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The association of serum interleukin-6 levels with clinical outcomes in antineutrophil cytoplasmic antibody-associated vasculitis

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ABSTRACT

Objective: To investigate serum IL-6 (sIL-6) levels during active disease, complete remission (CR), and relapse in antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV), and to explore the association of changes in sIL-6 with clinical outcomes.

Methods: sIL-6 levels were measured at baseline and longitudinally over 18 months, in 78 patients with AAV enrolled in a randomized controlled trial comparing treatment with either rituximab (RTX) or cyclophosphamide (CYC)/azathioprine (AZA). Outcome variables included baseline clinical features, ANCA specificity, disease activity (active disease *versus* CR), time to relapse events, B cell repopulation, and ANCA titer increases.

Results: At baseline, sIL6 levels were detectable in 81% of patients; 73% (n = 57) of subjects were proteinase 3 (PR3)-ANCA positive, sIL-6 levels were higher in subjects with PR3-ANCAs and positively correlated with their levels ($r_s = 0.36, p < 0.01$), but not with levels of myeloperoxidase (MPO)-ANCA ($r_s = -0.17, p = 0.47$). Higher baseline sIL-6 levels were associated with PR3-ANCA positivity, fever, pulmonary nodules/cavities, conductive deafness, and absence of urinary red blood cell casts ($p < 0.05$). Baseline sIL6 levels did not predict CR at month 6 ($p = 0.71$), and the median sIL-6 level declined from baseline with induction therapy, regardless of CR achievement. An increase in sIL-6 during CR was a predictor for subsequent severe relapse in RTX-treated patients (hazard ratio (HR):7.24, $p = 0.01$), but not in CYC/AZA-treated patients (HR:0.62, $p = 0.50$). In contrast, a sIL-6 increase did not predict B cell repopulation or ANCA titer increase in either treatment arm ($p > 0.05$).

Conclusion: At baseline, sIL-6 concentrations correlate with PR3-ANCA titers and are associated with specific clinical manifestations of AAV. Baseline sIL6 concentrations do not predict CR at 6 months, but the increase in sIL-6 concentrations during CR is associated with subsequent severe relapse among RTX-treated patients. Further investigation into the mechanistic role of IL6 in AAV might lead to identifying this pathway as a potential therapeutic target in this disease.

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1. Introduction

Interleukin (IL)-6 is a pleiotropic cytokine with a wide range of biological activities in inflammation, immune regulation, hematopoiesis, and oncogenesis [1]. The competency to produce and secrete IL-6 is shared by several immune and non-immune cells, in particular monocytes, endothelial cells, and mesangial cells [1–3]. B cells may also be involved in IL-6 production, mostly in an autocrine-paracrine fashion [1,4]. Among other biological activities, IL-6 induces synthesis of acute phase response proteins by hepatocytes and maturation of B cells into antibody-producing cells, leading to immunoglobulin production *in vivo* [1,2]. Therefore, deregulated overproduction of IL-6 has been implicated in inflammatory and antibody-mediated autoimmune diseases [5]. The IL-6 pathway is involved in several rheumatologic conditions, particularly rheumatoid arthritis and large-vessel vasculitis [6–8], in which elevated serum IL-6 correlates with disease activity, and targeting IL-6 signaling is effective therapeutically [9–11].

Small case series or case reports have described elevated IL-6 levels in blood of patients with ANCA-associated vasculitis (AAV) and its local production at sites of active vasculitis, leading investigators to postulate a role of IL-6 in the pathogenesis of AAV [12–18]. Studies in a mouse model of myeloperoxidase (MPO)-ANCA-associated rapidly progressive glomerulonephritis suggested that IL-6-mediated signaling may increase the severity of disease [19], and be involved in ANCA production [20]. Exploratory analyses have shown that levels of circulating IL-6 and other cytokines are elevated in patients with severe active AAV [21,22]. However, the role of IL-6 has not been investigated in AAV in detail.

This study was conducted using serum samples collected during the conduct of a large clinical trial to investigate the association of serum IL-6 levels (sIL-6) with disease activity in AAV and to explore associations of sIL-6 with disease relapses, repopulation of blood B cells, and ANCA titer increases.

2. Methods

2.1. Subject population and definitions

The Rituximab in ANCA-Associated Vasculitis (RAVE) study was a multicenter, double-blind, placebo-controlled trial that randomized 197 patients in a 1:1 ratio to receive either RTX (375 mg/m^2 intravenously each week for 4 weeks) or cyclophosphamide (CYC) (2 mg/kg for 3–6 months) followed by azathioprine (AZA) (2 mg/kg, up to 150 mg/day) [23,24]. Both groups received the same glucocorticoid regimen, and were followed for 18 months on protocolized therapy. Disease activity was measured using the Birmingham Vasculitis Activity Score for Wegener's Granulomatosis (BVAS/WG) [25].Complete remission (CR) was defined as a BVAS/WG of 0, following successful completion of the prednisone taper to 0 mg and regardless of the time it was reached. Disease relapse was defined as any new disease activity, with an increase in BVAS/WG ≥ 1 point after achievement of CR. Severe relapse was defined as a BVAS/WG ≥ 3 or the occurrence of at least one major BVAS/WG item following disease remission requiring re-treatment with either RTX or CYC [23].

Serum IL-6 was measured longitudinally. An IL-6 increase was defined as a change from undetectable to detectable (> 0.49 pg/ml), or as a greater than 50% increase over the preceding measurement. Similarly, an increase in CRP level was defined as a change from undetectable to detectable (> 0.50 mg/dL), or as a greater than 50% increase over the preceding time-point. ANCA and B cells were also measured longitudinally. Since there is not an accepted cut-off for IL-6 in the literature and given the exploratory nature of our study, we arbitrarily decided to use as a cut off the change from undetectable to detectable levels or an increase greater than 50% from the previous measurement, in order to register any possible clinical association with clinical outcome. To be consistent, we used the same criteria to define the CRP increase.

An ANCA titer increase was defined as a doubling from the preceding positive titer (≥20 units), or an increase of ≥40 units if the assay had previously become negative [26]. B cell redetection in RTX-treated patients was defined as at least 10 but less than 69 CD19$^+$ cells per microliter, and reconstitution as 69 or more CD19$^+$ cells per microliter or a return to baseline levels.

Because of cost constraints this exploratory study was conducted using a representative subset of 78 patients from the RAVE trial cohort: we included all patients with available sera who suffered severe relapses during 24 month of observation after having achieved CR at month 6th; the remaining were randomly chosen among the subjects of the RAVE cohort with available sera (Supplementary Table 1). All clinical and laboratory data were obtained from the trial database.

2.2. Study design

Clinical and experimental data were analyzed at each study visit including baseline (time 0), 2 weeks and 1, 2, 4, 6, 9, 12, 15, and 18 months after enrollment, and patients were followed until month 24 (Supplementary Fig. 1). The primary goals were (i) to identify associations of baseline sIL-6 levels with clinical disease manifestations, ANCA specificity (PR3-ANCA$^+$ *versus* MPO-ANCA$^+$) and titers, and the likelihood of achieving CR by 6 months (primary trial endpoint); (ii) to longitudinally determine any association between sIL6 increase and subsequent relapse, B cell redetection or reconstitution, and ANCA titer increases during follow-up.

2.3. Disease manifestations and disease phenotype categories at baseline

The organ manifestations at enrollment and at each study visit were recorded using the BVAS/WG instrument [25]. Two disease phenotype categories (granulomatous disease and capillaritis) were based on the BVAS/WG items recorded at the time of enrollment, as previously published elsewhere [26]; patients positive for at least one granulomatous disease BVAS/WG item and at least one capillaritis BVAS/WG item were classified as having granulomatous AND capillaritis phenotype.

2.4. Sample processing and assays

Serum from each of the 78 patients was assayed for IL-6 at all timepoints. Serum samples were processed and stored at each study site, then shipped to the University of Michigan (Michigan, USA) where the sIL-6 levels were measured. All samples remained frozen at −80 °C until the assays were performed. IL-6 protein concentrations were measured using a custom array of capture immunoassays [21], which used the same pair of monoclonal antibodies used in a commercial ELISA kit (human IL-6 catalogue number DY206, DuoSet ELISA Development kit, R&D Systems).

Standardized direct enzyme linked immunosorbent assays (ELISAs) for PR3-ANCA and MPO-ANCA (supplied by Euroimmun US, Inc.) were performed at the centralized laboratory on all serial serum samples from all patients [23,24].

C-reactive protein (CRP) was assayed at the participating sites at the time of each study visit, and data were retrieved from the clinical report forms.

2.5. Statistical analysis

Descriptive statistics (percentages, mean, etc.) were used to summarize the characteristics of the cohort. Since sIL-6 was not normally distributed, associations of sIL6 levels with continuous outcomes were evaluated using the Mann-Whitney *U* test for independent observations and Wilcoxon's signed rank test for paired observations. Fisher's exact test and non-parametric Spearman test were applied, as appropriate.

Hazard ratios were calculated using the Cox proportional hazards method to assess whether an increase in sIL-6 level was associated with a subsequent relapse (separate analyses were performed with the event of interest being "any" or "severe" relapse), B cell reappearance (separate analyses were performed for "redetection" or "reconstitution") or ANCA titer increase. Similarly, a Cox regression analysis to assess whether an increase in CRP level was associated with subsequent relapse was performed using the same model used for sIL6 analysis (see Supplementary Material). For this analysis, the date of the increase was used as time 0.

To further characterize the cumulative percentage of patients who experienced a relapse following an increase in sIL-6 level in each treatment arm, a Kaplan-Meier analysis that included only patients who experienced an increase in sIL-6 level was performed.

All analyses were performed using JMP and SAS software (SAS Institute, Cary, NC). Differences were considered significant when $p < 0.05$.

3. Results

3.1. Baseline analyses

3.1.1. Demographic, clinical and laboratory findings at baseline

Per protocol, all 78 subjects had active disease upon study enrollment (BVAS/WG score ≥ 3). Seventy-five percent (n = 58) of patients were diagnosed with GPA and 25% (n = 20) with MPA, 73% (n = 57) were PR3-ANCA positive and 27% (n = 21) MPO-ANCA positive; 46% (n = 36) had relapsing disease while 54% (n = 42) were newly diagnosed upon enrollment. Baseline sIL6 levels were detectable (> 0.49 pg/ml) in 63 of 78 (81%) patients.

Twenty-nine patients had severe relapse during the 24-month follow-up, 12 additional patients suffered only non-severe relapses, 8 patients that did not achieved CR at any time; the remaining 29 patients remained in remission after CR for the entire follow up. Forty-five patients were treated with RTX and 33 with CYC/AZA, and were followed for 24 months after randomization. There was no significant difference in sIL6 levels, CRP, BVAS/WG, or glucocorticoid/immunosuppressive therapy used at screening before blood sampling between the two treatment groups ($p > 0.05$). Additional baseline characteristics of this cohort are presented in Table 1.

3.1.2. Serum IL-6 levels and clinical characteristics at baseline

We evaluated whether baseline sIL-6 levels were associated with specific disease manifestations upon enrollment, as determined using the BVAS/WG. The median [IQR] sIL6 level of patients who only had BVAS/WG items that reflect granulomatous disease manifestations (20.42 [1.72, 98.30] pg/mL) was significantly higher compared to that of patients who only had BVAS/WG items reflecting capillaritis (1.16 [0.49, 4.86] pg/mL) ($p < 0.02$). Patients with both granulomatous manifestations and capillaritis had a significantly higher sIL6 (median 7.35 [0.73, 32.30]) compared to those with only capillaritis (Fig. 1A). In contrast, specific diagnosis (GPA *versus* MPA) and disease presentation (newly diagnosed *versus* relapsing patients) did not have a significant influence on sIL-6 levels (Fig. 1B–C). Median sIL-6 levels were higher in PR3-ANCA positive patients than those with MPO-ANCA at baseline (5.78 [0.96, 27.61] *versus* 1.0 [0.49, 4.11], p = 0.04) (Fig. 1D), but these differences were not maintained at subsequent time-points during follow-up ($p > 0.05$ in all comparisons; *not shown*). A positive correlation between baseline sIL-6 levels and PR3-ANCA titers was found ($r_s = 0.36$, $p < 0.01$), but not with MPO-ANCA titers ($r_s = -0.17$, $p = 0.47$). To further validate this finding, we took a complementary analysis approach by dividing the study population based on the median level of baseline sIL6 (i.e. patients below ("low") and above ("high") the median baseline sIL6 value in this cohort), and observed that the 50% of the cohort with higher serum IL6 levels present with more individual manifestations within the spectrum of granulomatous disease. Specifically, baseline clinical manifestations were compared between patients with "high" and "low" sIL6 levels, but only the presence of fever, conductive deafness, pulmonary nodules or cavities and the absence of red cell blood casts were associated with higher levels of sIL-6 ($p < 0.05$) (Supplementary Table 2).

3.2. Longitudinal analyses

3.2.1. Serum IL-6 levels and response to induction treatment

At month 6 after randomization, sIL-6 was significantly reduced in response to remission-induction therapy (n = 78, $p < 0.01$) (Fig. 2A), and CR was achieved in 76% (60/78) of the patients included in this study (RTX group 80% and CYC/AZA group 73%, $p = 0.59$). The percentage of patients achieving this endpoint was equivalent between treatment arms for both patients with GPA and MPA and for those with PR3-and MPO-AAV ($p > 0.05$ in both comparisons). Levels of sIL-6 at baseline, week 2, and months 2, 4, and 6 did not differ in patients who

Table 1
Baseline characteristics of the study subjects and clinical outcomes by treatment group.

Patient features	All	CYC/AZA (n = 33)	RTX (n = 45)	P value
Age at onset of symptoms, mean ± SD years	51 ± 16	52 ± 15	50 ± 17	*0.47*
Sex, % female	55	45	62	*0.17*
Race or ethnic group, % white	92	97	89	*0.23*
GPA/MPA[a], %	75/25	73/27	82/22	*0.41*
PR3-ANCA/MPO-ANCA, %	72/28	70/30	76/24	*0.61*
New-onset disease/relapse, %	46/54	55/45	40/60	*0.25*
Renal disease, %	31	33	29	*0.80*
Pulmonary manifestation, %	51	42	58	*0.25*
BVAS/WG at study entry, median (25%–75% IQR)	8 (6,10)	9 (6,10)	7 (6,9)	*0.18*
CRP at enrollment, median (25%–75% IQR) mg/dL	1.3 (0.0, 5.2)	2.0 (0.4, 7.5)	1.2 (0.5, 3.9)	*0.57*
Glucocorticoid before randomization, no.(%)	44 (56%)	21 (64%)	23 (51%)	*0.36*
Complete remission at 6 month, no. (%)	60 (77%)	24 (73%)	36 (80%)	*0.59*
Complete remission at any time, no. (%)	70 (90%)	31 (94%)	39 (84%)	*0.29*
Patients in complete remission whose disease flared, no./total no. Assessed (%)	41/70 (59%)	18/31 (58%)	23/39 (59%)	*1.00*
Severe flares, no.	29/70 (41%)	14/31 (45%)	15/39 (38%)	*0.63*
IL-6 levels median (25%–75% IQR) (pg/mL)	2.66 (0.76, 20.98)	5.78 (0.85, 23.63)	2.14 (0.61, 20.81)	*0.41*

Continuous variables were compared between groups using Mann-Whitney *U* test or Student's *t*-test. Categorical variables were compared between groups using Fisher's exact test.

RTX: rituximab, CYC/AZA: cyclophosphamide/azathioprine, GPA/MPA: granulomatosis with polyangiitis/microscopic polyangiitis, PR3-ANCA/MPO-ANCA: proteinase 3-ANCA/myeloperoxidase-ANCA, BVAS/WG: Birmingham Vasculitis Activity Score for Wegener's Granulomatosis, IL-6: Interleukin-6.

[a] One patient had indeterminate diagnosis (included in the RTX group), and was excluded from the percentage calculation.

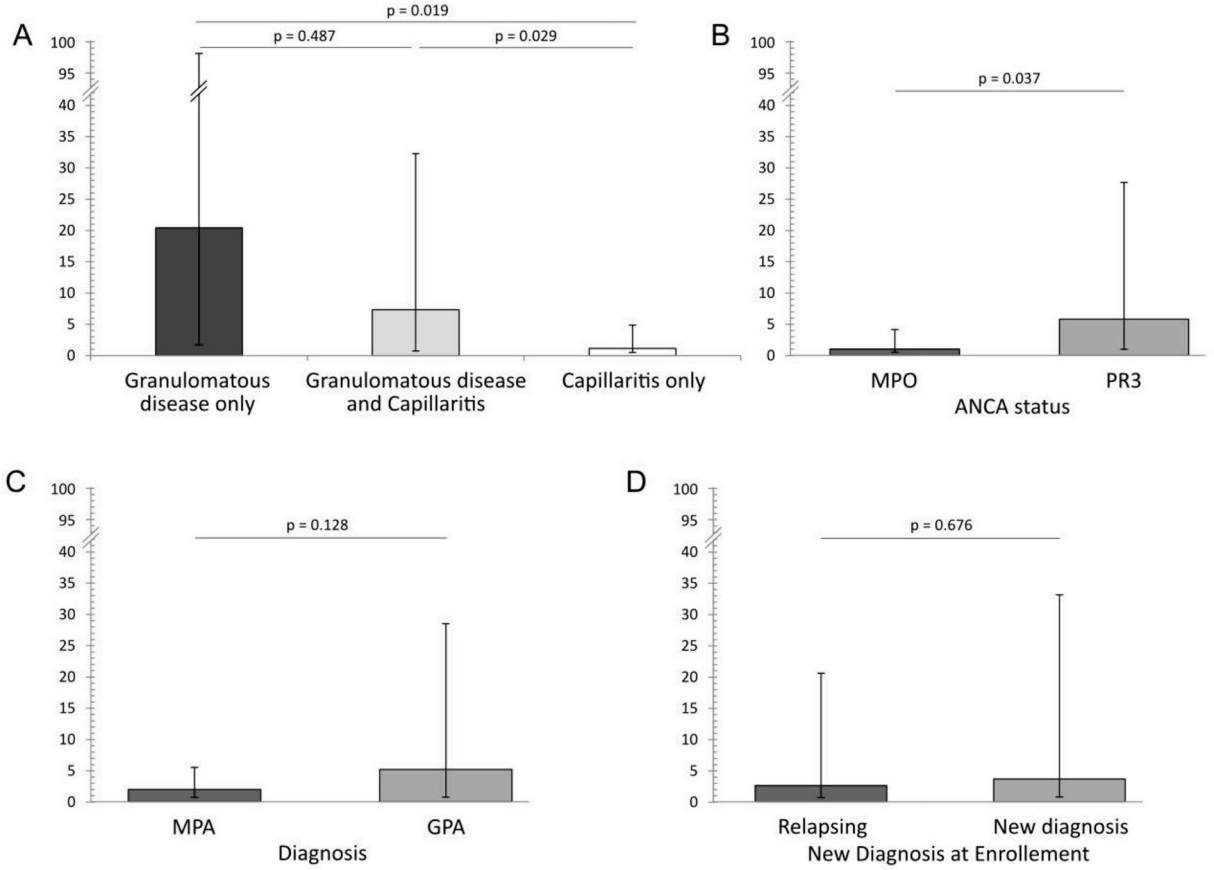

Fig. 1. Association of IL-6 levels and disease features at baseline according to BVAS/WG items and ANCA status. (A) Patients (n = 78) are grouped in three mutually exclusive subsets of patients; only granulomatous manifestation (n = 11), only capillaritis (no = 22), or both (n = 45) (B) Patients were then grouped in two mutually exclusive subsets according to diagnosis (MPA, n = 19; GPA, n = 58); (C) disease at presentation (relapsing, n = 42; newly diagnosed, n = 36); and (D) ANCA status (MPO-ANCA = 21, PR3-ANCA = 57). Bars show the median and interquartile range.
Footnotes. Granulomatous disease only: Birmingham Vasculitis Activity Score for Wegener's Granulomatosis (BVAS/WG) items reflecting underlying necrotizing granulomatous inflammation included mouth ulcers, retro-orbital mass/proptosis, bloody nasal discharge, sinus involvement, salivary gland enlargement, subglottic inflammation, conductive deafness, other major or minor ear, nose, and throat involvement, pulmonary nodule/cavity, endobronchial involvement, meningitis, and cord lesion. Capillaritis only: defined as the presence of one or more of the following BVAS/WG items: purpura, scleritis, retinal hemorrhage or exudate, sensorineural deafness, hematuria, red blood cell casts on urinalysis or glomerulonephritis, increase in creatinine level, alveolar hemorrhage, mesenteric ischemia, sensory peripheral neuropathy, or motor mononeuritis multiplex. Granulomatous disease and capillaritis: patients positive for at least one granulomatous disease BVAS/WG item and at least one capillaritis BVAS/WG item.

did or did not achieve CR by 6 months ($p > 0.05$ in all comparisons) (Fig. 2B). Univariate analyses showed that CR at 6 months was not achieved significantly more often in patients with or without detectable sIL-6 levels at baseline ($p = 0.71$). In 60% (47/78) of subjects, sIL-6 decreased below detectable levels within 2 months from randomization; the rate of decline of sIL-6 over time was similar in both treatment arms.

When median sIL6 levels of patients who achieved CR at any time (70/78) were compared to those of patients who did not (8/78), no difference was found at baseline ($p = 0.81$) or at any subsequent timepoint in the first 6 months ($p > 0.05$ in all comparisons, *data not shown*), suggesting that sIL-6 levels do not predict the likelihood of achieving CR at any time.

3.2.2. Serum IL-6 as a predictor of disease relapse, B cell reconstitution or ANCA titer increase

To determine whether an increase in sIL-6 levels predicted disease relapse (i.e. if an increase in sIL6 levels was identified at time points preceding the event, and increases detected at the same time of the event not being counted), we calculated the Hazard Ratios (HRs) for the sIL-6 increase in the entire cohort of patients and in each treatment arm (Table 2). Again, the eight patients who did not achieve CR were excluded from this analysis.

Fifty-nine percent of patients relapsed and 41% experienced a severe relapse, and proportions of relapse and severe relapses were similar in both treatment arms (Supplementary Fig. 2). The proportion of patients in the RTX arm who suffered severe relapses after CR following any sIL-6 increase was 25% (95% CIs: 5–40%) at 3 months, 40% (95% CIs: 16–59%) at 6 months, and 56% (95% CIs: 14–78%) at 9 months, whereas it was 13% (95% CIs: 0–29%) at 3 months, 24% (95% CIs: 0–45%) at 6 months, and 24% (95% CIs: 0–45%) at 9 months in the CYC/AZA arm.

Overall, when the entire cohort was analyzed, an increase in sIL-6 titer was not associated with a subsequent increased risk of any relapse ($p = 0.37$) or any severe relapse during follow-up ($p = 0.13$). However, when analyzed by treatment group, an increase in sIL-6 levels was associated with subsequent severe relapses in patients treated with RTX (HR 7.24, $p = 0.01$), but not in those treated with CYC/AZA (Fig. 3A–B). The majority of the severe relapses occurred within 6 months after the IL6 increase (Table 3), and no relapse occurred after month 9th.

When we replicated this analysis using CRP instead of sIL6 as potential predictor of relapse, CRP did not predict any relapse or severe relapse in the entire group (any relapse HR 1.84 [0.91–3.74] and severe

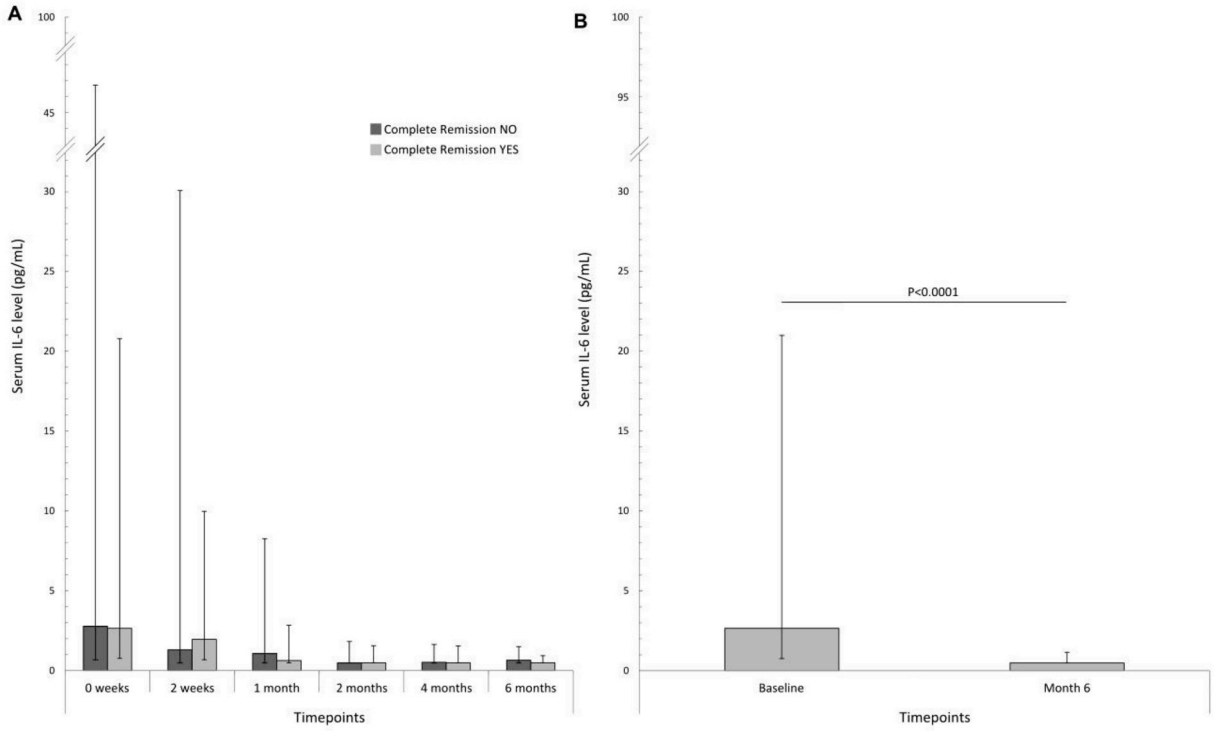

Fig. 2. Longitudinal IL-6 levels at selected time-points before and during induction of remission treatment. (A) Absolute value of serum IL-6 level in patients who achieved complete remission at 6 months (light grey, n = 60) and in those who did not (dark grey, n = 18) at baseline, 2 weeks, month 1, month 2, month 4, and month 6 after the initiation of treatment. Bars show the median and interquartile range ($p > 0.05$ at each time-points). (B) Absolute value of serum IL-6 level in all the cohort of patients (n = 78) at baseline and month 6th, showing a significant decrease as an effect of induction of remission therapy ($p < 0.01$). Bars show the median and interquartile range.

relapse HR 1.78 [0.78–4.09]), in the RTX arm (any relapse 1.99 [0.74–5.34] and severe relapse HR 1.82 [0.58–5.74]), or the CYC/AZA arm (any relapse 1.64 [0.58–4.64] and severe relapse HR 1.61 [0.48–5.37]).

As sIL6 is a recognized driver of B cell proliferation [27,28] and antibody production, we wanted to know whether a sIL6 increase during CR predicted the B cell reappearance (in the RTX-B cell depleted patients) or an ANCA titer increase (in the whole group of patients with AAV).Among patients treated with RTX, B cells were depleted ($CD19^+$ B cell/microliter < 10) in 100% of patients after treatment, and B cells reappeared (CD19 + B cells ≥10/microliter) in 92% (36/39) and reconstituted (CD19 + B cells ≥69/microliter) in 87% (34/39) during follow-up. In these patients, the rise of sIL-6 levels did not predict B cell reappearance, regardless of whether a cut-off of ≥10 CD19 + B cell/microliter (redetection) or of ≥69 CD19 + B cell/microliter (reconstitution) was considered (Table 2).

Twenty-six of 70 (37%) patients had at least one ANCA titer increase during the follow-up, 21 in RTX group and 5 in CYC/AZA group. Increases of sIL-6 did not predict subsequent ANCA titer increases (Table 2).

3.2.3. Timing of sIL-6 increases and B cell reappearance

Since sIL6 levels did not predict B cell reappearance in RTX treated subjects, we analyzed the timing of these two events and observed that the majority of subjects had reconstituted B cells when sIL6 increased. Among patients treated with RTX who experienced a severe relapse (n = 15), B cells were redetected before or at the time of the severe relapse in 100% of cases, with a median time between the redetection of B cells and relapse of 86 days (25%-75%IQR: 60–203; range 0–315 days). Eighty percent (12/15) of the patients in the RTX arm who subsequently had a severe relapse had a B cell redetection before or at the time of the IL-6 increase, with a mean time between the redetection of B cells and sIL-6 increase of 55 days (25%-75%IQR: 0–184.25; range 0–210 days). In the remaining 3 patients, an increase in sIL-6 level occurred between 86 and 189 day before B cells were detected. Among patients treated with RTX who did not experience a severe relapse (n = 24), B cells were redetected in 79% (n = 19/24) of cases during the 24 months of follow-up. sIL-6 increased in 80% (n = 20/24) of

Table 2
Relapse rate, ANCA titer increase, and B cell repopulation following an increase serum IL-6 level during the observational period.

Model	All subjects (n = 70)		CYC/AZA treatment arm (n = 31)		RTX treatment arm (n = 39)	
	HR (95%CI)	P value	HR (95%CI)	P value	HR (95%CI)	*P value*
Any Relapse	1.43 (0.66–3.11)	0.37	0.84 (0.26–2.72)	0.77	2.21 (0.75–6.46)	*0.15*
Severe Relapse	1.98 (0.82–4.80)	0.13	0.62 (0.15–2.52)	0.50	7.24 (1.50–34.87)	*0.01*
ANCA Titer Increase	2.56 (0.89–7.42)	0.08	[a]	[a]	1.97 (0.66–5.88)	*0.23*
CD19+/B cell ≥10 μL	–	–	–	–	0.97 (0.27–3.51)	*0.97*
CD19+/B cell ≥69 μL	–	–	–	–	1.50 (0.57–3.94)	*0.41*

IL-6: Interleukin-6, HR: Hazard Ratio, 95%CI: 95% confidence intervals, RTX: rituximab, CYC/AZA: cyclophosphamide/azathioprine.
[a] There were only 5 ANCA flares in CYC/AZA treatment arm, one of which occurred after IL-6 increase. Not enough information to calculate a HR.

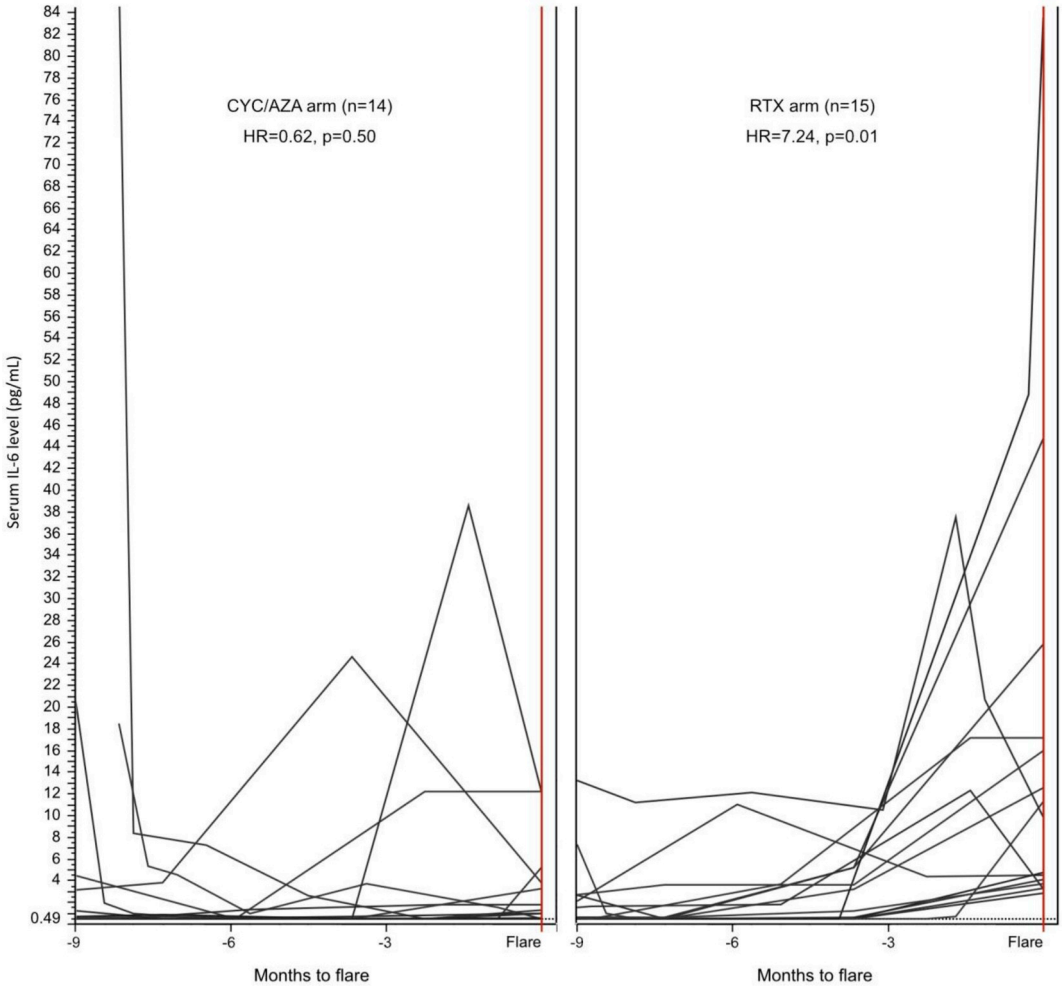

Fig. 3. Interleukin-6 levels prior to flare in patients with AAV who severely relapsed according to treatment group (CYC/AZA n = 14, RTX n = 15). Each line represents an individual patient.

these patients: in 11 cases before B cell redetection, in 4 cases at the same time or after B cell redetection, in 5 cases the sIL6 increase was not followed by B cell redetection during the observation period.

4. Discussion

This study demonstrates that among patients with AAV in the RAVE trial, higher sIL-6 levels were associated with the presence of PR3-ANCA and several disease manifestations at baseline, including fever, conductive deafness, and pulmonary nodules/cavities. Among subjects treated with RTX, but not those treated with CYC/AZA, a rise in sIL-6 levels occurring during CR conveyed a significantly increased risk for subsequent severe relapse occurring within 6 months after the sIL6 increase in the majority of patients. No previous study assessed sIL-6 in AAV using longitudinally-collected blood samples in a large cohort of patients. Whereas the observed association of higher sIL-6 levels with fever was not surprising, the associations with PR3-ANCA and the other specific clinical manifestations were unexpected [12,29–31].

The associations of sIL-6 with pulmonary granulomatous manifestations of AAV and PR3-ANCA serology observed in this human study contrast with the results of most preclinical studies, which showed sIL-6 involvement associated with MPO-ANCA and rapidly progressive glomerulonephritis [19,20]. This apparent contradiction may be partially explained by the fact that there is no established model of PR3-ANCA associated granulomatous inflammation in GPA/PR3-AAV, making a comparison between humans and mice difficult. Our results also suggest a potential contribution of sIL-6 to surrogate manifestations of granulomatous inflammation, providing support for further

Table 3
Relapse rate within the 6, 9 and 12 months following the first serum IL-6 levels increase. No relapse after month 9th was observed (for this reason data censored at 12 months were not represented, since identical to those at 9 months).

Censor	Model	All subjects (n = 70)		CYC/AZA treatment arm (n = 31)		RTX treatment arm (n = 39)	
		HR (95%CI)	P value	HR (95%CI)	P value	HR (95%CI)	P value
6 months	Any Flare	1.34 (0.60–3.00)	0.47	0.79 (0.23–2.74)	0.72	2.08 (0.69–6.25)	0.19
	Severe Flare	1.97 (0.81–4.80)	0.14	0.68 (0.17–2.72)	0.59	6.98 (1.43–34.02)	0.02
9 months	Any Flare	1.43 (0.66–3.11)	0.37	0.84 (0.26–2.72)	0.77	2.21 (0.75–6.46)	0.15
	Severe Flare	1.98 (0.82–4.80)	0.13	0.62 (0.15–2.52)	0.50	7.24 (1.50–34.87)	0.01

IL-6: Interleukin-6, HR: Hazard Ratio, 95%CI: 95% confidence interval.

investigations of therapeutic agents targeting the IL-6 pathway in these patients.

Serum IL-6 concentrations are associated with disease activity in several rheumatic diseases, including rheumatoid arthritis and large-vessel vasculitides [6–8,11]. In this study, reduction in sIL-6 paralleled reduction in disease activity during induction therapy, and the increase of sIL-6 levels, but not CRP levels, anticipated severe relapse in RTX-treated patients, supporting a potential contribution of IL-6 to triggering AAV relapses. Indeed, sIL-6 is a potent inducer of inflammation, and also plays an important role in the terminal differentiation of B cells [1,4,28], both processes likely involved in relapses of AAV. Alternatively, an increase in sIL-6 may merely be a reflection of early low-grade inflammatory activity associated with a relapse that remains undetected by other conventional laboratory tests or localizing symptoms.

In giant cell arteritis, a granulomatous vasculitis in which sIL6 plays a central role, IL6 production is promoted and maintained by T helper 1 cells at vasculitis sites, driving the granuloma formation and chronic inflammation [6–8,11]. Although we did not provide similar evidence on a molecular level for AAV, it is tempting to speculate that in GPA/PR3-ANCA positive patients, IL6 may promote granuloma formation in a similar fashion, explaining the difference we observed in baseline sIL6 levels when patients were grouped by ANCA specificity and by the presence of granulomatous manifestation *versus* capillaritis. Interestingly, when patients were divided by clinical diagnosis (GPA vs MPA), the IL6 levels did not differ, suggesting that ANCA specificity associates better with the presence of granulomatous manifestations compared to clinical diagnosis, further supporting this idea.

Non-specific disease manifestations including fatigue and malaise are known to be triggered by IL-6 [25]. In patients with AAV, a rise in the patient global assessment disease activity score during times defined by physicians as periods of remission was shown to be associated with subsequent occurrence of disease relapse [32]. Thus, many patients feel they are about to flare before a relapse is detected clinically by physicians using conventional disease activity detection tools. The finding that increases in sIL-6 levels may anticipate the occurrence of a severe relapse may in part explain patients' perceptions of imminent clinical worsening.

No association was observed between sIL-6 levels at baseline and achievement of CR by 6 months, the primary outcome of the RAVE trial, or at any time during the follow-up. The kinetics of sIL-6 levels was similar in both treatment arms, i.e. rapidly declining during induction of remission, an effect that may be attributable to therapy with sustained high-dose glucocorticoids during the first months of treatment [33].

Interestingly, a rise in sIL-6 levels after CR was associated with subsequent severe relapse only in RTX-treated patients. Although we cannot provide a definitive explanation for the observed discrepancies between treatment arms, different treatments seem to have different effects on sIL-6 producing cells. B cells targeted by RTX may be directly involved in the production of sIL-6 or indirectly influence monocytes to produce it [28]. Reconstituting B cells after RTX depletion may have different effects on conditioning of the surrounding molecular or cellular environment, compared to CYC/AZA treatment that does not deplete B cells.

Although IL-6 was discovered as a B cell stimulatory factor, has been shown to enhance humoral immunity [27] and to provide important survival and proliferative signals to different leukocyte populations [1,2,28], B cell repopulation usually preceded an increase of sIL-6 levels. Since most disease relapses are preceded by B cell repopulation and that ANCA-titer increases [24,26], it may be speculated that sIL-6 represents the final trigger of autoimmunity and/or inflammation in AAV after B cell return, ultimately leading to severe relapses in PR3-ANCA positive patients. We cannot conclude that IL-6 is the central molecular mediator driving the disease relapse in AAV, but –according to our results-it appears to precede it, usually within 6 months and after B cell reconstitution, suggesting a possible pathogenic role.

This study has several limitations. First, the severity of the relapse was evaluated based on BVAS/WG scores, thus relying on the judgment of different investigators, which is one of the intrinsic limits of this tool. Furthermore, our analysis compares a subjective clinical score (BVAS/WG) representing the aggregate of disease activity over the 28 days preceding the study visit to a biological measurement (sIL-6) reflecting the single point in time of the study visit. However, this is inherent to any such of comparison and not a weakness unique to our study. Second, the small cohort size limits the power to detect differences between small subgroups, and we therefore avoided comparisons of subgroups of patients that were too small for meaningful analyses. Third, we cannot make any meaningful statement about MPO-ANCA positive patients because their relapse rate is low compared to PR3-ANCA positive patients, and furthermore because, in part related to this difference in relapse propensity, fewer MPO-ANCA positive patients than PR3-ANCA positive patients were enrolled into the trial. However, these numbers reflect the clinical reality associated with AAV in the general population. Finally, in some of the analyses (i.e. correlations between individual BVAS/WG items and baseline serum IL6 levels), we did not comprehensively investigate possible mutual interactions between these clinical manifestations. However, the understanding of the reciprocal influence of these clinical manifestations is beyond the scope of our study.

5. Conclusions

In conclusion, sIL-6 reflects disease activity in AAV, rapidly declining when remission is induced. Prior to induction therapy, sIL-6 levels correlate with PR3-ANCA titers, are associated with the presence of fever and pulmonary nodules/cavities and with the absence of several features considered clinical surrogates of small-vessel vasculitis or capillaritis. Increases of sIL-6 levels are usually preceded by B cell reconstitution and ANCA titer increases in patients treated with RTX, rather than preceding them. The risk of relapse following an increase in sIL-6 after CR appears partially associated with the severity of relapse and treatment chosen to induce remission, with a significant association seen only for severe relapses in patients treated with RTX.

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Disclosure

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Appendix A. Supplementary data

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