Combination of B cell biomarkers as independent predictors of response in patients with RA treated with rituximab

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Abstract

Objective

Identification of B cell biomarkers predictive of response prior to therapy with rituximab (RTX) and evaluation of the efficacy of long-term treatment in patients with rheumatoid arthritis (RA).

Methods

302 RA patients failing one TNFi were treated with two applications of 1000 mg RTX (FIRST study). During the follow-up study (ReFIRST) the patients were treated for up to three more courses if they showed measurable clinical response but RA was still active. In a substudy on 154 RA patients peripheral B cell subsets were determined by flow cytometry before starting RTX. Rheumatoid factor (RF), RF-isotypes and anti-citrullinated protein antibodies (ACPA) were also measured.

Results

Based on multivariate analyses patients with positive RF and normal (>lower limit) levels of $CD19^+$ B cells (RF+/$CD19^+$) showed better treatment effects compared to patients who had only one or none of those parameters. Considering the RF status of the patients, analysis of B cell subpopulations yielded a correlation between higher ER rates and "double negative" $CD19^+CD27^-IgD^-$ B cells. Lowest ER rates were observed for RF negative patients in combination with low numbers of $CD19^+CD27^-IgD^-$ B cells as independent risk factors, thus defining a group with lower responses. Conversely, higher $CD19^+CD27^-IgD^-$ B cells identified a responder group within RF negative patients.

Conclusion

The data of this large biomarker study suggest that beyond RF positivity, normal levels of $CD19^+$ B cells together with increased $CD19^+CD27^-IgD^-$ B cells predict response to RTX in RA, in particular when all parameters were present.

Key words
rheumatoid arthritis, rheumatoid factor, rituximab

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Received on November 14, 2014; accepted in revised form on July 27, 2015.

Competing interests: none declared.

Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterised by clinical signs and consequences of synovitis of affected joints. It involves autoantibody production, cartilage and bone destruction, and systemic features including cardiovascular and skeletal disorders (1). Although the etiology and pathogenesis of RA remains unknown, an improved understanding of the immune mechanisms has led to the development of innovative therapies against the pathogenetic elements of the disease including monocytes/macrophages, B cells, T cells, endothelial cells, and fibroblasts (2). In addition, advanced characterisation of the cytokine networks responsible for inflammation in RA (3) resulted in therapies that target tumour necrosis factor α (TNF), interleukin-1, and interleukin-6 (4-6). Accumulating evidence suggests that B cells have multiple potential roles in the immunopathogenesis of RA, including antigen presentation, secretion of pro-inflammatory cytokines, production of autoantibodies, and regulation of dendritic cell function (7-12). It needs emphasis that blocking BAFF by belimumab as well as blocking BAFF/APRIL by atacicept did not result in clinical efficacy in RA and therefore B cell functions that are not controlled by BAFF and/or APRIL appear to be important in RA (13).

Targeting B cells in RA is the main mechanism of rituximab (MabThera®/Rituxan®). Rituximab (RTX), a chimeric mouse/human antibody directed against CD20, is a B cell surface antigen that is expressed on pre-B and mature B cells. It is not present on stem cells or plasma cells (14), thus allowing B cell recovery after treatment (15). By binding CD20 on the cell surface, cell-mediated and complement-dependent cytotoxicity as well as apoptosis are initiated (16-18).

It is well established that RA patients benefit from RTX therapy in combination with MTX if an anti-TNF-α therapy failed (19). However, several unmet needs remain: The optimal regimen for re-treatment of RTX continues to be a matter of investigation (20), and likewise the search for reliable predictive biomarkers for prognosis and therapeutic response to RTX is still ongoing (21). The FIRST and ReFIRST studies were performed to address these needs in a particular RA population that has failed one TNFi only. Previous studies indicated that repeated courses of RTX produce an improved degree of efficacy relative to original baseline, with no apparent cumulative toxicity in patients with inadequate response to TNF inhibitors (2, 15, 19, 22-24).

Patients and methods

Patients

FIRST and its subsequent study ReFIRST were exploratory, multicentre, open label, uncontrolled phase IIIb studies in Germany evaluating the efficacy of long-term treatment with RTX in RA patients with an inadequate response to a single TNF inhibitor. In the FIRST study 302 patients with active RA were included who had an inadequate response to a single TNF-α inhibitor (either etanercept or infliximab or adalimumab). Active RA was defined as DAS28 (Disease Activity Score in 28 joints) >3.2 and both a swollen joint count ≥4 and a tender joint count ≥4. The patients had to fulfil the 1987 American College of Rheumatology (ACR) criteria for classification of RA. Study patients were treated with two i.v. infusions of 1000 mg RTX applied 2 weeks apart. Prior to each RTX infusion i.v. methylprednisolone and antihistaminics were also taken as per label. Patients received stable background MTX (7.5–25 mg/week). No other DMARDs were allowed before starting RTX treatment. Patients were permitted to take glucocorticoids (≤10 mg/day of prednisone or equivalent) and oral non-steroidal anti-inflammatory drugs, both of which had to remain at a stable dose throughout the study.

We report the results of a substudy of 154 patients with assessment of B cell biomarkers and peripheral B cell subpopulations.

Retreatment study protocol (ReFIRST)

Patients could be included in the ReFIRST study if they had participated in the FIRST study, had completed the week 16 visit and had experienced a measurable clinical response. This

was defined as a decrease in DAS28 of at least >0.6 points at any time point between week 16 and 24 while still showing a DAS28 >2.6. In addition, for eligibility in the ReFIRST trial not more than 1 year should have passed between the patient's first RTX course (within FIRST) and the second course (within ReFIRST). Patients were eligible for subsequent (third and fourth) courses, if they had active disease and measurable clinical response as described above, with a minimum interval between treatment courses of 24 weeks. Patients were followed-up for up to 1 year after the last RTX course.

Efficacy and safety analysis

DAS28 and EULAR response (ER) criteria were determined at weeks 8, 16, and 24 after each RTX course. Changes were calculated relative to original baseline values in FIRST. The parameters age, sex, the failed TNF inhibitor, clinical RA specific factors, and blood parameters (RF, peripheral $CD19^+$ B cells [% and absolute numbers]) were analysed as potential predictive factors of ER. Efficacy analyses were performed on the 154 patients of the B cell biomarker substudy. For the analysis of predictive factors we used the baseline of RF. This baseline refers to the time point of the therapy decision. The stable+/stable- analysis was an additional subgroup analysis which was not extended further to the results of the overall analysis of predictive values.

Safety monitoring included the collection of all adverse events (AEs) and serious AEs (SAEs). In addition, infections and infestations were defined as AEs of special interest. All events were monitored throughout the studies and followed up until resolution. Safety aspects are not subject of this publication. As main result incidences of adverse events were not increased by repeated treatment courses of RTX. There were no new or unexpected safety findings as compared to previous studies.

Assessment of B cell biomarkers and peripheral B cell subpopulations

RF, RF-isotypes (RF IgA, RF IgM), ACPA and B cell subpopulations (defined by CD19, CD20, CD27, CD38, IgD, and CD10) were investigated to identify parameters predictive of response to RTX. RF, RF isotypes, and ACPA were determined at weeks 4, 8, 16, and 24 after each course. Peripheral blood samples for immunephenotyping were taken at baseline and early in repopulation (with $CD19^+$ B cells >1%) and were prepared as described previously (25). Immunephenotyping was done by four colour staining using a FACSCalibur (Becton Dickinson, San Jose, CA) with the following mAb: CD19 (APC), CD38 (PerCPCy5.5), CD10 (PE), CD27 (PE), CD20 (FITC), and anti-human IgD (FITC). As isotype control antibodies were used: Mouse G1/G2a (FITC/PE), Mouse IgG1 (PerCPCy5.5), Mouse IgG1 (APC). All antibodies were from Becton Dickinson (Heidelberg, Germany). Frequencies of $CD19^+$ cells were calculated using CellQuest software (Becton Dickinson). All RA sera were tested with specific ELISA systems detecting ACPA as well as RF activity (Generic Assays GmbH, Dahlewitz, Germany), including isotype autoantibodies. Due to transportation time (≥24 hours), plasmablast assessment was variable and was not used in this study.

If necessary, lab values were classified according to reference ranges of the local laboratories (RF), or of the central laboratory (RF IgA, RF IgM, ACPA, CD19, $CD19^+CD27^-IgD^-$) (Table I).

The studies were carried out in accordance with the Declaration of Helsinki. Informed consent was obtained from all patients prior to study entry. The study protocol was approved by the Ethics Committees of the University of Wuerzburg and Charité Berlin, Germany.

Statistical analysis

The impact of the parameters measured in the substudy on ER at week 16 of each course was analysed using

Table I. Baseline characteristics.

Baseline characteristics		n=154
Age [years]	Mean (min, max)	54.9 (26.77)
Sex [n (%)]	Male	33 (21.4%)
	Female	121 (78.6%)
Former TNF-α inhibitor (TNFi) [n (%)]	Adalimumab	84 (54.5%)
	Etanercept	43 (27.9%)
	Infliximab	27 (17.5%)
Reason for stopping TNFi [n (%)]	Loss of response	78 (50.6%)
	Lack of response	48 (31.2%)
	Intolerance	28 (18.2%)
DAS28 Baseline	Mean (±SD)	5.8 ± 1.0
Predictive factors	**Patients at baseline**	
	n (%*) Positive	n (%*) Negative
Rheumatoid factor (RF)**	110 (72.8%)	41 (27.2%)
RF IgA***	86 (57.7%)	63 (42.3%)
RF IgM***	105 (70.5%)	44 (29.5%)
ACPA antibodies***	112 (75.2%)	37 (24.8%)
	Above limit	Below limit
$CD19^+$ B cells (abs)***	96 (64,9%)	52 (35,1%)
$CD19^+$ B cells (%)***	103 (69,6%)	45 (30,4%)
$CD19^+CD27$-IgD- (abs)	Mean (±SD)	6.7 ± 7.1
$CD19^+CD27^-IgD^+$ (abs)	Mean (±SD)	90.0 ± 76.2
$CD19^+CD27^+IgD^-$ (abs)	Mean (±SD)	28.6 ± 31.0
$CD19^+CD27^+IgD^+$ (abs)	Mean (±SD)	20.9 ± 23.0
$CD19^+CD38^{++}IgD^-$ (abs)	Mean (±SD)	1.4 ± 2.1
$CD19^+CD10^+IgD^+$ (abs)	Mean (±SD)	6.1 ± 7.3

abs: absolute number. *Percentages refer to non-missing values.**Classification of RF as negative or positive according to definition of reference values of local laboratories. ***Classification according to definition of reference values of central laboratory (*i.e.* RF IgA ≥/<25 U/ml, RF IgM ≥/<10 U/ml, ACPA antibodies ≥/<30 U/ml, $CD19^+$ B cells (abs) >/≤100/µl, $CD19^+$ B cells (%) >/≤6%).

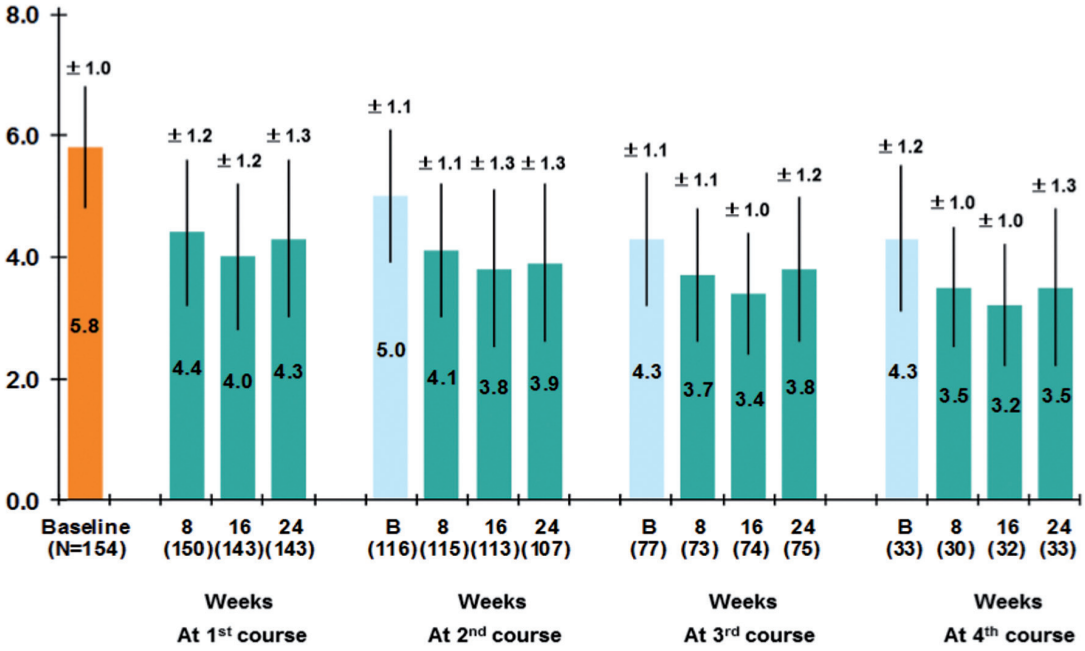

Fig. 1. Time course of DAS28 (Mean ± SD) Column B refers to the patients who entered the next treatment course. It displays the mean DAS28 at the time point when the decision to start a new treatment course was taken. Week 8, week 16, week 24 refer to the weeks after start of the respective treatment course. In brackets: Number of non-missing values.

univariate logistic regression. For univariately relevant parameters ($p<0.1$) multivariate stepwise forward logistic regression with entry and stay criterion of 10% was used. Odds ratios (OR) and their 95% confidence intervals (CI) were determined for the final models. Corrections for multiple comparisons were not necessary as all analyses were descriptive only.

Results

Clinical efficacy

An extended panel of serologic and particular B cell subsets was studied for their potential of prediction to response. Baseline values are given in Table I. The decrease in mean DAS28 score from original baseline (mean=5.8) was most pronounced at week 16 after each treatment course (Figure 1). In order to better control for drop outs during the retreatment study, EULAR responses were calculated for patient subgroups with a complete data set from 1 to 4 treatment courses, respectively (Table II). At week 16, ER (good and moderate) was determined after each course. After the first course an ER rate of 75.3% was observed. After 2, 3, and 4 courses of RTX, ER rates were 83.8%, 93.3%, and 97.0%, respectively. The increase was mainly due to an increase of good response (27.9%, 34.2%, 48.0%, and 51.5%, respectively), while moderate responses remain approximately the same (Table II).

Evaluation of RF and dose intensity as predictive factors in multiple courses

Since retreatment intervals during the REFIRST study were not fixed but left to the physicians' discretion, a straight forward analysis of predictive seromarkers was not amenable. Therefore we classified patients with repeat treatments in categories of always positive values for RF or always negative values during all applied courses. 51% of patients stayed always positive for RF, and 24% of patients stayed always negative. There were also changes from positive to negative values in 25% of patients. ER rates were higher in stable RF positive patients compared to stable RF negative patients (Fig. 2) although dose intensity (defined as cumulative dose/observation time) was higher for stable negative patients.

Predictive factors of EULAR response

Analysing all 154 patients, the following pre-treatment parameters were

Table II. EULAR response at week 16 after treatment in subgroups of patients with 1, 2, 3, and 4 treatment courses, respectively.

Treatment course (s)*	n**	ER after 1st course % of n	ER after 2nd course % of n	ER after 3rd course % of n	ER after 4th course % of n
		First line: overall response Second line: good / moderate response			
Course 1	154	75.3 27.9 / 47.4			
Course 1-2	117	82.1 28.3 / 53.8	83.8 34.2 / 49.6		
Course 1-3	77	82.7 29.4 / 53.3	92.0 34.7 / 57.3	93.3 48.0 / 45.3	
Course 1-4	33	84.8 27.2 / 57.6	97.0 39.4 / 57.6	100.0 51.5 / 48.5	97.0 51.5 / 45.5

*Subgroups of patients receiving at least one ("Course 1"), at least two ("Course 1-2"), at least three ("Course 1-3"), or at least four treatment courses ("Course 1-4").
**These subgroups encompass those patients who continued to the respective courses since not all patients with appropriate response continued in the retreatment study.

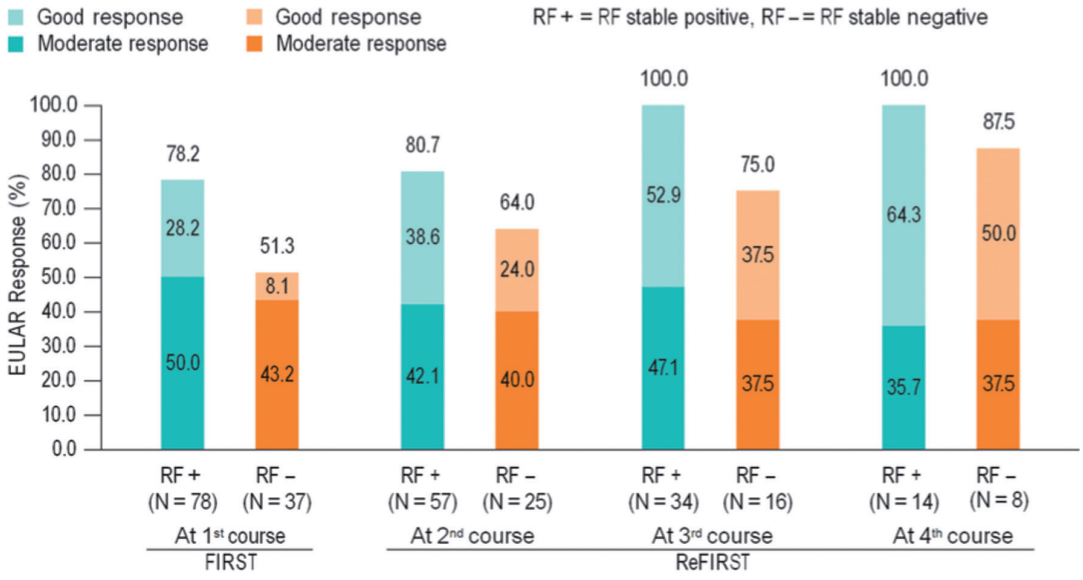

Fig. 2. EULAR response for RF stable positive or RF stable negative patients
EULAR response was calculated at week 16 of the 1st, 2nd, 3rd, or 4th treatment course always compared to the baseline value before the first RTX infusion within the FIRST study. (RF-unstable patients were not considered.)

evaluated as potential predictive factors of ER at week 16 in the first RTX course (p-value): age (p=0.0663), sex (p=0.6904), the failed TNF-α inhibitor (p=0.9353) and reason for stopping it (p=0.4020), RF (p=0.0202), RF IgA+ (p=0.0831), RF IgM$^+$ (p=0.0533), peripheral CD19$^+$ B cell count [absolute (p=0.0633) and % (p=0.2176)], and ACPA (p=0.3577). Following multivariate logistic regression analysis revealed that the presence of RF (p=0.0098, OR=3.1 [1.3, 7.3]) and normal absolute values of CD19$^+$B cells [defined as positive >100/B cells/μl] (p=0.0107, OR=1.7 [1.1, 2.6]) were related to ER with no interaction between both factors. These both parameters were used to define patient subgroups by combining in each case two of the independent parameters, found in the multivariate model. The observed respective ER rates after the first course were 87.3% for patients with positive RF and normal absolute numbers of CD19$^+$B cells (RF$^+$/CD19$^+$), 69.1% for patients with positive RF and low CD19$^+$ B cells/μl (RF$^+$/CD19$^-$), 52.5% for negative RF and normal CD19+B cells absolute (RF$^-$/CD19$^+$) and 44.4% for negative RF/CD19- B cell patients. DAS28 baseline values were not different between the groups RF positive (mean±SD=5.8±0.9) *versus* negative (5.6±1.1) (p=0.2568, t-test) nor between the groups CD19 positive (5.7±1.0) versus negative (5.8±1.0) (p=0.2971, t-test), indicating the differences in ER are not due to different baseline values.

B cell subpopulations as predictive factors for ER

To evaluate the value of potential B cell subpopulations as predictive factors for ER, further logistic regression analyses were carried out on all available data of 154 patients. The following absolute numbers of CD19 positive B cell subpopulations at baseline were univariately analysed for effect on ER (p-value): CD27$^+$IgD$^+$ (p=0.1493), CD27-IgD$^-$ (p=0.0020), CD27$^+$IgD$^-$ (p=0.0211), CD27-IgD$^+$ (p=0.1459), CD38^{++}IgD$^-$ (p=0.0663), and CD10$^+$IgD$^+$ (p=0.0472). Those parameters with p<0.1 were further investigated multivariately using stepwise forward logistic regression, always including the covariable RF ±. The final model yielded higher ER rates for the "double negative" CD19$^+$CD27-IgD$^-$ memory B cell population (≥5/μL blood, *i.e.* the rounded median of the population) (p=0.0013, OR=2.2 [1.4, 3.5]) and for positive RF (p=0.0028, OR=4.1 [1.6, 10.2]).

DAS28 baseline values were not different between the groups CD19+CD27-IgD- ≥5/μL (mean ± SD=5.9±0.9) versus <5/μl (5.7±1.0) (p=0.3079, t-test),

Table III. EULAR response at week 16 in subgroups of patients with RF+/- and combinations with CD19$^+$CD27$^-$IgD$^-$ ±.

Parameter(s) at baseline		ER after 1st course % (of N*)	ER after 2nd course % (of N*)	ER after 3rd course % (of N*)	ER after 4th course % (of N*)
RF**					
Negative		62.5% (of 40)	67.9% (of 28)	82.4% (of 17)	88.9% (of 9)
Positive		81.1% (of 111)	88.5% (of 87)	96.5% (of 57)	100.0% (of 24)
Missing data		3	2	3	0
p value§		0.0291	0.0114	0.0674	0.2727
RF**	CD19$^+$CD27$^-$IgD$^-$***				
Negative	Low	41.2% (of 17)	53.3% (of 15)	85.7% (of 7)	75.0% (of 4)
Negative	High	75.0% (of 20)	91.7% (of 12)	88.9% (of 9)	100.0% (of 5)
Positive	Low	72.5% (of 51)	92.1% (of 38)	96.0% (of 25)	100.0% (of 8)
Positive	High	93.3% (of 45)	89.7% (of 39)	96.2% (of 26)	100.0% (of 12)
Missing data		21	13	10	4

*Total number of patients in the respective subgroup and with at least one, two, three or four treatment courses, respectively. The number of not included patients due to missing data in the biomarkers are indicated as 'missing' data. **RF at baseline negative or positive according to definition of reference values of local laboratories.***CD19$^+$CD27$^-$IgD$^-$ at baseline low (<5/μL blood) or high (≥5/μL blood) according to the rounded median.

indicating the differences in ER are not due to different DAS28 baseline values.

Combinations of predictive factors and B cell subpopulations

Combining RF and absolute $CD19^+$ CD27-IgD^- B cell counts resulted in observed ER rates for RF positive patients of 93.3% when $CD19^+CD27$-IgD^- cells were ≥5/μL and 72.5% when $CD19^+CD27$-IgD^- B cells were <5/μl, respectively. In RF negative patients the $CD19^+CD27$-IgD- ≥5/μL group achieved 75.0% ER and the $CD19^+CD27$-IgD^- <5/μl group 41.2% ER (Table III). After receiving two courses these baseline parameters were also predictive for ER. Although the numbers of patients receiving 3 or 4 courses were low, the same trend was seen. ER of patients who show both RF negative and $CD19^+CD27$-IgD^- <5/μl compared to the ER of the other patients are significantly different (p=0.0010, Fisher test) after the first course. The same is true after the 2nd course (p=0.0011).

Discussion

In RA patients failing one prior TNF-α inhibitor, repeated courses of RTX resulted in consistent and sustained efficacy in the present study. For the most important parameters DAS28 and EULAR response the improvement was clinically significant in each treatment course. Changes in DAS28 as well as rates of ER were in good agreement with data of the SERENE, MIRROR and IMAGE studies as well as with the extension analysis reported by Keystone and colleagues (2, 26-29).

ER rates as well as improvements in DAS28 increased from course to course. Most likely this was due to a positive selection of responders over time. However, the subanalysis of patients completing various courses of RTX nevertheless suggests a sustained and even further improving responsiveness (see Table II).

Over the last years several groups evaluated biomarkers as predictors of response to RTX in order to individualise therapy. Rheumatoid factor positive patients were shown to have a better response to immune modulation with RTX than seronegative patients (19, 30). This was recently confirmed by pooled data of 10 European registries in 2019 RA patients treated with one course of RTX (31). Apart from serological parameters also cellular markers on the B cell level were evaluated (32-34). In the SMART trial the absence of autoantibodies (RF) and reduced B cell activity, measured by serum IgG concentration below the upper limit of normal was linked to a decreased probability of (EULAR) response to RTX (35). In our study we could confirm RF as serological biomarker predictive of response to RTX. Furthermore, in a multivariate analysis we could identify that negative RF and low absolute numbers of $CD19^+$ B cells are associated with a poorer outcome. The analysis of B cell subpopulations has been an important issue with regard to RTX responses. In 2008 we reported in a small cohort of RA patients treated with RTX that non-responders show a larger fraction of $CD27^+IgD^+$ memory B cells and that a "high memory burden" at baseline was predictive of an early relapse (36). In the meantime, these observations have been confirmed and extended. Möller and co-workers reported a correlation between lower numbers of repopulating $CD27^+$ memory B cells and a good response to RTX (33), and Sellam and colleagues found lower baseline $CD27^+$ B cells were associated with greater clinical response to RTX (32). Moreover it was reported that the extent of B cell depletion after the first infusion (34) and the depletion of $CD27^+$ memory B cells in BM and in the periphery is predictive of the clinical outcome to RTX (37). A prospective study could recently show that relapse seems to occur less frequent in the B cell depletion phase. In that study a significant majority of patients relapsed within 4 months following repopulation of total $CD19^+$ B cells, transitional and memory B cells (38). Although rituximab acts by depleting B cells, not all B cell subgroups are equally sensitive to this agent, therefore a monitoring of especially memory B cells is pertinent. On the molecular level elevated baseline mRNA levels of IgJ, a marker for antibody secreting plasmablast was associated with a poorer outcome (39). In contrast to studies conducted in RA patients a recently published study on myositis patients treated with rituximab showed no correlation of peripheral CD20 depletion B cells with clinical response. However B cell subclasses were only separated in naïve ($CD19^+$/$CD20^+$/$CD5^+$) and memory B cells ($CD19^+$/$CD20^+$/$CD27^+$) and no detailed analyses of the memory compartment was reported (40).

Summarising these results it seems pertinent that the characteristics of the memory B cell compartment are crucial for the therapeutic response to RTX. We extend these findings in our study by showing that another memory B cell subpopulation, the $CD27^-IgD^-$ ("double negative") memory B cell subset, seems to have an influence on RTX responses as well. The ontogenesis and the function of $CD27^-IgD^-$ B cells is to date not well characterised. They are assumed to belong to the memory compartment since, like convential $CD27^+$ memory B cells, they show typical somatic hypermutations in their Ig receptors (41). In systemic lupus erythematosus (SLE), these $CD27^-IgD^-$ B cells are expanded (42) and associated with a higher disease activity (43), although mainly restricted to $CD95^+$/$CD27^-IgD^-$ B cells (44). Their function in RA is so far not diligently studied. Brezinschek and colleagues found $CD27^-IgD^-$ B cells elevated in RA which may candidate them as an interesting target for therapy (45). Our study now provides evidence that higher absolute numbers (≥5/μl blood) of $CD27^-IgD^-$ B cells yielded better response rates to RTX and therefore suggests that these cells candidate as important target in responding RA patients.

Even though the interest in biomarkers has been very high during the last years, so far no clinically meaningful approach could be derived which allows defining low or high responders in unselected patients in daily practice. Combining biomarkers which show independent correlations in multivariate analyses may allow superior definitions of RTX responsiveness. Our study shows how this can be applied to define particularly low responder groups of patients treated with RTX. Patients with low absolute numbers of $CD19^+CD27^-$

IgD$^-$ memory B cells and negative RF showed an ER of only 41.2%. In contrast, an EULAR response rate of 93.3% can be seen in patients with higher numbers of CD19$^+$CD27$^-$IgD$^-$ B cells and positive RF. It seems that in addition selecting RF and absolute CD19 count baseline proportions did also help in defining responder patients. Patients with low level of CD19 B cells and negative RF achieved the lowest ER rates (44.4%). On the other hand determining the number of CD19$^+$ B cells or the number of CD19$^+$CD27$^-$IgD$^-$ B cells in RF negative patients may also prove useful to select responding patients in the seronegative subgroup. RF negative patients with either normal or elevated CD19$^+$ B cells or higher CD19$^+$CD27$^-$IgD$^-$ B cells yielded an ER rate of 52.5% and 75.0%, respectively. In daily clinical practice RF has not really been accepted to decide in a single patient if he can be treated with RTX since it is obvious that a significant proportion of RF negative patients do show meaningful responses to RTX. Therefore, combining statistically defined biomarkers may prove more helpful for these difficult therapeutic decisions.

The analysis of biomarkers for repeat RTX treatments is very sparse in the literature. Our retreatment study (Re-FIRST) has some major limitations for that purpose since the protocol did not specify defined retreatment intervals. In our study a diminishing retreatment interval has been observed over the study period. It is very likely that this is mainly due to an improved confidence in RTX therapy by the investigators over time. Nevertheless also during multiple treatments RF or ACPA positivity seems to be associated with improved responses to RTX. When patients with constant negative RF or ACPA were compared to patients who stayed positive for either autoantibody through all RTX courses, clinical responses appeared to be lower in autoantibody negative patients even though these patients received a higher dose intensity in our trial.

In conclusion, RTX showed good efficacy and safety after repeated courses in RA patients who had previously failed one anti-TNF agent. Combination of independent predictive factors may allow an improved stratification of patients before starting RTX therapy in order to identify patients who may have a lower response. Using the combination of RF positivity, normal levels of peripheral CD19$^+$ B cells, or higher numbers of CD19$^+$CD27$^-$IgD$^-$ double negative memory B cells, patient groups can be identified which will probably gain enhanced benefit also in repeat courses of RTX.

Key messages

- Rituximab induces EULAR response rates of 75.3%, 83.8%, 93.3%, and 97.0% at week 16 after 1, 2, 3, and 4 treatment courses, respectively.
- Negative RF in combination with low numbers of CD19$^+$ B cells as well as negative RF in combination with low numbers of CD19$^+$CD27$^-$IgD$^-$ B cells define low response groups.
- Patient characterisation regarding the biomarkers RF, CD19, and CD27$^-$IgD$^-$ at baseline allows improved prediction of EULAR response even after several treatment courses.

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