., case characteristics, temporality of reporting, and completeness of the reports) between natalizumab and rituximab, given the distinct regulatory pathways over time, different indications, and treatment populations of these two products.

RESULTS

Case characteristics

A total of 375 and 287 spontaneous reports on suspected drug-induced PML were retrieved for natalizumab and rituximab, respectively, from EudraVigilance, the European database for collection of ADR reports. As shown in **Table 1**, substantial differences were observed in patient and treatment characteristics between cases of natalizumab-associated and rituximab-associated PML. Compared with patients with rituximab-associated PML, patients with natalizumab-associated PML were, on average, younger (45 vs. 65 years; $P < 0.001$), and were more frequently women (69.3% vs. 44.3%; $P < 0.001$). The median time to onset from treatment initiation was 36 months (interquartile range (IQR), 26–48 months) for natalizumab-associated PML, and 12 months (IQR, 4–24 months) for rituximab-associated PML ($P < 0.001$). The outcome of the PML reaction was more frequently fatal for rituximab-associated PML ($n = 114$; 39.7%) as compared to natalizumab-associated PML ($n = 40$; 10.7%; $P < 0.001$).

For each case of natalizumab-associated PML, on average, 3 reports (IQR, 2–5 reports) had been received over time, involving either follow-up reports from the initial reporter, or multiple reports from different reporters on the same case. For rituximab-associated PML the average number of reports per case was 2 (IQR, 1–3). Medical doctors were most frequently involved in the reporting of PML cases for both natalizumab ($n = 348$; 92.8%) and rituximab ($n = 251$; 87.5%). Notably, patients were involved in the reporting of 89 cases (31.0%) for rituximab, and in 50 cases (13.3%) for natalizumab.

Temporal trends in reporting

The first spontaneous PML report for natalizumab was received in July 2008, approximately two years after its initial marketing approval in Europe and being back on the market in the United States (June 2006; see **Figure 1**). The first spontaneous report on rituximab-associated PML was received in December 2004, respectively, 6.5 and 7 years after receiving initial marketing approval in Europe (June 1998) and the United States (November 1997).

The change-point analysis showed that the PML reporting rate was low (0.1 reports/month) for both natalizumab and rituximab in the year(s) after the first spontaneous report, but increased over time. Overall, as shown in **Figure 1**, a total of, respectively, four and three change points in reporting rates were found for natalizumab and rituximab over time. For natalizumab, the reporting rate increased from 0.1 reports/month (until 9 September 2009) up to 24.2 reports/month from 13 November 2012 onward. For rituximab, the reporting rate was more constant over time, increasing from 0.1 reports/month (until 14 September 2006) up to 4.3 reports/month from 21 November 2008 onward. However, a peak in reporting (11.3 reports/month) was noted between 30 September 2008 and 20 November 2008, with a higher proportion of reports originating from the United States ($n = 12$) relative to Europe ($n = 5$) and other regions ($n = 3$). This peak occurred shortly after a direct healthcare professional communication had been sent on rituximab-induced PML in the United States (September 2008).

Figure 2 shows the lag time between the occurrence of the PML reaction and the receive date of the initial spontaneous report. Overall, the lag time was significantly shorter for cases of natalizumab-associated PML (median, 1 month; IQR, 0–1 month), as compared to cases of rituximab-associated PML (median, 3 months; IQR, 1–8 months; $P < 0.001$). Notably, six cases of rituximab-associated PML in EudraVigilance had occurred before the receive date of the first spontaneous reports (December 2004), but were reported only thereafter. This was not observed for natalizumab. The peak in reporting for rituximab that was identified through change-point analysis, in particular, involved cases with a long lag time (median, 8 months; IQR, 1–51 months) between reaction and reporting.

Table 1 Characteristics of spontaneous reports on suspected drug-induced PML for rituximab and natalizumab in EudraVigilance

	Natalizumab (n = 375)	Rituximab (n = 287)
Patient and treatment information		
Age, median (IQR)	45 (37–52)	65 (57–73)
Missing, no. (%)	28 (7.5)	62 (21.6)
Sex, no. (%)		
Female	260 (69.3)	127 (44.3)
Male	113 (30.1)	140 (48.8)
Missing	2 (0.5)	20 (7.0)
Indication, no. (%)[a]		
Multiple sclerosis	341 (90.9)	–
Lymphoid neoplasm	–	218 (76.0)
Rheumatoid arthritis/ other autoimmune disorder	–	29 (10.1)
Other condition	2 (0.5)	9 (3.1)
Missing	33 (8.8)	34 (11.8)
Time to onset, median mo (IQR)	36 (26–48)	12 (4–24)
Missing, no. (%)	92 (24.5)	125 (43.6)
Treatment duration, median mo (IQR)	36 (25–48)	6 (3–20)
Missing, no. (%)	75 (20.0)	126 (43.9)
No. of concomitant drugs (%)		
0 concomitant drugs reported	221 (58.9)	76 (26.5)
1–5 concomitant drugs reported	109 (29.1)	139 (48.4)
6–10 concomitant drugs reported	27 (7.2)	51 (17.8)
>10 concomitant drugs reported	18 (4.8)	21 (7.3)
Outcome of PML reaction, no. (%)		
Fatal	40 (10.7)	114 (39.7)
Not recovered	205 (54.7)	82 (28.6)
Recovered	29 (7.8)	6 (2.0)
Recovering	4 (1.1)	7 (2.4)
Missing	97 (25.9)	78 (27.2)
Report information		
Reporter, no. (%)[a]		
Physician	348 (92.8)	251 (87.5)
Pharmacist	9 (2.4)	20 (7.0)
Other health professional	151 (40.3)	94 (33.8)
Patient/nonhealth professional	50 (13.3)	89 (31.0)
Missing	1 (0.3)	–
Reporter region, no. (%)		
Europe	233 (62.1)	161 (56.1)
United States	119 (31.7)	79 (27.5)
Other	23 (6.1)	47 (16.4)
Reports per PML event, median (IQR)	3 (2–5)	2 (1–3)

PML, progressive multifocal leukoencephalopathy; IQR, interquartile range.
[a]Because of the possibility of multiple indications for rituximab/natalizumab therapy per patient and the possibility of multiple reporters per event, these variables do not add up to 100%.

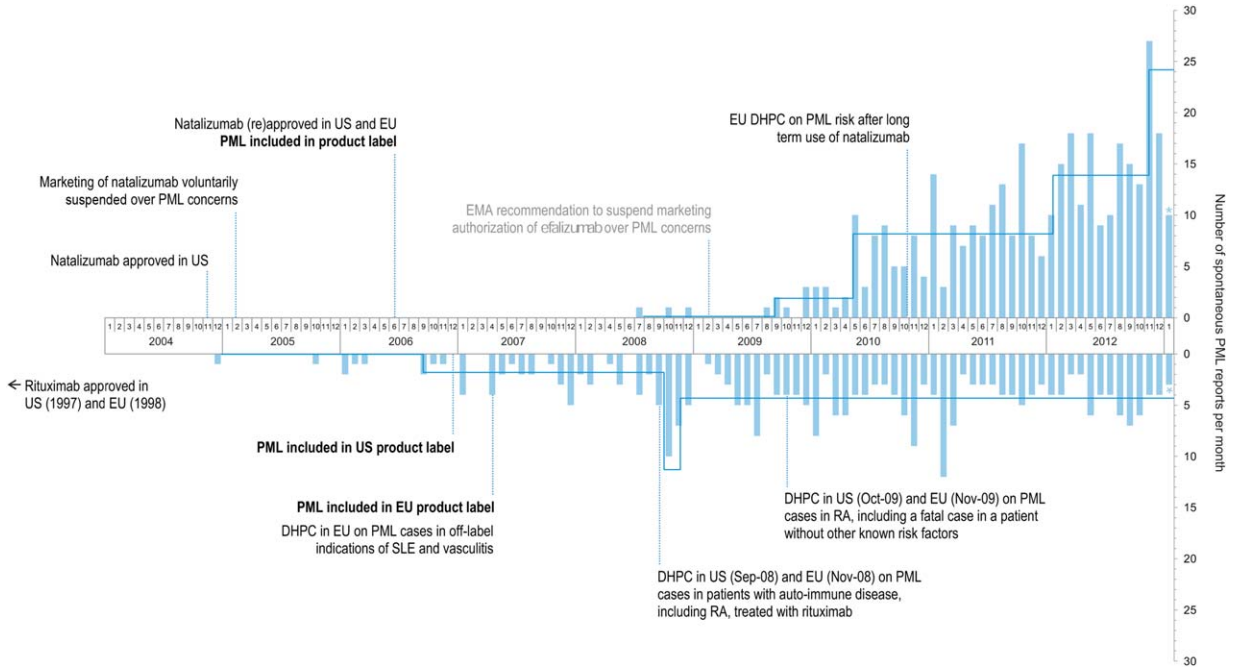

Figure 1 Temporal trends in the spontaneous reporting of cases suspected of drug-induced progressive multifocal leukoencephalopathy (PML) for natalizumab (above) and rituximab (below), according to the absolute number of reports per calendar-month (bars) and average number of reports per interval as found by the change-point analysis (lines). EMA, European Medicines Agency; DHPC, Direct Healthcare Professional Communication; SLE, system lupus erythematosus; RA, rheumatoid arthritis. *Data on January 2014 is incomplete because of study end.

Trends in reporting completeness

The completeness of PML reports was assessed along the availability of data for eight predefined variables on patient, treatment, and reaction details. Overall, taking into account any follow-up information received over time, the data completeness was 79% for natalizumab cases (median, 87.5%; IQR, 62.5–100%), as compared to 60% for rituximab cases (median, 62.5%; IQR, 37.5–75%; $P < 0.001$). As shown in **Table 2**, the indication of therapy was frequently available for both natalizumab (91%) and rituximab (88%), but particular differences were

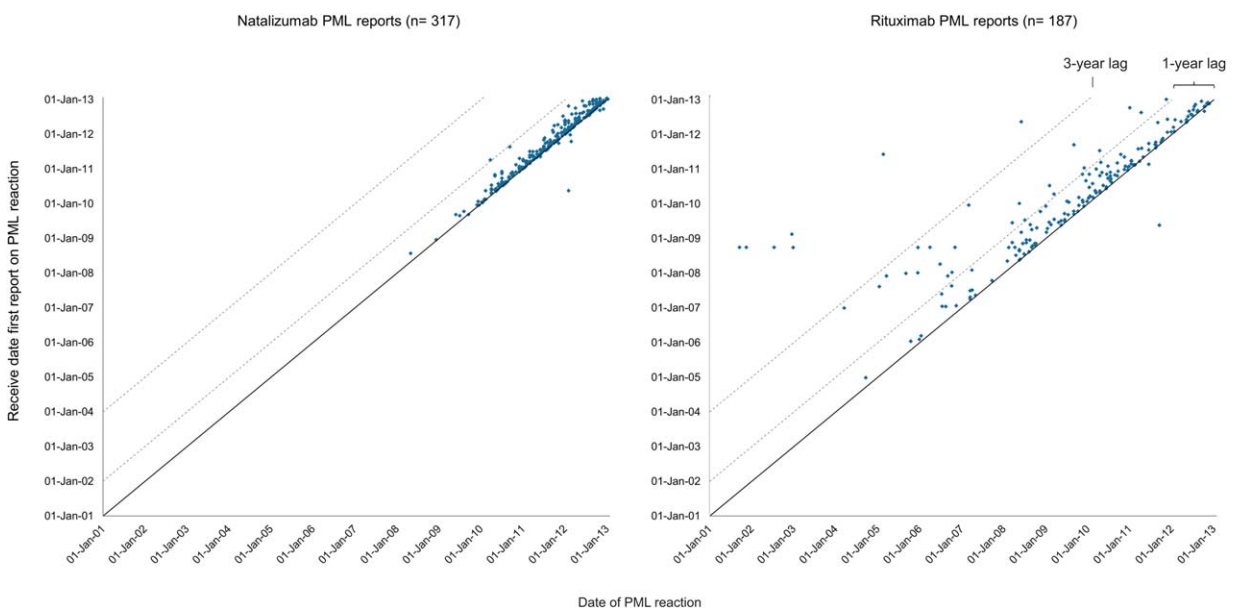

Figure 2 Lag time between occurrence of suspected drug-induced PML reaction (X-axis) and reporting (Y-axis), for natalizumab (left) and rituximab (right). Each bullet in the graphs marks a spontaneous report. The diagonal line indicates a zero lag time. *In a number of cases (n = 8 for natalizumab, n = 2 for rituximab) the date of the PML reaction was after the receive date of the first report on the case.

Table 2 Completeness of spontaneous reports on suspected natalizumab-induced and rituximab-induced PML

	Natalizumab (n = 375)		Rituximab (n = 287)	
	No. of reports	%	No. of reports	%
Age and gender provided	345	92	223	78
Start date of therapy	316	84	190	66
End date and/or duration of therapy	300	80	161	56
Indication of therapy	342	91	253	88
Previous drug history	210	56	63	22
Start date of PML reaction	317	85	186	65
Outcome of PML reaction	278	74	209	73
Diagnostic test results for PML	261	70	89	31
Total		79		60

PML, progressive multifocal leukoencephalopathy.

observed with regard to the patient's previous drug history (56% and 22%, respectively) and availability of diagnostic test results for the PML reaction (70% and 31%, respectively).

For both natalizumab and rituximab, the completeness of the PML reports declined over time with sequence of reporting (**Figure 3**). The decline was particularly pronounced for the

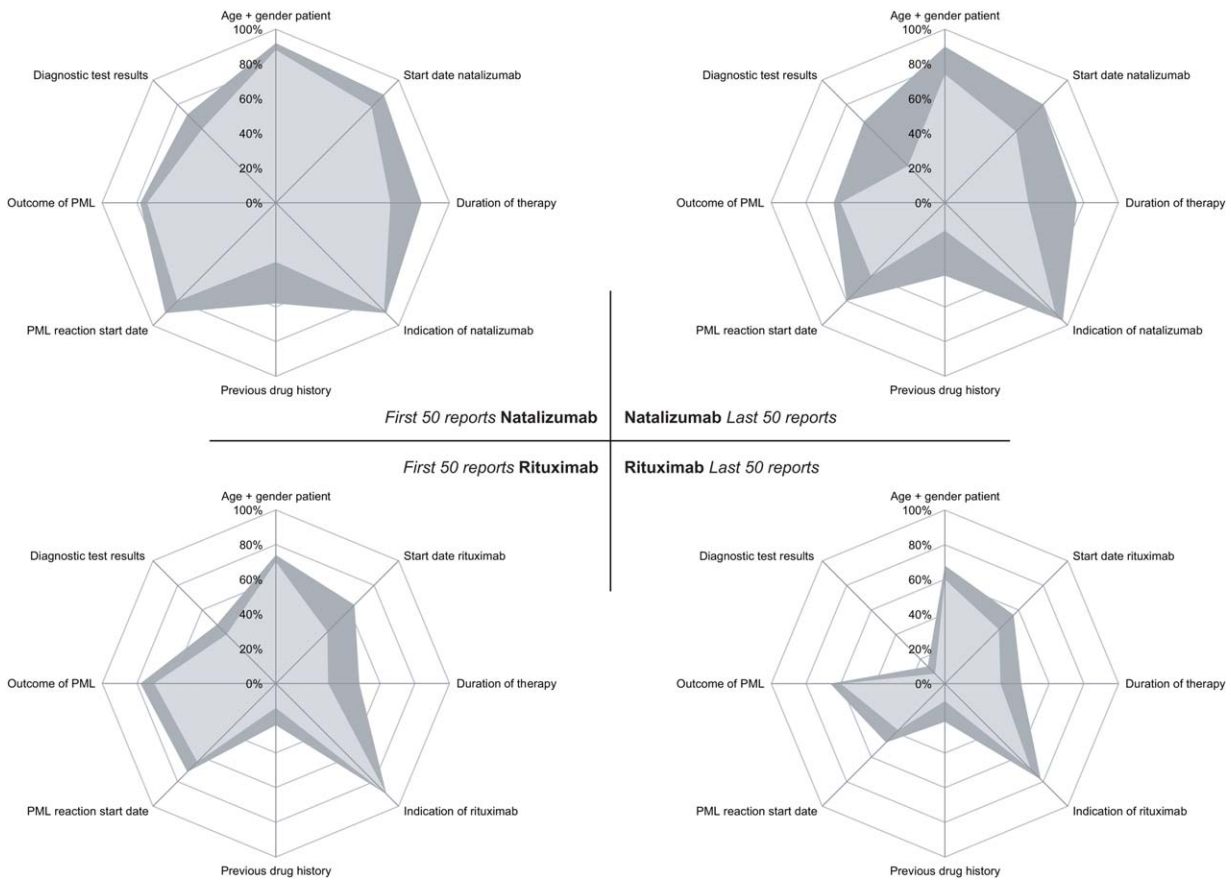

Figure 3 Completeness of spontaneous reports on natalizumab-associated (above) and rituximab-associated (below) PML, stratified by first 50 case reports received (left) and latest 50 case reports (right). The inner plot reflects the first information received (initial report) and the outer plot the most recent ("master" report) on the same cases. The radar chart shows the availability of data on the eight predefined variables in the reports. For example, 90% completeness of "start date natalizumab" indicates that, in 90% of the reports, the start date of natalizumab had been provided by reporter.

initial reports (i.e., the first information received from the initial reporter). For natalizumab, the completeness of the initial reports declined from 71% (median, 75%; IQR, 50–87.5%) for the first 50 reports to 55% (median, 62.5%; IQR, 37.5–75%; $P = 0.002$) for the last 50 reports. Similarly, for rituximab, the completeness declined from 53% (median, 62.5%; IQR, 37.5–75%) to 40% (median, 37.5%; IQR, 12.5–62.5%; $P = 0.013$). As further shown in **Figure 3**, differences were observed in the quality of the information on individual variables across the reports received over time. For rituximab, a particular decline in the reporting of information on diagnostic test results was observed over time: from 48% for the first 50 reports to 14% for the latest 50 reports.

DISCUSSION

This study highlights several differences in ADR reporting patterns for suspected cases of drug-induced PML between natalizumab and rituximab. We found that, apart from the differences in clinical characteristics (age, gender, indication of therapy, time to event, duration of use before event, and fatality rate subsequent to onset of PML), PML reports for natalizumab were more complete and were received sooner after occurrence, as compared to reports for rituximab. Furthermore, a time gap in reporting of suspected cases of rituximab-induced PML was observed within the first seven years after approval. Several factors may account for these observed differences, including variability in patient and treating physician populations (health status, confounding by indication, and clinical monitoring), as well as temporal issues related to the general awareness regarding the risk of drug-induced PML.

The recognition of unexpected associations between drug exposure and clinical events is one of the main challenges in pharmacovigilance.[20,21] Rare but serious ADRs may therefore go unnoticed until first reported by a small number of observant clinicians. In this study, the observed absence of spontaneous reports for rituximab-associated PML until seven years after approval (December 2004), and low reporting rate until September 2006 (0.1 report/month), also indicate that initial cases of drug-induced PML may not have been recognized. As shown in **Supplementary Figure 1**, in which the PML reporting trends have been put into context of the use of natalizumab and rituximab, the PML reporting curves of both products are not proportional to the increase in patient exposure over time. With a median time to onset of 36 months, it is expected that the PML reporting of natalizumab lags behind a couple of years to the exposure curve. Although a shorter delay may be expected for rituximab, in view of the shorter time to onset (median 12 months), a larger delay was observed instead. Notably, by the time of receipt of the first PML report for rituximab (December 2004), an estimated 540,000 patients had been exposed to the product in clinical practice.[22] This suggests that particularly initial cases of rituximab-induced PML may not have been recognized, as further supported by our finding that six cases of suspected rituximab-induced PML in EudraVigilance occurred before 2004, but were only reported thereafter.

The reason for the delay in recognition of PML as a potential drug-induced event of rituximab could be twofold: cases may either not have been diagnosed as PML, or may not have been attributed to rituximab but seen as a consequence of the disease (i.e., confounding by indication), or concomitant therapy. Rituximab was initially approved as third-line treatment for stage III to IV lymphoma. Diagnosing PML in this population can be particularly challenging because PML symptoms may be falsely interpreted as symptoms of central nervous system infiltration of lymphoma cells in relation to disease progression,[23] or as symptoms of high-dose chemotherapy toxicity.[24] On the other hand, the elucidation of the causal involvement of rituximab presented a challenge, as lymphoma is an independent risk factor for PML,[2] and rituximab was administered as third-line therapy in heavily pretreated patients. By contrast, PML in patients with multiple sclerosis is predominantly a phenomenon associated directly with natalizumab therapy.

The recognition of PML as a drug-induced event of rituximab may thus relate to a shift in product usage; from third-line to first-line treatment of lymphoma; and from use in the oncology setting to use in autoimmune diseases that have not traditionally been associated with PML, including rheumatoid arthritis.[2] On the other hand, safety learning between products could also have been important. Dissemination of information on the suspended marketing of natalizumab over PML concerns may have contributed to the awareness among health professionals regarding the potential risk of rituximab-associated PML. Notably, regulatory authorities had received only one spontaneous report for rituximab-associated PML before the suspension of marketing of natalizumab over PML concerns, although, before this, observant clinicians had described a number of cases in medical literature.[25–27] The reporting of suspected rituximab-induced PML subsequently peaked after a safety alert had been issued, a phenomenon known as the "notoriety effect."[28] Although not evaluated in this study, also media coverage may result in temporal increase in ADR reporting.[29] This may include social media coverage, particularly among patients with multiple sclerosis, a patient group known to be very active on the internet. Apart from these effects, efforts to characterize and minimize the risk of drug-induced PML, including the establishment of a common case definition[30] and the formation of a global research agenda,[14] may also have contributed to safety learning between products.

Safety learning across and between products has repeatedly been coined as a key activity for pharmacovigilance. Subsequent to the first documentation of QT-prolongation with quinidine therapy in 1964,[31] the risk has been identified for multiple other drugs, and eventually became the single most common reason for withdrawal and restriction of use of marketed drugs.[32] As a result, testing for drug-induced QT-prolongation is now a routine requirement for new drug approvals. More recently, subsequent to the identification of the risk of osteonecrosis of the jaw for the intravenous bisphosphonate zoledronic acid, and the hereto-related pharmacovigilance efforts,[33] the whole class of bisphosphonates came under scrutiny,[34] and a task force was formed to further characterize, prevent, and treat this drug-induced condition.[35] Over time, evolving regulatory pathways and learning

within and between regulatory systems may result in earlier recognition and more timely regulatory actions for safety concerns like PML.

Apart from the observed differences in temporal reporting patterns, we found that individual cases of suspected natalizumab-induced PML were generally received sooner after occurrence, when compared to cases of suspected rituximab-induced PML. This may be the result of routine magnetic resonance imaging monitoring and evaluation of neurological status during natalizumab therapy as recommended by the risk management plan,[12,13] and the potential for earlier diagnosis of PML in patients with multiple sclerosis given the significant overlap in clinical signs and, therefore, earlier diagnostic workup. By contrast, as rituximab is primarily prescribed in oncology, in patients with more severe disease and the potential for disease progression and other side effects, physicians may be less likely to timely, if at all, investigate and report suspected cases of drug-induced PML. In view of the observed differences in reporting patterns, it is important to consider that spontaneous reports can only be used to quantify and compare the incidence of PML across products over time with great caution. Other pharmacovigilance databases should, therefore, be considered to further characterize and quantify the risk of PML, including patient registries, database of medical records, and claims databases.

Differences in patient and treating physician populations may also have been important in the observed differences in completeness of the PML reports. Quality and completeness of spontaneous reports is critical to efficient pharmacovigilance. Detailed information on individual cases may not only contribute to the timely identification but also further characterization of new risks, allowing the implementation of appropriate risk minimization strategies. For natalizumab, an algorithm was proposed to calculate an individual's risk of developing drug-induced PML on the basis of information from spontaneous reports.[36] Previous immunosuppressant use was one of the factors that increases the PML risk. The relative low availability and further decline of information on previous drug use in spontaneous reports for rituximab hampers the possibility to identify similar risk stratification factors if they would be present. The relative low availability of diagnostic test results is another aspect with potential undesirable consequences. Apart from clinical symptoms, PCR for John Cunningham virus DNA in cerebrospinal fluid, brain magnetic resonance imaging, and brain biopsy/autopsy results form the basis to determine the diagnostic certainty of PML.[30] The Pharmacovigilance Risk Assessment Committee of the European Medicines Agency (EMA) has recently proposed a labeling strategy for PML in which ascertainment of this diagnostic certainty plays a crucial role.[37] Paucity of diagnostic information hampers appropriate and consistent labeling resulting in suboptimal risk minimization.

In this study, we also observed substantial differences in clinical characteristics between cases of suspected natalizumab-induced and rituximab-induced PML. In accordance with previous case series,[6,7] we found that patients with rituximab-associated PML had a significantly higher case fatality rate, as compared with patients with natalizumab-associated PML. The difference in mortality rate may first be explained by the difference in overall health status between the patient groups, including disease severity, prior treatments, and concomitant medication use. Apart from these patient-related factors, it has also been reported that PML associated with natalizumab is somewhat dissimilar from PML associated with human immunodeficiency virus and hematologic disease,[38] as it more frequently affects the frontal lobes, and is more commonly heralded by cognitive and behavior disturbances. Furthermore, the potential for early diagnosis of PML in multiple sclerosis, as described above, may also contribute to a better prognosis.

CONCLUSION

We have contrasted the occurrence of drug-induced PML for two biologicals with their own unique characteristics, temporal features, and challenges. Despite all well-documented limitations, spontaneous reports remain critical to pharmacovigilance. This study serves as an important reminder that lumping uncharacterized PML reports together without taking into account variability in reporting patterns over time, differences in patient populations, and treating physicians, may result in biased comparisons and flawed conclusions about differential safety.

METHODS

Setting

We used data from the EudraVigilance database of the EMA. EudraVigilance contains reports of suspected (serious) ADRs to medicines licensed in Europe, including reports from clinical studies, literature reports, and reports from postmarketing use by health professionals and patients. As required by European Union law, EudraVigilance contains all serious ADR reports that occur in the European Union, and all serious unexpected ADR reports occurring in the rest of the world. As of 31 December 2013, more than 4.5 million unique case reports are stored in the EudraVigilance database from worldwide reporting sources.[39]

Selection of PML cases

We included cases from postmarketing use ("spontaneous reports") for natalizumab and rituximab in which the reaction term involved "progressive multifocal leukoencephalopathy." Other types of reports, including cases emerging from clinical studies and literature, were excluded from the present study.

Data extraction

For each case, both the report from the initial reporter was retrieved, as well as the "master" report, which contains the most recent information on the same case, including any follow-up information received over time.

Information on the following standardized data elements[40] was extracted from the reports: administrative details (source country, receive date, reporter qualification, number of reports per case); patient characteristics (date of birth, age, sex); reactions details (reaction, reaction start date, reaction outcome); results of tests and procedures relevant to investigation of the patients, including autopsy results; and drug information (current and past drug use, indication for use, therapy start and stop dates, duration of use).

The data extraction was carried out at the EMA. Although ADR reports from the EudraVigilance database are published on http://www.adrreports.eu, these publicly available data contain too limited details to fulfill the aim of the present study. The data lock point for data extraction was 28 January 2013. This date matched the cutoff date of a quality check of PML reports in EudraVigilance, in which duplicate reports were detected and handled according to a predefined algorithm,[41] and followed by a manual deduplication step.

Data classification and analysis

Case characteristics. Information on patient, therapy, and report characteristics was retrieved from the most recent available ("master") reports. For cases in which the age was not reported, the age was calculated using the date of birth and the reaction start date (where available) or the receive date of the report. The treatment duration was calculated using the therapy start and stop dates, and the time to onset was calculated using the therapy and reaction start dates. When only the month and year had been provided, the 15th of the month was used by default. Indications for rituximab therapy were categorized into the following groups: lymphoid neoplasms, unspecified neoplasms, rheumatoid arthritis or other autoimmune disorders, and other conditions. For natalizumab, we categorized the indications into multiple sclerosis and other conditions. The source country was categorized into Europe, the United States, and other.

Temporal trends in the reporting. We used the receive date to calculate temporal trends in reporting of suspected cases of drug-induced PML. The receive date comprises the date on which the report was received by the initial stakeholder (i.e., regulatory agency or pharmaceutical company), thus, before the report is actually transmitted to the EudraVigilance database. The reporting trend was calculated by the number of reports per calendar month and graphically presented over time.

Changes in reporting rates for each product over time were identified by a previously described change-point analysis.[42] This method assumes that the number of reports follows a Poisson distribution with constant intensities between two subsequent change points. The procedure involves a multistep approach. In the first step, all data over the entire interval (0, T) are used to test the model of no change points (M0), against the model of one single change point (M1). If the data best fits model M0, the intensity is considered constant over the entire interval. If, however, model M1 is preferred over model M0, the procedure is repeated within the two newly formed intervals. The Bayesian information criterion approximation to the Bayes factor was used for model selection. An interval comprised a minimum of five reports and/or seven calendar days. The reporting rates in each interval between two change points were calculated by the average number of reports per month in the respective interval.

Cumulative patient exposure. Data on the estimated cumulative number of patients exposed to natalizumab and rituximab over time was extracted from publicly available sources, including literature articles, US Food and Drug Administration safety communications, and from the websites of the marketing authorization holders and was used to put the trends in reporting into context of the trends in patient exposure (see **Supplementary Material**).

Lag time between reaction and reporting. The lag time between onset of PML and initial reporting was calculated using the reaction date and receive date. When only the month and year was provided for the reaction date, the 15th of the month was used by default, unless this was before the receive date. Cases with lacking information on the reaction date, or only referring to the year of onset, were excluded from this analysis.

Completeness of case reports. For each case, we assessed the completeness of both the initial and the "master" report. The completeness was assessed along the availability of data for the following eight predefined variables: age and gender of the patient; therapy start date; therapy end date and/or duration of therapy; indication of therapy; previous drug history; start date of PML reaction; outcome of PML reaction; and diagnostic test results. Regarding the latter, in line with a recently proposed case definition for PML,[30] we assessed the availability of results for any of the following diagnostic tests: brain magnetic resonance imaging characteristic of PML; positive test result for John Cunningham virus DNA in cerebrospinal fluid; or evidence from an autopsy.

We assessed the trends in data completeness by comparing the first 50 cases (both the initial and master report) to the last 50 cases (both the initial and master report). For this analysis, we included only cases reported before 28 July 2012 (i.e., a half year before the data lock point of the data extraction) to allow for sufficient time for collection of any follow-up information.

Statistical methods

Data were presented as means, medians, or proportions, as appropriate. Normality was tested using Shapiro-Wilk's test. Independent sample *t* tests were used to compare normally distributed variables, and nonparametric testing was performed using the Mann-Whitney test. Categorical data were tested using Pearson's χ^2 tests.

Additional Supporting Information may be found in the online version of this article.

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AUTHOR CONTRIBUTIONS

M.L.D.B., N.S.V., S.S., A.K.M.T., A.H.S., T.E., and H.G.M.L. wrote the manuscript. M.L.D.B., N.S.V., S.S., A.K.M.T., T.E., and H.G.M.L. designed the research. M.L.D.B. and N.S.V. performed the research. N.S.V. analyzed the data.

CONFLICT OF INTEREST/DISCLAIMER

The authors declared no conflict of interest. The views expressed in this article are the personal views of the authors and may not be understood or quoted as being made on behalf of or reflecting the position of the MEB, or the EMA or one of its committees or working parties.

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