The potential risk of infections during (prolonged) rituximab therapy in rheumatoid arthritis

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Introduction: Biologicals are a fast expanding group of new drugs and rituximab (RTX) is one of them. Long-term efficacy and safety constantly need addressing as little is known about these factors. In rheumatoid arthritis, RTX is used for active disease that is not responding to other therapies. Since RTX acts by depleting B-cells, concerns regarding the long-term safety of this drug have been raised.

Areas covered: This review covers 10 manuscripts on RTX safety in rheumatoid arthritis published between January 2004 and July 2010. Expert opinion: In present literature RTX appears to be safe for up to five courses. In this review, important drawbacks of current research are discussed. Longer follow-up time is needed to make relevant conclusions on RTX safety with regard to infectious complications. Prolonged RTX therapy causes subsequent B-cell depletion. Eventually, plasma cells disappear, causing hypogammaglobulinemias and subsequent problems in immunity. The formation of new plasma cells is halted due to a lack of B-cells. Attention needs to be focused on the status of immunoglobulins and the role this plays in the occurrence of infections. Until a complete, long-term safety profile of RTX is available, it cannot be considered safe with regard to the incidence of infectious complications.

Keywords: immunoglobulin, infection, rheumatoid arthritis, rituximab, serious infection

1. Introduction

Rheumatoid arthritis (RA) is a potentially severe disabling condition. It is a systemic auto-immune disease, clinically characterized by symmetrical inflammation of joints. Usually, hands and feet are affected. The disease results in inflamed, swollen and painful joints, ultimately causing their destruction. First presentation is most commonly between the ages of 25 and 50. However, the disease can present at any age. The prevalence of RA is ~1% of the general population. The disease affects women more than men in the ratio of 2 to 3 persons to 1 [1,2]. The major symptom of RA is peripheral arthritis, which may also occur in other inflammatory joint diseases such as ankylosing spondylitis and psoriatic arthritis. RA is clinically diagnosed based on signs and symptoms. The diagnosis is supported by laboratory tests that show raised inflammatory markers such as the erythrocyte sedimentation rate (ESR) and elevated C-reactive protein (CRP) levels as well as serological markers such as a positive rheumatoid factor (RF+) or antibodies to citrullinated proteins (anti-CCP+). After the diagnosis, the rheumatologist follows severity and course of the individual patient with clinical disease-specific severity tests, that is, disease activity score (DAS) [3], biochemical parameters of inflammation and radiological progression.
The potential risk of infections during (prolonged) rituximab therapy in rheumatoid arthritis

1.1 Treatment
On diagnosis of RA, patients start pharmacotherapy, and symptomatic treatments such as physical therapy as well as ergotherapy are advised. General practitioners primarily prescribe NSAIDs [4]. These compounds are able to slow down the inflammation process by inhibiting prostaglandin production. However, no influence on disease progression in terms of joint damage is obtained with NSAID therapy. Currently, after diagnosis of RA, disease-modifying antirheumatic drugs (DMARDs) are started immediately. At present, methotrexate (MTX) is the cornerstone of DMARD therapy. It is proven to modify disease activity as well as retard or even hold back the radiologic progression of damage. MTX is a reversible inhibitor of dihydrofolate reductase and antagonizes folic acid. It causes interference with DNA synthesis, repair and cellular replication. MTX at a dose of 7.5 – 25 mg/week is well tolerated in a population of RA patients with a powerful antirheumatic effect generally [4]. Low-dose glucocorticosteroids (GCs; 5 – 10 mg/day p.o.), a known suppressor of the immune system, can be added at this or later steps [4]. When complete remission of RA is not obtained, a combination of DMARDs is started. In ~ 10 – 30% of cases a biological is needed in order to obtain remission. At present, several biological response modifiers are available: TNF-α antagonists (adalimumab, certolizumab pegol, etanercept, golimumab and infliximab) but also a B-cell depleting antibody (rituximab or RTX), a modulator of the costimulation of T-cells (abatacept) and inhibitors of IL-1 (anakinra) and IL-6 (tocilizumab).

1.2 Biologicals
Over the last few years many new treatment options have become available, most of them belonging to the group of biologicals. Biologicals are produced from cell lines by using recombinant DNA techniques. The process results in antibodies that have more or less animal protein fragments incorporated, potentially causing allergic reactions or reduced efficacy in some patients. This problem can be reduced by producing more human-like proteins. These compounds provide excellent therapeutic options for the treatment of RA patients. They have in common the fact that they affect compounds of the accelerated inflammatory pathways in RA. They slow down the accelerated immune process by blocking cytokine activity (IL and TNF) or intercellular signaling pathways (the costimulatory signaling pathway), or by depleting B-cells (humoral immunity). Since biological compounds were introduced relatively recently, long-term effects and safety issues still need to be addressed in detail. The risk of developing malignancies [5] and the incidence of infection are currently studied. This review focuses on the risk of infection in RA patients during treatment with RTX, which is a B-cell depleting antibody. By depleting B-cells, concerns about the risk of bacterial infection due to disturbed humoral immunity are raised.

1.3 Infections in RA
Patients suffering from RA have a higher risk of infection than healthy controls. The overall infection rate is 20/100 person-years for patients with RA versus 13/100 person-years in the general population, with a relative risk (RR) of 1.53 (1.41, 1.65). These data reflect established infections by culture or imaging. Even corrected for confounders, such as GC use, a significantly higher infection risk was found in RA patients [6].

1.3.1 Infections during RA treatment
As discussed earlier, most RA patients are treated with a regimen including NSAID, MTX and/or prednisolone; most biologicals are preferred as adjuvant therapy alongside MTX. However, biologicals can be used as monotherapy when MTX is not tolerated or contra-indicated. NSAID use is not related to an increased risk of infection. A review on MTX [7] suggests that there might be a minimally increased infection risk when patients use MTX, although most data in this review do not support an increased infection risk. Seven of the nine reviewed papers show no increased infection risk, whereas one study shows an RR of 1.16 (95% CI 1.18,1.97) for pneumonia and another shows an RR of 1.52 (95% CI 1.04,2.22) for any infection and 2.19 (95% CI 1.45,3.31) for skin infections. A review on GC and infection risk in RA patients [8] showed a small increase in risk of infection for patients on low-dose GC (< 10 mg/day) compared to RA patients not treated with GC. The increase was seen in two out of eight reviewed reports and in one of three reviewed trials. One report showed RRs that ranged from 1.32 (95% CI 1.06,1.63) for low-dose prednisone (< 5 mg/day) to 1.95 (95% CI 1.53 – 2.46) for higher-dose prednisone (6 – 10 mg/day). This trial showed a hazard ratio (HR) of 1.7 (95% CI 1.5 – 2.0) for infections for RA patients on GC therapy.
These data are generally supported by a retrospective longitudinal study by Lacaille et al. [9]. The risk of infection during use of non-biological DMARDs and GCs was studied using data of 27,710 patients. The data were compared with infection data on patients with no DMARD and no GC. For mild infections they found an RR of 1.12 (95% CI 1.08 – 1.16) during the use of a DMARD + GC, an RR of 0.9 (95% CI 0.88 – 0.93) during the use of a DMARD and an RR of 1.15 (95% CI 1.11 – 1.19) during the use of a GC alone. For serious infections, an RR of 1.63 (95% CI 1.5 – 1.77) was reported during the use of a DMARD + GC, of 0.9 (95% CI 0.85 – 1.0) during the use of a DMARD alone and of 1.9 (95% CI 1.75 – 2.05) during the use of a GC alone.

1.3.2 Infection and biological treatment
As mentioned earlier, long-term effects and safety issues for biologicals in RA still need to be addressed because of their immune-suppressive activity and their relatively recent introduction.

A meta-analysis by Bongartz et al. showed an odds ratio of 2.0 (95% CI 1.3 – 3.1) for serious infections when comparing TNF-blocking therapy with placebo in patients with RA. The number-needed-to-harm was 59 (95% CI 39 – 125) during a 3- to 12-month treatment period [10]. A cohort of 8,659 patients treated with TNF-blockers by the British Society for Rheumatology Biologics Register was compared with 2,170 conventional DMARD users for serious infection risk in a study by Dixon et al. [11]. They found an RR of 1.22 (95% CI 0.88 – 1.69) for TNF-blockers versus conventional DMARDs. If the treatment period was limited to the first 90 days after start of treatment, the RR was 4.6 (95% CI 1.8 – 11.9). The incidence of serious infections in this cohort during treatment was 5.6 per 100 patient-years. Another meta-analysis on TNF-blockers by Bernatsky et al. [12] from 2010 showed an RR of 1.37 (95% CI 1.18, 1.60) for serious infections.

On considering abatacept, an inhibitor of T-lymphocyte costimulation, comparable data are revealed. A double-blind, placebo controlled study by Genovese et al. [13] with 391 patients during 2 years shows 89 infections and 5.0 serious infections per 100 patient-years during abatacept therapy. Another abatacept study by Kremer et al. [14] with 539 patients reports on an infection and serious infection risk of consecutively 78 and 4.3 events per 100 patient-years. A review on the IL-1 antagonist anakinra by Mertens et al. [15] showed no significantly increased risk of infection. This was supported by a meta-analysis by Salliot et al. [16]. In this analysis, however, the risk of infection was increased for high doses of anakinra (> 100 mg/day), with an OR of 9.63 (95% CI 1.31,70,91) compared with placebo. In both reports follow-up duration was limited to ≤ 24 weeks.

A meta-analysis on the IL-6 antagonist tocilizumab by Nishimoto et al. showed no increased infection risk in long-term treatment [17]. This report on 601 patients, with a total of 2,188 patient-years and median treatment duration of 3.8 years, showed an infection rate of 6.22 per 100 patient-years. This number did not increase in long-term treatment.

In conclusion, data show an increased infection rate of biologicals, excluding RTX, compared with placebo for patients with RA on non-biological DMARD therapy.

1.4 RTX and infection risk
1.4.1 Introduction to RTX
RTX is a chimeric (part mouse, part human) mAb directed against CD20+ B-cells [18]. It acts by binding the Fab-fragment to the CD20-antigen on B-lymphocytes. The RTX Fc domain activates different immunological effector functions resulting in B-cell lysis. Possible B-cell lysis mechanisms comprise complement-dependent cytotoxicity (CDC), antibody-dependent cellular cytotoxicity (ADCC) and apoptosis.

In 1997, RTX was approved for non-Hodgkin's lymphoma (NHL). Registration for the use in RA followed in July 2006 [18,19]. According to the registration file and international rheumatological treatment guidelines the patient needs to have at least moderate disease activity despite MTX. Additionally, at least one TNF-blocker had to be ineffective or contraindicated before RTX may be used. RTX may be used alone or in combination with MTX [20]. For treatment of RA it is given in courses. A 1-g gift is followed by a second 1-g gift 2 weeks later. It is recommended to pre-treat the patient with methylprednisolone 100 mg i.v. to reduce the risk of developing acute infusion reactions [18]. Amelioration of disease activity is seen 12 – 16 weeks after completion of the first course. The effect duration is 6 – 12 months on an average; however, large interindividual variation in response duration is observed [18]. Timing of re-treatment is marked by a flare in RA activity. For pragmatic reasons, arguments for fixed dosing intervals could be considered, for example, to prevent possible damage during the short flare preceding re-treatment.

1.4.2 Safety
RTX use in NHL has been documented for > 1,000,000 patient years. Safety of RTX in RA is still being researched. Especially, effects on immunity and incidence of infection are studied. It only sounds reasonable that taking away key blocks of the immune system causes problems in immunity. One of the major functions of B-cells is immunoglobulin production, which is a key feature of the immune system. RTX depletes all CD20+ B-cells in the body. However, plasma cells, formed after maturation of B-cells, are CD20-. Plasma cells produce specific immunoglobulin (IgG, IgA and IgE) against antigens. The current population of plasma cells is not depleted by RTX, with specific immunoglobulin production remaining generally intact, at least for the short term. Plasma cells have lifetimes of > 100 days [21,22]. Effective RTX therapy in RA may take several years of...
repeated RTX administration. During RTX therapy B-cells remain depleted most of the time (23). Repeated RTX therapy causes long-term B-cell depletion in almost all patients for many months or years. This B-cell depletion stops the formation of novel plasma cells directed against recently encountered antigens, reducing the immunological response against vaccinations and recent infective agents (24,25). This may eventually result in loss of plasma cells and absolute decreases of all types of immunoglobulin starting with IgM and IgA and ultimately IgG. When this proves to be the case, prolonged RTX therapy may cause impaired humoral defense resulting in an increased infection rate.

2. Methods

2.1 Search for literature
A search was conducted in Medline and EMBase for the period from January 2004 to 15 July 2010. The following terms were used: ‘Rituximab (RTX), rheumatoid arthritis (RA)’ completed with ‘safety’, ‘adverse events’ or ‘infection’. Screening for applicability was done via abstracts. One of the authors (IB) judged the article as relevant or useful, based on the presence of data on (serious) infections or immunoglobulin level. Complete articles were obtained. Further manuscripts were retrieved from the reference lists of the selected articles. Randomized controlled trials (RCTs) or papers describing controlled clinical trials and prospective cohort studies investigating infection risk during use of RTX in RA were considered relevant. Since all information obtained from clinical trials and cohort studies on infection risk is deemed useful at this stage, no special requirements on patient numbers or power were made prospectively. However, clear statistics on infection rates were considered necessary. In three of the published studies 500 mg of RTX and five courses, respectively. These numbers, however, two open-label extension studies show a higher point estimate for serious infections after the last course (four and five courses, respectively). These numbers, however, show large confidence intervals probably due to small patient numbers.

3. Results

3.1 Studies included in this review
Ten studies were included in this review (Table 1); six double-blind, RCT studies (A, B, C, G and J), two open-label extension studies mostly based on the controlled studies (E, F) and two prospective observational studies in daily practice (D, I).

3.2 RTX-treatment characteristics in the studies
3.2.1 RTX monotherapy versus combination therapy
The consensus statement states that RTX should be used in combination with MTX (19). However, in clinical practice RTX is often used as monotherapy. The Air Registry (26), which is a study of daily clinical practice, reports the use of RTX without MTX in ≥ 516 (39.6%) of the patients studied.

3.2.2 Dose considerations
The registration text and the consensus statement prescribe the use of 1,000 mg of RTX. Re-treatment is possible if necessary. In three of the published studies 500 mg of RTX was used (27-29). Table 1 states the treatment courses used per trial.

3.2.3 Follow-up duration
Follow-up time differs in the selected studies, ranging from 24 weeks in the RCTs to > 5 years in the open-label extensions. On average, patients received one – four courses within the time frame studied. All patients were treated according to the consensus statement (20), except the Air Registry patients (26). For all patients, data are limited on five courses or more, resulting in low power on these data.

3.3 Infections
Table 1 shows the number of infections reported in the articles. Infections were not reported in a uniform fashion. Sometimes percentages are given, reflecting the amount of patients experiencing an infectious episode. In other cases the number of infectious episodes per 100 patient-years is given. The difference in definition of infectious parameters is the reason why direct comparison of data from the studies and meta-analysis cannot be performed.

Infections in RTX users are reported in 10 – 65% of patients and at a yearly incidence rate of 0.8 – 1.55 events per patient-year. Serious infections in RTX users are reported in 0 – 5.4% and at an incidence rate of 0.038 – 0.08 events per patient-year.

The published data show neither increased infection rate nor serious infection rate in the RTX treatment groups versus the comparator (placebo or other DMARDs). Only three studies (30,31,26) report data on infectious episodes depending on the duration of RTX treatment. They report a stable infection and serious infection rate across all courses. However, two open-label extension studies show a higher point estimate for serious infections after the last course (four and five courses, respectively). These numbers, however, show large confidence intervals probably due to small patient numbers.

3.4 Immunoglobulin status
The extended analyses by Keystone (30) and van Vollenhoven (31) report on longer-term effects on immunoglobulin levels. Their data show that IgM levels drop below the lower limit of normal (LLN) in 10% of patients after the first course, in 19% after the second course, in 21 – 24% after the third course, in 29% after the fourth course and in 40% after the fifth course. Data on IgG levels shows that IgG levels are below the LLN in 1.5 – 2.8% of patients after the first course, in 3.4 – 4.3% after the second course, in 2.8 – 5.9% after the third course, in 4.7% after the fourth course and in 5.7% after the fifth course. So their data show progressively lower levels of immunoglobulins starting with IgM followed by IgG in line with the biology of
### Table 1. Studies relevant for this review (plus group description plus follow-up time).

<table>
<thead>
<tr>
<th>Name</th>
<th>Type study</th>
<th>Patient number</th>
<th>Group description</th>
<th>Follow-up time</th>
<th>Infection</th>
<th>Serious infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Edwards June 2004</td>
<td>Randomized, double-blind,</td>
<td>161</td>
<td>1. Oral MTX (≥10 mg/week): 26 pts</td>
<td>24 weeks (a. primary analysis)</td>
<td>1a. 1* (2.5%)</td>
<td>1b. 0* (0%)</td>
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<td></td>
<td>controlled study</td>
<td></td>
<td>2. RTX (1,000 mg on days 1 and 15): 32 pts</td>
<td>1. 7* (19%)</td>
<td>2. 6* (16%)</td>
<td>2a. 2* (5.3%)</td>
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<td></td>
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<td></td>
<td>3. RTX (1,000 mg on days 1 and 15) + CY (750 mg on days 3 and 17): 34 pts</td>
<td>3. 3.4* (11%)</td>
<td>3b. 0* (0%)</td>
<td>4a. 0* (0%)</td>
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<td></td>
<td></td>
<td></td>
<td>4. RTX (1,000 mg on days 1 and 15) + MTX: 38 pts</td>
<td>4. 4* (10%)</td>
<td>3a. 2* (5.4%)</td>
<td>4b. 1* (2.6%)</td>
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<td>All treatment groups received placebo for the medication not given in their</td>
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<td>treatment group. All patients receiving i.v. methylprednisolone premed. and oral</td>
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<td>prednisolone during 17 days</td>
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<td>All three groups were divided, each subgroup receiving either (a) placebo glucose</td>
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<td>corticoids, (b) i.v. methylprednisolone premed., (c) i.v. methylprednisolone premed.</td>
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<td>+ oral prednisone (2 weeks); all patients received MTX (10−25 mg/week)</td>
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<tr>
<td>B. Emery May 2006</td>
<td>Randomized, double-blind,</td>
<td>465</td>
<td>1. Placebo (days 1 and 15): 149 pts</td>
<td>24 weeks</td>
<td>1. 28%</td>
<td>1. 2* (1%)</td>
</tr>
<tr>
<td></td>
<td>controlled study</td>
<td></td>
<td>2. RTX (500 mg on days 1 and 15): 124 pts</td>
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<td>2. 35%</td>
<td>2. 0* (0%)</td>
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<td></td>
<td>3. RTX (1,000 mg on days 1 and 15): 192 pts</td>
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<td>3. 3.5%</td>
<td>3. 3.4* (2%)</td>
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<td>All three groups were divided, each subgroup receiving either (a) placebo glucose</td>
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<td>corticoids, (b) i.v. methylprednisolone premed., (c) i.v. methylprednisolone</td>
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<td>premed.+ oral prednisone (2 weeks); all patients received MTX (10−25 mg/week)</td>
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<tr>
<td>C. Cohen September</td>
<td>Randomized, double-blind,</td>
<td>500</td>
<td>1. RTX (1,000 mg on days 1 and 15) + MTX (10−25 mg/week): 300 pts</td>
<td>24 weeks, post-treatment period with follow-up every 2 months, for an overall</td>
<td>24 months</td>
<td>1. 41% (138.2/100</td>
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<tr>
<td>2006 REFLEX [40]</td>
<td>placebo-controlled study</td>
<td></td>
<td>study duration of 24 months</td>
<td>patient-years)</td>
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<td>patient-years)</td>
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<td></td>
<td>2. Placebo (days 1 and 15) + MTX (10−25 mg/week): 200 pts</td>
<td>2. 38% (154.6/100 patient-years)</td>
<td></td>
<td>patient-years)</td>
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<td></td>
<td>All patients receiving i.v. methylprednisolone premed. and oral prednisolone</td>
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<td>patient-years)</td>
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<td></td>
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<td></td>
<td>during 17 days</td>
<td></td>
<td></td>
<td>patient-years)</td>
</tr>
<tr>
<td>D. Popa December</td>
<td>Observational study</td>
<td>37</td>
<td>Dosing regimes ranging from a start with 300 mg RTX on day 1 followed by 3,000 mg</td>
<td>14 patients 3−5 years, 22 patients &gt; 5 years, 19 patients remained on the</td>
<td>No clear description</td>
<td>No clear description</td>
</tr>
<tr>
<td>2006 [23]</td>
<td></td>
<td></td>
<td>RTX gift for 3 weeks, to 1,000 mg RTX on days 1 and 8. Some patients received</td>
<td>program</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>CY. Most patients received concomitant steroids but not all. Overall, the study</td>
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<td></td>
<td></td>
<td></td>
<td>groups are not clearly defined</td>
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<tr>
<td>E. Keystone December</td>
<td>Open-label extension study,</td>
<td>1,039</td>
<td>Re-treatment was possible if effect was present. Time of re-treatment was</td>
<td>570 patients were followed for two courses, 191 for three, 46 for four and 3 for 839 patients were followed for &gt; 1 year, 139 &gt; 2 years and 89 for &gt; 3 years</td>
<td>570 patients were followed for two courses, 191 for three, 46 for four and 3 for 839 patients were followed for &gt; 1 year, 139 &gt; 2 years and 89 for &gt; 3 years</td>
<td>1. 2.6 (100 patient-years)</td>
</tr>
<tr>
<td>2007 Extension analysis [30]</td>
<td>based in part on study A and C in this list</td>
<td></td>
<td>investigated, but ≥16 weeks after previous gift. Re-treatment consisted of RTX (1,000 mg on days 1 and 15). Patients received concomitant i.v. methylprednisolone premed. and oral prednisolone. Patients received stable MTX (10−25 mg/week) background</td>
<td></td>
<td></td>
<td>2. 6.2 (100 patient-years)</td>
</tr>
</tbody>
</table>

**Number of patients.**

**Number of patients with a (serious) infection.**

CY: Cyclophosphamide; DAS: Disease activity score; MTX: Methotrexate; NCI-CTC: National cancer institute, common toxicity criteria (different versions used); RTX: Rituximab; SI: Serious infection.
Table 1. Studies relevant for this review (plus group description plus follow-up time) (continued).

<table>
<thead>
<tr>
<th>Name</th>
<th>Type study</th>
<th>Patient number</th>
<th>Group description</th>
<th>Follow-up time</th>
<th>Infection</th>
<th>Serious infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>F. Vollenhoven</td>
<td>Global clinical trial program, consisting of nine clinical trials, including extension studies</td>
<td>2,578</td>
<td>Multiple options possible depending on study. Courses are defined as RTX 1,000 mg on days 1 and 15 or RTX 500 mg on days 1 and 15. MTX background was present. Most patients received concomitant i.v. methylprednisolone premed. and oral prednisolone. Time of re-treatment was 6 months in some studies and was decided by the investigator in others</td>
<td>One course 2,578* Two courses 1,890* Three courses 1,043* Four courses 425* Five courses 133* Six courses 50* &gt; 1 year 2,244* &gt; 2 years 851* &gt; 3 years 720* &gt; 4 years 317* &gt; 5 years 97*</td>
<td>Infections presented per 100 patient-years. Course one: 98 Course two: 95 Course three: 101 Course four: 99 Course five: 89</td>
<td>SIs presented per 100 patient-years. Course one: 4.5 Course two: 3.8 Course three: 4.8 Course four: 3.8 Course five: 6.8</td>
</tr>
<tr>
<td>G. Mease</td>
<td>Randomized double-blind, controlled study</td>
<td>559</td>
<td>All patients received one course open-label RTX (1,000 mg on days 1 and 15). From week 24, patients, if DAS remission was not achieved, were randomized, double-blind to receive either (a) another course RTX (1,000 mg on days 1 and 15) or (b) placebo (days 1 and 15) in a ratio of 2:1. They were followed until week 48. All patients received i.v. methylprednisolone premed. All patients received MTX (10 – 25 mg/week) DMARD rescue treatment was allowed between weeks 16 and 23.</td>
<td>Infections during randomization period 1. 120* (38%) 2. 59* (38%)</td>
<td>1. 1* (&lt; 1%) 2. 2* (2%)</td>
<td></td>
</tr>
<tr>
<td>H. Emery</td>
<td>Randomized, double-blind, controlled study</td>
<td>512</td>
<td>Initial randomization to 1. 500 mg RTX (days 1 and 15) 2. 1,000 mg RTX (days 1 and 15) 3. Placebo (days 1 and 15). After 24 weeks, unblinding was performed. If no remission was achieved: groups 1 and 2 received the same course again (1a and 2a) and group 3 received 500 mg RTX (days 1 and 15) (1a). Patients continued to receive MTX (10 – 25 mg/week) DMARD rescue treatment was allowed between weeks 16 and 23. All patients received i.v. methylprednisolone premed.</td>
<td>48 weeks</td>
<td>1a. 96* (57%) 2a. 85* (50%)</td>
<td>1a. 3* (2%) 2a. 3* (2%)</td>
</tr>
</tbody>
</table>

* Number of patients.
1 Number of patients with a (serious) infection.

Cy: Cyclophosphamide; DAS: Disease activity score; MTX: Methotrexate; NCI-CTC: National cancer institute, common toxicity criteria (different versions used); RTX: Rituximab; SI: Serious infection.
Table 1. Studies relevant for this review (plus group description plus follow-up time) (continued).

<table>
<thead>
<tr>
<th>Name</th>
<th>Type study</th>
<th>Patient number</th>
<th>Group description</th>
<th>Follow-up time</th>
<th>Infection</th>
<th>Serious infection</th>
</tr>
</thead>
</table>
| I. Gottenberg              | Prospective cohort study    | 1,303          | No clear group description possible. Most patients received courses of 1,000 mg MTX on days 1 and 15, with re-treatment by discretion of the physician. MTX, oral prednisolone and other extra medication was continued. Most patients received i.v. methylprednisolone premed. Patients were assessed at 3 months from start, at 6 months from start and every 6 months, or at disease relapse. | Total 1629 patients years  
3 – 6 months 177*  
6 – 12 months 406*  
12 – 18 months 303*  
> 18 months 417*  
2 cycles 466*  
3 cycles 176*  
4 cycles 45*  
≥ 5 cycles 25* | X                       | Total 5.0 per 100 patient-years  
First cycle*: 1 56  
Second cycle*: 2 22  
Third cycle*: 3 65 (79%) of infections occurred in 6 months following last RTX cycle  
42 (51%) before third month  
23 (28%) between 3 and 6 months  
17 (21%) between 6 and 12 months |  

| J. Rubbert-Roth            | Randomized, double-blind, controlled study | 378            | 1. Start with RTX (500 mg on days 1 and 15); at 24 weeks, again RTX (500 mg on days 1 and 15)  
2. Start with RTX (500 mg on days 1 and 15); at 24 weeks again RTX (1,000 mg on days 1 and 15)  
3. Start with RTX (1,000 mg on days 1 and 15); at 24 weeks again RTX (1,000 mg on days 1 and 15)  
Patients continued to receive MTX (10 – 25 mg/week). All patients received i.v. methylprednisolone premed. | 48 weeks  
Data are taken over total 48 weeks  
1. 75* (56%)  
2. 73* (61%)  
3. 60* (65%) |  

*Number of patients.
†Number of patients with a (serious) infection.
CY: Cyclophosphamide; DAS: Disease activity score; MTX: Methotrexate; NCI-CTC: National cancer institute, common toxicity criteria (different versions used); RTX: Rituximab; SI: Serious infection.
B-cells and plasma cells as discussed earlier in this article. No statistically significant increased infection risk was reported in their analysis.

4. Conclusion

The reviewed literature shows a trend towards, but not a statistically significant, increased infection risk for RTX use in RA. The raised infection risk seen in the first weeks after the gifts in a course could be a nadir of the intravenous methylprednisolone pre-medication, as suggested previously by Gottenberg et al. [26]. The data, however, do point towards a higher serious infection risk after multiple courses of RTX exposure. It must be mentioned that the number of observations beyond the fifth course are limited and thus are underpowered. Long-term follow-up is really important in this matter. The mechanism of gammaglobulin production related to plasma cells and B-cells needs to be considered seriously when using RTX for periods longer than five courses. Looking at the mechanistic considerations and the current data, it seems too early to declare that RTX is a safe (long-term) drug when considering infectious complications. For now, the use of RTX seems to be safe with regard to infections when used for five courses or less.

5. Expert opinion

The manuscripts reviewed conclude that no increased risk of (serious) infections is seen in patients with RA during RTX exposure (up to four courses) in comparison with placebo or non-biological DMARDs. Point estimates of infection rates and serious infection rates are 80 – 101 and 3.8 – 8.0 per 100 patient-years, respectively. These point estimates are comparable with those seen in TNF-blockers, anakinra and abatacept. Biologicals may be associated with a slightly increased risk beyond four courses.

5.1 Sponsor bias

All RCTs are initiated and paid for by the manufacturer. The Air Registry was also funded by the producer of RTX. This means a potential sponsor bias needs to be taken into account.

5.2 Duration of follow-up

Prolonged complete B-cell depletion will inevitably lead to immunoglobulin depletion. As seen in most long-term studies [23,30,31] drops in immunoglobulin levels are reported. It is shown that in line with the mechanism of the humoral immune system immunoglobulin level decline starts with IgM, sometimes even until it is undetectable [23], followed by drops in IgG levels. This is a pattern that may be expected, taking into account that B-cells produce IgM and that plasma cells produce IgG. B-cell depletion thus directly inhibits IgM production, but not IgG production. If B-cell depletion continues beyond the lifetime of plasma cells, drops in IgG levels will occur due to depletion of IgG-producing plasma cells resulting in hypo- or agammaglobulinemia. The relatively short follow-up period may be part of the explanation why no increase in infection rates is seen in current literature. In our opinion this is not highlighted enough in current literature.

The reviewed articles have a maximum follow-up time of 5 years, corresponding with five – seven courses. Given the nature of RA, it may be expected that RTX therapy needs to be continued for years. In this light, a long-term analysis, favorably performed prospectively by an independent institution, should comprise longer periods than the current maximum of 5 years to make reliable estimations on the potential increase in infection rates.

5.3 Immunoglobulin

Considering the increasing percentage of IgG/IgM deficient patients with consecutive RTX gifts, infectious problems with RTX will probably become evident after the fifth infusion cycle.

Potentially, the consequences of hypogammaglobulinemia or even agammaglobulinemia could become similar to hereditary agammaglobulinemias. These conditions, requiring intravenous immunoglobulin replacement therapy to avoid recurrent serious infections, are accompanied by an increased serious infection rate. Rates rise up to more than one serious infectious episode per patient per year. Most of these serious infectious episodes need hospitalization [32-36]. Supporting evidence for the serious nadirs of low immunoglobulin levels comes from research on immunoglobulin levels and serious infection incidence rates in lymphoid malignancies. If IgG levels in patients with chronic lymphocytic leukemia drop to < 7 mg/ml (an arbitrary chosen LLN), the serious infection rate rises from 36 to 71%, a twofold increase [32,37]. The referent LLN of immunoglobulin levels differs slightly depending on the laboratory, but a global LLN for IgG is 6 mg/ml and for IgM is 0.45 mg/ml [32]. These LLN immunoglobulin levels are approached after five courses of RTX, with the lower quartile ranges already below the LLNs [31]. If IgG and IgM levels keep dropping during prolonged exposure to RTX, eventually well below the LLN, the serious infection rate could rise two-fold. Starting from the 6.8 serious infections per 100 patient-years found after the fifth course by van Vollenhoven et al. [31], this could mean > 13 serious infections per 100 patient-years. These theoretical considerations are supported by research by van Assen et al. [24,25], which both show impaired response to certain vaccination in RA patients using RTX. This finding is possibly caused by impaired IgM production due to B-cell depletion. It shows that problems in immunity against novel antigens arise during RTX therapy, which could get worse with longer-lasting therapy.

5.4 Dosing and dosing interval

The consensus statement [19] and the FDA label [38] state that RTX should be given as two 1,000 mg gifts separated by
15 days. However, results from the Dancer [27] and Serene [28] studies show that RTX courses of two 500 mg gifts separated by 15 days are as effective as the 2 × 1,000 mg regimen. These findings could have important implications for future guidelines and deserve more attention currently given. Besides the economic benefits, lower doses might result in fewer side effects and less production of human antichimeric antibodies (HACA). Lower doses may be associated with a lower infection risk. We think this is not the case since RA disease activity probably returns before B-cells have formed into plasma cells. This necessitates a re-treatment with RTX before problems in immunity have been resolved.

Although subject to debate, current guidelines state that re-treatment could be considered in RTX responders, directly after a flare in RA activity (≥16 weeks after the last course) [19,38,39]. The guidelines further state that for patients with residual disease activity after response to RTX, courses systematically given every 6 months can be considered [19,38,39]. Another option, currently not advised due to lack of evidence, could be to re-treat on B-cells status [19]. Whether on demand, periodically or based on B-cell status, we think re-treatment should also be based on absence of hypoglogulinemia, that is, immunoglobulin status.

### 5.5 Power considerations

Due to the relative short-term follow-ups there are problems with power of the statistics of the studies. The extended analysis by van Vollenhoven et al. [31] states that RTX is well tolerated over multiple courses. Gottenberg et al. [26] claim that the risk for infection is similar to that reported in clinical trials. The former study makes a remark about a large confidence interval for data on serious infections after the fifth course and the latter only has data on 25 patients with five or more courses. We suppose these studies lack sufficient power to make statements on safety on infectious complications. Larger groups and long-term follow-up is mandatory to resolve this issue.

### 5.6 Definition of (serious) infections

A few words on the definition of infection are necessary. Most papers [27,40,30,31,29] start with the definitions stated in the Common Terminology Criteria for Adverse Events [42,43]. Often an additional remark is made that an infection requiring intravenous antibiotics is also regarded as serious. For some papers [44,40,30,41] the definition of a serious adverse event is “an event that is fatal, life-threatening, required hospitalization or prolongation of hospitalization (except when exacerbation of RA was causative), caused disability or incapacity, caused a congenital abnormality or a birth defect, was medical significant or required intervention to prevent any of the above mentioned”. Other definitions solely state that hospitalization or intravenous antibiotics are needed for the classification ‘severe’ or sometimes the definition is not clear. These classifications can be disputed. Why should an infection requiring oral or topical antibiotic use automatically be classified as non-serious? None of the literature studied in this review [A-J section results] speaks of oral or topical antibiotics. Any infection needing antibiotic therapy could become serious if not treated. Moreover the antibiotic burden on the patient increases when this type of therapy is necessary. A study in 188 RTX users with lupus erythematosus confirms the thought that the infection rate rises to 19% when a different definition for infection is used [45]. The definition included that empirical antibiotic therapy was started and the infection was confirmed by bacterial culturing. When using a different definition for infection and following the described line of thoughts in setting up a trial, different results may arise. Therefore, uniform definitions of serious infections are essential to compare data between trials.

### 5.7 Trial setting versus clinical practice

Two issues with the current evidence are methodological problems. First, as Gottenberg et al. [26] pointed out, most data originate from open-label extended pivotal trials. Most trials (except C, D and I) have exclusion criteria stating that patients that went through multiple infections in the past or are currently going through an infection are not eligible for inclusion. Therefore, most data in the extension analyses are based on these exclusion criteria. Patients included in the trials could differ substantially from RA patients in daily clinical practice. This may lead to safety results that cannot be directly translated to daily clinical practice.

### 5.8 Withdrawal due to infections

Second, most literature report on withdrawal due to adverse events (AE), but not very clear or complete. Keystone [30] and van Vollenhoven [31] both report on 3% withdrawal due to AEs after the first course and 2% after the second course with <1% withdrawal for subsequent courses. Van Vollenhoven et al. report that 15 out of 123 patients withdrew due to an infection, but the specifics are unclear. Keystone [30] reports no data at all. We believe these trials only report on spontaneously reported infections. The infection numbers when specifically looked at could be much larger. We think the reasons for RTX withdrawal deserve more attention, especially when due to an infection.

### 5.9 Case reports

Although case reports were not included in this review, it should be mentioned that some concerns were raised on cases during RTX use. As indicated by a black box warning by the FDA [38], progressive multifocal leukoencephalopathy (PML) is reported after RTX use [46–48]. PML is caused by the JC virus and is a fatal disease in 90% of the cases [47]. Although incidence after RTX in RA is rare, PML should be considered as a serious side effect of RTX in RA. When patients on RTX present with neurological manifestations, PML should be considered and RTX use should be ceased.
5.10 Some practical considerations

- It could be argued that a carry-over effect of infection risk after TNF-blocker use exists for RTX starters and vice versa. Theoretically, the nadirs of RTX can still be present when TNF-blockers are started. On the contrary, the relatively short-effect half-life of TNF-blockers makes problems when starting RTX after TNF blocking therapy less likely. The guideline by Smolen et al. [19] advises caution when RTX and TNF-blockers are used after one another. However, a recent review by Buch [49] reports that no safety signals are currently present with biological sequencing.

- With regard to vaccination, the advice in current guidelines is to use non-live vaccines (influenza, pneumonia) 4 weeks prior to an RTX course, while live vaccines should not be used at all [19,38]. Recent research by Arad et al. [50] confirms this by showing that cellular response to influenza vaccination is preserved in RA patients treated with RTX, making this vaccination useful. They showed that although humoral immunity was severely impaired, the cellular response was present. Reviewing the evidence, it seems advisable to vaccinate RA patients on RTX in programs against influenza. With regard to prophylactic use of other vaccinations little evidence exists and thus no clear advice can be given.

- An answer to the problems arising with dropping immunoglobulin levels could be, theoretically, immunoglobulin replacement with intravenous immunoglobulin (IVIG). Whether this is a permanent solution or not remains to be seen. However, if IVIG treatment is about to be introduced, the decision should not only be based on immunoglobulin levels but also on manifest infections being resistant to antibiotic therapy, that is, the patient’s clinical status. This is also stated as a condition to the indication in the leaflets of IVIG products.

- As for IVIG, it could be argued that antibiotic prophylaxis could be a solution for recurrent infections. No current guideline advises to do this. We also think it is not advisable since it does not solve the underlying reason for the recurrent infections. Moreover, it will increase antibiotic use with only growing resistance as a long-term result.

5.11 Future research

To resolve a few problems mentioned earlier, some recommendations can be made for future research.

- Focus needs to be on longer-term effects than the periods described in current literature.
- The definition of an infection or serious infection needs to be readdressed and standardized.
- More attention needs to be focused on the immunoglobulin status of patients and the role this plays in development of (serious) infections.
- IVIG supplementation needs to be looked into as an option to prevent problems in immunity.

The extent to which other parts of the immune system are able to support immunity lacking B-cells and their function needs to be studied in order to elucidate the working mechanism and possible harms of RTX. Special attention can be given to immunoglobulin subclasses and specific antibody titers, which is indicative for immunity or absence of immunity against new types of pathogens [51].

Novel, possibly less partial CD20+ depleting therapies, such as ofatumumab [52], have become available for treatment nowadays. These compounds act similarly as RTX but are fully humanized mAbs and bind more potently to different epitopes of CD20. Recently, the development of ocrelizumab [53] was halted [54]. Early analysis showed an increased risk for serious, fatal infections. This finding could have implications for the safety record of RTX.

Until further research is done and a complete safety profile of RTX is available, doctors need to be aware of problems in immunity when treating patients with RTX for long periods.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.
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