Development of anti-rituximab antibodies in rituximab-treated patients: Related parameters & consequences

Fatemeh Saffari¹ & Abdollah Jafarzadeh¹,²

¹Department of Immunology, Medical School, Kerman University of Medical Sciences, Kerman & ²Molecular Medicine Research Center, Rafsanjan University of Medical Sciences, Rafsanjan, Iran

Received February 17, 2019

The utilization of the monoclonal antibodies (mAbs) as therapeutic agents is one of the most favourable fields in immunotherapy. The immunogenicity of mAbs is one of the major parameters that may restrict their therapeutic and diagnostic applications. Rituximab, a chimeric mAb against CD20, is attached to the B-cell membrane-linked CD20 and is used to treat some B-cell-related malignancies, a number of autoantibody-mediated autoimmune disorders and improvement of graft survival. The risk of anti-rituximab antibody (ARA) development and ARA-related adverse events are low in rituximab-treated patients with lymphoma. No important association was reported between the ARA positivity and drug levels, and drug efficacy in rituximab-treated patients with lymphoma. The patients with autoimmune disorders exhibit greater risk of ARA development and ARA-related adverse events. In autoimmune diseases, ARA positivity may have no significant impact on either the drug level or its efficacy, (i.e.), it may reduce drug levels without influencing drug efficacy and, vice versa, or may reduce both drug level as well as its efficacy. The characterization of the parameters affecting the production of ARA can be used to design strategies to reduce the immunogenicity of mAb and promote its efficacy in humans. In this review, the host and therapeutic programme-related parameters affecting the development of the ARA have been discussed to suggest novel insights to reduce ARA-associated adverse events and enhance the drug efficacy.

Key words Anti-rituximab antibody - immunogenicity - immunotherapy - rituximab

The targeting of cancerous cells and the strengthening of anti-tumour immune mechanisms are among the strategies that are frequently considered in cancer immunotherapy¹. Monoclonal antibodies (mAbs) exhibit promising therapeutic potentials in cancer immunotherapy and treatment of autoimmune diseases as they bind specifically to antigenic targets. The therapeutic effects of mAbs are exerted through a number of mechanisms such as the killing of target cells, receptor-ligand inhibition and receptor blocking²-⁶.

The clinical application of a mAb has been challenged by a number of problems, especially its immunogenicity. The administration of a non-humanized mAb to humans may stimulate the production of antibodies to some regions of that mAb such as fragment of antigen binding, fragment
of crystallizable and complementarity-determining regions (CDRs)\(^2,7\). Further, fully human mAbs can contain epitopes in their CDRs which may cause an antibody response through the network of idiotypes/anti-idiotypes\(^2\). The produced anti-drug antibody (ADA) limits the binding of mAb to target antigens and promotes its clearance largely through hepatic and splenic macrophages\(^2,7\). In addition, the ADA may interfere with immunodiagnostic techniques leading to false results and incorrect diagnosis, and therefore, inappropriate treatment\(^8\).

Several factors can affect the immunogenicity of therapeutic mAbs such as protein structure, doses, treatment programme, patient co-medication, immune status of the patients, genetic predisposition of the patients, underlying disease and, age and gender of the patients\(^9\). Antibodies targeting cell membrane-linked molecules may have a higher risk of immunogenicity compared with antibodies targeting soluble molecules\(^2,15\). This phenomenon may be attributed to the antigen internalization into target cells and subsequently its processing and presentation to patient’s specific T lymphocytes, which then enable B-cells to produce high-affinity ADA\(^2,15\). When a target antigen is present on the cell membrane, mAbs bind to the target antigen and are quickly internalized along with the target antigen, leading to rapid uptake of mAbs into the cell\(^16\). Interestingly, the disappearance of CD20 and internalization of CD20-rituximab have been reported in some rituximab-treated patients with CLL\(^17,18\). The internalized mAb which then acts as an antigen, is processed and eventually presented to T cells through interaction between the T cell receptor and the major histocompatibility complex II-antigen complex on antigen-presenting cells (APC), resulting in ADA production through a T-cell-dependent manner. In these circumstances, the Th cell-derived cytokines help B cells to produce high-affinity ADA from various isotypes, such as IgG and IgE\(^19\). The mAb-related epitopes may directly cross-link the surface immunoglobulins of the specific B-cells, resulting in the production of anti-drug IgM in a T-cell-independent manner\(^19,20\). As there are different ADA isotypes, these may also cause various side effects in their recipients, such as allergic reactions, serum sickness and renal failure\(^19,20\).

Rituximab is a human/murine chimeric mAb that is composed of the human kappa and IgG1 constant regions connected to the murine light- and heavy-chain variable parts, respectively\(^21\). Rituximab specifically binds to the CD20 marker that is expressed on the B lymphocytes and exerts its cytotoxicity through induction of the apoptosis, complement activation, and antibody-dependent cell-mediated cytotoxicity\(^22-27\). Rituximab is used for the treatment of some malignancies such as CD20\(^+\) B-cell non-Hodgkin’s lymphoma and chronic lymphocytic leukaemia, autoimmune disorders associated with the presence of autoantibodies, including systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA), or improvement of the graft survival\(^28,29\).

The characterization of parameters affecting the production of anti-mAb antibodies can be used to design strategies to reduce the immunogenicity of a mAb and promote its efficacy in humans. There are many studies on the rituximab immunogenicity and its sequels, but it is necessary to provide a comprehensive description of this subject. In this review, a comprehensive insight regarding the host and therapeutic programme-related parameters that influence the development of the anti-rituximab antibody (ARA) are provided and novel insights to reduce ARA-associated adverse events and enhancement of drug efficacy are suggested.

**The possible parameters influencing anti-rituximab antibody production**

*The effect of number of rituximab infusions on anti-rituximab antibody formation:* The number of rituximab infusions may be associated with the production of ARA. In one study, the ARA was detected in about 33 per cent of the rituximab-treated multiple sclerosis (MS) patients\(^30\). Among patients with relapsing-remitting MS (RRMS) and primary progressive MS (PPMS), a negative correlation was also observed between the infusion numbers of rituximab and ARA positivity\(^30\). Furthermore, the ARA was not detectable in rituximab-administered patients with relapsed mantle cell lymphoma shortly after the second and third course of treatment\(^31\). There was also a significant association between serum concentrations of rituximab, serum ARA titre, B-cell count and clinical responses\(^31\). The mentioned studies display an inverse correlation between number of rituximab infusions and risk of ARA development. The diminished count of B-cells or immune tolerance to rituximab may be considered as possible reasons for decreased ARA titre in subsequent administrations in rituximab-treated patients. Nevertheless, there was no significant relationship between the injection numbers of rituximab and development of the ARA in rituximab-treated patients with lymphoma or
leukaemia in one of our previous studies\textsuperscript{32}. There was also no significant relationship between the number of infusions and ADA concentrations in infliximab-treated patients with Crohn’s disease\textsuperscript{7}.

The impact of age and gender on anti-rituximab antibody formation: The relationship between age and ARA production has rarely been studied. In one study performed on infliximab-administered patients with Crohn’s disease, the ARA was detected in 2.7 per cent and 11 per cent of children and adults, respectively\textsuperscript{33}. In another study, the ARA was detected in 37 per cent of rituximab-administered patients with lymphoma and 26 per cent of rituximab-treated patients with progressive forms of MS. However, association was found between ARA production and the age or gender of MS patients\textsuperscript{30}.

The effects of disease type on anti-rituximab antibody formation: It has been demonstrated that the disease type influences the immunogenicity of rituximab in mAb-administered subjects. Therefore, variable ARA positivity was reported in rituximab-treated patients with different diseases. The development of ARA was reported in about 2.7 per cent of patients with non-Hodgkin’s lymphoma\textsuperscript{44-37}, in <4 per cent patients with diffuse large B-cell lymphoma\textsuperscript{38-40} and in 19.8 per cent patients with follicular B-cell lymphoma\textsuperscript{41}. It seems that rituximab exhibits differential immunogenicity in different types of B-cell lymphoma (Table I)\textsuperscript{42,43}. In a study\textsuperscript{44} on 166 rituximab-treated patients with relapsed low-grade or follicular lymphoma, ARA was detected only in one patient on day 50. Also, no association was observed between the ARA seropositivity and laboratory or clinical abnormalities. Similarly, in 11 rituximab-administered patients with relapsed B-cell lymphoma, no patients were found to develop ARA\textsuperscript{34}. Moreover, ARA was not quantifiable in 15 rituximab-administered patients with B-cell lymphoma\textsuperscript{35}. In our recent study, the development of the ARA was found in four out of 32 (12.5\%) rituximab-treated patients with lymphoma or leukemia\textsuperscript{32}. It was also observed that the chemotherapy may influence the development of the ARA in patients with lymphoma or leukemia\textsuperscript{32}.

Regarding autoimmune diseases, ARA was detected in 7 - 37 per cent of patients with RRMS\textsuperscript{30,44,45}, 26.5 per cent patients with PPMS, 1.8 - 21.7 per cent of patients with RA, 16.6 - 50 per cent patients with SLE\textsuperscript{40,47}, 27 per cent patients with Sjögren’s syndrome\textsuperscript{48} and in 18.18 per cent patients with Pemphigus vulgaris\textsuperscript{49}, who were treated with rituximab (Table II). The development of ARA was also indicated in 21 per cent of rituximab-treated patients with Crohn’s disease\textsuperscript{50}. It is obvious that the rate of ADA positivity was higher in rituximab-administered patients with autoimmune diseases compared to patients with lymphoma. Therefore, in active autoimmune diseases, a mAb tends to exhibit greater immunogenicity, regardless of the type of disease. However, the results from an investigation suggest a greater rate of ARA positivity in patients with RRMS than patients with PPMS (37 vs. 26\%). The reason for this differential immunogenicity of rituximab in patients with various patterns of MS is not clear. However, higher intensity of immune responses during relapsing stages may influence this parameter\textsuperscript{30}.

Some immunological disorders such as defects in the effector T-cell-mediated anti-tumour immune response and hyper-activation of regulatory T cells have been reported in patients with malignancies\textsuperscript{57-61}. Therefore, lower immunogenicity of a mAb in malignant patients, such as B-cell malignancy may be due to the general immunosuppression that dampens the B-cells responsible for ADA production\textsuperscript{11,62}. Since mAbs against B cell-related markers suppress the B cells responsible for ADA production, it may be postulated that mAbs against B cell-related markers would be inherently less immunogenic than other therapeutic mAbs\textsuperscript{63}.

The association of the B-cell number with anti-rituximab antibody formation: The results of a study on rituximab-administered patients with MS indicated that there was a powerful association between ADA positivity and higher B-cell count. The ARA titre and positivity were greater in patients with lower B-cell depletion\textsuperscript{30}. Similarly, ARA development was associated with reduced B-cell depletion in rituximab-treated patients with SLE\textsuperscript{46}. The aforementioned studies clearly indicate an inverse correlation between serum levels of ARA and the circulating number of B-cells. However, no association was found between ARA titres with circulating B cell numbers, mAb-related harmful events, or clinical response rate in ARA-positive patients with RA\textsuperscript{64}.

The effects of rituximab types on anti-rituximab antibody formation: The results of a study on RA patients showed that the ARA was similarly developed in patients treated with rituximab biosimilar forms such as rituximab-Pfizer (PF-05280586),...
Table 1. Production of anti-rituximab antibody and related complications in rituximab-treated patients with lymphoma

| Disease type                      | Number of patients | Rituximab type | Infusion numbers | Infusion programme | Rituximab doses | ARA monitoring times | Per cent ARA positivity | ARA relation to adverse events | ARA relation to drug efficacy | ARA relation to serum drug levels | Reference |
|----------------------------------|--------------------|----------------|------------------|--------------------|-----------------|---------------------|------------------------|--------------------------------|-------------------------------|--------------------------------|-----------|
| Non-Hodgkin’s lymphoma           | 11                 | Rituximab      | 4                | Weekly intervals   | 250 or 375 mg/m²| Weekly (4 wks) and then monthly (2 months) | 0.0<sup>a</sup> | NR                             | NR                            | NR                            | 34        |
| Non-Hodgkin’s lymphoma           | 37                 | Rituximab      | 4                | Weekly intervals   | 375 mg/m²       | Weeks 0, 1, 2, 3, 4 and certain times until year 4 | 2.7<sup>a</sup> | NSR                           | NSR                           | NSR                           | 35        |
| Non-Hodgkin’s lymphoma           | 37                 | Rituximab      | 1                | Single infusion    | 10, 50, 100, 250, 500 mg/m² | Months 1, 2, 3 | 0.0<sup>a</sup> | NR                             | NR                            | NR                            | 36        |
| Non-Hodgkin’s lymphoma           | 37                 | Rituximab      | 8                | Weekly intervals   | 375 mg/m²       | Wk 2, 4, 8 & 12 | <4.0<sup>a</sup> | NSR                           | NSR                           | NSR                           | 37        |
| Diffuse large B-cell lymphoma    | 127                | BS (RTXM83)    | 1-6              | 3 wk intervals     | 375 mg/m²       | 18 wk              | <4.0<sup>a</sup> | NSR                           | NSR                           | NSR                           | 38        |
| Diffuse large B-cell lymphoma    | 124                | Rituximab      | 1-6              | 3 wk intervals     | 375 mg/m²       | 18 wk              | <4.0<sup>a</sup> | NSR                           | NSR                           | NSR                           | 37        |
| Diffuse large B-cell lymphoma    | 136                | BS (RTXM83)    | 6                | 3 wk intervals     | 375 mg/m²       | 24 wk              | 2.3<sup>a</sup> | NSR                           | NSR                           | NR                            | 39        |
| Diffuse large B-cell lymphoma    | 136                | Rituximab      | 6                | 3 wk intervals     | 375 mg/m²       | 24 wk              | 3.2<sup>a</sup> | NSR                           | NSR                           | NR                            | 37        |
| Diffuse large B-cell lymphoma    | 76                 | BS (DRL)       | 6                | Every 21 days      | 375 mg/m²       | 18 months          | 1.3                   | NSR                           | NSR                           | NSR                           | 40        |
| Diffuse large B-cell lymphoma    | 75                 | Rituximab      | 6                | Every 21 days      | 375 mg/m²       | 18 months          | 2.6                   | NSR                           | NSR                           | NSR                           | 40        |
| Follicular lymphoma              | 196                | BS (PF05280586)| 4                | Weekly intervals   | 375 mg/m²       | 52 wk              | 22.1<sup>a</sup> | NSR                           | NSR                           | NR                            | 41        |
| Follicular lymphoma              | 198                | Rituximab      | 4                | Weekly intervals   | 375 mg/m²       | 52 wk              | 19.8<sup>a</sup> | NSR                           | NSR                           | NR                            | 41        |
rituximab-EU and rituximab-US. In another study, it was also demonstrated that the immunogenicity of biosimilar GP2013, rituximab-EU and rituximab-US was similar in patients with active RA. Moreover, similar immunogenicity was reported for CT-P10 (a rituximab biosimilar) and rituximab in RA patients. Similar immunogenicity was also reported for Kikuzubam (Rituximab biosimilar) and MabThera (Rituximab). Collectively, the findings from different studies summarized in Tables I and II indicate that in most circumstances, the rituximab and its biosimilar drugs exhibit similar immunogenicity in patients with lymphoma or autoimmune diseases. However, a discrepancy was observed in some situations considering the immunogenicity of rituximab and its biosimilar (Tables I and II).

The association between patients’ genetic profile and anti-rituximab antibody formation: The genetic background is an essential patient-related parameter affecting the antigenicity of a biological therapeutic agent. The polymorphisms in human leukocyte antigen (HLA) are related to the likelihood of ADA formation. For instance, the HLA-DR1 locus was associated with a greater prevalence of the ADA against infliximab in patients with Crohn’s disease. Around 81 per cent of ADA-positive patients displayed DRβ S13 residue, in comparison with 50 per cent of ADA-negative patients. To date, there is no way to distinguish the producers of ARA from non-producers of ARA before treatment with a rituximab. As immune response genes, especially human leukocyte antigen (HLA)-related genes play a fundamental role in the induction of antibody response to a given antigen, the clarification of the association between the HLA genes and development of ARA needs to be considered in future studies. If the association of some HLA genes with the development of ARA is confirmed, then the risk of drug immunogenicity may be predictable prior to rituximab treatment.

The association between the rituximab efficacy and anti-rituximab antibody development

The ADA development may have important clinical consequences in patients with autoimmune and malignant diseases treated with mAbs. However, no significant association was reported between the ARA positivity and serum drug levels in rituximab-treated patients with lymphoma (Table I). Furthermore, ARA positivity did not significantly influence the drug efficacy in rituximab-administered patients with lymphoma (Table I).
| Disease type                      | Number of patients | Rituximab type | Infusion numbers | Infusion programme | Rituximab doses | ARA monitoring times | Per cent ARA positivity | ARA relation to adverse events | ARA relation to drug efficacy | ARA relation to serum drug levels | Reference |
|----------------------------------|--------------------|----------------|------------------|-------------------|-----------------|---------------------|------------------------|-------------------------------|-------------------------------|--------------------------------|-----------|
| RRMS                             | 238                | Rituximab      | 1–8              | 6 months          | 500 or 1000 mg  | >5 months post-infusion | 37.0                | NSR                           | NSR                           | NSR                           | 30        |
| PPMS                             | 101                | 1–8            |                  |                   |                 |                      | 26.5                | NSR                           | NSR                           | NSR                           | 30        |
| RRMS                             | 69                 | Rituximab-EU   | 2                | Days 1 and 15     | 1000 mg         | At baseline, wk 24 & 48 | 24.6                | NSR                           | NSR                           | NSR                           | 44        |
| RRMS                             | 292                | Rituximab      | 8                | Wk 0, 2, 24, 26, 48, 50, 72, 74 | 1000 mg         | Wk 2, 4, 24, 26, 48, 50, 72, 74 | 7.0                 | NSR                           | NR                            | NSR                           | 45        |
| SLE (low grade)                  | 6                  | Rituximab      | 1                | Single infusion   | 100 mg          | Wk 3, 6, 9 & 12    | 16.6                | NSR                           | Was observed                  | Fast drop in drug level | 46        |
| SLE (intermediate grade)         | 7                  | Rituximab      | 1                | Single infusion   | 375 mg          | Wk 3, 6, 9 & 12    | 42.8                | NSR                           | Was observed                  | Fast drop in drug level | 46        |
| SLE (high grade)                 | 4                  | Rituximab      | 4                | Weekly intervals  | 375 mg          | Wk 3, 6, 9 & 12    | 50.0                | NSR                           | Was observed                  | Fast drop in drug level | 46        |
| Rheumatoid arthritis             | 316                | Rituximab      | 2                | Days 1 and 15     | 500 or 1000 mg  | 4 wk intervals between baseline and wk 24 | 4.2 and 2.7          | NSR                           | NSR                           | NSR                           | 47        |
| Sjögren’s syndrome               | 15                 | Rituximab      | 4                | Weekly intervals  | 375 mg          | Wk 5, 12, 24, 48   | 27.0                 | 3 of 4 ARA+patients exhibited serum sickness | NSR                           | NR                            | 48        |
| Pemphigus vulgaris               | 11                 | Rituximab      | 4                | Weekly intervals  | 375 mg          | 3, 9 and 15 months 1st first infusion | 18.18               | NR                            | Was observed                  | NSR                           | 49        |
| Disease type          | Number of patients | Rituximab type | Infusion numbers | Infusion programme | Rituximab doses | ARA monitoring times | Per cent ARA positivity | ARA relation to adverse events | ARA relation to drug efficacy | ARA relation to serum drug levels | Reference |
|----------------------|--------------------|----------------|------------------|--------------------|-----------------|----------------------|--------------------------|-------------------------------|--------------------------------|--------------------------------|-----------|
| Rheumatoid arthritis | 74                 | Rituximab-EU   | 2                | Days 1 and 15      | 1000 mg         | Days 1, 15, 29, 57, 85, 169 | 13.5±††                 | NSR                           | NSR                           | NSR                           | 50        |
|                      | 73                 | Rituximab-US   | 2                | Days 1 and 15      | 1000 mg         | Days 1, 15, 29, 57, 85, 169 | 12.3±††                 | NSR                           | NSR                           | NSR                           |           |
|                      | 73                 | BS (PF-05280586) | 2               | Days 1 and 15      | 1000 mg         | Days 1, 15, 29, 57, 85, 169 | 9.5±††                  | NSR                           | NSR                           | NSR                           |           |
| Rheumatoid arthritis | 51                 | Rituximab      | 2                | Days 1 and 15      | 1000 mg         | Wk 8, 16, 24          | 17.6±                  | NSR                           | Was observed†                  |                                | 51        |
|                      | 102                | BS (CT-P10)    | 2                | Days 1 and 15      | 1000 mg         | Wk 8, 16, 24          | 17.6±                  | NSR                           | Was observed†                  |                                |           |
| Rheumatoid arthritis | 23                 | Rituximab      | 4                | Days 1 and 15, weeks 24–48 | 500 or 1000 mg | Wk 8, 16, 24 after last infusion | 21.7±                 | NR                            |                                |                                | 52        |
|                      | 60                 | BS (CT-P10)    | 4                | Days 1 and 15, weeks 24–48 | 500 or 1000 mg | Wk 8, 16, 24 after last infusion | 20.0±                 | NR                            |                                |                                |           |
| Rheumatoid arthritis | 87                 | Rituximab-EU   | 2                | Days 1 and 15      | 1000 mg         | 4, 8, 16, 24 wk after 1st infusion | 15.1±                 | NR                            | Was observed†                  |                                | 53        |
|                      | 92                 | Rituximab-US   | 2                | Days 1 and 15      | 1000 mg         | 4, 8, 16, 24 wk after 1st infusion | 15.1±                 | NR                            | Was observed†                  |                                |           |
|                      | 133                | BS (GP2013)    | 2                | Days 1 and 15      | 1000 mg         | 4, 8, 16, 24 wk after 1st infusion | 16.5±                 | NR                            | Was observed†                  |                                |           |
| Rheumatoid arthritis | 53                 | BS (GP2013)    | 2                | 2 weeks intervals  | 1000 mg/m²     | Wk 2, 12, 24         | 0.0                    | NSR                           | NSR                           | NR                             | 54        |
|                      | 54                 | Rituximab      | 2                | 2 weeks intervals  | 1000 mg/m²     | Wk 2, 12, 24         | 1.8                    | NSR                           | NSR                           | NR                             |           |

Contd...
The association of the ARA positivity and drug levels and efficacy was also reported in a number of autoimmune disorders with inconsistent results (Table II). For example, no significant difference was found between ADA-positive and ADA-negative MS patients concerning the efficacy of rituximab. The results from an investigation revealed that the ADA positivity did not influence the drug level and efficiency in rituximab-treated patients with SLE. The results from a multinational study indicate that ARA-positive patients with RA exhibit lower drug levels compared with ARA-negative patients, but without influencing the drug efficacy. However, it was reported that ARA-positive patients with SLE exhibit lower drug levels along with lower drug efficacy compared to ARA-negative patients. In addition, the ARA positivity suggestively influences the treatment efficacy in rituximab-treated patients with Pemphigus negatively. Furthermore, higher titre of ARA was accompanied by higher disease activity at baseline in rituximab-administered patients with SLE. The presence of ADA may reduce the serum levels of administrated mAb. The RA patients who were positive for ADA had lower levels of administrated mAb and higher clearance as compared to patients who were negative for ADA.

### Anti-rituximab antibody-related adverse clinical consequences

Immune response-linked adverse events are the most frequent side-effects in mAb-treated patients, which mainly affect the skin and gastrointestinal tract with less frequent manifestations in the liver, endocrine and nervous organs. The side effects of a mAb may be partly attributed to its immunogenicity. The results from studies summarized in Table 1 indicate that there was no significant association between ARA positivity and expression of adverse events in rituximab-treated patients with lymphoma. Moreover, no significant association was reported between ARA positivity and expression of adverse events in rituximab-treated patients with autoimmune diseases such as MS and RA, and pemphigus vulgaris (Table II). In studies carried out on rituximab-administered MS patients, the presence of ARA was not related to the type or severity of harmful events during the study. No significant differences were reported between rituximab-treated RA patients with positive or negative ARA status concerning the serious adverse events. No correlation was reported between ARA production and elevated risk of infusion-associated adverse
reactions in rituximab-treated patients with pemphigus vulgaris.

However, the expression of serum sickness events was reported in some rituximab-treated patients with SLE or Sjögren’s syndrome (Table II). Four serum sickness events (3 of the 4 patients were positive for ARA) were observed in the 169 rituximab-administered patients with SLE compared with no event in the 88 placebo-treated subjects. In another study carried out on the 15 rituximab-administered patients with Sjögren’s syndrome, four out of 15 patients (27%), were positive for ARA, of these three exhibited serum sickness. The results of a systematic review on 25 studies indicated 33 cases with rituximab-mediated serum sickness. The expression of the serum sickness occurred mainly after the second dose in the first cycle of infusion. Further, the rituximab-mediated serum sickness is more prevalent (> 12 times) in patients with autoimmune disorders compared to patients with haematological malignancies. However, the reasons for higher development of the serum sickness in patients with autoimmune diseases remains to be clarified in the future. Serum sickness has been observed in patients with concomitant presence of hypergammaglobulinemia and rheumatoid factor. The concomitant chemotherapy used for treatment of the malignancies may be protective against serum sickness in rituximab-treated subjects.

The B cells act as efficient APCs, because they express HLA class II molecules. The internalization of CD20-rituximab was reported in some rituximab-treated patients with CLL. Therefore, B cells can present rituximab-derived peptides to specific Th cells in association with the HLA class II molecules. Then, Th cell-derived cytokines help B cells to produce high-affinity ADA from various isotypes, such as IgG and IgE. The antibody response to rituximab as an antigenic protein can be influenced by numerous parameters including host-related factors (HLA gene, cytokine gene polymorphisms, age, gender, immunosuppression, disease type and concomitant medication). Some other host-related parameters may also affect the ADA development, such as weight, nutrition, psychological stress and smoking. The treatment-related factors such as route of administration, dose, infusion numbers and duration of treatment also influence the ADA production.

The findings presented in this study indicate that the risk of ARA development and ARA-related adverse events is low in rituximab-treated patients with lymphoma. No significant association was reported between the ARA positivity and serum levels and drug efficacy either in rituximab-treated patients with lymphoma (Table I). However, the patients with autoimmune disorders exhibit a greater risk of ARA development and ARA-related adverse events. Therefore, it is required to outline the major criteria to predict the rituximab immunogenicity before starting the drug treatment. In autoimmune diseases, ARA positivity may have no significant impacts on either the drug level or its efficacy, so it may reduce drug levels without influencing its efficacy, or may reduce the drug efficacy without influencing the levels, or may reduce both drug level as well as its efficacy. The exact evaluation of both the host- and treatment-related parameters, and the characterization of ARA are, hence, necessary to clarify regarding factors influencing the drug levels and its efficacy in rituximab-treated patients with autoimmune disorders. Structural modifications in a drug to decrease its immunogenicity, combinational therapy using an appropriate B-cell modulator, and removal of the ADA may be considered as strategies to increase the efficacy of a mAb.

Moreover, various immunoassay methods such as enzyme-linked immunosorbent assay (ELISA), electrochemiluminescence (ECL) and colorimetric assays were used to detect ARA, with inconsistent results in some cases. The affinity capture elution-ELISA technique has been introduced as a valid method for the detection of ARA in which the rituximab–ARA complexes were dissociated by adding an acidic reagent to serum. It has been also reported that the ECL method exhibits more sensitivity than the ELISA method for detection of ARA. The standardization of the methods for ARA detection need further consideration.

Financial support & sponsorship: None.

Conflicts of Interest: None.

References
1. Sathyanarayanan V, Neelapu SS. Cancer immunotherapy: Strategies for personalization and combinatorial approaches. Mol Oncol 2015; 9: 2043-53.
2. Harding FA, Stickler MM, Razo J, DuBridge RB. The immunogenicity of humanized and fully human antibodies: Residual immunogenicity resides in the CDR regions. MAbs 2010; 2: 256-65.
3. Glassman PM, Balthasar JP. Mechanistic considerations for the use of monoclonal antibodies for cancer therapy. Cancer Biol Med 2014; 11 : 20-33.

4. Scott AM, Allison JP, Wolchok JD. Monoclonal antibodies in cancer therapy. Cancer Immun 2012; 12 : 14.

5. Neves H, Kwok HF. Recent advances in the field of anti-cancer immunotherapy. BBA Clin 2015; 3 : 280-8.

6. Weiner LM, Surana R, Wang S. Monoclonal antibodies: Versatile platforms for cancer immunotherapy. Nat Rev Immunol 2010; 10 : 317-27.

7. Baert F, Noman M, Vermeire S, Van Assche G, D’Haens G, Carbonza A, et al. Influence of immunogenicity on the long-term efficacy of infliximab in Crohn’s disease. N Engl J Med 2003; 348 : 601-8.

8. Klee GG. Human anti-mouse antibodies. Arch Pathol Lab Med 2000; 124 : 921-3.

9. Strand V, Al-Bali A, Al-Saleh J, Barile-Fabris L, Horiuchi T, Takeuchi T, et al. Immunogenicity of biologics in chronic inflammatory diseases: A systematic review. BioDrugs 2017; 31 : 299-316.

10. Schellekens H. Factors influencing the immunogenicity of therapeutic proteins. Nephrol Dial Transplant 2005; 20 (Suppl 6) : vi3-9.

11. Mirick GR, Bradt BM, Denardo SJ, Denardo GL. A review of human anti-globulin antibody (HAGA, HAMA, HACA, HAHA) responses to monoclonal antibodies. Not four letter words. Q J Nucl Med Mol Imaging 2004; 48 : 251-7.

12. Farrell RJ, Alshahi M, Jeen YT, Falchuk KR, Peppercorn MA, Michetti P. Intravenous hydrocortisone premedication reduces antibodies to infliximab in Crohn’s disease: A randomized controlled trial. Gastroenterology 2003; 124 : 917-24.

13. Hanauer SB, Wagner CL, Bala M, Mayer L, Travers S, Diamond RH, et al. Incidence and importance of antibody responses to infliximab after maintenance or episodic treatment in Crohn’s disease. Clin Gastroenterol Hepatol 2004; 2 : 542-53.

14. Garcia S, Demengeot J, Benito-Garcia E. The immunogenicity of anti-TNF therapy in immune-mediated inflammatory diseases: A systematic review of the literature with a meta-analysis. Ann Rheum Dis 2013; 72 : 1947-55.

15. Kuriakose A, Chirmule N, Nair P. Immunogenicity of biotherapeutics: Causes and association with posttranslational modifications. J Immunol Res 2016; 2016 : 1298473.

16. Haraya K, Tachibana T, Igawa T. Improvement of pharmacokinetic properties of therapeutic antibodies by antibody engineering. Drug Metab Pharmacokinet 2019; 34 : 25-41.

17. Jilani I, O’Brien S, Manshuri T, Thomas DA, Thomazy VA, Imam M, et al. Transient down-modulation of CD20 by rituximab in patients with chronic lymphocytic leukemia. Blood 2003; 102 : 3514-20.

18. D’Auria F, Guariglia R, Villani O, Mansuetto G, Greico V, Zonno A, et al. Modulation of CD20 antigen expression after rituximab treatment: A retrospective study in patients with chronic lymphocytic leukemia. Clin Ther 2010; 32 : 1911-6.

19. Talotta R, Rucci F, Canti G, Scaglione F, Pros and cons of the immunogenicity of monoclonal antibodies in cancer treatment: A lesson from autoimmune diseases. Immunotherapy 2019; 11 : 241-54.

20. Yin L, Chen X, Vicini P, Rup B, Hickling TP. Therapeutic outcomes, assessments, risk factors and mitigation efforts of immunogenicity of therapeutic protein products. Cell Immunol 2015; 295 : 118-26.

21. Salles G, Barrett M, Foà R, Maurer J, O’Brien S, Valente N, et al. Rituximab in B-cell hematologic malignancies: A review of 20 years of clinical experience. Adv Ther 2017; 34 : 2232-73.

22. Miranda-Hernández MP, López-Morales CA, Ramírez-Ibáñez ND, Piña-Lara N, Pérez NO, Molina-Pérez A, et al. Assessment of physicochemical properties of rituximab related to its immunomodulatory activity. J Immunol Res 2015; 2015 : 910763.

23. Weiner LM, Dhodapkar MV, Ferrone S. Monoclonal antibodies for cancer immunotherapy. Lancet 2009; 373 : 1033-40.

24. Tsushima N, Hinton PR, Kumar S. Design of humanized antibodies: From anti-Tac to Zenapax. Methods 2005; 36 : 69-83.

25. Breedveld FC. Therapeutic monoclonal antibodies. Lancet 2000; 355 : 735-40.

26. Borregaarde CA, Carlsson R. Human therapeutic antibodies. Curr Opin Pharmacol 2001; 1 : 404-8.

27. Shan D, Ledbetter JA, Press OW. Signaling events involved in anti-CD20-induced apoptosis of malignant human B cells. Cancer Immunol Immunother 2000; 48 : 673-83.

28. Leandro MJ. B-cell subpopulations in humans and their differential susceptibility to depletion with anti-CD20 monoclonal antibodies. Arthritis Res Ther 2013; 15 (Suppl 1) : S3.

29. van Meerten T, Hagenbeek A. CD20-targeted therapy: The next generation of antibodies. Semin Hematol 2010; 47 : 199-210.

30. Dunn N, Juto A, Ryner M, Manouchehrinia A, Piccoli L, Fink K, et al. Rituximab in multiple sclerosis: Frequency and clinical relevance of anti-drug antibodies. Mult Scler 2018; 24 : 1224-33.

31. Maeda T, Yamada Y, Tawara M, Yamasaki R, Yakata Y, Ramírez-Ibáñez ND, Piña-Lara N, Pérez NO, Molina-Pérez A, et al. Assessment of physicochemical properties of rituximab related to its immunomodulatory activity. J Immunol Res 2015; 2015 : 910763.

32. Saiffari F, Jafarzadeh A, Kalantari Khandani B, Saiffari F, Soleimanyamoli S, Mohammadi M. Immunogenicity of rituximab, trastuzumab, and bevacizumab monoclonal antibodies in patients with malignant diseases. Int J Cancer Manag 2018; 11 : E64983.

33. Fasanmade AA, Adedokun OJ, Blank M, Zhou H, Davis HM. Pharmacokinetic properties of infliximab in children and
adults with Crohn’s disease: A retrospective analysis of data from 2 phase III clinical trials. *Clin Ther* 2011; 33 : 946-64.

34. Tobinai K, Kobayashi Y, Narabayashi M, Ogura M, Kagami Y, Morishima Y, et al. Feasibility and pharmacokinetic study of a chimeric anti-CD20 monoclonal antibody (IDEC-C2B8, rituximab) in relapsed B-cell lymphoma. The IDEC-C2B8 Study Group. *Ann Oncol* 1998; 9 : 527-34.

35. Maloney DG, Grillo-López AJ, White CA, Bodkin D, Schilder RJ, Neidhart JA, et al. IDEC-C2B8 (Rituximab) anti-CD20 monoclonal antibody therapy in patients with relapsed low-grade non-Hodgkin’s lymphoma. *Blood* 1997; 90 : 2188-95.

36. Maloney DG, Liles TM, Czerwinski DK, Waldichuk C, Rosenberg J, Grillo-Lopez A, et al. Phase I clinical trial using escalating single-dose infusion of chimeric anti-CD20 monoclonal antibody (IDEC-C2B8) in patients with recurrent B-cell lymphoma. *Blood* 1994; 84 : 2457-66.

37. Piro LD, White CA, Grillo-López AJ, Janakiraman N, Saven A, Beck TM, et al. Extended Rituximab (anti-CD20 monoclonal antibody) therapy for relapsed or refractory low-grade or follicular non-Hodgkin’s lymphoma. *Ann Oncol* 1999; 10 : 655-61.

38. Candelaria M, Gonzalez D, Gómez FJF, Paravisisi A, Del Campo Garcia A, Pérez L, et al. Comparative assessment of pharmacokinetics, and pharmacodynamics between RTXM83™, a rituximab biosimilar, and rituximab in diffuse large B-cell lymphoma patients: A population PK model approach. *Cancer Chemother Pharmacol* 2018; 81 : 515-27.

39. Candelaria M, González DE, Delamain MT, Bár DO, Beniwal SK, Dasappa L, et al. Rituximab biosimilar RTXM83 versus reference rituximab in combination with CHOP as first-line treatment for diffuse large B-cell lymphoma: A randomized, double-blind study. *Leuk Lymphoma* 2019; 60 : 3375-85.

40. Viswabandya A, Shah S, Mukhopadhyay A, Nagarkar RV, Batra SS, Lopez-Lazaro L, et al. Randomized, double-blind, pharmacokinetic equivalence trial comparing DRL-rituximab with MabThera® in patients with diffuse large B-cell lymphoma. *J Glob Oncol* 2019; 5 : 1-13.

41. Sharan JP, Liberati AM, Ishizawa K, Khan T, Robbins J, Alcasid A, et al. A randomized, double-blind, efficacy and safety study of PF-05280586 (a rituximab biosimilar) compared with rituximab reference product (MabThera®) in subjects with previously untreated CD20-positive, low-tumor-burden follicular lymphoma (LTB-FL). *BioDrugs* 2020; 34 : 171-81.

42. Poddubnaya IV, Alekseev SM, Kaplanov KD, Lukavetskyy LM, Rechtman GB, Dolai TK, et al. Proposed rituximab biosimilar BCD-020 versus reference rituximab for treatment of patients with indolent non-Hodgkin lymphomas: An international multicenter randomized trial. *Hematol Oncol* 2020; 38 : 67-73.

43. McLaughlin P, Grillo-López AJ, Link BK, Levy R, Czuczman MS, Williams ME, et al. Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: Half of patients respond to a four-dose treatment program. *J Clin Oncol* 1998; 16 : 2825-33.

44. Hauser SL, Waubant E, Arnold DL, Vollmer T, Antel J, Fox RJ, et al. B-cell depletion with rituximab in relapsing-remitting multiple sclerosis. *N Engl J Med* 2008; 358 : 676-88.

45. Hawker K, O’Connor P, Freedman MS, Calabresi PA, Antel J, Simon J, et al. Rituximab in patients with primary progressive multiple sclerosis: Results of a randomized double-blind placebo-controlled multicenter trial. *Ann Neurol* 2009; 66 : 460-71.

46. Looney RJ, Anolik JH, Campbell D, Felgar RE, Young F, Arend LJ, et al. B cell depletion as a novel treatment for systemic lupus erythematosus: A phase I/II dose-escalation trial of rituximab. *Arthritis Rheum* 2004; 50 : 2580-9.

47. Emery P, Fleischmann R, Filipowicz-Sosnowska A, Schechtman J, Szczepanski L, Kavagnaugh A, et al. The efficacy and safety of rituximab in patients with active rheumatoid arthritis despite methotrexate treatment: Results of a phase IIB randomized, double-blind, placebo-controlled, dose-ranging trial. *Arthritis Rheum* 2006; 54 : 1390-400.

48. Pipe J, Van Imhoff G, Spijkervet F, Roodenburg JL, Wolbink GJ, Mansour K, et al. Rituximab treatment in patients with primary Sjögren’s syndrome: An open-label phase II study. *Arthritis Rheum* 2005; 52 : 2740-50.

49. Schmidt E, Hennig K, Mengede C, Zillikens D, Kromminga A. Immunogenicity of rituximab in patients with severe pemphigus. *Clin Immunol* 2009; 132 : 334-41.

50. Cohen S, Emery P, Greenwald M, Yin D, Becker JC, Melia LA, et al. A phase I pharmacokinetics trial comparing PF-05280586 (a potential biosimilar) and rituximab in patients with active rheumatoid arthritis. *Br J Clin Pharmacol* 2016; 82 : 129-38.

51. Yoo DH, Suh CH, Shim SC, Jeka S, Cons-Molina FF, Hrycay P, et al. A multicentre randomised controlled trial to compare the pharmacokinetics, efficacy and safety of CT-P10 and innovator rituximab in patients with rheumatoid arthritis. *Ann Rheum Dis* 2017; 76 : 566-70.

52. Yoo DH, Suh CH, Shim SC, Jeka S, Molina FFC, Hrycay P, et al. Efficacy, safety and pharmacokinetics of up to two courses of the rituximab biosimilar CT-P10 versus innovator rituximab in patients with rheumatoid arthritis. *Ann Rheum Dis* 2017; 76 : 1598-602.

53. Smolen JS, Cohen SB, Tony HP, Scheinberg M, Kivitz A, Balanescu A, et al. A randomised, double-blind trial to demonstrate bioequivalence of GP2013 and reference rituximab combined with methotrexate in patients with active rheumatoid arthritis. *Ann Rheum Dis* 2017; 76 : 1598-602.

54. Tony HP, Krüger K, Cohen SB, Schulze-Koops H, Kivitz AJ, Jeka S, et al. Brief report: Safety and immunogenicity of rituximab biosimilar GP 2013 after switch from reference rituximab in patients with active rheumatoid arthritis. *Arthritis Care Res (Hoboken)* 2019; 71 : 88-94.

55. Merrill JT, Neuwelt CM, Wallace DJ, Shanahan JC, Latinis KM, Oates JC, et al. Efficacy and safety of rituximab in...
moderately-to-severely active systemic lupus erythematosus: The randomized, double-blind, phase II/III systemic lupus erythematosus evaluation of rituximab trial. *Arthritis Rheum* 2010; 62: 222-33.

56. Maser EA, Villela R, Silverberg MS, Greenberg GR. Association of trough serum infliximab to clinical outcome after scheduled maintenance treatment for Crohn’s disease. *Clin Gastroenterol Hepatol* 2006; 4: 1248-54.

57. Sheikhi A, Jafarzadeh A, Kokhai P, Hojjat-Farsangi M. Whole tumor cell vaccine adjuvants: Comparing IL-12 to IL-2 and IL-15. *Iran J Immunol* 2016; 13: 148-66.

58. Ancuceanu R, Neagu M. Immune based therapy for melanoma. *Indian J Med Res* 2016; 143: 135-44.

59. Khalife E, Khodadadi A, Talaeizadeh A, Rahimian L, Nemati M, Jafarzadeh A. Overexpression of regulatory T cell-related markers (FOXP3, CTLa-4 and GITR) by peripheral blood mononuclear cells from patients with breast cancer. *Asian Pac J Cancer Prev* 2018; 19: 3019-25.

60. Guerrouahen BS, Maccalli C, Cugno C, Rutella S, Akporiaye ET. Reverting immune suppression to enhance cancer immunotherapy. *Front Oncol* 2019; 9: 1554.

61. Kim JH, Kim BS, Lee SK. Regulator T cells in tumor microenvironment and approach for anticancer immunotherapy. *Immunome Net* 2020; 20: e4.

62. Moss A, Brinks V, Carpenter J. Immunogenicity of anti-TNF biologics used for cancer therapy. *Front Immunol* 2015; 6: 135-44.

63. Jafarzadeh A, Bagheri-Jamebozorgi M, Nemati M, Golsaz-Shirazi F, Shokri F. Human leukocyte antigens influence the antibody response to hepatitis B vaccine. *Iran J Allergy Asthma Immunol* 2015; 14: 233-45.

64. Bar-Or A, Calabresi PA, Arnold D, Markowitz C, Shafer S, Kasper LH, et al. Rituximab in relapsing-remitting multiple sclerosis: A 72-week, open-label, phase I trial. *Ann Neurol* 2008; 63: 395-400.

65. Emery P, Deodhar A, Rigby W, Isaacs JD, Combe B, Racewicz AJ, et al. Efficacy and safety of switching from innovator rituximab to biosimilar CT-P10 compared with continued treatment with CT-P10: Results of a 56-week open-label study in patients with rheumatoid arthritis. *BioDrugs* 2017; 31: 369-77.

66. Park W, Suh CH, Shim SC, Molina FFC, Jeka S, Medina-Rodriguez FG, et al. Efficacy and safety of switching from innovator rituximab to biosimilar CT-P10 compared with continued treatment with CT-P10: Results of a 56-week open-label study in patients with rheumatoid arthritis. *BioDrugs* 2017; 31: 369-77.

67. Deegan PB. Fabry disease, enzyme replacement therapy and the significance of antibody responses. *J Inherit Metab Dis* 2012; 35: 227-43.

68. Magira E, Lind C, Baldassano R, Monos D. 236-P: The HLA DRβ13 residue is associated with the development of human anti-chimeric antibody in some Crohn’s patients treated with infliximab. *Hum Immunol* 2009; 70: S131.

69. Jafarzadeh A, Bagheri-Jamebozorgi M, Nemati M, Golsaz-Shirazi F, Shokri F. Human leukocyte antigens influence the antibody response to hepatitis B vaccine. *Iran J Allergy Asthma Immunol* 2015; 14: 233-45.

70. Bar-Or A, Calabresi PA, Arnold D, Markowitz C, Shafer S, Kasper LH, et al. Rituximab in relapsing-remitting multiple sclerosis: A 72-week, open-label, phase I trial. *Ann Neurol* 2008; 63: 395-400.

71. Emery P, Deodhar A, Rigby W, Isaacs JD, Combe B, Racewicz AJ, et al. Efficacy and safety of switching from innovator rituximab to biosimilar CT-P10 compared with continued treatment with CT-P10: Results of a 56-week open-label study in patients with rheumatoid arthritis. *BioDrugs* 2017; 31: 369-77.

72. Guan M, Zhou YP, Sun JL, Chen SC. Adverse events of monoclonal antibodies used for cancer therapy. *Biomed Res Int* 2015; 2015: 428169.

73. Karmacharya P, Poudel DR, Pathak R, Donato AA, Ghimire S, Giri S, et al. Rituximab-induced serum sickness: A systematic review. *Semin Arthritis Rheum* 2015; 45: 334-40.

74. Bayer G, Agier MS, Lepelley M, Zenut M, Lanoue MC, et al. Rituximab-induced serum sickness is more frequent in autoimmune diseases as compared to hematological malignancies: A French nationwide study. *Eur J Intern Med* 2019; 67: 59-64.

75. Rubin SJS, Bloom MS, Robinson WH. B cell checkpoints in autoimmune rheumatic diseases. *Nat Rev Rheumatol* 2019; 15: 303-15.

---

For correspondence: Dr Abdollah Jafarzadeh, Department of Immunology, Medical School, Kerman University of Medical Sciences, Kerman, Iran

e-mail: jafarzadeh14@yahoo.com