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Short report

Safety of breast feeding during rituximab treatment in multiple sclerosis

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ABSTRACT

Background There are limited data on the safety of breast feeding during rituximab therapy. Our objective is to determine exposure from breast feeding and biological effects of rituximab in breastfed infants.

Methods In our case series of six mother–infant pairs, the nursing mothers with relapsing-remitting multiple sclerosis received rituximab during breast feeding. As part of clinical follow-up, six serial breast milk samples, and blood samples from both mothers and infants, were collected and analysed.

Results The median average rituximab concentration (C_{avg}) in breast milk was 0.04 µg/mL and the estimated relative infant dose (RID) was 0.07%. The highest measured concentration of rituximab in the breast milk samples was 0.25 µg/mL, giving an estimated RID of 0.26%.

All infant serum rituximab concentrations were below 0.01 µg/mL. The CD19 +B cell count values were within the 10th– 90th percentiles of reported normal ranges in healthy infants.

Conclusions We found minimal transfer of rituximab into breast milk and could not reliably detect levels of rituximab in infant serum. B cell counts in infants were unaffected.

INTRODUCTION

Rituximab is an anti-CD20 IgG_1, and binding to its ligand on B-cells results in cytotoxicity. It is widely used to treat autoimmune diseases, though often off-label,[1] and it is particularly effective against relapsing-remitting multiple sclerosis (RRMS).[2] For mothers with MS, the first 3 months post partum is associated with an increased risk of MS relapses,[3] making early restart of high-efficacy disease-modifying therapy important. There are well-known health benefits with breast feeding for mother and infant, and for mothers with MS, exclusive breast feeding is associated with lower relapse rate post partum.[4] Therefore, the lack of data on safety of breast feeding during rituximab therapy is challenging for both the parents and healthcare providers.[5]

A cohort study reported low rituximab concentrations in breast milk in serial samples from four women treated with rituximab.[6] There are two other case reports,[7 8] reporting low concentrations in breastmilk, and the latter includes two serum samples from the breastfed infant where rituximab was not detected. However, the timing of collecting serum was not optimised to capture transfer of rituximab to the infant. To the best of the authors' knowledge, there are no studies with reports on systematically serial collections of serum from the infants. Hence, whether rituximab is safe during breastfeeding remains unclear.

We present six mothers with RRMS who breastfed their infant after rituximab infusion. Serial breast milk and blood samples were collected from each mother–infant pair. Analyses included concentration of rituximab, lymphocyte counts and immunoglobulin levels. We could thus evaluate infant exposure with unique data and reliable estimates, and potential haematological effects in the newborn.[9]

METHODS

The mothers were patients receiving rituximab for RRMS at the Department of Neurology, Haukeland University Hospital (HUS), Norway. They received treatment as soon as considered safe post partum. After being informed of the current knowledge of the transfer of rituximab to breast milk, the mothers chose to breastfeed. They were advised not to breastfeed the first 24 hours after infusion, due to pretreatment with intravenous methylprednisolone. As part of clinical follow-up, mother–infant serum and breast milk samples were collected to monitor child exposure throughout the anticipated period between infusion and complete elimination of rituximab from the mothers' serum.

Blood samples from the mothers and their infants, and mature breast milk samples, were collected before rituximab infusion, 2 days after infusion, after 1 week and after 1, 3 and 5 half-lives of rituximab (around day 22, 66 and 110). To secure good quality of the samples and to minimise their trauma, most of the infants' samples were drawn by phlebotomists at the Department of obstetrics at HUS. Analyses of blood cell counts, lymphocyte cell counts and immunoglobulin levels were all performed at the department for immunology and transfusion medicine at HUS. Samples for determination of rituximab concentrations were placed into polypropylene tubes (1–2 mL), frozen at –20°C and shipped overnight on dry ice for ELISA-based analyses at Sanquin Diagnostic Services (Amsterdam, the Netherlands). Measures of rituximab concentrations below the lower limit of detection were converted to correspond to the lower detection limit, 0.005 µg/mL, to avoid underestimating infant exposure.

Table 1 Characteristics of the mothers, their treatment and breast milk rituximab concentrations

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Rituximab infusions prior delivery, no	2	2	0	3	1	2
Time from last infusion to delivery, months	11	12.4	–	10.7	12	10.4
Time after delivery to infusion, days	13	20	23	30	26	31
Rituximab dose, mg	500	500	1000	500	500	500
AUC_{0-Tmax}, µg.d/mL	1.93	3.55	5.32	4.56	3.40	4.75
C_{avg}, µg/mL	0.017	0.031	0.048	0.041	0.035	0.043
C_{max}, µg/mL	0.09	0.1	0.25	0.1	0.2	0.12
Average daily infant dose, µg/kg/day	2.58	4.75	7.25	6.22	5.25	6.42
Absolute maximum daily infant dose, µg/kg/day	13.5	15	37.5	15	30	18.5
RID from C_{avg}, %	0.036	0.067	0.051	0.087	0.074	0.090
RID from C_{max}, %	0.19	0.21	0.26	0.21	0.42	0.26
Timing of samples after infusion, day	0, 2, 9, 22, 66, 112	0, 2, 8, 22, 67, 112	0, 4, 8, 22, 64, 110	0, 2, 8, 22, 69, 110	0, 3, 7, 21, 66, 97	0, 3, 8, 27, 84, 111

AUC, area under the curve; C_{avg}, average rituximab concentration measured in breast milk; C_{max}, maximum rituximab concentration measured in breast milk post infusion; RID, relative infant dose.

A simple index to assess the likelihood of dose-dependent effects in otherwise healthy breastfed infants, is the relative infant dose (RID): dividing the infant's daily dose via milk, to the mother's dose, both relative to weight.[10 11] We used the trapezoidal rule to calculate the area under the breast milk concentration/time curve (AUC) for each mother. The AUC was divided by the number of days between infusion and the last sample of breast milk was collected, to calculate the average breast milk rituximab concentration (C_{avg}) throughout the exposure interval. Assuming an infant drinks 150 mL/kg/day, we multiplied this with the C_{avg} in the breast milk, and found the absolute average infant dose over 24 hours. These numbers were divided by the mother's dose (500 or 1000 mg), relative to her weight and multiplied by 100, resulting in RID of C_{avg}, %. As their weights was not registered at time of infusion or when the samples were drawn, we used an estimation of 70 kg (based on average height and body mass index for women in Norway after giving birth).

RESULTS

The mothers were aged 27–38 years, and had a disease duration of three to 12 years, with EDSS-scores from 0.0 to 2.0. Their treatment history are shown in online supplemental table 1. Their deliveries were between July 2018 and March 2019. All the infants were healthy, and none born preterm. Three of the mothers breastfed exclusively, while the other three predominantly breastfeed with infant formulas alongside. The mothers' clinical characteristics are shown in table 1.

The C_{max} in breast milk was reached on average 4.5 days after infusion. The median C_{avg} in breast milk was 0.04 µg/mL and the corresponding estimated RID was 0.07%. The highest measured rituximab concentration among the breast milk samples was 0.25 µg/mL, giving an estimated RID of 0.26%, and was a sample from the mother who had received 1000 mg rituximab 4 days earlier. Individual measures of rituximab concentrations are shown in figure 1A (breast milk) and figure 1B (serum).

All rituximab concentration levels analysed from the infants' serum were below 0.01 µg/mL. Among these, the majority (80.6%) were below the limit of quantification of 0.005 µg/mL. Rituximab concentrations just above the lower limit were measured in baseline serum samples from infant number 2 and 4, 0.007 µg/mL and 0.008 µg/mL, respectively.

Well-established CD19+ B cell count reference values for infants are not determined, especially for early ages. However, our values are within the 10th– 90th percentiles (300–2000 cells/µL aged 0–3 months, 430–3000 cells/µL aged 3–6 months) of values found in a healthy infant population described by Shearer *et al*[12] (figure 1C). We found, as expected for healthy infants, that the B cell numbers increased the first months of life. The immunoglobulin levels in the mothers' serum were within the normal range, except for the three first IgG level measured from mother number four, which were lower. These were the baseline sample, and the samples taken on day 2 and eight postinfusion: 5.23, 5.58 and 5.58 g/L, respectively (online supplemental figure 1). All the infants' immunoglobulin levels were within the normal range, except for the IgM level of infant number three, which was higher than the reference range (online supplemental figure 2).

DISCUSSION

We found minimal transfer of rituximab to breast milk, with a median average rituximab concentration of 0.04 µg/mL in breast milk throughout five times the expected serum rituximab half-life. The infants' exposure to rituximab from breast milk were, on average, 0.07% (RID) of the maternal dose. The highest estimated RID of C_{max} was 0.42%. Both estimates are far below the commonly accepted threshold of concern of 10%. We could not reliably detect levels of rituximab in the infants' serum, and their B cell counts were unaffected. The immunoglobulin levels were mostly within the reference range.

Our findings are consistent with a cohort study of nursing mothers treated with rituximab for MS.[6] Serial samples from four of these mothers found a median C_{avg} of rituximab in breast milk of 0.063 µg/mL, and an estimated RID of 0.08%, very similar to ours. Another case report[7] described a nursing mother treated with 1000 mg of rituximab for granulomatosis with polyangiitis. Samples of the mother's serum and her breast milk were collected on four consecutive days, starting on day 7 after infusion. They found breast milk rituximab concentrations between 0.4 and 0.6 µg/mL and serum concentrations between 110 and 130 µg/mL. The serum concentration in the mother was similar to the patient who received 1000 mg in our group, but the breast milk concentrations were more than twice the concentration in our samples. However, the breast milk rituximab concentration can still be characterised as low. A more recent case report describes a mother with myeloperoxidase antineutrophil cytoplasmatic antibody (MPO-ANCA) positive vasculitis who received rituximab 500 mg 4 months post partum because of increased MPO-ANCA titre.[8]

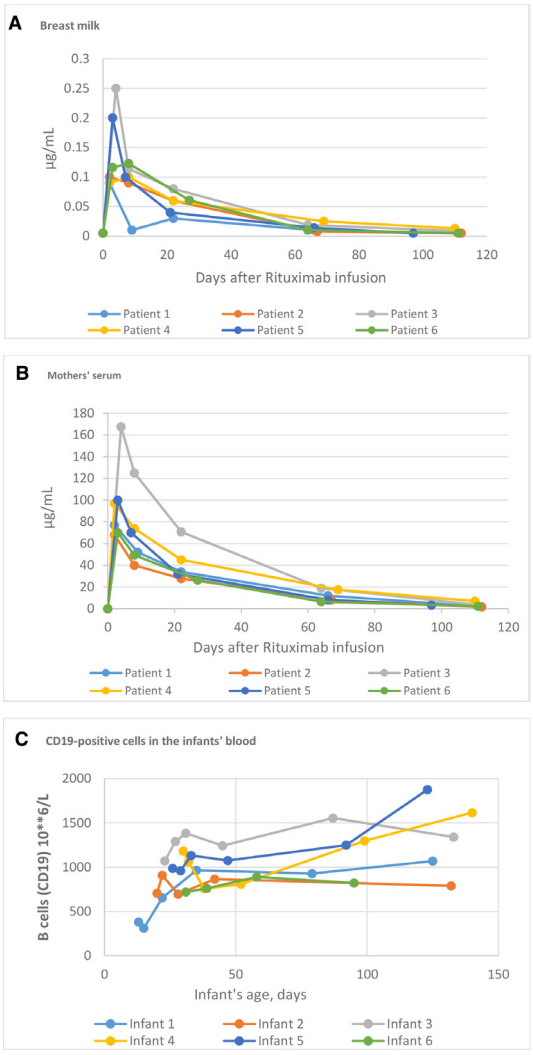

Figure 1 Rituximab concentrations and CD19+ positive cells. (A) In the breast milk samples. Each line represents individual rituximab concentrations (µg/mL) of the patients' breast milk samples from the day of rituximab infusion (baseline, x=0) and up to five half-lives of rituximab (about 110 days). (B) In the mothers' serum samples. Each line represents individual serum concentrations of rituximab of the patients from the day of rituximab infusion (baseline, x=0) and up to five half-lives of rituximab (about 110 days). Patient number 3 received a dose of 1000 mg rituximab intravenous, while the other patients received a dose of 500 mg. (C) CD19+ cells in the infants' blood. Each line represents individual levels of CD19+ cells in the infants' blood. Infant number 1 is the child of patient number 1, and so on. The first point on every line represents the test taken the same day their mothers received rituximab infusion, x days after birth. 10th–90th percentiles among healthy infants: 300–2000 CD19+ cells/µL aged 0–3 months, 430–3000 CD19+ cells/µL aged 3–6 months.[12]

Breast milk samples four consecutive days after infusion showed an average rituximab concentration of 0.0037 µg/mL (maximum 0.004), thus relatively low compared with our results. Serum samples from the infant were drawn after four and 24 hours, with no detectable levels, though these time points for the tests might be too early to capture any rituximab transfer.

Newborns are dependent on the transfer of immunoglobulins from the mother to provide systemic immunity. Most of newborns' IgG is of maternal origin, transferred by selective, active transplacental transport late in pregnancy. There is some transfer of immunoglobulins via breast milk, but mainly IgA, and only 5%–6% IgG.[13] When ingested, most immunoglobulins are denatured due to acidic pH or proteolytic digestive enzymes in the gastrointestinal tract. There is little or no absorption of the immunoglobulins through the infant's intestinal mucosa to its circulation.[14] With this in mind, our findings are as expected, as rituximab is an antibody of the IgG isotype.

The strength of our case series is the complete collection of serial blood samples from the mothers and their infants, and of breast milk. The analyses allow us to estimate RID and to evaluate potential biological effects from rituximab exposure in infants: effects on blood cell counts including specific B cell counts and immunoglobulin levels. To the best of the authors' knowledge, these are unique results that have not been described previously.

However, our case series has some limitations. To estimate the RID, we need the mothers' weight. As this was not registered, we used an estimation, and this makes our RID estimations less accurate. The baseline samples from infant 2 and 4 showed rituximab concentrations just above the lower threshold for detection. These are likely to be false positives, as these mothers received therapy more than 10 months before the samples were drawn. Further, one should be aware that our results reflect transfer through mature breast milk. Colostrum and transitional milk, produced the first 2 weeks post partum, was not analysed and might have a different concentration. Similarly, prematurely born infants are not included, and one cannot out rule the possibility of larger ingestion of drugs through an immature gut. Also, mastitis, or possibly other systemic inflammations, may allow passage of larger molecules to breast milk, and greater drug passage than predicted.[15] The immunoglobulin levels in serum in our case series are mostly within the reference ranges, but for mothers with secondary hypogammaglobulinaemia and their infants, the case might be different.[16] Future studies on the breast milk compound of these mothers and their infants' development are needed.

In conclusion, we found that the level of rituximab ingested by the breastfeeding infants over a 110-day interval postinfusion is minimal, with no detectable biological effect on infant blood cell counts. Our results indicate that treatment with rituximab during the postpartum period does not exclude mothers from breast feeding their infants.

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Contributors Conception of project: LB and SW. Data acquisition: SW. Data analysis and interpretation: BER and SW. Drafting the manuscript: BER. Critical revision of the manuscript for important intellectual content: ØT, K-MM, LB and SW. All authors revised the manuscript and approved the final draft.

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Competing interests BER reports no disclosures. ØT received speaker honoraria from and served on scientific advisory boards for Biogen, Sanofi-Aventis, Merck and Novartis. K-MM has received scientific advisory board or speaker honoraria from Biogen, Novartis and Roche, and has participated in clinical trials organised by Biogen, Merck, Novartis, Roche and Sanofi. LB has received unrestricted research grants to his institution and speaker honoraria from Almirall, Biogen, Genzyme, Merck, Novartis, Roche, and Teva, and has participated in clinical trials organised by Biogen, Merck, Novartis, Roche and Genzyme. SW has received honoraria from Biogen, Novartis and Sanofi.

Patient consent for publication Consent obtained directly from patient(s).

Ethics approval This study involves human participants but the Regional Committees for Medical and Health Research Ethics of Western Norway waived the need for ethics approval in the present case series as it is not subject of the Federal

Act on Research involving Human Beings (Helseforskningsloven, §§2 and §4a, 20 June 2008). Reference number: 469578. Participants gave informed consent to participate in the study before taking part.

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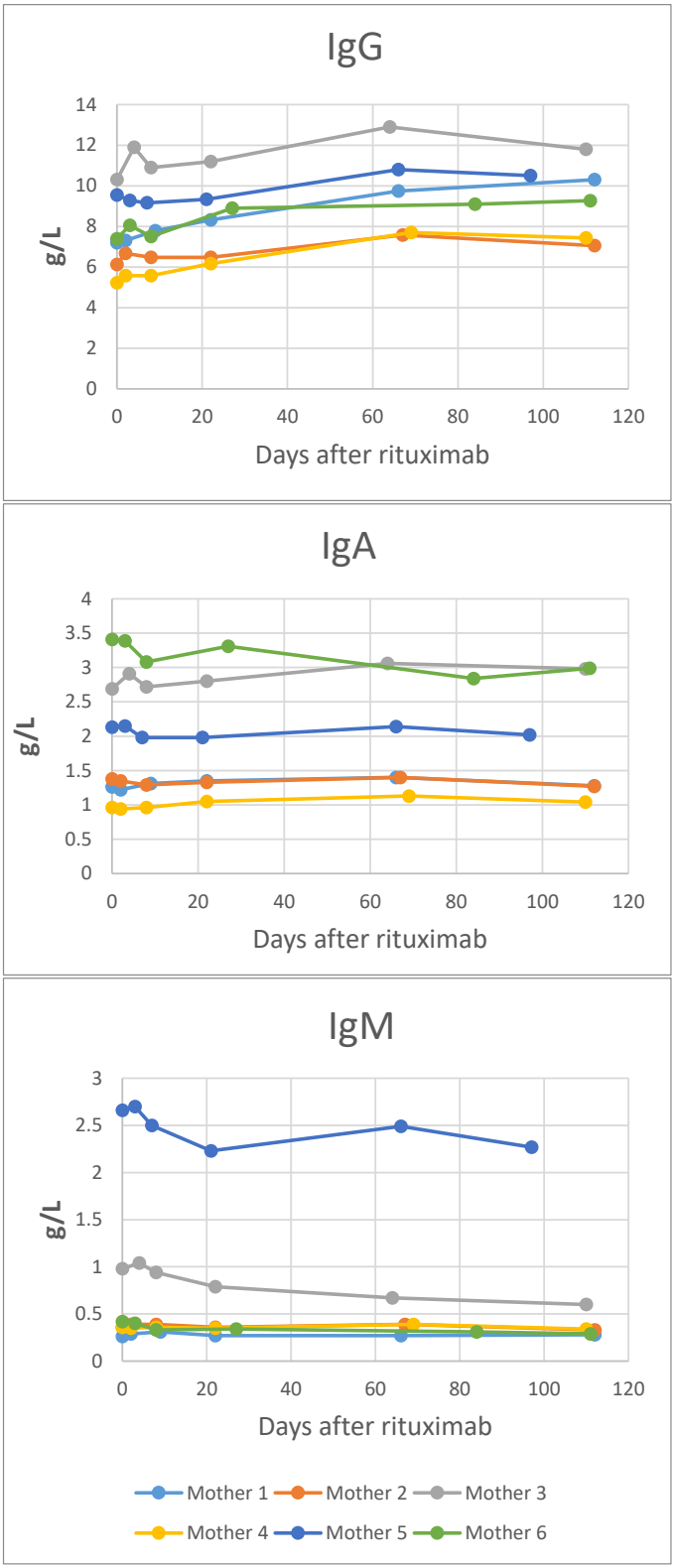
SUPPLEMENTAL MATERIAL

Supplementary figure 1: Immunoglobulin levels in the mothers' serum



Reference ranges for serum immunoglobulin for adults:

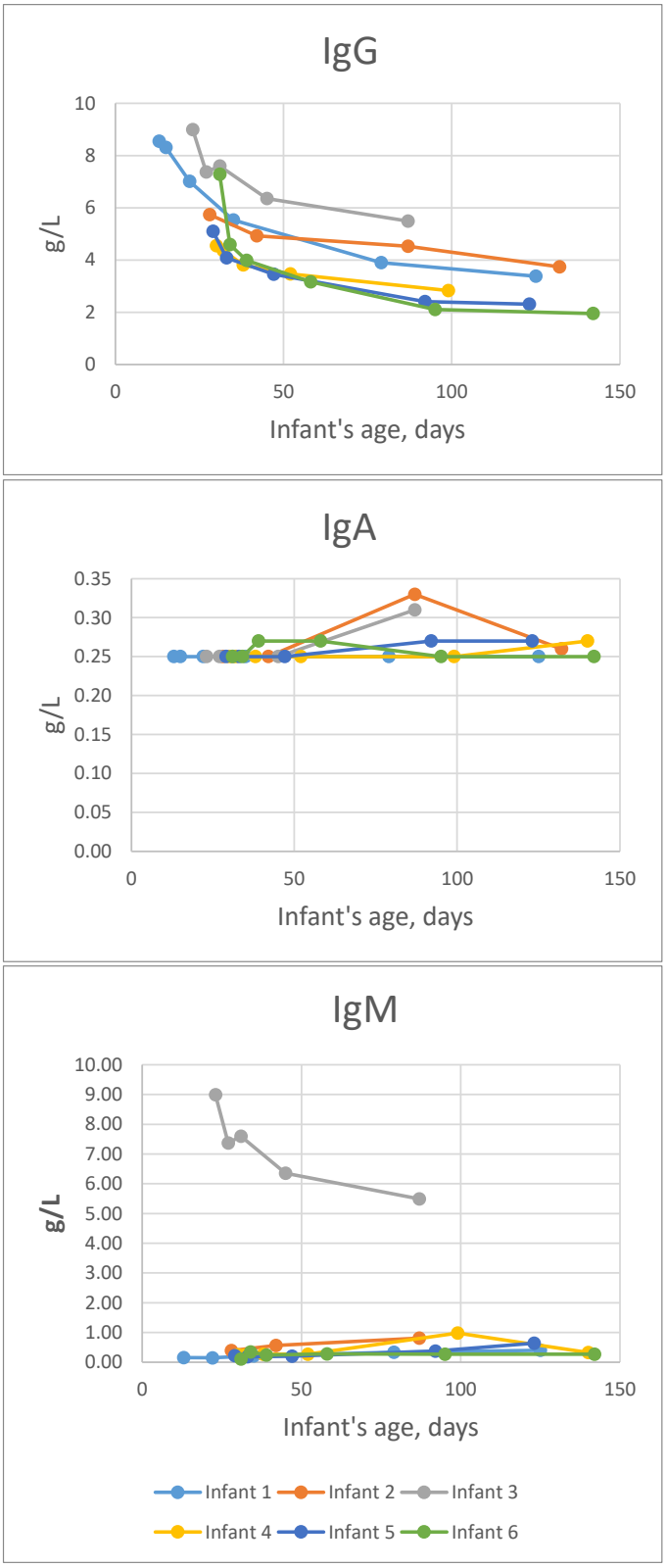
IgG: 6.0–15.3 g/L

IgA: 0.8–4.0 g/L

IgM: 0.3–2.3 g/L

The levels are within the reference ranges, except from the three first IgG values of the serum samples taken of mother number four: on day 0, 2 and 8. They were 5.23, 5.58 and 5.58 g/L, thus not far from the lower range of 6 g/L.

Supplementary figure 2: Immunoglobulin levels in the infants' serum



Age-related reference ranges used for immunoglobulins at HUS:

IgG

0–30 days: 6–13 g/L
30–90 days: 2.1–9.4 g/L
90–180 days: 1.7–8.5 g/L

The IgG levels are high in the newborn period, but decrease until their sixth month of life.[1]

IgA

0–30 days: 0–0.1 g/L
30–90 days: 0–0.6 g/L
90–180 days: 0–0.85 g/L

Note: The IgA levels <0.25 g/L are here marked as 0.25 g/L.

IgM

0–30 days: 0–0.1 g/L
30–90 days: 0.1–1.1 g/L
90–180 days: 0.1–1.2 g/L

IgM levels are within the ranges, except for the high IgM levels in infant number 3.

Supplemental table 1: Disease-modifying treatments prior to rituximab

Patient 1	Interferon beta-1b, fingolimod, dimethyl fumarat and alemtuzumab
Patient 2	Fingolimod, natalizumab and dimethyl fumarat
Patient 3	None.
Patient 4	Interferon beta-1a, glitrameracetat and dimethyl fumarat
Patient 5	Dimethyl fumarat
Patient 6	Interferon beta-1b and natalizumab

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