Clinical Relevance of Rituximab Immunogenicity in Rheumatoid Arthritis

A pilot study

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Data about immunogenicity of rituximab (RTX), a chimeric monoclonal antibody that targets BCD20+ cells, and its clinical relevance in patients with rheumatoid arthritis (RA) remains controversial. We performed a cross-sectional study in a cohort of forty-three active RA treated with RTX, aiming to systematically assess anti-RTX antibodies (RTX ADA) and drug level (DL). Evaluation for efficacy, safety and immunogenicity was performed in all patients enrolled as follows: disease activity scores, clinical outcomes and adverse events were evaluated at baseline and during the study visit, while RTX ADA and serum drug concentrations were collected as a single-point data. Lab assays (IgG-anti-RTX and DL) were measured using ELISA Progenika kits, with cut-off values of 140 AU/mL for ADA and 0.75µg/mL for drug concentration, respectively. Although the level of residual RTX was undetectable in more than half of cases, RTX ADA were reported only in two out of 43 patients (4.7%). Treatment failure was demonstrated in 4/43 RA, strongly associated with ADA status ($p<0.01$) and drug level ($p<0.01$), with a specific profile for secondary non-responders meaning ADA positivity at higher titers (184.75 vs 147.54AU/mL anti-RTX, $p<0.05$), lower serum RTX (1.10 vs 3.66µg/mL, $p<0.05$), lower biologic exposure (32.2 vs 38.3 months; $p<0.05$), higher DAS28-ESR at baseline (6.14 vs 6.7, $p<0.05$). Low immunogenicity rate was reported in active RA under RTX, potentially associated with impaired drug efficacy. Although anti-RTX antibodies monitoring is relevant only in selected cases, it may represent a key finding towards optimizing biological therapeutics in RA during long-term follow-up.

Keywords: immunogenicity, rituximab, rheumatoid arthritis, anti-drug antibodies, drug level

The introduction of biological agents (TNFα inhibitors, anti-BCD20+ antibodies, IL-6 inhibitors) in the therapeutic armamentarium of rheumatoid arthritis (RA), has essentially changed the evolution and prognosis of the disease [1-10]. Furthermore, sustained remission or low disease activity became feasible with their routine application in patients sub-optimally controlled or intolerant to synthetic disease modifying anti-rheumatic drugs (DMARDs) [1-16].

However, a significant proportion of patients (up to 40%) fails to accomplish with the treat-to-target strategy and are classified as non-responders or develop adverse events, requiring a distinct, personalized therapeutic approach [1-16].

It is widely accepted that immunogenicity represents one of the factors potentially involved in the mechanism of failure to biologic DMARDs [1-16]. Known as the development of anti-drug antibodies (ADA) with significant consequences on serum drug level (DL), immunogenicity is a complex process described with all biologics, influenced by different drug, disease, treatment and patient-related factors, as well [1-16]. The clinical relevance of immunogenicity obviously depends on the type of anti-drug antibodies (neutralizing or non-neutralizing ADA) and account for specific effects on drug efficacy, safety (hypersensitivity reactions), dosing and drug survival [1-16].

Different methods are currently available to assess ADA and DL, with various specificities and sensitivities, such as the standard ELISA techniques, bridging assays, antigen-binding tests based on a radioimmunoassay (RIA) with either fluid-phase or solid-phase [1-16].

Immunogenicity of TNF inhibitors, either monoclonal antibodies (e.g. infliximab, adalimumab) or TNF receptors (etanercept), is by far the most extensively studied. Thus, neutralizing antibodies against both chimeric (infliximab) and human monoclonal (adalimumab) anti-TNF antibodies are commonly linked with restricted drug bioactivity by preventing their binding to TNF, and are consequently associated with reduced serum drug concentrations, loss of therapeutic response, adverse events, and treatment discontinuation [1-16]. In addition, it is well recognized that low ADA concentrations usually modulate the clinical drug efficacy, while high levels are able to promote safety reactions [1-16].

One potential application of immunogenicity is a personalized treatment in active RA using serum DL as the development of ADA impairs the bioactivity or bioavailability of the drug [11]. The decision to cycle in the same class of biologics (e.g. the case of switching between TNF

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inhibitors) or to swap with a different class of biotherapeutics (e.g., anti-BCD20+ antibodies, anti-IL-6) in RA with failure to their first biologics is currently made on empirical basis. Conversely, different authors have already published personalized algorithms to be applied in special RA settings, the therapeutic strategy being stratified according to ADA and/ or DL [1-16].

For other classes of biologic DMARDs including rituximab (RTX) less information about immunogenicity is available. RTX is a chimeric monoclonal antibody that targets mature B cells by binding to CD20 molecule on their surface; the drug was approved by the FDA initially for the management of relapsed or refractory low-grade or follicular CD20 positive B-cell non-Hodgkin's lymphoma and about ten years later, in moderate to severe active RA, as a second line biologic agent [1, 12, 13, 15]. Since RTX is considered a low immunogenic drug, with anti-RTX antibodies described in 4.3% to 11% of RA patients, their clinical relevance is unfamiliar [1, 12, 13, 15, 16]. On the other hand, the synthesis of human anti-chimeric antibodies in RTX treated patients as well as posttranslational modifications that can developed during the drug lifecycle may result in loss of efficacy in selected cases [1, 12, 13, 15].

Since the clinical relevance of rituximab immunogenicity in patients with RA is still controversial, we aimed to systematically assess the anti-rituximab antibodies (RTX-ADAs) and serum rituximab level (RTX-DL) and the influence on clinical disease outcomes in active disease.

Experimental part

Material and method

We performed a cross-sectional study in a cohort of forty-three consecutive patients with moderate to severe active RA (defined as DAS28-ESR more than 3.2) receiving rituximab (MabtheRa®) for their disease, followed-up in an academic outpatient rheumatology department during a 5 months interval (January 2015 to May 2015).

RTX was given as per the classical protocol, meaning two infusions of 1000 mg administered at day 1 and day 15, with a re-treatment cycle after 24 weeks until 48 weeks based on clinical response and residual disease activity. All patients receiving at least one cycle of RTX were eligible for the final analysis.

The study comprised two visits, the baseline visit (V1) before initiating RTX (data collected retrospectively from patients files) and the second one (V2), across the study, when data about immunogenicity of RTX were recorded.

Patients were assessed according to a predefined protocol including demographics, individual parameters or patient reported outcomes (tender and swollen joints count on a 28 joints basis, pain on a 0-100 mm visual analogue scale), inflammatory parameters (ESR- erythrocyte sedimentation rate and CRP- C reactive protein levels), immunologic profile (RF, rheumatoid factor, and ACPA, anti-cyclic citrullinated peptide antibodies), anti-rituximab antibodies and antidrug level. Disease activity defined by Disease Activity Score (DAS28-ESR), Clinical Disease Activity Index (CDAI), Simplified Disease Activity Index (SDAI), clinical outcomes (EULAR- European League Against Rheumatism response criteria) and adverse events were evaluated at baseline and during V2, while serum RTX and anti-RTX levels were collected as a single-point data (V2).

We were interested in detection of anti-drug antibodies taking into account their presence (detectable or undetectable) and titer, as well as the serum concentration of RTX expressed as arbitrary unit per milliliter.

Serum drug and antidrug levels were measured just before a new administration; lab assays (IgG-anti-RTX and serum RTX levels) were measured following the manufacturer's instructions using an enzyme linked immunosorbent assay (ELISA) Progenika kits (Promonitor-RTX, Promonitor-anti-RTX), with detection limit set at 140 AU/mL for anti-RTX and 0.75µg/mL RTX serum concentration, respectively.

Statistical analysis was conducted in SPSS statistical software, version 19.0 ($p<0.05$), with a subgroup analysis based on detectable and undetectable drug level, detectable and undetectable ADA, drug efficacy (responder and non-responder) and based on history of previous biological drug exposure (bio-experimented and bio-naïve RA).

The study was approved by the hospital Ethics Committee and all patients gave written informed consent before the study was started.

Results and discussions

RA-related parameters at baseline and visit 2

Patients were selected from a total of 91 RA treated with biologicals (TNF antagonists and non-TNF biologicals) in which we assessed immunogenicity during an ample program studying the clinical relevance of immunogenicity and the impact on clinical decision making.

Baseline characteristics and V2 characteristics are summarized in table 1.

Table 1

PATIENT CHARACTERISTICS AT BASELINE AND STUDY VISIT

Parameter*	Value
anti-RTX Ab (AU/mL)	150.53± 49.37
drug level (microg/mL)	3.43±7.76
age (years)	61.95±9.95
RA duration (years)	15.74±9.02
persistence on drug (months)	44.30±21.61
baseline DAS28-ESR	5.37±0.85
actual DAS28-ERS	3.83±0.51
disease activity at final point	3.23 ±0.71
SDAI	14.47±4.73

*average values

The majority of patients were women (83.7%), in their middle age (61.95+9.95 years), with established RA (disease history 15.74+9.02 years), mainly seropositive (RF positivity in 95.3%, ACPA positivity in 76.7%) and erosive (97.7%) disease subtype.

36 of patients were already bio-experimented, with a minimum one TNF antagonist in their therapeutic history, while seven RA were bio-naïve (RTX given as first line biologic option due to specific contraindications in TNF inhibitors).

Anti-rituximab antibodies

Mean serum level of anti-RTX antibodies was 150.53+49.37 AU/mL; positive anti-drug antibodies were detected only in two cases (4.7%), all the others presenting with undetectable concentrations.

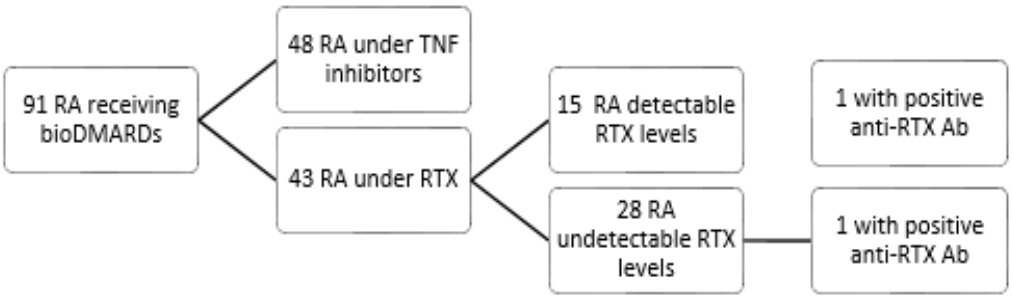

Fig. 1. Patient distribution according to drug levels and anti-drug antibodies levels

	Detectable drug levels (15) (34.89%)	Undetectable drug levels (28) (65.11%)	p
Anti-drug Ab	151.93±46.21	149.8±51.8	<0.05
DAS28 baseline	4.99±0.49	5.57±0.93	<0.05
DAS28 actual	3.84±0.39	3.83±0.57	<0.05
Delta DAS28	1.20 ±0.57	1.76±0.89	<0.05
SDAI actual	13.49 ±3.2	14.98± 5.35	<0.05
HAQ-DI	1.8±0.34	1.73±0.24	<0.05
ACPA status	100%	71%	<0.05

Table 2
STATISTICAL SIGNIFICANT DIFFERENCES AMONG GROUPS BASED ON DETECTABLE OR UNDETECTABLE RTX LEVEL

Serum rituximab levels

15 out of 43 RA (34.9%) had detectable RTX level, while in 28 cases RTX was undetectable during the standard evaluation.

A closer look to the distribution of patients based on RTX and anti-RTX levels is presented in figure 1.

Treatment failure meaning loss of response (secondary non-responder) or the presence of side effects was demonstrated in 4 out 43 RA, strongly associated with ADA status ($p<0.01$) and drug level ($p< 0.01$).

Patients were classified according to the presence of residual drug activity and data about disease activity, sero-positivity, drug exposure and EULAR response were collected and analyzed (table 2).

We reported several characteristic of RA with detectable RTX levels as follows: lower baseline disease activity (DAS28-ESR), lower final DAS2-ESR and SDAI scores, better response at V2 meaning significant more patients achieving treat-to-target (remission or low disease activity) and a smaller proportion of RA maintaining moderate and high disease activity. In addition, we demonstrated more ACPA positive RA among responders. Finally, patients with positive RTX concentration had more than two TNF inhibitors in their therapeutic background. Conversely, only one patient in those with undetectable RTX level developed anti-drug antibodies. All the above mentioned parameters were significantly ($p<0.05$) different in patients with detectable compared with those with undetectable drug.

A specific profile for secondary non-responders meaning anti-drug antibodies positivity at higher titers (184.75 vs 147.54AU/mL anti-RTX, $p<0.05$), lower serum titers of rituximab (1.10 vs 3.66µg/mL, $p<0.05$), lower exposure to biologic DMARD (32.2 vs 38.3 months; $p<0.05$) as well as higher activity (DAS28-ESR) at baseline (6.14 vs 6.7, $p<0.05$) were demonstrated.

We systematically evaluated serum drug levels and anti-drug antibodies in a cohort of consecutive RA patients receiving RTX for their active disease. All cases were followed up in a single academic rheumatology department according to a standard protocol, comprising data about drug efficacy and immunogenicity parameters as well.

Although the level of residual RTX was undetectable in more than half of our cases (65.1%), anti-drug antibodies were reported only in two out of 43 patients (4.7%), suggesting that immunogenicity is not the only trigger for low levels of drug in such patients. Moreover, only four patients were classified as non-responders at visit 2, one case with a surprisingly high level of anti-RTX antibodies (414 AU/mL), while the other without detectable antibodies against RTX. Furthermore, the generation of anti-drug antibodies din not seems to significantly influence the clinical efficacy of RTX.

The topic of immunogenicity with biologic therapies, particularly TNF inhibitors, received increased emphasis during the last years, as anti-drug antibodies are potentially responsible for loss of clinical response or impaired safety profile in patients with different RA settings [1-16]. Moreover, several strategies for the management of treatment failure including switching to a another drug in the same class or swapping to a different class based on immunogenicity profile have already advanced, shaping the way to treat in a more personalized way rheumatologic patients [1-16].

The generation of antibodies targeting RIX was reported during lupus erythematous, Sjogren's syndrome, vasculitis and pemphigus. However, only few studies addressed the particular issue of immunogenicity with RTX in patients with RA [12-16].

Denoel et al (2015) have performed a kinetic analysis of RTX immunogenicity and clinical consequences in severe active RA suggesting that anti-drug antibodies detected in up to 10% of cases did not appear to influence the ability of the drug to deplete CD19+B cell subpopulation [15]. Although the peak incidence of anti-RTX antibodies was reported in the year after the first injection, only two patients had persistent antibodies [15].

Mazilu et al. (2014) have published their results about the biologic drug levels (TNF inhibitors and RTX) and anti-drug antibodies collected at first inadequate response in RA, suggesting the importance of monitoring drug immunogenicity in routine practice, especially in long-term use of biologic agents [16]. Detectable levels of RTX correlated with a better clinical response at follow-up, while no significant difference between patients with and without detectable drug levels regarding DAS28 and SDAI was reported. Interestingly, significant difference in RTX serum levels depending on ACPA profile was demonstrated in this

	Responders (39)	Non-responders (4)	P
Serum RTX level	3.66µg/mL	1.10µg/mL	<0.05
anti-RTX Ab	147.54AU/mL	184.75AU/mll	<0.05
DAS28-ESR baseline	6.14±1.2	6.7±1.5	<0.05
Persistence on RTX	38.3 months	32.2 months	<0.05

Table 3
COMBINED ADA-DRUG LEVEL AND CLINICAL OUTCOME IN RESPONDERS VERSUS NON-RESPONDERS

study, suggesting a better clinical response on RTX based on sero-positivity status [16].

Resuming our study, we also performed a complex subgroup analysis, based on drug level (detectable or undetectable), anti-RTX antibodies status (positive or negative), responders and non-responders, and specific patient profiles were identified accordingly. Our results showed also a higher clinical effectiveness for cases with a specific RA background.

However, further studies with RTX are required in order to correctly ascertain the role of antibodies against the drug in targeting clinical outcomes in different settings of RA.

In another paper was studied the evolution of inflammatory Biochemical markers within periodontal therapy to patient with rheumatoid arthritis [17].

Conclusions

Low immunogenicity rate was reported with RTX therapy in moderate to severe active RA, potentially associated with impaired drug efficacy. Although monitoring of anti-RTX antibodies is relevant only in selected cases, it may represent a key finding towards optimizing biological therapeutics in rheumatoid arthritis during long-term follow-up.

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