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ORIGINAL ARTICLE

Lymphocyte counts in patients with ANCA-associated vasculitis

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Abstract How lymphocyte counts relate to treatment-response in patients with ANCA-associated vasculitis (AAV) is controversial, and data on short-term variability of lymphocyte counts are lacking. Retrospective single center evaluation of disease activity and lymphocyte counts in patients with AAV, and of lymphocyte counts in kidney transplant-recipients, were done; both at the University Hospital Basel, Switzerland. Twenty-three patients with AAV were included. Remission was achieved in all patients. Ten patients experienced a relapse after a median of 66 weeks (range 15–189 weeks). Median lymphocyte counts at diagnosis were significantly higher than at remission (1.38×10^9/L vs. 0.99×10^9/L; $P = 0.007$). By contrast, median lymphocyte counts at remission and relapse did not differ significantly. However, intra-individual variability of lymphocyte counts early after diagnosis was high [median lymphocyte variability-range during the first 3 weeks of treatment 1.57 (range 0.27–3.95), $n = 17$]. This variability was not specific to patients with AAV, but was also observed in patients after kidney transplantation [variability of 1.76 (range 0.74–3.95, $n = 31$)]. The significantly higher median lymphocyte counts at diagnosis of AAV make lymphocyte counts a valuable surrogate for the treatment-efficiency in clinical studies. By contrast, on a patient-level, variability of lymphocyte counts impedes meaningful interpretation of individual measurements.

Keywords Vasculitis · ANCA · Lymphocytes · AAV · Prognostic · Variability

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Introduction

Therapeutic options for severe AAV are limited, and therapy—mostly cyclophosphamide (CYC)-based—remains a balancing act between disease- and treatment-related morbidity and mortality [1, 2]. Factors predicting treatment-response would hence be important.

Lymphocytes, both of the T- and B-cell lineage, are thought to be involved in the pathogenesis of AAV [3–6]. How circulating lymphocyte counts relate to disease activity, however, remains controversial. One study reported that treatment-induced lymphopenia ($\leq 0.5 \times 10^9$/L) is associated with a high likelihood to achieve sustained remission [3, 7]. By contrast, another group suggested that, analogous to patients with systemic lupus erythematodes, lymphopenia in AAV reflects disease activity, with lymphocyte counts actually increasing in patients achieving remission [8, 9]. While the reason for these contradictory findings remain unclear, neither study has taken into account short-term variability of lymphocyte counts and how this might impact study results.

Here, we investigated how in AAV-patients (1) median lymphocyte counts relate to disease activity on a population

level, and (2) assessed, in these same individuals, short-term lymphocyte count variability.

Patients and methods

Study populations

After obtaining approval by the institutional review board, disease activity and lymphocyte counts in patients with AAV (diagnosed between 1/1992 and 12/2007), and lymphocyte counts in kidney transplant-recipients (transplanted within the living-donor program between 1/2004 and 12/2006) were retrospectively assessed in patients treated at the University Hospital Basel, Switzerland. Medical charts from all patients with a definite diagnosis of AAV were reviewed to obtain demographic, clinical and laboratory data.

Diagnosis and follow-up of AAV

AAV was diagnosed according to American College of Rheumatology (ACR) criteria [10], the Birmingham Vasculits Activity Score (BVAS) [11] was calculated initially and for every subsequent visit.

Laboratory measurements

Lymphocyte counts were determined via routine flow-cytometry (ADVIA® Hematology system, Siemens), ANCA titers were measured using indirect immunofluorescence (INOVA®, San Diego), and ANCA were specified [myeloperoxydase (MPO) vs. proteinase3 (PR3)], and quantified using enzyme linked immunosorbent systems (DLD Diagnostika®, Hamburg).

Outcome parameters

Remission of AAV was defined as complete absence of clinical symptoms and a daily prednisone-dose $\leq$7.5 mg, according to published criteria [12]. Relapse was defined as de novo disease activity according to the BVAS [11].

Statistical analysis

Discrete variables are expressed as counts (percentage), continuous variables as medians and ranges. Frequency comparison was done by Chi-square test. Two-group comparison of normally distributed data was performed by paired or unpaired Student's *t* test. For not normally distributed

Table 1 Patients with AAV: disease manifestation, treatment and response to treatment

Sex	Diagnosis	Age at diagnosis (years)	Manifestation	Initial BVAS	Initial therapy	TTR weeks	TSR weeks
m	WG	43	K, P, I, M, A, S, E	35	CYC	32	16+
m	WG	63	K, A, E	14	CYC	16	2+
m	WG	68	K, M	13	CYC	31	343+
m	WG	66	K, P, A, S, E	26	CYC	28	438+
m	WG	44	K, P, M, A, S, N	23	CYC	25	32
m	WG	29	K, P, M	10	CYC	34	223+
m	WG	55	K, P, M, A, S, N	13	CYC	58	110+
f	WG	70	P, A, S	9	PRED	19	1+
f	WG	65	K, M, A, N	22	AZA	17	189
f	WG	47	K, P, A, S	14	CYC	24	254+
f	WG	77	K, P, M, N	18	CYC	83	15
f	WG	33	M	6	MTX	10	93
f	WG	22	P, M, A, S	12	CYC	87	38
f	WG	71	K, I, M	28	CYC	13	122+
f	WG	51	K, M, A	21	CYC	42	167
f	WG	27	P, M, S	13	MTX	17	5+
f	WG	60	M, A, S, N	15	MTX	38	18
f	WG	60	M, A, E	9	MTX	19	168
m	CSS	33	P, M, N	17	CYC	26	35
f	CSS	67	P, M, A	6	CYC	25	179
f	CSS	51	P, S, N	10	CYC	29	294+
m	AAV nc	58	K	12	CYC	40	15+
f	AAV nc	44	K, P, A	12	AZA	13	66+

Abbreviations: *WG* Wegener granulomatosis, *CSS* Churg–Strauss syndrome, *AAV nc* ANCA-associated vasculitis not otherwise classified. Manifestation: organ involvement at presentation, *K* kidney, *P* lung, *I* Intestinal, *M* sinus/mucous, *A* arthritis, *S* skin, *E* eye, *N* neurologic, *BVAS* Birmingham Vasculitis Activity Score, *CYC* cyclophosphamide, *MTX* methotrexat, *PRED* prednisone alone, *AZA* azathioprin, *TTR* time-to-remission in weeks, *TSR* time since remission, + indicating ongoing remission

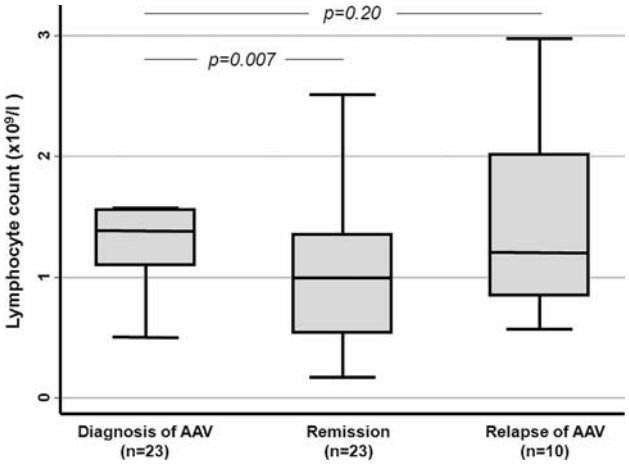

Fig. 1 Lymphocyte counts at diagnosis, in remission and at relapse of AAV. *Boxes* denote median and interquartile ranges; *whiskers* denote ranges

data, the Mann–Whitney *U* test for unpaired data and the Wilcoxon's matched pair signed rank test for paired data were used. All testing was two-tailed, and *P* values <0.05 were considered to indicate statistical significance. For all statistical analysis STATA 9.2 (Stata Corp®, College Station, TX) was used.

Results

Patient's characteristics

Of the 33 AAV patients identified, 23 (70%) were included in the analysis, 10 patients (30%) had to be excluded from the analysis because of missing data, being lost during follow-up, or due to concurrent diagnoses other than AAV necessitating immunosuppressive therapy. Wegener's granulomatosis was diagnosed in 18/23 patients (78%), Churg–Strauss Syndrome in 3/23 patients (13%), two patients had AAV not otherwise classified. A total of 14/23 patients were female (61%), the median age at diagnosis was 55 years (range, 22–77 years). Relevant baseline characteristics of the AAV study population are summarized in Table 1. Median BVAS at diagnosis was 13 (range 6–35). Sixteen out of twenty-three patients had CYC as part of their induction therapy. Median CYC dose-to-remission was 13.5 g (range, 1.5–76.5 g). All 23 patients (100%) achieved remission [median time-to-remission, 26 weeks (range 10–87 weeks)], 10/23 patients (43%) experienced a relapse [median time-to-relapse: 66 weeks (range 15–189)]. The control-cohort of kidney transplant-recipients ($n = 31$), transplanted consecutively between 2004 and 2006) received immunosuppressive regimens containing glucocorticoids, mycophenolate mofetil, and tacrolimus. Ten/31 (33%) transplant-recipients were female; the median age at transplantation was 50 years (range, 19–68 years).

Lymphocyte counts

Lymphocyte counts were analyzed (1) cross-sectional (i.e., on a population level) at diagnosis and in remission, and (2) longitudinally (i.e., intra-individually) during the first 3 weeks after diagnosis. On a population level, median lymphocyte counts at diagnosis [first measurement 1.38×10^9 L (range: 0.5–3.11)] were significantly higher than median lymphocyte counts in remission [first measurement in remission, 0.99×10^9 L (range, 0.17–3.1), $P = 0.007$] (Fig. 1). Relapsing and non-relapsing patients had similar lymphocyte counts both at diagnosis and in remission [median lymphocyte counts at diagnosis, relapsing patients: 1.54 (range 0.77–3.11), non-relapsing patients: 1.34 (range 0.5–2.56), $P = 0.15$, median lymphocyte counts in remission, relapsing patients: 0.94 (range 0.17–3.1), non-relapsing patients: 0.99 (range 0.41–2.82), $P = 0.88$]. Time-to-remission was independent of initial lymphocyte counts ($P = 0.8$, Cox regression).

However, serial lymphocyte counts early after diagnosis [defined here as ≥4 (range 4–17) measurements during the first 3 weeks after diagnosis; available in $n = 17$ AAV-patients] revealed a high intra-individual variability of lymphocyte counts, even if measured in very short (daily) intervals [median intra-individual variability-range; 1.57

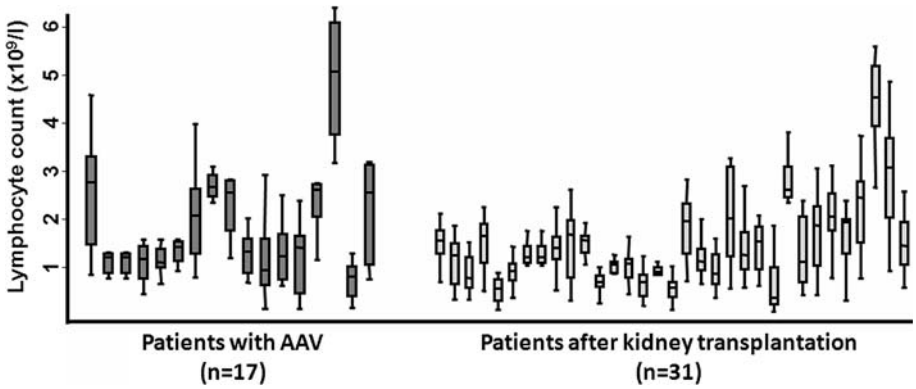

Fig. 2 Variability of serial lymphocyte counts during the first 3 weeks of therapy in patients with AAV (*left panel*) and in patients after kidney transplantation (*right panel*). *Boxes* denote median and interquartile ranges; *whiskers* denote ranges

(range 0.27–3.599)]. The lymphocyte count variability in patients with AAV was compared to the variability in a cohort of kidney transplant-recipients during the first 3 weeks after transplantation ($n = 31$, 4–21 measurements). The median lymphocyte count at transplantation was 0.95×10^9/L (range, 0.12–2.8), the median intra-individual variability-range 1.76 (range 0.74–3.95). Variability of lymphocyte counts in AAV-patients and transplant-recipients was similar, excluding an AAV-specific phenomenon ($P = 0.48$) (Fig. 2).

Discussion

The key observations of this study are that (1) in patients with AAV median lymphocyte counts on a cohort level are higher at presentation than in remission, and (2) in a given AAV-patient intra-individual short-term variability of lymphocytes is making their value as an indicator of treatment-efficiency questionable. The major limitations of our study are its retrospective design, and the small number of patients that were studied. Despite these limitations, the range of the observed short-term lymphocyte count variability—which is essentially unaffected by the study design—may have implications possibly extending beyond the field of clinical AAV-research.

In the context of AAV, our data—on a cohort level—are in-line with the plausible notion stemming from a relatively large study stating that lymphocyte counts inversely associate with sustained remission [7]. However, by combining cohort-derived data and analyses in individual patients, our results indicate that the value of individual lymphocyte counts in reflecting treatment-efficiency is limited.

While for now remaining a provocative hypothesis, short-term (day-by-day!) variability of lymphocyte counts may also influence clinical decision making when judging immuno-competence, e.g., in patients infected with HIV or in transplant-recipients.

In summary, our study establishes a surprising short-term variability of lymphocyte counts both in patients with AAV, and in a heterogeneous group of kidney transplant-recipients. Larger, ideally prospective studies integrating short-term variability of peripheral lymphocyte counts and disease activity are needed to more definitely establish the value of lymphocyte counts as biomarkers for the treatment-efficiency in patients suffering from AAV.

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