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Original article

Long-term maintenance rituximab for ANCA-associated vasculitis: relapse and infection prediction models

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Abstract

Objectives. Following a maintenance course of rituximab (RTX) for ANCA-associated vasculitis (AAV), relapses occur on cessation of therapy, and further dosing is considered. This study aimed to develop relapse and infection risk prediction models to help guide decision making regarding extended RTX maintenance therapy.

Methods. Patients with a diagnosis of AAV who received 4–8 grams of RTX as maintenance treatment between 2002 and 2018 were included. Both induction and maintenance doses were included; most patients received standard departmental protocol consisting of 2× 1000 mg 2 weeks apart, followed by 1000 mg every 6 months for 2 years. Patients who continued on repeat RTX dosing long-term were excluded. Separate risk prediction models were derived for the outcomes of relapse and infection.

Results. A total of 147 patients were included in this study with a median follow-up of 63 months [interquartile range (IQR): 34–93]. Relapse: At time of last RTX, the model comprised seven predictors, with a corresponding C-index of 0.54. Discrimination between individuals using this model was not possible; however, discrimination could be achieved by grouping patients into low- and high-risk groups. When the model was applied 12 months post last RTX, the ability to discriminate relapse risk between individuals improved (C-index 0.65), and once again, clear discrimination was observed between patients from low- and high-risk groups. Infection: At time of last RTX, five predictors were retained in the model. The C-index was 0.64 allowing discrimination between low and high risk of infection groups. At 12 months post RTX, the C-index for the model was 0.63. Again, clear separation of patients from two risk groups was observed.

Conclusion. While our models had insufficient power to discriminate risk between individual patients they were able to assign patients into risk groups for both relapse and infection. The ability to identify risk groups may help in decisions regarding the potential benefit of ongoing RTX treatment. However, we caution the use of these prediction models until prospective multi-centre validation studies have been performed.

Key words: ANCA, vasculitis, rituximab, relapse, infection, prediction

CLINICAL SCIENCE

Rheumatology key messages

- Benefits of relapse prevention with long-term RTX must be weighed against the risk of RTX-induced immunodeficiency.
- These prediction models can identify risk groups for both relapse and infection outcomes following RTX.
- The ability to assign patients into risk groups may help with decisions regarding ongoing treatment.

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Introduction

ANCA-associated vasculitis (AAV) is an organ and life-threatening multisystem autoimmune disease that often follows a relapsing and remitting course. B cell-derived ANCAs are implicated in the pathogenesis [1] and evidence from randomized trials supports the use of rituximab (RTX), an anti-CD20 monoclonal antibody that depletes B cells, as both a remission induction and maintenance agent [2–4]. Fixed-interval repeat-dose RTX infusions over a 2-year period is a commonly used approach to maintain remission and prevent relapses [2, 5]. However, after a maintenance course of RTX, relapses occur on cessation of therapy, and further dosing is considered where the benefits of relapse prevention must be weighed against the risk of RTX-induced immunodeficiency and susceptibility to infections.

Patient-specific and disease-specific characteristics exist that can influence an individual's risk of relapse and risk of infectious complications [6–8]. For instance, a consistent finding from observational studies and clinical trials is that having circulating ANCAs against proteinase 3 (PR3) rather than myeloperoxidase (MPO) is a significant risk for relapsing disease [9, 10]. The disease phenotype also influences relapse risk as patients with granulomatosis with polyangiitis (GPA) tend to have more relapses than patients with microscopic polyangiitis (MPA), as do those with involvement of the upper and lower airways. In addition, patients who have had previous relapses, tend to relapse again, and some studies have shown increased relapse risk in patients with better renal function, persistent ANCA positivity and nasal colonization of *Staphylococcus Aureus* [6, 11, 12]. Prior and current immunosuppressive treatments, both in terms of the agent used and the duration of therapy, may also influence relapse risk [6].

Elderly patients are at increased risk of infectious complications associated with immunosuppressive therapy, as are those with impaired renal function, lung damage and diabetes [13–15]. An over-suppressed immune system may be indicated by leukopenia and hypogammaglobulinaemia; the former being more commonly associated with CYC use, whereas the latter has been seen in patients with AAV, both prior to and in association with RTX use [16–18]. Overall immunosuppressive burden including the use of other agents such as prednisolone, CYC and mycophenolate may contribute to hypogammaglobulinaemia. However, there is not a clear association between cumulative RTX exposure, low immunoglobulin levels and infection risk, suggesting complex interplay of many patient, disease and treatment related factors [19]. This heterogeneity between individual patients makes predicting the occurrence and severity of RTX-induced hypogammaglobulinaemia challenging.

In clinical practice, clinicians must weigh up potential relapse and infection risk factors when deciding whether or not an individual patient will benefit from ongoing RTX treatment. Although previous observational studies and clinical trials have identified risk factors for relapse and infection, this is the first study to attempt to generate risk prediction models to help guide decision making regarding extended RTX maintenance therapy in AAV beyond a 2-year RTX treatment course.

Methods

All patients with a diagnosis of AAV (GPA or MPA) who received between 4 and 8 g of RTX at Addenbrooke's Hospital (Cambridge, UK) between January 2002 and January 2018 were included in this study. Both induction and maintenance doses were included; most patients received standard departmental protocol consisting of $2\times$ 1000 mg 2 weeks apart, followed by 1000 mg every 6 months for 2 years; however, 21 (14%) patients were participants in the RITAZAREM trial, in which they received 4 weekly doses of 375 mg/m^2 followed by 1000 mg every 4 months for 20 months. Patients who received ongoing fixed-interval RTX beyond 2 years from the initial induction dose for high perceived relapse risk were excluded ($n = 47$). Concomitant use of CYC or another immunosuppressant including azathioprine, methotrexate, or mycophenolate mofetil was permitted; however, in the majority immunosuppression was discontinued at RTX initiation. Clinical and laboratory data were collected retrospectively using electronic patient records. In accordance with the UK National Health Service Research Ethics Committee guidelines, ethics approval was not required because this work comprises retrospective data, and all treatment decisions were made before our evaluation.

Definitions

Predictors

Diagnosis of clinical phenotype (GPA *vs* MPA) followed the definitions from the Chapel Hill Consensus Conference, 2012 [20]. ANCA positivity was defined based on the reference ranges provided by the manufacturer ($>$1.9 iU/l for PR3-ANCA, $>$3.4 iU/l for MPO-ANCA) using commercial EliA fluoro enzyme immune assay test reagents and the Phadia instrument 2500/5000. B cell return was defined as detectable CD19+ cells in the blood ($\geq 0.01 \times 10^9$/l). The Disease Extent Index [21] was used to score disease activity and organ involvement at time of first RTX dose. Involvement of each organ system scores 2 points; constitutional symptoms score 1 point (total possible score = 21). A patient was classified as having diabetes if the diagnosis was documented in their medical notes or the patient was taking long-term anti-diabetic medications. Structural lung disease was defined as the presence of either obstructive lung disease (endobronchial stenosis, bronchiectasis, emphysema) or restrictive lung disease (fibrosis; not pleural disease). Age was dichotomized at 60 for the relapse models (the median age) and at 70 for infection

models (chosen as older patients are known to have a greater risk of infection [22]). The threshold for infectious events during RTX treatment was defined as either one serious infection (requiring intravenous antibiotics and/or hospital admission) or at least three non-serious infections (requirement of oral antimicrobials in the community). A complete list of all candidate predictors entered into the original models is shown in Tables 1 and 2.

Outcomes

Relapse: time to first relapse was defined as the occurrence of any new manifestations attributable to active vasculitis that required escalation of immunosuppressive therapy beyond a temporary increase in oral corticosteroids in a patient previously in remission. Infection: a clinically relevant definition for infectious events was chosen, which included the composite of either time to first serious infection or third non-serious infection.

Statistical analysis

Full details of the statistical methods can be found in the supplementary materials section (Introduction, Supplementary Figs S1–3, Supplementary Tables S1–3, all available at *Rheumatology* online). From the time of the last dose of RTX, separate risk prediction models were derived for the outcomes of relapse and infection. Time to relapse was censored by death or last follow-up. Time to infection was censored by death, last follow-up or relapse (due to confounding effect of additional immunosuppression). Multivariable Cox proportional hazards models were fitted to each outcome using clinically relevant baseline predictors. Continuous predictors were normalized to improve the stability of estimated coefficients. Proportional hazards assumptions were assessed using Schoenfeld residual plots. Due to a relatively small amount of missing values across all predictors, complete-case analyses were performed.

The predictive performance of each model was assessed using the C-index and the calibration slope. The C-index [23] captures the ability of a model to distinguish between high-risk and low-risk patients (0.5 represents no discrimination and 1 represents perfect discrimination). The calibration slope reflects the agreement between observed and estimated risk (ideal is 1; values below 1 represent over-fitting). When dealing with relatively small sample sizes, it makes more sense to use all of the available data in both training and testing a model rather than splitting the dataset into independent training and test sets, as this would reduce the ability to develop reliable prediction models. Thus, a non-parametric bootstrap procedure (1000 iterations for each analysis), incorporating the variable selection using backwards elimination, was used to correct for over-optimism from assessing predictive performance on the same dataset used to fit the model. Optimism-corrected validation statistics were computed (Supplementary Table S2, available at *Rheumatology* online), and the final model was derived by multiplying the estimated coefficients by a shrinkage factor.

Prediction models for both relapse and infection were then refitted at 12 months following the last dose of RTX. These models used all of the same baseline variables; however, the model for relapse included updated values for serum creatinine (continuous) and ANCA status (positive or negative), as well as the return of B cells within 12 months (binary). Updated values for the infection model at 12 months included serum creatinine, serum immunoglobulin G (IgG) level and total lymphocyte count, each as a continuous variable. Missing values of the latter two variables (>30%) were imputed using their baseline values (correlation is 0.78).

Risk scores were calculated by multiplying 1 or 0 (for binary variables) or the actual value (for continuous variables) by the $\log^{10}$ of the hazard ratio for each variable. The sum of the risk scores for each model represents the linear predictor for the outcome, i.e. an individual's risk score. Risk groups could be assigned by dividing the distribution of all linear predictors by the median (low- and high-risk groups).

Patient population, definition of predictors and outcomes and statistical analysis were planned a priori with the exception of the infection outcome, which was modified post hoc to include the third non-serious infection (in a composite infection outcome) as a meaningful measure of infection-related morbidity after a relatively low event rate was identified with serious infection alone. A power analysis was not conducted because all eligible patients attending the clinic were included in the study.

All analyses were performed in R version 3.5.3 (packages: rms [24] and survminer [25]) with fully reproducible scripts (supplementary, github, available at *Rheumatology* online).

Results

One hundred and forty-seven patients were included in this study with a median follow-up after last RTX of 63 months (IQR: 34–93). Eighty patients experienced a relapse, with a median time to relapse of 45 (IQR: 23–97) months following last RTX. There were 88 infectious events (26 had a serious infection; 62 had ≥3 non-serious infections) with a median time to infection of 44 (IQR: 23–88) months. Ten relapses and 8 infectious events occurred within 12 months, and 7 patients had <12 months follow-up. Therefore, the relapse and infection risk assessment at 12 months post RTX was performed on 130 patients and 122 patients, respectively (see Supplementary Fig. S1, available at *Rheumatology* online, for more details). Clinically relevant predictors are summarized in Table 1 for relapse and Table 2 for infection. Proportional hazard assumption was checked for all models (Supplementary Fig. S2, available at *Rheumatology* online).

Table 1 Candidate predictors for relapse (at time of last RTX and 12 months after the last RTX)

Predictors of relapse	Prediction at time of last RTX			Updated prediction 12 months post last RTX		
	Total (*N* = 147)	No relapse (*N* = 67)	Relapse (*N* = 80)	Total (*N* = 130)	No relapse (*N* = 60)	Relapse (*N* = 70)
Gender, *n* (%)						
Female	76 (52)	34 (51)	42 (52)	70 (54)	32 (53)	38 (54)
Male	71 (48)	33 (49)	38 (48)	60 (46)	28 (47)	32 (46)
Age strata, *n* (%)						
<60	74 (50)	31 (46)	43 (54)	64 (49)	27 (45)	37 (53)
≥60	73 (50)	36 (54)	37 (46)	66 (51)	33 (55)	33 (47)
Disease subtype, *n* (%)						
GPA	122 (83)	51 (76)	71 (88)	107 (82)	45 (75)	62 (88)
MPA	25 (13)	16 (24)	9 (12)	23 (18)	15 (25)	8 (12)
ANCA subtype, *n* (%)						
Negative	8 (5)	4 (6)	4 (5)	8 (6)	4 (7)	4 (6)
MPO	23 (16)	14 (21)	9 (11)	21 (16)	13 (22)	8 (11)
PR3	116 (79)	49 (73)	67 (84)	101 (78)	43 (72)	58 (83)
ANCA positive at end RTX, *n* (%)						
No	104 (71)	49 (73)	55 (69)	–	–	–
Yes	43 (29)	18 (27)	25 (31)	–	–	–
Indication for RTX, *n* (%)						
New disease/refractory	39 (27)	18 (27)	21 (26)	35 (27)	16 (27)	19 (27)
Relapse	108 (73)	49 (73)	59 (74)	95 (73)	44 (73)	51 (73)
ENT involvement, *n* (%)						
No	34 (23)	26 (39)	8 (10)	31 (24)	24 (40)	7 (10)
Yes	113 (77)	41 (61)	72 (90)	99 (76)	36 (60)	63 (90)
Serum creatinine at end RTX, μmol/l						
Median (IQR)	82 (67, 111)	80 (65, 115)	83 (69, 108)	–	–	–
Concomitant CYC or oral IS, *n* (%)						
No	133 (90)	60 (90)	73 (91)	116 (89)	53 (88)	63 (90)
Yes	14 (10)	7 (10)	7 (9)	14 (11)	7 (12)	7 (10)
Cumulative RTX, g						
Median (IQR)	6.0 (5.0, 6.0)	6.0 (5.0, 6.0)	6.0 (5.0, 6.25)	6.0 (5.0, 6.0)	6.0 (5.0, 6.0)	6.0 (5.0, 6.75)
Cumulative CYC prior to 1st RTX, g						
Median (IQR)	6.0 (0.0, 10.0)	4.5 (0.0, 9.0)	7.2 (0.0, 12.0)	6.0 (0.0, 10.0)	4.5 (0.0, 9.0)	7.2 (0.0, 12.0)
Steroid dose at end RTX, mg/day						
Median (IQR)	1.0 (0.0, 5.0)	0.0 (0.0, 5.0)	1.25 (0.0, 5.0)	–	–	–
ANCA status 12 months post last RTX, *n* (%)				**(*N* = 117)**	**(*N* = 55)**	**(*N* = 62)**
Negative	–	–	–	81 (69)	45 (82)	36 (58)
Persistently positive	–	–	–	23 (18)	7 (12)	16 (23)
Negative–positive switch	–	–	–	13 (11)	3 (5)	10 (16)
B cell return within 12 months, *n* (%)				**(*N* = 97)**	**(*N* = 47)**	**(*N* = 50)**
No	–	–	–	41 (42)	25 (53)	16 (32)
Yes				56 (58)	22 (47)	34 (68)
Serum creatinine 12 months post last RTX, μmol/l				**(*N* = 125)**	**(*N* = 57)**	**(*N* = 68)**
Median (IQR)	–	–	–	84 (71, 111)	83 (66, 115)	85 (74, 107)
Steroid dose 12 months post last RTX, mg/day				**(*N* = 130)**	**(*N* = 60)**	**(*N* = 70)**
Median (IQR)	–	–	–	0.75 (0.0, 5.0)	0.0 (0.0, 5.0)	1.75 (0.0, 5.0)

RTX, rituximab; GPA, granulomatosis with polyangiitis; MPA, microscopic polyangiitis; ENT, ear, nose and throat; IS, immunosuppression; IQR, interquartile range.

Table 2 Candidate predictors for infection (at time of last RTX and 12 months after the last RTX)

Predictors of infection	Prediction at time of last RTX			Updated prediction 12 months post last RTX		
	Total (*N* = 147)	No infection (*N* = 59)	Infection (*N* = 88)	Total (*N* = 122)	No infection (*N* = 54)	Infection (*N* = 68)
Gender, *n* (%)						
Female	76 (52)	23 (39)	53 (60)	63 (52)	21 (39)	42 (62)
Male	71 (48)	36 (61)	35 (40)	59 (48)	33 (61)	26 (38)
Age strata, *n* (%)						
<70	110 (75)	40 (68)	70 (80)	91 (75)	37 (69)	54 (79)
≥70	37 (25)	19 (32)	18 (20)	31 (25)	17 (31)	14 (21)
Structural lung disease, *n* (%)						
No	107 (73)	51 (86)	56 (64)	92 (75)	47 (87)	45 (66)
Yes	40 (27)	8 (14)	32 (36)	30 (25)	7 (13)	23 (34)
Diabetes, *n* (%)						
No	120 (82)	54 (92)	66 (75)	102 (84)	50 (93)	52 (76)
Yes	27 (18)	5 (8)	22 (25)	20 (16)	4 (7)	16 (24)
Cumulative CYC prior to 1st RTX, g						
Median (IQR)	6.0 (0.0, 10.0)	5.0 (0.0, 9.0)	7.0 (0.0, 12.0)	6.0 (0.0, 10.0)	5.0 (0.0, 9.0)	7.0 (0.0, 11.2)
Concomitant CYC or oral IS, *n* (%)						
No	133 (90)	55 (93)	78 (89)	109 (89)	51 (94)	58 (85)
Yes	14 (10)	4 (7)	10 (11)	13 (11)	3 (6)	10 (15)
Cumulative RTX, g						
Median (IQR)	6.0 (5.0, 6.0)	6.0 (5.0, 6.5)	6.0 (5.0, 6.0)	6.0 (5.0, 6.0)	6.0 (5.0, 6.7)	6.0 (5.0, 6.0)
Steroid dose at end RTX, mg/day						
Median (IQR)	1.0 (0.0, 5.0)	0.0 (0.0, 5.0)	1.7 (0.0, 5.0)	0.7 (0.0, 5.0)	0.0 (0.0, 5.0)	1.2 (0.0, 5.0)
On antibiotic prophylaxis at end RTX, *n* (%)						
No	84 (57)	30 (51)	54 (61)	67 (55)	26 (48)	41 (60)
Yes	63 (43)	29 (49)	34 (39)	55 (45)	28 (52)	27 (40)
Infections during RTX, *n* (%)[a]						
No	128 (87)	55 (93)	73 (83)	112 (92)	50 (93)	62 (91)
Yes	19 (13)	4 (7)	15 (17)	10 (8)	4 (7)	6 (9)
Serum creatinine at end RTX, μmol/l						
Median (IQR)	82 (67, 111)	81 (67, 142)	83 (68, 105)	–	–	–
Nadir serum IgG level during RTX, g/l						
Median (IQR)	6.50 (5.43, 8.10)	7.00 (5.60, 8.41)	6.40 (5.10, 7.80)	6.60 (5.47, 8.39)	7.00 (5.55, 8.42)	6.4 (5.2, 7.9)
Serum IgG level at end RTX, g/l						
Median (IQR)	7.2 (5.9, 9.1)	7.8 (6.4, 9.7)	6.9 (5.7, 8.6)	–	–	–
Total lymphocyte count at end RTX, $\times 10^9$/l	**(*N* = 143)**	**(*N* = 58)**	**(*N* = 85)**	**(*N* = 119)**	**(*N* = 53)**	**(*N* = 66)**
Median (IQR)	1.3 (0.9, 1.6)	1.2 (0.8, 1.5)	1.3 (0.9, 1.7)	1.26 (0.9, 1.6)	1.1 (0.8, 1.5)	1.37 (1.0, 1.7)
Serum creatinine 12 months post RTX, μmol/l				**(*N* = 119)**	**(*N* = 51)**	**(*N* = 68)**
Median (IQR)	–	–	–	84 (70, 111)	85 (67, 142)	84 (72, 99)
Serum IgG level 12 months post RTX, g/l				**(*N* = 94)**	**(*N* = 38)**	**(*N* = 56)**
Median (IQR)	–	–	–	7.2 (5.9, 9.5)	7.7 (6.6, 9.7)	6.7 (5.7, 9.3)
Total lymphocyte count 12 months post RTX, $\times 10^9$/l				**(*N* = 86)**	**(*N* = 35)**	**(*N* = 51)**
Median (IQR)	–	–	–	1.3 (1.0, 1.6)	1.2 (0.8, 1.5)	1.4 (1.1, 1.9)

[a]Clinically relevant infections = ≥1 serious or ≥3 non-serious infections. RTX, rituximab; ENT, ear nose and throat; IS, immunosuppression; IQR, interquartile range.

Relapse

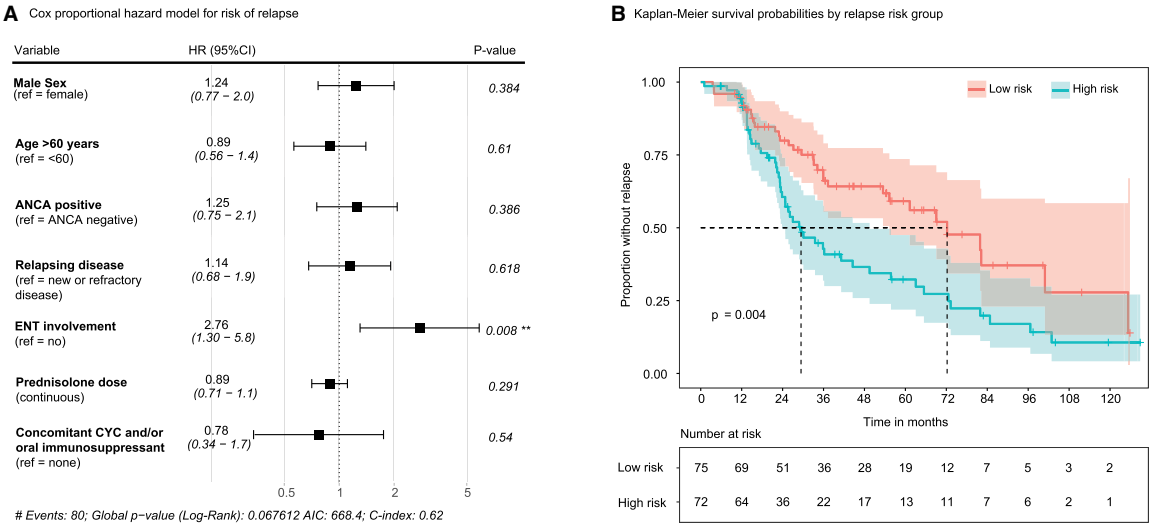
At time of last RTX, 11 baseline predictors were entered into the original model, of which 7 were retained in the final model. ANCA subtype, serum creatinine at end of RTX, cumulative RTX dose and cumulative CYC exposure before first RTX treatment were dropped in the bootstrap backwards elimination procedure (Supplementary Table S1, available at *Rheumatology* online). ENT involvement was found to be associated with a higher risk of relapse [unshrunken hazard ratio (HR) = 2.76 (95% CI: 1.3, 5.8); $P = 0.008$] and the contribution (unshrunken coefficients) of other predictors are shown in Fig. 1A. The optimism-corrected C-index was low (C-index = 0.54), indicating that discrimination between individuals was poor; however, discrimination could be achieved by grouping patients into low-risk and high-risk groups, which have a median time to relapse of 72.2 months and 29.4 months, respectively (Fig. 1B).

For prediction performed 12 months post last RTX, ANCA positivity became a strong predictor of a relapse at this time point [unshrunken HR = 2.73 (95% CI: 1.56, 4.80); $P < 0.001$] while gender, age group, concomitant CYC (or oral immunosuppressant) and return of B cells were dropped from the model due to their limited contribution (Fig. 2A). As a result, the ability of the updated model to discriminate relapse risk between individual patients improved (optimism corrected C-index = 0.65). Furthermore, grouping of patients into low and high risk of relapse was possible with clear separation highlighting the ability of the model to discriminate between these groups. Median time to relapse was 69.6 months and 22 months for the low- and high-risk group, respectively (Fig. 2B). Both relapse models (Supplementary Table S3, available at *Rheumatology* online) were well calibrated (Supplementary Figs S3 A1, A3, A5; B1, B3, B5, available at *Rheumatology* online).

Infection

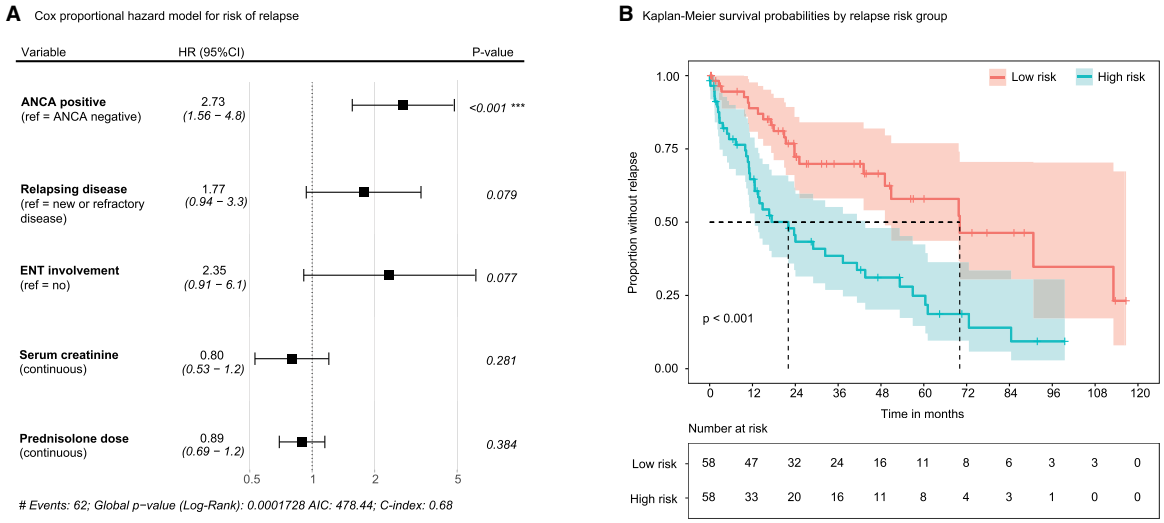
At time of last RTX, a total of 5 (out of 13) predictors were retained in the final model. The presence of structural lung disease [HR = 1.83 (1.17–2.90); $P = 0.008$], diabetes [HR = 2.72 (1.65–4.50); $P < 0.001$], the occurrence of infections during RTX treatment [HR = 2.32 (1.29–4.20); $P = 0.005$] and lower serum IgG level at the end of RTX [HR = 0.71 (0.56–0.90); $P = 0.005$] were significantly associated with infection (Fig. 3A). Age group, use of concomitant CYC (or another oral immunosuppressant), use of antibiotic prophylaxis at the end of RTX, cumulative CYC dose before first RTX, cumulative RTX dose, prednisolone dose at end of RTX, serum creatinine at end of RTX and total lymphocyte count at the end of RTX were not selected for the final model. The optimism-corrected C-index was 0.64 allowing discrimination between low and high risk of infection groups. Median time to infection was 74.8 months and 29 months for the low- and high-risk group, respectively (Fig. 3B).

Fig. 1 Relapse prediction at time of last RTX



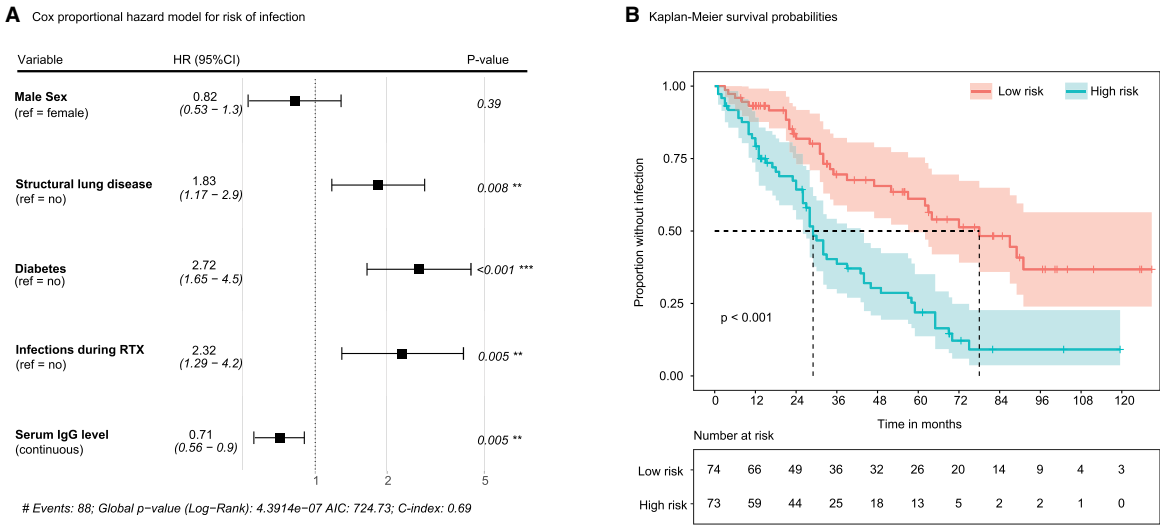
(**A**) Unshrunken multivariable hazard ratios from the Cox proportional hazard model for risk of relapse after the last RTX treatment ($N = 147$). Apparent concordance index (C-index) = 0.62 (optimism corrected C-index = 0.54). (**B**) Estimated survival probabilities by patient risk groups based on the final model using shrunken coefficients. Kaplan-Meier survival probabilities of patients in the low- (below median risk) and high-risk (above median risk) groups of relapse. *P*-value is derived from the non-parametric log-rank test for the differentiability of survival curves. *P*-values <0.05 indicates survival curves were statistically differentiable between groups.

Fig. 2 Relapse prediction 12 months post last RTX



(**A**) Unshrunken multivariable hazard ratios from the Cox proportional hazard model for updated risk of relapse 12 months after the last RTX treatment ($N = 114$). Apparent concordance index (C-index) = 0.68 (optimism corrected C-index = 0.65). (**B**) Estimated survival probabilities by patient risk groups based on the final model using shrunken coefficients. Kaplan-Meier survival probabilities of patients in the low- (below median risk) and high-risk (above median risk) groups of relapse after 12 months post last RTX treatments. *P*-value is derived from the non-parametric log-rank test for the differentiability of survival curves. *P*-values <0.05 indicates survival curves were statistically differentiable between groups.

Fig. 3 Infection prediction at time of last RTX



(**A**) Unshrunken multivariable hazard ratios from the Cox proportional hazard model for risk of infection after the last RTX treatment ($N = 146$). Apparent concordance index (C-index) = 0.68 (optimism corrected C-index= 0.64). (**B**) Estimated survival probabilities by patient risk groups based on the final model using shrunken coefficients. Kaplan-Meier survival probabilities of patients in the low- (below median risk) and high-risk (above median risk) groups of infection. *P*-value is derived from the non-parametric log-rank test for the differentiability of survival curves. *P*-values <0.05 indicates survival curves were statistically differentiable between groups.

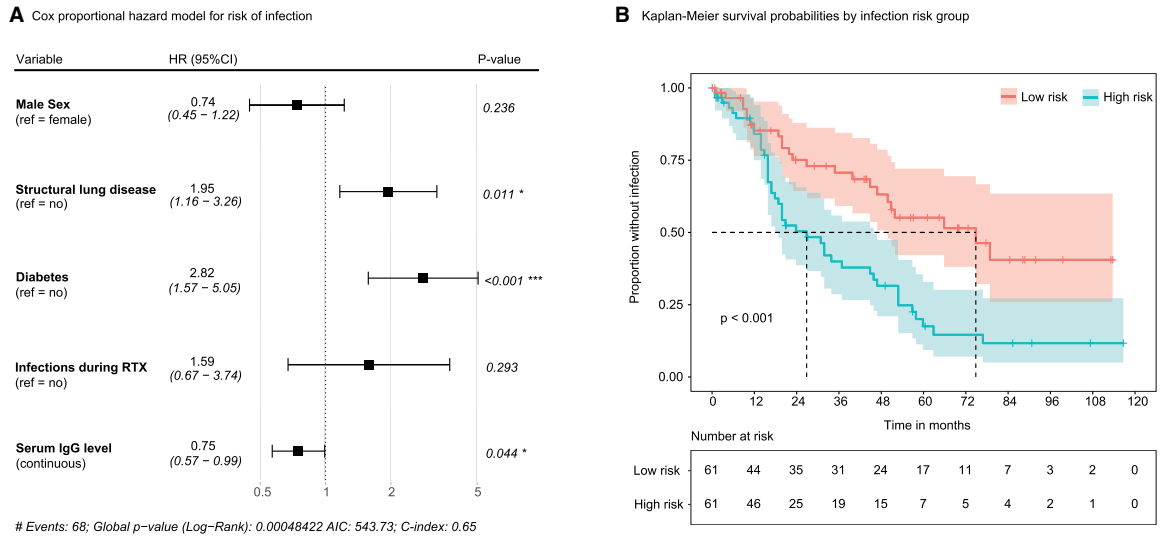
At 12 months post RTX, the predictive power of the presence of lung disease [HR = 1.95 (1.16–3.26); P = 0.011], diabetes [HR = 2.82 (1.57–5.05); P =<0.001] and lower serum IgG level [HR = 0.75 (0.57–0.99); P = 0.044] was strong but the discriminability of the final model was marginally worse than previously (optimism-corrected C-index = 0.63) (Fig. 4A). Once again, clear separation of patients from two risk groups was observed, where median time to infection was 74.8 months and 26.8 months for the low- and high-risk group, respectively (Fig. 4B). The final infection models (Supplementary Table S3, available at *Rheumatology* online) with shrunken coefficients were both well calibrated (Supplementary Figs S3 C1, C3, C5; D1, D3, D5, available at *Rheumatology* online).

Discussion

The aim of this study was to develop relapse and infection risk prediction models to help guide decision making regarding extended RTX maintenance therapy beyond a 2-year treatment course for patients with AAV. Cox proportional hazard models were fitted for each outcome using clinically relevant predictors at two key time points: first, at the time of last RTX and again 12 months after the last RTX. The relapse prediction model when assessed at the time of last RTX performed poorly in terms of its ability to discriminate risk of relapse between individual patients but could discriminate between high- and low-risk groups. The strength of the model improved when performed 12 months later with additional data, once again allowing discrimination into low- and high-risk groups. The improvement in the model was largely driven by the contribution of ANCA positive status, which was associated with a much higher risk of relapse 12 months after RTX but not immediately following RTX. While there is ongoing debate about the clinical utility of ANCA status for predicting relapse, there is growing evidence suggesting greater relevance in the context of B cell-targeted therapy with RTX compared with other less specific immunosuppressive treatments [11, 26]. Contrary to the findings of others [27], B cell return within 12 months of RTX was not associated with earlier relapse. While the relative infrequency and variability of time between measurements of returning B cells among individuals may have limited the strength of this association in our study, a recent randomized trial evaluating the usefulness of B cells and ANCA to inform treatment decisions (RTX given for re-emergence of B cells or ANCA reappearance/rise in titre *vs* fixed-interval RTX administrations) [28], also did not provide strong support for the biomarker-based regimen, highlighting the limitations of these commonly measured biomarkers.

The infection risk models were also able to clearly discriminate between low- and high-risk groups at both last RTX and 12 months after last dose; however, once again, discrimination between individual patients was not possible (C-index 0.64 and 0.63, respectively, for

Fig. 4 Infection prediction 12 months post last RTX



(**A**) Unshrunken multivariable hazard ratios from the Cox proportional hazard model for updated risk of infection 12 months after the last RTX treatment (N = 122). Apparent concordance index (C-index) = 0.71 (optimism corrected C-index = 0.63). (**B**) Estimated survival probabilities by patient risk groups. Kaplan-Meier survival probabilities of patients in the low- (below median risk) and high-risk (above median risk) groups of infection after 12 months post last RTX treatments. P-value is derived from the non-parametric log-rank test for the differentiability of survival curves. P-values <0.05 indicates survival curves were statistically differentiable between groups.

each time point). Previously reported factors driving up the infective risk were the presence of structural lung disease, diabetes and hypogammaglobulinaemia [8, 16, 29], all of which were associated with infection in this cohort and were retained in the final models at both time points. Importantly, infections during RTX were predictive of future infections when assessed immediately after RTX. In contrast to other studies [7], renal impairment and older age did not contribute to infection risk in this study.

Infections are common in patients with diabetes [14]. The hyperglycaemic environment can directly induce immune dysfunction at a cellular level, and complications such as neuropathy, gastrointestinal and urinary dysmotility predispose these patients to more frequent and/or serious infectious events. Diabetes has been shown to be an independent risk factor for infection in patients with autoimmune diseases including AAV, and systemic immunosuppression probably increases the infective risk further [8, 30]. This effect has been observed with conventional therapies and is therefore probably not specific to RTX treatment [8, 31]. Nevertheless, as the strongest predictor of infection in both models, this study highlights the importance of taking diabetes into account when assessing an individual's infective risk.

The association between structural lung disease and infections is also well recognized [15]. Patients with structural lung disease are often colonized with potentially pathogenic microorganisms that predispose to recurrent lower respiratory tract infections. Importantly, chronic infections have also been implicated as triggers as well as persistent drivers of various autoimmune diseases including AAV [32, 33]. However, this paradigm was not supported by the present study when an alternative model was fitted using structural lung disease and previous infections as candidate predictors for relapse as well as infection: structural disease was a risk factor for infection but was not associated with relapse, and no significant association was observed between previous infections and later relapse (data not shown).

Consistent with randomized controlled trials, average IgG levels of this cohort are within population norms [4, 34]. Greater infection risk has been identified in those with moderate to severe hypogammaglobulinaemia [18, 35]. The observed association between IgG levels and risk of infection in this study highlights the impact of immunoglobulin levels on infection risk in patients with AAV following RTX. Extrapolated from its use in the common variable immunodeficiency setting, antibiotic prophylaxis and immunoglobulin replacement has been used in this setting to reduce the risk of infection in this population.

When developing a risk prediction model, a rule of thumb based on the events per variable (EPV) ratio is often used to determine the sample size, where an EPV ratio of 10 or more is needed to avoid the problem of overfitting [36]. When the EPV ratio is <10, the effect of overfitting is pronounced. In the present study the EPV of all four models was >10; however, despite meeting this widely accepted criteria for EPV ratio the strength of our models is limited by the small sample size, highlighted by the wide CIs observed. Nonetheless, a notable strength of our approach was that any over-optimism in apparent performance statistics was considered and adjusted for accordingly. Beyond the methodological limitations of this study, we also acknowledge that the variables themselves may not be such strong predictors of our chosen outcomes. Although previous studies have identified factors such as PR3-ANCA (*vs* MPO-ANCA), GPA (*vs* MPO), lower serum creatinine levels, and a history of prior relapse to be associated with relapse [37–39], we know a proportion of newly diagnosed patients with PR3-ANCA and GPA do not relapse, and conversely a subset of patients with MPO-ANCA and MPA do relapse. Such heterogeneity within AAV limits the accuracy of relapse prediction and may explain why discrimination between individual patients' risk was so difficult to achieve.

Both relapse and infection models are subject to unmeasured bias common to retrospective observational datasets. The exclusion of 47 patients who were given ongoing fixed-interval RTX beyond 2 years as they were deemed to have the highest risk of relapse represents a selection bias that likely weakened the strength of the relapse models. Bias also exists for the infection outcome: it is recognized that the presence of diabetes or structural lung disease and lower lymphocyte and IgG levels are associated with high infection risk [8, 16, 29, 40]. It is likely that the presence of one or more of these risk factors would concern the treating clinician who may take measures to mitigate risk including more frequent clinic follow-up appointments, reduction in corticosteroid dose or use of prophylactic antibiotics, or the use of immunoglobulin replacement therapy. Additionally, non-serious infections treated in the community and serious infection treated in local hospitals were potentially underreported to the specialist clinic. Randomized clinical trials are the gold standard for defining the risk of drug-related adverse effects; however, the published clinical trials of maintenance RTX in AAV [4] are too small to provide reliable risk prediction models and extrapolating risk from induction trials is problematic as outcomes are confounded by differences in the treatment regimens and use of higher doses of corticosteroids when compared with maintenance regimens. A further limitation of the study is the lack of generalizability to other populations given the small sample size derived from a single institution and the comparatively low representation of patients with MPA. While this reflects the more frequent use of maintenance RTX for relapsing GPA, RTX maintenance strategies are used for MPA and thus relapse and infection risk evaluation are of great importance for this group of patients and should be addressed in future studies.

While maintenance protocols have been shaped by the evidence provided by two landmark trials [4, 34]

supporting the use of fixed-interval maintenance RTX (500 mg every 6 months for 18 months in MAINRITSAN and 1000 mg every 4 months for 20 months in RITAZAREM), evidence supporting extended RTX maintenance is lacking, and common practice is to stop therapy after 2 years. Thus, the time points chosen for assessment of relapse and infection risk in the present study (after a 2-year course and again 12 months later) represent important and clinically relevant time points. However, we acknowledge that relapse and infection risk prediction is equally important following a single induction course of RTX, as there is a subgroup of patients who remain in remission for long periods after induction therapy and do not require repeat-dose maintenance RTX. Further studies and more reliable biomarkers are needed to help identify these patients who would benefit from a more personalized and tailored treatment strategy.

Conclusion

To our knowledge these are the first published relapse risk and infection risk prediction models with RTX in AAV. While our models had insufficient power to discriminate risk between individual patients they were able to assign patients into risk groups for both relapse and infection. The ability to identify risk groups may help in decisions regarding the potential benefit of ongoing RTX treatment. However, we caution the use of these prediction models until prospective multi-centre validation studies have been performed.

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Data sharing statement

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Supplementary data

Supplementary data are available at *Rheumatology* online.

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