Lack of efficacy of rituximab in Wegener’s Granulomatosis with refractory granulomatous manifestations.

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KEYWORDS:
Wegener’s granulomatosi, B-cells, refractory, rituximab, granuloma
Abstract

**Objective**: The intention of this prospective open-label study was to investigate the safety and efficacy of rituximab in patients with refractory Wegener’s granulomatosis (WG).

**Methods**: Eight consecutive patients with active refractory WG were included. In all patients disease activity persisted despite standard therapy with cyclophosphamide and prednisolone (CYC and CS), added by TNF-alpha blockade three months before study inclusion. Patients had particular granulomatous manifestations like retroorbital granulomata (n=5), nodules of the lungs (n=1) and subglottic stenosis (n=2). Rituximab was administered intravenously every 4th week in combination with the standard therapy in 5 and with methotrexate in 2 other patients. Disease extent and activity were monitored clinically by an interdisciplinary care, immunodiagnostics (ANCA-serology, B-cells by flow cytometry) and by Magnetic Resonance Imaging.

**Results**: Any beneficial response with the decline of disease activity was seen in only 3 patients, two of which went into complete remission. In 3 other patients, disease activity remained unchanged while a further progress was seen in the remaining 2 patients. In all patients peripheral blood B-cells declined to zero within the course of the treatment with rituximab. C-ANCA-titers remained unchanged in all except one patient.

**Conclusion**: In this pilot study, B-lymphocyte depletion was not associated with a change of the ANCA-titers or obvious clinical improvement of refractory granulomatous disease in patients with WG. Further studies are needed to evaluate the role of Rituximab in WG.
Cyclophosphamide (CYC) and corticosteroids (CS) remain the treatment of first choice in severe Wegener’s granulomatosis (WG), but there is some uncertainty about the rescue therapy in cases with persistend or refractory disease activity. With a better understanding of the pathogenesis of WG in the past decade, it has become possible to develop therapeutic strategies that utilise (biological) agents to target specific elements of the inflammatory response. Data from smaller pilot studies suggest that the addition of TNF-α blocking agents like infliximab or etanercept to standard therapy, anti-CD4-antibody or novel agents like 15-desoxypergualin may be successfully applied in refractory cases of WG. Despite great efforts, most of the therapies are limited by infectious complications or the absence of a lasting response. The evidence for the role of ANCA’s in amplification of inflammatory signals in vitro has led to attempts to inhibit production of these antibodies specifically. Rituximab, a chimeric monoclonal antibody that binds to CD20 expressed on the surface of B cells, leads to a B-cell depletion by complement-mediated activities and through antibody-dependent cellular cytotoxicity. Preliminary results of the use of rituximab in patients with ANCA-associated vasculitides suggest that rituximab-induced depletion of CD20+ B-cells can inhibit ANCA-production to some extent and appears to be capable to induce disease remission. However, the results of a recent pilot study were somewhat biased by other concomitant therapies making it difficult to work out the effect of rituximab in relation to other confounders. We report about our experience of an open-label study of eight WG patients with mainly granulomatous manifestations refractory to standard therapy and TNF-α blockade which were subsequently treated with rituximab according to a standardized protocol.

**Patients and methods:**
Patients were followed by an interdisciplinary approach in a single tertiary referral center as previously described. All patients fulfilled the definitions of the Chapel Hill Consensus Conference and of the ACR criteria for WG. ANCA against Proteinase-3 (Anti-PR3-ANCA) were tested positive in all patients. Clinical diagnosis was confirmed by the presence of characteristic histopathologic features in all patients. Patients underwent a regular set of interdisciplinary clinical, serological, immunological examinations of disease activity and extent and for therapy related side effects as reported earlier. Activity was assessed by the Birmingham Vasculitis Activity Score (BVAS), which has been validated for it’s use in WG as outlined elsewhere. Disease extent was assessed by using the Disease Extent Score (DEI), as described and validated by the authors. Remission was defined as a BVAS score that indicated the absence of signs of new or worse disease activity, and persistent disease activity for no more than one item. Relapse was defined as the recurrence or first appearance of at least one item on the BVAS score; if indicating a life or organ threatening dysfunction of a vital organ (lung, brain, eye, motore nerve, gut, or kidney) it was defined as a major relapse.

Rituximab (RTX) (Mabthera®, F. Hoffmann.-La Roche LTD) was applied in addition to standard therapy with cyclophosphamide (2mg/kg every day p.o. or 15-20mg/kg every 18-21 days) or methotrexate (0,3mg/kg every week i.v.). Rituximab dosage was calculated by body surface area (375mg/m²) and administered intravenously every 4th week. Methylprednisolone (100mg), clemastin as antihistaminic prophylaxis and a histamine-receptor antagonist were applied additionally 30 to 60 minutes prior to RTX to prevent hypersensitivity and other reactions. During and 120 minutes after
the infusion, patients were monitored on the intensive care unit. On the day before
the first RTX infusion was given, a test dosage of 50mg rituximab in 50ml NaCl 0,9%
was administered to test for an allergic reaction to the protein. Patients were followed
up for a median of 18-months (range 6-28 months) after the last rituximab infusion.
B lymphocytes were counted by flowcytometry (FACS) and ANCA were determined
by indirect immunofluorescence and direct enzyme-linked immunosorbent assays
(ELISA) as earlier described.[12]

Results:

Patient characteristics  The major reason for escalation therapy in five of the eight
patients was a progress of retroorbital granulomas documented by the
ophthalmologist and MRI despite standard therapy with CYC and CS for a median of
16 months (range: 6-48). In one patient (No. 6), pulmonary granuloma and
progressive granulomatous sinusitis with osseous destruction had developed during
standard therapy. Two patients had subglottic stenoses and severe dyspnea (No 7.
and 8). Despite the addition of infliximab (5mg/kg/month) to CYC and CS (n=6),
respectively etanercept (25mg twice a week s.c.) to MTX (n=1) or mycophenolate
mofetil (n=1) for three months, granulomatous inflammation progressed in seven
patients and persisted in one (No. 5). Concomitant scleritis improved in one patient
(No. 4), but persisted in three others (No. 1, 2, 5). In three patients bloody nasal
discharge, considered as active disease on ENT examination, persisted. After
insufficient response to standard therapy (median cumulative CYC dosage: 76g,
range: 6-163g) and four pulses of infliximab, treatment was switched to RTX in all
eight patients. While CYC was continued in five, therapy was switched to
methotrexate in two and to mycophenolate mofetil in one other patient despite active
disease. Reasons for not continuing CYC was hemorrhagic cystitis or a toxic bone
marrow after CYC therapy. The dosage of CS was raised from a median of 10 mg to
32 mg in 6 patients and remained unchanged in 2 patients. (table 1)

Outcome  All patients received four pulses of rituximab (375mg/m²) in four-weekly
intervals. Treatment was well tolerated by all patients. Figure 1a/b displays disease
activity (BVAS) and disease extent (DEI) during the course of treatment. Four weeks
after completion of the fourth rituximab pulse, two patients were in remission (No. 6
and 8), three had a unchanged disease activity (No. 1, 4 and 5) while a further
progress was seen in the remaining three patients (No. 2, 3 and 7). Active scleritis
persisted in all three patients. In three of the five patients with retroorbital granulomas
a further enlargement of the retroorbital masses was documented by MRI, while the
size remained unchanged in the two other patients. Progress of retroorbital
granuloma was associated with further visual impairment and decreasing eye motility
in all of the three patients. Bloody nasal discharge considered as active disease on
ENT examination persisted in 3 patients. Patient No. 6 showed a beneficial clinical
response to RTX (No. 6) and had a decreased disease activity after the first two
courses. Constitutional symptoms disappeared and granulomatous inflammation as
well as cephalgia diminished. After completion of the fourth rituximab course, no new
or worse disease activity was detected and only little inflammation of the sinus
persisted. Dyspnea and subglottic stenosis improved significantly in patient Nr. 8, but
the effect was not remarkable before the fourth RTX pulse.

In all patients peripheral blood lymphocyte counts became undetectable following
rituximab therapy. (Figure 2) Titers of C-ANCA and ANCA-specific direct enzyme-
linked immunosorbent assay (ELISA) remained unchanged in all except two
patients. (Figure 3) The mean levels of CRP decreased from 3,8 mg/dl (25th and 75th
percentiles: 1.6-8.2 mg/L) to 1.4 mg/dl (25th and 75th percentiles: 0.6-2.8 mg/L), while mean ESR dropped from 70 mm/hr to 32 mm/hr during the course. In comparison with the period before introducing RTX, CS dosage was decreased in 3 patients, kept stable in one and was increased in 4 other patients due to active disease. Five patients refractory to RTX were afterwards successfully treated with azathioprine-pulse therapy as published recently.[13] Two patients have been followed up for more than one year and showed promising results.

Discussion:
We report about eight patients with WG refractory to standard therapy and TNF-α blockade, of which five also failed to respond to the treatment with RTX. In this pilot-study, B-lymphocyte depletion was not associated with a change of the ANCA-titers or obvious clinical improvement of refractory granulomatous disease. Recently and in contrast to the presented results, the successful use of RTX in 11 patients with ANCA-associated vasculitis (WG n=10, MPA n=1), who had either refractory disease or contraindication for the further use of CYC has been reported.[6] The results of the recent study lead to the assumption, that a B-cell depletion by rituximab leads to a decrease of C-ANCA titers and correlates directly with the remission of the disease. Because of the varied concomittant immunosuppressive therapies, it can not be ruled out that the potential effect of rituximab might be influenced in the herewith reported patients. But two major factors are more likely to account for the striking differences in the outcome in the present study compared to the cohort reported by Keogh and coworkers.

First, it needs to be noted, that unlike other published series of refractory WG, the patients reported here had more prominent granulomatous manifestations rather than vasculitis or glomerulonephritis. In contrast, in the vast majority of patients reported by Keogh and coworkers, active organ involvement at the time of initiation of RTX therapy was mainly related to severe vasculitis (e.g. alveolar hemorrhage) or glomerulonephritis. The lack of response to CYC, infliximab and RTX in the patients reported in here suggests that refractory granulomatous disease represents a subset of patients particularly difficult to treat and are likely to be pathogenetically different from the vast majority of WG patients with predominantly vasculitic manifestations.

Granulomatous lesions in WG are made up of monocyte derived tissue macrophages, giant cells, neutrophils, CD4+CD28- T cells and B cells.[14][15] Immunohistochemical studies have shown that those CD4+CD28- T cells appear to be the major source of interferon-γ and TNF-α secreted in the granulomatous lesions.[16] CD4+CD28- T cells may be recruited into granulomatous lesions from the blood via CD18 interaction and may subsequently promote monocyte accumulation and granuloma formation through their cytokine secretion in WG. Further on, we were able to prove follicle-like B lymphocytic infiltrates within the granulomatous lesions of endonasal speciments of WG patients.[17] The respective immunoglobulin-encoding genes indicated that these B cells are potential PR3-ANCA producers. However, despite the local presence of CD20 positive B cell clusters within the granulomatous lesions of untreated WG patients, there is yet no direct evidence that B cells or ANCA play a pathogenic role for the granulomatous inflammation in WG; in a recently published animal model, local inflammation induced by intradermal injection of TNF-α triggered a strong subcutaneous panniculitis in the presence of passively transferred systemic murine proteinase 3-ANCA’s, but granulomatous inflammation was not seen.[18]
In the herewith reported patients, it has to be assumed that the granulomata reflect a variable histopathological picture of inflammation, fibrinoid necrosis and in particular excessive fibrosis, but exceptional vasculitis.[19] Fibrous tissue might replace areas of acute inflammation and necrosis as a kind of a healing response, that even temporarily exceed the normal amount. In contrast to most granuloma found in other organ systems, the unusual portion of fibrosis might explain that granulomatous lesions of the orbita shrink up to a certain degree during immunosuppressive therapy in some patients, but sometimes do not vanish entirely. Orbital socket contracture is a complication of inflammatory orbital disease and the excessive fibrosis.[19] However, in the herewith demonstrated cases, the new development or rapid increase of the circumference as well as an obvious enhancement in the MRI suggests a continuing active inflammatory process rather than predominant fibrosis. Additionally, a significant decrease of the granulomatous inflammation after the switch to high dose azathioprine pulse therapy or desoxysperguarline in 4 patients who did not respond to RTX either, confirm a prevailing inflammatory process. [20] One other patient did not respond to azathioprine pulse therapy either and had to be enucleated finally. The histologic specimen of the enucleated eye and retroorbital tissue revealed a relevant retroorbital active granulomatous inflammation in addition to a dense fibrosis, like it was seen in other WG studies of patients with inflammatory orbital disease.[19] (Image 2)

Second, the data presented here do not support the hypothesis that anti-CD20 mediated B cell blockade via RTX results in an effective suppression of ANCA production by plasma cells. As it was seen in the previous studies, peripheral B-lymphocytes became undetectable in all our patients within the period of treatment. However, ANCA titers remained mostly unchanged in our patients during and after treatment with RTX. Moreover, no correlation of the percentage of B cells and ANCA-titer, nor on the disease activity was found. Thus, the sustained or increased vasculitic manifestations in five of our patients could be a result of the unchanged ANCA-titer despite peripheral B-cell depletion. Careful analysis of the data reported by Keogh and coworkers raises additional concern about the potential of RTX to abolish ANCA production. In three of the five WG patients presented by Keogh et al. ANCA-titer did not become undetectable after re-treatment with RTX alone, suggesting that the previous decline of ANCA-titer in these and other patients might not be solitary due to RTX, but rather be related to the simultaneously increase of glucocorticoids (1g methylprednisolone per day for 3 days) or concomitant plasma exchange. A persisting ANCA-production after one or more cycles of RTX might indicate an incomplete B cell depletion. Other reasons might be the persistence of long-living plasma-cells, that do not get harmed by rituximab because of the lack of CD20 expression.[21][22][23][24] There is mounting evidence that long-lived plasma cells with life spans of months to years are an important source of autoantibody production in different autoimmune diseases.[25][26] Remaining ANCA-titers despite cytotoxic treatment in patients with WG and the persistence of normal immunoglobuline levels after treatment with rituximab implies the existence of long-lived plasma cells.[27]

Incidentally, the lack of efficacy of the previous treatment with infliximab in seven of our patients is in contrast to results from a previous pilot study, suggesting a beneficial effect in refractory cases of WG.[1] The individually different response to TNF-α blockade and other therapies rather implicates the existence of pathogenically distinct disease subsets. The exceptional amount of fibrosis distinguish retroorbital
granulomata from other granulomatous manifestations of WG and might influence the response to therapy significantly. Our data show that certain subsets (or disease stages) of WG are particularly difficult to treat, although the underlying differences of responders and non-responders have remained for the most part indefinite in clinics, up to now.

In summary, the data presented here do not provide evidence that B cell depletion using RTX is sufficient effective for treatment of retroorbital granulomata in WG, which are refractory to standard therapy. In respect to the small number of patients studied, our data do not exclude a potentially beneficial effect on vasculitic manifestations or less aggressive disease courses as it has been reported by others before. Thus, further studies are needed to assess the role of B-cells in the pathogenesis of WG and the effect of RTX.
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<tr>
<th>Pat.-No.</th>
<th>sex, age</th>
<th>active organ involvement at time of rituximab</th>
<th>Failing previous therapy at time of RTX introduction</th>
<th>C-ANCA before / after RTX</th>
<th>B-cells (%) before / after RTX</th>
<th>BVAS 1 before / after RTX</th>
<th>DEI before / after RTX</th>
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<tr>
<td>1</td>
<td>f / 54 y</td>
<td>E, Ey, B</td>
<td>CYC 20mg/kg every 3rd week PRD 10 mg od*</td>
<td>CYC 1.2g every 3rd week iv PRD 25mg od</td>
<td>64 / 64</td>
<td>1 / 0</td>
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<tr>
<td>2</td>
<td>f / 33 y</td>
<td>E, Ey</td>
<td>MTX 20 mg/wk PRD 30mg od* Inflixiab 5mg/kg/month for 3 mo.</td>
<td>MTX 20 mg/wk PRD 30 mg od</td>
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<td>5 / 0</td>
<td>8 / 10</td>
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<td>3</td>
<td>m / 43 y</td>
<td>E, Ey</td>
<td>CYC 100 mg od PRD 10 mg od* Inflixiab 5mg/kg/month for 3 mo.</td>
<td>CYC 100 mg od PRD 10 mg od</td>
<td>32 / 32</td>
<td>7 / 0</td>
<td>10 / 7</td>
</tr>
<tr>
<td>4</td>
<td>m / 46 y</td>
<td>E, Ey, B</td>
<td>CYC 12mg/kg every 3rd week PRD 30 mg od*</td>
<td>CYC 12mg/kg every 3rd week PRD 15mg od</td>
<td>128 / 128</td>
<td>20 / 0</td>
<td>7 / 7</td>
</tr>
<tr>
<td>5</td>
<td>f / 51 y</td>
<td>E, Ey, B</td>
<td>CYC 100 mg od. PRD 10 mg od* Inflixiab 5mg/kg/month for 3 mo.</td>
<td>CYC 100 mg od PRD 20 mg od</td>
<td>128 / 128</td>
<td>1 / 0</td>
<td>9 / 9</td>
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<tr>
<td>6</td>
<td>m / 27 y</td>
<td>E, L, B</td>
<td>CYC 100 mg od PRD 17 mg od* Inflixiab 5mg/kg/month for 3 mo.</td>
<td>CYC 100mg od PRD 15 mg od</td>
<td>128 / 128</td>
<td>2 / 0</td>
<td>9 / 0</td>
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<tr>
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<td>m / 36 y</td>
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<td>Leflunomide 30mg od PRD 17 mg od* Etanercept 25mg twice a wk</td>
<td>MTX 25mg/wk PRD 20 mg od</td>
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<td>6 / 11</td>
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<td>8</td>
<td>m / 38 y</td>
<td>E, L, K, A, B</td>
<td>Mucophenolate mofetil 2g od PRD 10mg od* Etanercept 25mg twice a wk</td>
<td>Mucophenolate mofetil 2g od PRD 10mg od*</td>
<td>128 / 128</td>
<td>14 / 0</td>
<td>5 / 0</td>
</tr>
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Table 1. Clinical characteristics of the study population at baseline. * latest prednisolone dosage before switch to rituximab
Figure 1 a
Figure 1 b
Figure 2
Figure 3
Image 1

**MR imaging of orbital granulomas** (Pat No.1) before (a, b) and after treatment (c, d) with Rituximab. In T2-weighted imaging (a,c), typical hypointense masses in both retrobulbar spaces (white arrows) are found which are even enlarged at follow up after 3 months. Contrast-enhanced, fat-suppressed T1-weighted MRI sequences (b, d) reveal persistence of diffuse bright signals in the orbitae due to granulomatous inflammation. In addition, extensive granulomatous processes in the ethmoidal cells are not improved after therapy.

Image 2

**Retroorbital granuloma (Pat No. 1 after treatment with Rituximab):**
Typically ill-defined Wegener’s granuloma with multiple epitheloid cells centered around a capillary within a dense background of granulocytes, lymphocytes and plasma cells (H&E, x200)


Figure 1a
Figure 1b
Figure 2

B-cells (%) vs Rituximab

previous | after
Figure 3

C-ANCA titer in IFT

previous after

1:128
1:64
1:32
1:32

Rituximab

C-ANCA titer in IFT
Lack of efficacy of Rituximab in Wegener's Granulomatosis with refractory granulomatous manifestations

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