

# Rituximab Serum Concentrations and Anti-Rituximab Antibodies During B-Cell Depletion Therapy for Myalgic Encephalopathy/Chronic Fatigue Syndrome

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## ABSTRACT

**Purpose:** Previous Phase II trials indicated clinical benefit from B-cell depletion using the monoclonal anti-CD20 antibody rituximab in patients with myalgic encephalopathy/chronic fatigue syndrome (ME/CFS). The association between rituximab serum concentrations and the effect and clinical relevance of antidrug antibodies (ADAs) against rituximab in ME/CFS is unknown. We retrospectively measured rituximab concentrations and ADAs in serum samples from patients included in an open-label Phase II trial with maintenance rituximab treatment (KTS-2-2010) to investigate possible associations with clinical improvement and clinical and biochemical data.

**Methods:** Patients with ME/CFS fulfilling the Canadian criteria received rituximab (500 mg/m<sup>2</sup>) infusions: 2 infusions 2 weeks apart (induction), followed by maintenance treatment at 3, 6, 10, and 15 months. The measured rituximab concentrations and ADAs in serum samples included 23 of 28 patients from the trial.

**Findings:** There were no significant differences in mean serum rituximab concentrations between 14 patients experiencing clinical improvement versus 9 patients with no improvement. Female patients had higher mean serum rituximab concentrations than male patients at 3 months ( $P = 0.05$ ). There was a significant negative correlation between B-cell numbers in peripheral blood at baseline and rituximab serum concentration at 3 months ( $r = -0.47$ ;  $P = 0.03$ ). None of the patients had ADAs at any time point.

**Implications:** Clinical improvement of patients with ME/CFS in the KTS-2-2010 trial was not related to rituximab serum concentrations or ADAs. This finding is also in line with a recent randomized trial questioning the efficacy of rituximab in ME/CFS. Rituximab concentrations and ADAs still offer supplemental information when interpreting the results of these trials. (*Clin Ther.* 2018;■:1–9) © 2018 Published by Elsevier Inc.

**Key Words:** antidrug antibodies, B-cell depletion, chronic fatigue syndrome, myalgic encephalopathy, rituximab, rituximab concentrations.

## INTRODUCTION

Myalgic encephalopathy/chronic fatigue syndrome (ME/CFS) is a disease of unknown etiology affecting ~0.2% of the population.<sup>1</sup> Patients with ME/CFS report a very low quality of life.<sup>2</sup> The main symptoms are profound fatigue, postexertional malaise, sleep disturbances with inadequate restitution, pain, impaired cognitive function, and several symptoms related to autonomic dysfunction and to the immune system.<sup>3</sup> Presently, there is no established standard interventional drug treatment for ME/CFS. Several observations support a role of immune disturbance in a subset of patients with ME/CFS: the female preponderance (3–4 times more common in women), an often abrupt start after

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infection (~70%), a genetic predisposition,<sup>4</sup> and studies indicating that partly overlapping syndromes such as postural orthostatic tachycardia syndrome<sup>5</sup> or complex regional pain syndrome<sup>6</sup> may have an autoimmune basis. A possible role of autoimmunity in ME/CFS has been suggested.<sup>7–10</sup>

Rituximab is a chimeric immunoglobulin G (IgG) monoclonal therapeutic antibody that targets CD20 and promotes a rapid and prolonged but reversible peripheral B-cell depletion,<sup>11</sup> with proven efficacy in lymphomas and in several rheumatic and autoimmune disorders.<sup>12</sup> B-cell depletion is associated with target-mediated elimination of rituximab.<sup>13</sup> Antidrug antibodies (ADAs) can also promote more rapid clearance of rituximab and change of clinical effect.<sup>14</sup> We have previously suggested a clinical benefit from B-cell depletion in patients with ME/CFS using the monoclonal anti-CD20 antibody rituximab in a small, randomized, placebo-controlled study (KTS-1-2008).<sup>7</sup> Prolonged responses were then shown in an open-label Phase II trial with maintenance rituximab treatment (KTS-2-2010).<sup>15</sup> However, we recently completed a multicenter, randomized, double-blind Phase III trial investigating rituximab maintenance treatment versus placebo (RituxME [B-Lymphocyte Depletion Using the Anti-CD20 Antibody Rituximab (Mabthera®) in Myalgic Encephalopathy/Chronic Fatigue Syndrome]; [ClinicalTrials.gov](https://clinicaltrials.gov) identifier NCT02229942) and concluded that there were no significant differences in outcome measures between the rituximab and placebo groups (submitted).<sup>16</sup> The relationships between serum rituximab concentrations and efficacy have been studied in lymphomas<sup>17–20</sup> and in systemic autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus.<sup>21–23</sup> The associations of rituximab serum concentrations to effect, and the clinical relevance of antidrug antibodies (ADAs) against rituximab in ME/CFS, have not been described. A possible association would have been important when deciding doses and making new protocols for B-cell depletion in the future, should the treatment principle demonstrate clinical efficacy. However, rituximab is not an established treatment for ME/CFS, nor is B-cell depletion a proven cause of symptom improvement in these patients. Based on the available knowledge at the time of the study, and as part of a broader approach to better understanding the disease mechanisms and possible reasons why a subgroup of patients reported benefit after rituximab

treatment, we analyzed serum samples from patients included in the KTS-2-2010 clinical trial.<sup>15</sup> We retrospectively measured rituximab concentrations and ADAs in serum samples harvested during follow-up to investigate possible associations with clinical improvement of ME/CFS symptoms, sex, and B-cell numbers in peripheral blood.

## PATIENTS AND METHODS

### Ethics, Trial Design, and Patient Cohorts

The clinical trial, including one amendment, was approved by the Regional Ethical Committee in Norway (no. 2010/1318-4) and by the National Medicines Agency. All patients gave written informed consent. The trial was conducted in accordance with Good Clinical Practice. The design and results of the rituximab maintenance trial have been previously reported.<sup>15</sup> KTS-2-2010 was a single-center, open-label, one-armed Phase II study (NCT01156909) that included 29 patients. The treatment schedule was rituximab (500 mg/m<sup>2</sup>; maximum, 1000 mg) 2 infusions 2 weeks apart (induction), followed by maintenance rituximab infusions (same dose) after 3, 6, 10, and 15 months and with follow-up for 36 months. The inclusion criteria were a diagnosis of ME/CFS according to the Fukuda 1994 criteria<sup>24</sup> and age 18–66 years. All patients also fulfilled the Canadian criteria.<sup>3</sup> Further characterization of the inclusion and exclusion criteria is included in the trial results previously published.<sup>15</sup>

The present study analyzed serum rituximab concentrations in 23 patients for whom samples were still available in the biobank from the 28 patients who received rituximab maintenance infusions in the KTS-2-2010 trial. Six patients were not included for serum rituximab measurements: 2 pilot patients (no biobank sampling), 2 patients who withdrew from the study during follow-up (1 due to an allergic reaction and 1 due to intercurrent disease), 1 who changed treatment to the anti-CD20 antibody ofatumumab due to an allergic reaction during the third rituximab infusion, and 1 due to missing biobank samples. Of the 23 patients, 15 received six rituximab infusions, 6 received five infusions, and 2 patients received four infusions (Table 1). This scheme was according to protocol because patients with no signs of clinical improvement at 10 months of follow-up could forgo the planned rituximab infusions at 10 and/or 15

Table 1. Rituximab (RTX) serum concentrations and clinical data for 23 patients with myalgic encephalopathy/chronic fatigue syndrome (ME/CFS) in the KTS-2-2010 trial.

Sex, Age (y)	BSA (m <sup>2</sup> )*	ME/CFS Duration (y) and Severity <sup>†</sup>	RTX Dose (mg)	No. of RTX Infusions	RTX Level (µg/mL) <sup>‡</sup>		RTX Level (µg/mL) <sup>‡</sup>		B Cells <sup>§</sup> at Baseline	Clinical Improvement <sup>  </sup>	Response at End of Study (3 Years)
					At 3 Months	At 6 Months	At 10 Months	At 15 Months			
F, 44	1.83	5 y, sev	925	6	16.0–16.0	10.0–10.0	1.1–5.2	4.8–1.1	201	Yes	No
F, 37	1.55	20 y, mod	800	5	9.3–8.2	11.0–9.7	3.5–3.1	1.2–1.1	282	No	No
M, 58	2.12	17 y, mod	1000	6	11.0–11.0	4.7–4.7	1.3–1.4	0.9–0.8	137	Yes	Yes
F, 26	1.79	3 y, mild/mod	900	5	10.0–10.0	8.6–8.6	2.3–2.0	0.4–0.4	851	No	No
F, 22	1.48	5 y, mod	750	6	14.0–14.0	11–9.7	2.5–2.8	0.7–0.8	146	Yes	Yes
M, 49	1.66	17 y, mod/sev	850	6	14.0–14.0	5.7–5.0	1.6–1.6	0.4–0.4	117	Yes	Yes
M, 20	1.80	8 y, mild	900	6	1.3–1.3	23–23.7	11.0–10.7	4.9–5.2	436	Yes	Yes
F, 28	1.71	12 y, mod	850	6	41.0–41.0	5.1–5.3	1.4–1.2	0.5–0.5	217	Yes	Yes
F, 37	1.75	10 y, mild	850	6	15.0–15.0	7.1–7.1	2.5–2.5	0.1–0.4	156	Yes	No
F, 32	1.55	9 y, mod/sev	800	6	11.0–11.0	4.7–4.7	1.6–1.6	13.0–0.6	m	No	No
F, 42	1.92	5 y, mild	950	4	12.0–10.9	7.2–6.4	1.5–1.3	m	365	No	No
F, 20	1.62	7 y, mod	800	6	22.0–14.2	5.3–7.4	1.3–1.2	0.7–0.7	110	No	No
M, 48	2.12	12 y, mod	1000	6	1.4–1.4	3.4–3.4	1.1–1.1	0.5–0.5	48	Yes	Yes
F, 46	1.61	13 y, mod	800	6	14.0–14.0	7.4–7.4	1.5–1.5	0.7–0.8	619	Yes	Yes
F, 25	1.74	11 y, mod	850	5	7.5–7.5	0.4–2.9	0.6–1.2	m	1151	Yes	Yes
F, 55	1.75	8 y, mod	850	5	20.0–20.0	12.0–7.7	3.2–3.9	m	m	No	No
M, 59	2.04	3 y, mod/sev	1000	6	5.3–5.3	3.5–4.5	1.1–0.9	0.3–0.4	286	Yes	No
F, 37	1.87	20 y, mild	900	6	11.0–11.0	2.0–3.1	9.5–2.5	1.3–1.4	79	Yes	Yes
F, 49	1.70	13 y, mod	850	4	14.0–14.0	11.0–17.1	m	m	222	No	No
F, 56	1.79	5 y, sev	875	5	26.0–22.9	11.0–11.0	5.8–5.8	m	293	No	No
M, 26	1.80	8 y, mild/mod	900	6	19.0–19.0	13.0–10.4	2.7–3.4	m	159	Yes	Yes
F, 47	1.63	4 y, mod	825	6	24.0–21.2	20.0–10.3	6.6–7.3	13.0–2.6	165	Yes	No
M, 50	2.08	1 y, mild	1000	5	10.0–10.0	3.3–1.7	3.5–2.8	m	241	No	No

BSA = body surface area; F = female; M = male.

\* According to the Du Bois method.

<sup>†</sup> Categorized as mild, mild/moderate (mild/mod), moderate (mod), moderate/severe (mod/sev), or severe (sev).

<sup>‡</sup> RTX serum concentrations, measured concentration - concentration adjusted to median time interval since last dose.

<sup>§</sup> B-cell numbers in peripheral blood (×10<sup>6</sup>/L) at baseline (m indicates missing sample).

<sup>||</sup> Clinical improvement according to predefined criteria in the KTS-2-2010 rituximab maintenance trial (ie, fatigue score >4.5 for 6 consecutive weeks; fatigue score with scale 0–6, in which 3 is no change from baseline and higher scores indicate less fatigue).

months. The characteristics of the patients and response data are shown in the [Table 1](#).

### Measurement of Rituximab Concentrations and ADAs

All serum samples used for rituximab measurements were gathered immediately before the next scheduled rituximab infusion and frozen at  $-80\text{ }^{\circ}\text{C}$  according to the standardized biobank procedure in the trial protocol.<sup>15</sup> For all 23 included patients, serum samples were available at 3 and 6 months' follow-up. At 10 months, there were 22 samples available, and at 15 months, 16 serum samples were available. In the protocol for the KTS-2-2010 trial, the interval between the maintenance doses (at 3, 6, 10, and 15 months) could vary 1–2 weeks, and in some patients, doses were postponed due to concomitant disease or other circumstances. Due to this naturalistic setting, the dosing interval and thus the sampling time could vary between patients at each new rituximab maintenance dose. Measured drug concentrations were adjusted according to an estimated median  $t_{1/2}$  of 22 days according to the Summary of Product Characteristics for rituximab [https://www.ema.europa.eu/documents/product-information/mabthera-par-product-information\\_en.pdf](https://www.ema.europa.eu/documents/product-information/mabthera-par-product-information_en.pdf).\*

Assays for serum rituximab concentrations and ADAs were performed by the Biologicals Laboratory, Diagnostic Services Sanquin (Amsterdam, the Netherlands). Measurements were performed according to the International Organisation for Standardization 15189 guideline. Rituximab concentrations were determined by using sandwich ELISA. In short, anti-rituximab-idiotype antibodies were generated in rabbits by immunization with rituximab F(ab)2. After purification of IgG by using Protein A Sepharose (GE Healthcare, US), reactivity against human IgG was removed by passage over a Sepharose-4B column. IVIG is a therapeutic intravenous IgG preparation prepared from >1000 blood donors. Antibodies that did not bind to the column were unreactive with serum IgG but showed strong binding to rituximab but not to adalimumab, infliximab, or natalizumab. They were used for

coating the ELISA plate and, after biotinylation, also as a detecting agent.

The detection limit of the assay is  $\sim 0.8\text{ }\mu\text{g/L}$ . Because sera are tested at 1:10 dilution or higher, the detection limit in serum is  $8\text{ }\mu\text{g/L}$ . The accuracy of the test is 110% (precision, 11.3%). ADAs were detected in an antigen-binding test using Protein A Sepharose for catching patient serum IgG and  $^{125}\text{I}$ -radiolabeled rituximab F(ab)2. Samples containing IgG antibodies against rituximab did not yield positive results in assaying for anti-adalimumab, anti-infliximab, or anti-natalizumab antibodies.

### Statistical Analyses

Serum rituximab concentrations from patients with different dosing intervals were made comparable by an estimated median  $t_{1/2}$  of 22 days according to the product monograph of rituximab. All blood samples were withdrawn  $\geq 3$  half-lives after each dose. Assuming similar rituximab terminal elimination kinetics between patients, and using the actual measured rituximab dose at the specified interval since last dose, we calculated adjusted rituximab concentrations corresponding to the median time intervals for each patient. We used the formula  $N(t) =$

$$N_0 \left( \frac{1}{2} \right)^{\frac{t}{t_{1/2}}}, \text{ where } N_0 \text{ is the initial concentration}$$

(calculated from  $t_{1/2}$  and time interval), and  $N(t)$  is the estimated concentration after time ( $t$ ). This assessment was performed to generate comparable rituximab concentrations corresponding to the same time interval since the last rituximab dose, and these data were used for analyses.

Serum rituximab concentrations (adjusted) were correlated to B-cell numbers in peripheral blood at baseline and through follow-up. General linear model for repeated measures (GLM) was used, with the interaction term (time\*group) assessing differences in course of adjusted serum rituximab concentrations, between patients with clinical improvement versus no improvement, and female patients versus male patients. Greenhouse-Geisser corrections were used. For GLM, samples from 22 patients at 3, 6, and 10 months were included; 15-month data were excluded because of missing samples. The Mann-Whitney U test for independent samples was used to assess differences in adjusted serum rituximab concentrations between groups at specific time points during follow-up, not

\* Trademark in Norway: MabThera<sup>®</sup> (Roche, Basel, Switzerland).

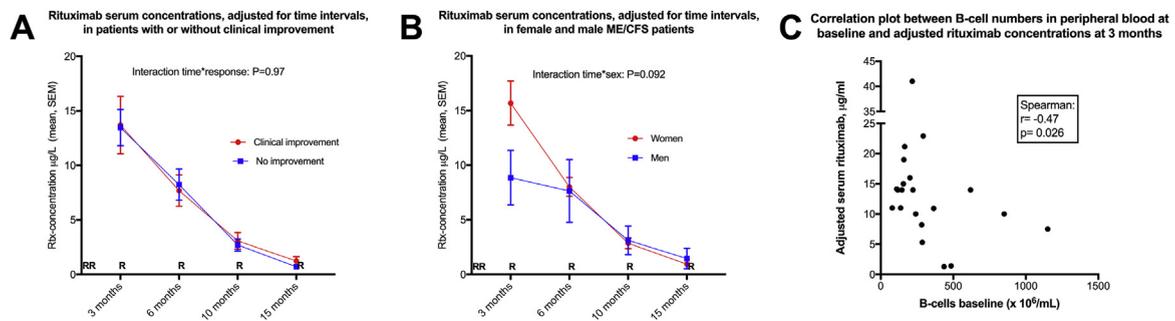


Figure 1. Rituximab serum concentrations during follow-up, adjusted for time intervals, in (A) 14 patients with and 9 without clinical improvement and in (B) 16 female and 7 male patients with myalgic encephalopathy/chronic fatigue syndrome (ME/CFS). The rituximab serum concentrations were assessed in samples taken at 3, 6, 10, and 15 months' follow-up, immediately before the scheduled infusion. The "R" in panels A and B indicate time points for rituximab infusions according to the trial protocol. P values from the general linear model for repeated measures are also shown. Error bars indicate mean with SEM. C, Correlation plot between B-cell numbers in peripheral blood at baseline and adjusted rituximab serum concentrations at 3 months' follow-up. Spearman correlations analysis between B-cell numbers in peripheral blood at baseline versus adjusted serum rituximab concentrations at 3 months' follow-up are shown in 21 patients with ME/CFS with available data.

taking into account repeated measures. Spearman analyses were used to assess correlations between serum rituximab concentrations and B-cell numbers in peripheral blood. A 2-sided  $P$  value  $< 0.05$  was considered statistically significant.

## RESULTS

Rituximab serum concentrations and clinical data for the 23 patients with ME/CFS are shown in the Table 1. Both measured value for rituximab serum concentrations and adjusted values to median time interval since last dose are presented. There were large interindividual differences in adjusted serum rituximab concentrations at all time points. Using GLM repeated measures (including 3, 6, and 10 months), there was no difference in repeated measures of adjusted serum rituximab concentrations between patients with clinical improvement versus no improvement during follow-up ( $P = 0.97$ ), not for the course through follow-up nor at any of the specific time points of 3, 6, 10, or 15 months (Figure 1). None of the 23 patients had ADAs at any time point.

The adjusted serum concentrations of rituximab at 3, 6, 10, and 15 months according to sex are shown in the Figure 1. There was a trend for difference in

repeated measures of adjusted serum rituximab concentrations between women and men assessed according to the interaction time\*sex ( $P=0.092$ ), with higher mean serum rituximab concentrations in female patients at 3 months ( $P = 0.05$ ).

Higher B-cell numbers in peripheral blood at baseline correlated significantly with lower rituximab serum concentrations at 3 months ( $r = -0.48$ ;  $P = 0.03$ ). Correlation analyses (Spearman) revealed negative but not significant correlations between B-cell numbers in peripheral blood at 15 months and rituximab serum concentrations at 3 months ( $r = -0.29$ ;  $P = 0.22$ ), 6 months ( $r = -0.03$ ;  $P = 0.94$ ), and 10 months ( $r = -0.04$ ;  $P = 0.88$ ) of follow-up.

## DISCUSSION

The current study is the first to examine the associations between rituximab serum concentrations and clinical improvement after B-cell depletion among patients with ME/CFS. The main finding was that any clinical effect of rituximab in patients with ME/CFS was not associated with serum concentrations or ADAs. We found a large interindividual variability for serum rituximab concentrations at the different time points, in

accordance with findings from other studies.<sup>18,25,26</sup> All patients had detectable serum concentrations of rituximab at 15 months (ie, 5 months after the last infusion). The lack of ADAs suggests a low risk of immunogenicity of rituximab in ME/CFS. Furthermore, ADAs could not explain the variability of rituximab concentrations or clinical effect.

Lack of associations between rituximab concentrations and clinical effect suggest that a concentration–effect relationship does not explain previously observed beneficial effects of the drug.<sup>15</sup> A recently completed (submitted) multicenter, randomized, double-blind Phase III trial investigating rituximab maintenance treatment versus placebo concluded that there were no significant differences in outcome measures between the rituximab and placebo groups.<sup>16</sup> This outcome casts doubt on the effects of rituximab intervention in ME/CFS in previous trials as well,<sup>7,15</sup> in which the improvements of ME/CFS symptoms could also have been caused by either placebo mechanisms or by natural variation over time. However, presently, we cannot exclude the possibility that selection mechanisms in previous trials could also be a relevant factor and that there may be a small subgroup of patients with ME/CFS with disease responsive to B-cell depletion. Thus, the assessment of associations between serum rituximab concentrations and clinical status of patients characterized as either responders or nonresponders, and presence of ADAs, is still interesting and offers supplemental information when interpreting the results.<sup>7,15</sup> In our opinion, it is highly relevant to include drug measurements when treating a new patient group off-label in clinical research.

Studies in patients with indolent lymphoma have suggested an association between higher serum rituximab concentrations and progression-free survival interval.<sup>17–19</sup> Serum trough concentrations of rituximab and AUC-time curves were higher for responders than for nonresponders in a study of aggressive B-cell lymphoma.<sup>20</sup> Results of studies in patients with rheumatoid arthritis have been inconclusive for the associations between rituximab serum concentrations and clinical responses. One study concluded that the variability in rituximab serum concentrations and ADA formation was not related to the clinical responses to rituximab,<sup>26</sup> whereas another study concluded that clinical

responses depended on the degree of B-cell depletion but not on the rituximab doses given.<sup>27</sup>

Although the number of patients in the current study was low, we can now assume that the concentration of rituximab and the degree of B-cell depletion is not the main mechanism for symptom improvement in the patients with ME/CFS. This observation does not exclude the involvement of B cells or the immune system in the disease mechanisms. Body surface area (BSA) is mainly used for calculating induction and maintenance doses when treating lymphoma patients with rituximab intravenous infusions, whereas for the subcutaneous rituximab formulation, a fixed rituximab dose is common.<sup>13</sup> In systemic rheumatic diseases, different rituximab dosing regimens exist, but fixed doses with 6-month intervals are often used. One study concluded that sex and BSA explained ~32% of the interindividual variance for clearance, and 42% of the variance for the distribution volume.<sup>25</sup> In the KTS-2-2010 trial, we used BSA<sup>28</sup> when dosing rituximab; however, wide interindividual ranges of drug concentrations at each time point remained during follow-up.

Interestingly, female patients with ME/CFS had higher serum rituximab concentrations at 3 months of follow-up compared with male patients. Higher rituximab concentrations are known to be associated with female sex both in lymphoma treatment<sup>13</sup> and in rheumatoid arthritis.<sup>25</sup> Higher rituximab serum concentrations have previously been observed in women with rheumatic diseases, believed to be due to a higher distribution volume of the drug in men.<sup>25,29</sup> Data suggest that female patients with lymphoma benefit from rituximab-containing regimens more than men, possibly due to higher serum concentrations throughout induction and maintenance.<sup>13</sup>

None of the study patients had antibodies (ADAs) to rituximab at any time point. Antibody production represents an adaptive response and usually takes days to weeks following treatment exposure. The presence and extent of immunogenicity after monoclonal antibody administration vary and depend on several factors, most of which are related to the patients themselves, the antibodies, or the treatment regimen.<sup>30</sup> Monoclonal antibodies (mAbs) that deplete B cells, thereby attenuating the immune response, seem to be at the lower end of the

immunogenicity scale from other mAbs.<sup>31</sup> We only analyzed for ADA of IgG type, which are responsible for the majority of the ADA responses. The pharmacokinetic variability of mAbs is usually large and can partly be explained by ADAs, which accelerate mAb elimination,<sup>27</sup> but this theory could not explain the large interindividual variability in serum concentrations between patients in our cohort. A review article described no immunization with ADAs in patients with B-cell malignancies treated with rituximab, but a few patients with rheumatoid arthritis developed ADAs.<sup>27</sup> A study that compared intravenous and subcutaneous administration of rituximab in patients with follicular lymphoma detected ADAs in only 1 of 278 patients.<sup>32</sup>

B-cell numbers in peripheral blood at baseline were inversely correlated to rituximab serum concentrations at 3 months of follow-up. The association between a higher B-cell count before intervention and subsequent lower serum rituximab concentrations is expected and has been described by others,<sup>25</sup> possibly due to increased presence of the CD20 target and thus more rapid clearance of rituximab. Also, the effective B-cell depletion and reduction of CD20-positive cells after the first infusion result in a decrease in rituximab clearance following subsequent infusions due to the very low number of B cells present.<sup>33</sup> There were negative, but not significant, correlations between rituximab serum concentrations at 3 or 6 months and B-cell numbers in peripheral blood at 15 or 20 months of follow-up. However, the very low numbers of B cells at 15 months in most patients ( $0-2 \times 10^6/\text{mL}$ ) makes these analyses uncertain. In the present study, both ME/CFS patients with or without clinical improvement during follow-up had adequate B-cell depletion, defined as  $<5.0 \times 10^6/\text{mL}$  CD19 + cells in peripheral blood.<sup>22</sup>

The strengths of the current study include a well-defined patient population with comprehensive follow-up according to the protocol for the clinical trial, standardized biobank sampling, and validated methods for determination of serum rituximab concentrations and of ADAs. The study was based on published clinical data with some limitations. It was not designed for the purpose of drug measurements and assessing the pharmacokinetic variables of rituximab in patients with ME/CFS. No blood samples were taken shortly after rituximab infusions to capture peak concentrations but

immediately before the next scheduled dose for assessment of trough concentrations. The intervals between the doses were gradually increased during follow-up, with the latest sample taken at 15 months (5 months after the last infusion), which means that rituximab concentrations at this point were low. The differences in rituximab serum concentrations caused by minor differences in time intervals between rituximab doses were adjusted presuming a rituximab  $t_{1/2}$  of 22 days in all patients and presuming a linear phase of elimination (all measurements at least 3 half-lives after the preceding dose).

## CONCLUSIONS

The present study is the first to examine the associations between rituximab serum concentrations, ADAs, and clinical responses among patients with ME/CFS. The results are complementary to a recent trial<sup>16</sup> that questions the benefit of rituximab among patients with ME/CFS and adds to the search for disease mechanisms, effective drug therapy, and mechanisms related to improvement of ME/CFS symptoms.

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IGR and JS were responsible for the design of the study; ØF and OM were responsible for the design and conduct of the clinical KTS-2-2010 trial; KA and KR acquired the biobank serum samples; AdV analyzed the rituximab concentrations and ADAs; IGR, ØF, JS, and KS analyzed and interpreted the data; and IGR, ØF, JS, KS, KA, KR, and AdV wrote the manuscript and/or revised it critically for important intellectual content. All authors approved the final manuscript as submitted.

## CONFLICTS OF INTEREST

The funders played no role in study design, data collection or analysis, decision to publish, or preparation of the manuscript. Haukeland University Hospital has patents and pending patent applications on the issue of B-cell depletion therapy for chronic fatigue syndrome (ME/CFS). The authors OM and ØF are mentioned as inventors in these applications. The authors have indicated that they have no other conflicts of interest regarding the content of this article.

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