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Original Article

ANCA titres, even of IgG subclasses, and soluble CD14 fail to predict relapses in patients with ANCA-associated vasculitis

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Abstract

Background. Antineutrophil cytoplasmic autoantibodies (ANCA) are presumed to reflect disease-activity and to be useful for guidance of immunosuppressive therapy of ANCA-associated systemic vasculitis (AASV), but with respect to conventional ANCA assays this is controversial. ANCA titres, measured in the IgG3 subclass and modern capture ELISAs, have been said to be superior predictors of relapses of AASV.

Methods. In this retrospective study serial measurements of ANCA parameters and soluble CD14 (sCD14) were performed in 169 consecutive sera over a median of 21 months in 18 patients with AASV and related to disease activity, assessed by Birmingham Vasculitis Activity Score (BVAS) for new or deteriorated (BVAS1), and for chronic disease activity (BVAS2). Fourteen patients had Wegener's granulomatosis (WG) and were C-ANCA positive with Pr 3-antibodies and four patients had microscopic polyangiitis (MPA) with P-ANCA and MPO-antibodies. In WG patients ANCA by IIF, Pr 3-ELISA for IgG, IgG1, IgG3, IgG4 and sCD14 were measured, as well as capture ELISA for Pr 3, and in MPA patients ANCA by IIF, MPO-ELISA for IgG and IgG1, IgG3, IgG4, and sCD14 respectively. In eight patients, data collection started at diagnosis, in 10 patients at remission.

Results. The parameters predicted neither the nine major relapses (increase of immunosuppression necessary), nor the 26 minor relapses (increase of BVAS1 >2) with sufficient sensitivity ($>80\%$) or specificity ($>90\%$), and they also failed to predict relapses within the following 2 months. ANCA-IgG3 and capture ELISA for Pr 3 were not advantageous for prediction of relapses (sensitivity 0.45 and 0.19 respectively), and sCD14 remained elevated in all samples irrespective of disease activity.

Conclusions. There is no rationale for serial measurements of ANCA in AASV. For changes of therapy, the ANCA parameters should only be used in conjunction with clinical information.

Keywords: IgG subclasses; prognostic value of ANCA; relapses; soluble CD14; systemic vasculitis

Introduction

Antineutrophil cytoplasmic autoantibodies (ANCA) are specifically associated with small-vessel systemic vasculitis and are excellent diagnostic markers for these diseases [1]. Wegener's granulomatosis (WG), microscopic polyangiitis (MPA), and the renal limited variant of the latter are therefore together categorized as ANCA-associated systemic vasculitides (AASV). In WG patients, ANCA are usually directed against the enzyme proteinase 3 (Pr 3-ANCA), and in MPA patients against myeloperoxidase (MPO-ANCA) or against Pr 3. When detected by indirect immunofluorescence on ethanol-fixed neutrophils, Pr 3-ANCA yield the diffuse cytoplasmic C-ANCA pattern and MPO-ANCA the perinuclear P-ANCA-pattern.

Apart from their diagnostic value, ANCA are presumed to reflect disease activity in AASV and are helpful for monitoring patients and guide immunosuppressive treatment. Already when ANCA were first described in patients with WG, it was observed that they usually became negative at the time of clinical remission [2].

Subsequently, the usefulness of serial ANCA measurements for monitoring of AASV patients was studied more systematically and it was hoped that ANCA titres would reliably predict relapses. The results of these studies have been conflicting and the debate on this issue is continuing [3,4].

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Today this situation has become even more complex because of the availability of sophisticated ANCA measurements with presumed advantages over ANCA measurement by indirect immunofluorescence (IFF) and the longer established ELISA for ANCA antigens, which were used in most published studies.

It has been argued that measurements of complete ANCA-IgG are not precise enough to indicate relapses of AASV, but that the autoantibody is more closely linked to disease activity when it appears in the IgG3 subclass [5,6]. ANCA of the IgG3 subclass may have a pronounced pathogenetic capacity in vasculitis according to *in vitro* data [7]. However, clinical studies proving the usefulness of ANCA-IgG3 monitoring in AASV-patients are still lacking.

In this retrospective study we have therefore studied the association of disease activity in AASV patients with serial ANCA testing of different methodology, including IgG subclass measurements. With soluble CD14 we included another parameter, which was proposed to be useful [8] but hitherto not studied in AASV.

Subjects and methods

Patients

Sera were obtained from 14 patients with WG and Pr 3-ANCA and from four patients with MPA and MPO-ANCA. The diagnosis was based on clinical criteria and histological confirmation in at least one organ. Two patients, one with MPA and one with WG, had already received cadaveric renal transplants.

Ten WG-patients had generalized disease with renal involvement, four had limited disease confined to the upper respiratory tract. Eight patients were followed from diagnosis onward (5 WG, 3 MPA), and in 10 patients (9 WG, 1 MPA) data collection started when they were already in remission. Organ involvement by AASV at time of diagnosis is shown in Table 1.

Active disease was treated in all but one patients with oral cyclophosphamide and oral corticosteroids. For induction of newly diagnosed AASV additional medication was given in seven patients: three plasma exchange, six i.v. boluses of corticosteroids. In one patient antithymocyte antibodies and deoxyspergualin were given because of disease progression and cyclophosphamide-intolerance. For maintenance treatment, low-dose oral corticosteroids combined either with cyclophosphamide ($n=9$), azathioprine ($n=3$), or mycophenolate mofetil ($n=6$) in tapering dosages were used. Patients with persisting ANCA-titres and/or grumbling disease activity (BVAS2 >0) were maintained on prolonged immunosuppression. In all other patients, immunosuppression was withdrawn 15 months after the first remission. In the particular time frame of this study, six patients were off immunosuppressive treatment for some time.

Assessment of disease activity and definition of relapse

Disease activity was assessed within the same time frame as sera collection, by using the standardized Birmingham Vasculitis Activity Score (BVAS) [9]. BVAS is calculated from symptoms and signs attributable to AASV in nine separate organ systems that are weighted according to their relative contribution on mortality and morbidity of AASV. BVAS1 reflects acute activity and is calculated from new or deteriorated symptoms and signs of disease activity within the previous month; BAVS2 for grumbling or persistent disease activity is calculated from items recorded to be present, but neither new nor worse.

Table 1. Diagnosis, ANCA-status and organ involvement at time of diagnosis. In patients 1–8 serial ANCA measurements began at the time of diagnosis, in patients 9–18 during remission

Patient	Diagnosis	ANCA	K	A	B	L	E	EY	S	P	GI
1	MPA	P-ANCA	X	X	X	X				X	
2	MPA	P-ANCA	X	X	X	X					
3	MPA	P-ANCA	X	X	X	X					
4	WG	C-ANCA	X	X	X	X	X	X	X	X	X
5	WG	C-ANCA	X	X	X	X	X				
6	WG	C-ANCA	X	X							
7	WG	C-ANCA	X	X	X	X		X	X		
8	WG	C-ANCA	X	X			X				
9	MPA	P-ANCA	X				X	X	X		
10	WG	C-ANCA	X	X	X	X	X	X	X	X	
11	WG	C-ANCA		X	X	X	X	X			
12	WG	C-ANCA	X	X	X	X	X	X	X		
13	WG	C-ANCA	X	X		X		X		X	
14	WG	C-ANCA		X		X	X				
15	WG	C-ANCA				X	X				
16	WG	C-ANCA	X		X		X			X	
17	WG	C-ANCA		X		X	X				
18	WG	C-ANCA	X	X		X			X		

E, nose, sinusitis, retrobulbar granuloma, ear, hearing impairment; A, arthralgia/myalgia, arthritis; B, temp $>38.5^{\circ}$C, loss of body weight >5 kg, night sweats; L, infiltrate, nodule, pleurisy, haemoptysis, other; EY, red eye, retinal changes, visual impairment; K, nephritic sediment, new rise in creatinine, RPGN/other; S, purpura, ulcerations, gangrene, other; P(NS), PNP sensory, PNP motor; C(NS), path. MRI/CSF, symptomatic; GI, gastrointestinal involvement.

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In active disease, the data were collected every 2nd week, in times of remission the intervals varied from 4 to 6 weeks. For remission, new or deteriorated disease activity had to be absent (BVAS1 = 0), but signs of grumbling disease activity, e.g. persistent proteinuria in patients with renal involvement were allowed to be present (BVAS2 > 0).

For the purpose of this retrospective study a minor relapse was defined as a rise in BVAS1 score by two or more. A major relapse was defined as an increase of disease activity that necessitated an increase of immunosuppression. Minor relapses prompted closer monitoring of the patients without modifications of the immunosuppressive regimen.

Sera and definition of increase in ANCA-parameters

We investigated 169 sera (124 Pr 3-ANCA-positive sera and 45 MPO-ANCA-positive sera) collected during a median follow-up of 21 months (4–30 months). Sera were stored at −20°C and were tested collectively. In all sera the following measurements were performed: ANCA by indirect immunofluorescence (IIF), anti-Pr 3- or anti-MPO ELISA for complete IgG, IgG1, IgG3, IgG4, and ELISA for soluble CD14 (sCD14). In WG patients Pr 3-ANCA was also measured by capture ELISA for Pr 3.

A significant rise in ANCA IIF titres was defined as a rise by at least two titre steps. A significant rise in Pr 3-ANCA and MPO-ANCA was defined as an increase by at least 50%. The same criteria were applied for the IgG-subclasses assays and for sCD14.

Laboratory methods

Indirect immunofluorescence (IIF). ANCA were detected by IIF on ethanol-fixed granulocytes using FITC-conjugated rabbit anti-human IgG (Dako A/S Glostrup, Denmark), diluted 1 : 20 in PBS. The preparations were considered to be positive for C-ANCA if the majority of neutrophils showed a diffuse fluorescence in the cytoplasm at a serum dilution of at least 1:16 and positive for P-ANCA if they showed a perinuclear staining. If the test was positive, serum titrations were performed.

Anti-Pr 3 and anti-MPO ELISA. A commercial ELISA-kit (Wielisa-kit, Wieslab, Lund, Sweden) was used. The wells of the microtitre strips are coated with purified Pr 3 or MPO respectively. During the first incubation, specific antibodies in diluted serum will bind to the antigen coating. The wells are then washed to remove unbound antibodies. A conjugate of alkaline phosphatase-labelled antibodies to human IgG binds to the antibodies in the wells in this second incubation. After a further washing step, detection of specific antibodies is obtained by incubation with substrate solution. The amount of bound antibodies correlates to the colour intensity and is measured in terms of absorbance. The absorbance is then calculated against a calibrator curve and the results are given in arbitrary units. The results were regarded as negative if < 10 U, neutral at 10–20 U, and positive if > 20 U.

C-ANCA positive sera were also studied using a capture ELISA (Wieslab, Lund, Sweden) with proposed superior sensitivity for Pr-3 [10]. The test was performed as described by Baslund *et al.* [11]. Wells of the microtitre strips were coated with anti-Pr 3 monoclonal antibody and purified Pr 3. During the first incubation, specific antibodies in diluted serum bind to the antigen coating. The wells are then washed to remove unbound antibodies. A conjugate of alkaline phosphatase-labelled antibodies to human IgG binds to the antibodies in the wells during the second incubation. After a further washing step, detection of specific antibodies is obtained by incubation with substrate solution. The amount of bound antibodies correlates to the colour intensity and is measured in terms of absorbance (optical density). The absorbance is then calculated against a calibrator curve and the results are given in arbitrary units. Anti-Pr 3 capture ELISA was regarded as negative if < 10 U, neutral at 10–20 U, and positive if > 20 U.

ELISA for ANCA IgG subclasses. Assays were performed using ELISA strips coated with purified Pr 3 and MPO obtained from Pharmacia and Upjohn, Freiburg, Germany. Sera were applied to wells for 1 h at room temperature in four dilutions (for complete IgG, 1 : 50–1 : 400; for IgG1, 1 : 25–1 : 200; for IgG3, 1 : 6.25–1 : 50; for IgG 4, 1 : 12.5–1 : 100). After washing, mouse monoclonal antibodies directed against human IgG (clone MH161M), IgG1 (clone MH161–1M), IgG3 (clone MH163–1M) and IgG4 (clone MH164–1M) (Central laboratory of the Blood-Transfusion Service, Amsterdam, The Netherlands) were applied at a dilution of 1 : 1000 in PBS buffer. IgG2 was not studied since ANCA in this subclass are known to be scarce [12]. After washing, alkaline phosphatase-conjugated goat anti-mouse immunoglobulins (Dako A/S Glostrup, Denmark) were added at a dilution of 1 : 1000 in PBS. After 1 h of incubation and washing p-nitrophenyl-phospate disodium (Sigma Chemicals Co, St Louis, MO, USA) was added as substrate. The plates were read at 405 nm with an automated multiscanner and values were expressed in OD units after subtraction of the blanks.

A dose-response curve generated from the experimental absorbance values in the four dilutions of each sample was processed. A function of the form $y = mx + b$ was fitted to the absorbances, using the method of the least squares sum. The new calculated OD value is m. This calculated OD value was expressed in arbitrary U/ml using a dilution curve of a reference serum (100 U/ml) measured on every ELISA plate.

Soluble CD 14 ELISA. A commercial sandwich ELISA was used to measure soluble CD 14 (IBL, Hamburg, Germany). The normal range is 1.4 to 4.5 μg/ml.

Statistics

Sensitivity and specificity of increases in any laboratory parameter for relapses (BVAS1 increase > 2) were calculated as A/(A + C) (sensitivity) and D/(D + B) (specificity), according to Table 2. To calculate sensitivity and specificity of laboratory-parameters to predict relapses, BVAS1 increases of > 2 within the following 8 weeks after the elevation of the laboratory parameter counted.

For correlation between conventional Pr 3 ELISA and capture ELISA for Pr 3 the chi-square method, and Spearman rank correlation were used for calculation of the correlation coefficient (rho-value). The term significant was used when $P < 0.01$.

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Results

Patients included with active disease

In eight patients included at diagnosis, BVAS1 activity declined as a result of immunosuppressive therapy from 25 (10–34) to 0 within 8 (4–13) weeks. These patients were highly ANCA positive at diagnosis and the fall of ANCA titres paralleled the treatment-response (Table 3a, b). In none of the patients did ANCA by IIF remain positive at remission, not even in patient no. 5 who later relapsed. In this patient, however, Pr 3-ELISA in the IgG and in the IgG3 assay remained positive. Two major relapses occurred in this patient with WG. Neither of the relapses was preceded or accompanied by a significant increase in any of the laboratory parameters.

Moreover, seven increases of BVAS1 of more than 2 (minor relapses) occurred in these patients. On follow-up, a number of significant increases of ANCA parameters occurred that were neither accompanied nor followed by significant changes in disease activity. From these data, sensitivity/specificity of changes in laboratory-parameters to indicate or predict relapses were calculated (Table 4a, b).

Patients included at remission

In patients included at remission (BVAS1 = 0) ($n = 10$), seven major relapses occurred in patient 9 with MPA and in patients 11, 13, 14, 17 with WG. In six patients ANCA persisted (three with relapses; three without relapses) (Table 5).

Major relapses were neither preceded nor accompanied by a rise in any of the laboratory parameters. Furthermore, 19 minor relapses occurred in this group of patients, as well as a number of unrelated increases in laboratory parameters; both of them were included in the calculation of sensitivity and specificity (Table 4a, b).

At the time of inclusion significant chronic disease activity (BVAS2 > 0) was present in 9 of 10 patients, and this was associated with higher ANCA parameters (Table 4). Neither BVAS2, nor persistence of ANCA, however, predicted the relapses that occurred during follow-up.

Distribution of ANCA in the IgG-subclasses

The study allowed only limited longitudinal observations of ANCA changes in the IgG1, 3, and 4 subclasses. In patients who became negative at the time of remission, ANCA disappeared in the IgG4 and IgG3 subclasses almost simultaneously, and thereafter in the IgG1 subclass. In WG patients with persistent ANCA and grumbling disease activity, ANCA appeared

Table 2. Sensitivity and specificity increases

	BVAS 1 increase >2	No BVAS 1 increase
Increase of parameter	A	B
No increase of parameter	C	D

Table 3a. Initial BVAS1, ANCA by IIF, complete IgG, and IgG3 ELISA for Pr3 or MPO in patients included from diagnosis onward

Patient	Diagnosis/ANCA specificity	BVAS1	IIF	Anti Pr3/MPO IgG (U)	Anti Pr3/MPO IgG3 (U)
1	MPA/P-ANCA	20	1 : 256	14.1	2.8
2	MPA/P-ANCA	27	1 : 256	20	5.6
3	MPA/P-ANCA	25	1 : 512	24.9	0
4	WG/C-ANCA	29	1 : 128	32	13
5	WG/C-ANCA	34	1 : 64	11.8	6.9
6	WG/C-ANCA	20	1 : 256	31.4	0.6
7	WG/C-ANCA	10	1 : 64	49.4	16.5
8	WG/C-ANCA	12	1 : 128	22.9	4.3

Table 3b. ANCA by IIF, complete IgG, and IgG3 ELISA for Pr3 or MPO after induction of remission (BVAS1 = 0)

Patient	Diagnosis/ANCA specificity	IIF	Anti Pr3/MPO IgG (U)	Anti Pr3/MPO IgG3 (U)
1	MPA P-ANCA	0	1.3	0
2	MPA P-ANCA	0	1.2	0
3	MPA P-ANCA	0	0	0
4	WG C-ANCA	0	0	0
5	WG C-ANCA	0	9.7	4.2
6	WG C-ANCA	0	4.6	0
7	WG C-ANCA	0	3.2	0.6
8	WG C-ANCA	0	3.4	1.6

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Table 4a. Sensitivity and specificity of increases of laboratory parameters to **predict** relapses (BVAS1 increase > 2) of AASV

	Sensitivity	Specificity
IIF	0.18	0.86
IgG-ELISA	0.25	0.83
Capture-ELISA (only anti Pr3 cases)	0.19	0.78
Subclasses-ELISAs		
IgG1	0.36	0.75
IgG3	0.45	0.78
IgG4	0.32	0.85
SCD14	0.34	0.69

Table 4b. Sensitivity and specificity of increases of laboratory parameters to **indicate** relapses (BVAS increase > 2) of AASV

	Sensitivity	Specificity
IIF	0.37	0.72
IgG-ELISA	0.32	0.64
Capture-ELISA (only anti Pr3 cases)	0.43	0.68
Subclasses-ELISAs		
IgG1	0.60	0.68
IgG3	0.40	0.72
IgG4	0.44	0.81
SCD14	0.36	0.57

predominantly in two subclasses, either as ANCA-IgG1 and IgG3, or as IgG1 and IgG4. In only one patient an approximately even distribution among the three subclasses occurred. High levels of ANCA-IgG3 were indeed found in WG patients with ANCA persistence and multiple organ involvement, as well as significant grumbling disease activity (see Table 5).

Correlation of conventional ELISA with capture ELISA for Pr 3

In patients with WG and Pr 3-ANCA, results obtained with the new capture-ELISA were correlated with the conventional ELISA. The correlation coefficient for the two tests was $r = 0.85$. In two WG patients who were studied from diagnosis onward and who became ANCA negative during the first 3 months of treatment, seroconversion by capture ELISA happened a few weeks later than by conventional ELISA.

Sensitivity and specificity of increases in laboratory parameters for ongoing or subsequent relapses

For none of the measured parameters was satisfactory sensitivity (> 80%) or specificity (> 90%) to indicate ongoing or to predict subsequent relapses found (see Table 4a, b). In particular, there was no superiority of Pr3 capture ELISA or IgG3-ELISA over conventional ELISAs in this respect (Table 4a, b).

sCD-14 levels are elevated irrespective of disease activity

Soluble CD14 was clearly elevated in all samples, usually to about 2–4 times above the normal level, irrespective of diagnosis or disease activity. There was no significant fluctuation of sCD14 levels throughout the study and no association with disease activity.

Discussion

The role of serial ANCA testing for monitoring patients with AASV has been debated since the first description of these autoantibodies which have undoubted diagnostic value.

Maintenance treatment of AASV aims at prevention of relapses by a minimum of immunosuppressive drugs to avoid side-effects of these agents. Any parameter predicting relapses with precision would be highly welcome. It would allow clinicians to intensify their treatment just for the time when this is required.

In this study we have examined retrospectively if relapses of patients with AASV are indicated by a rise in ANCA, and we have included in our analysis not only classical ANCA assays, but ANCA assays in the IgG-subclasses, as well as a new capture-ELISA for Pr 3. In addition, we measured soluble CD14, a marker not hitherto studied in AASV.

The main finding of the study is the failure of any of the measured laboratory parameters to predict or indicate the nine major relapses that occurred. On the other hand, there was a lot of noise by significant changes in the parameters that were neither paralleled, nor followed by relapses. This means that the parameters are not only not sensitive enough, but they are also not specific enough to be used for guidance of immunosuppressive therapy.

These results are in line with the majority of previous studies on this issue and come as no surprise [13,14]. However, what is new and particularly disappointing is the finding that even sophisticated measurements of ANCA failed to indicate disease activity.

The usefulness of ANCA monitoring in WG was first studied systematically by Cohen-Tervaert *et al.* [15], who found that ANCA-titres correlated closely with disease activity and predicted all 17 relapses in a prospective study. In a subsequent study in a small number of WG patients, the same authors were able to prevent relapses of WG by treatment based on ANCA-titres [16].

Most other studies on the value of ANCA monitoring in WG have been much less enthusiastic, starting with the study by Kerr *et al.* [13], who found that in WG patients, changes in serial ANCA titres correlated in only 64% with disease activity and that an increase in ANCA preceded clinical exacerbation in only 24%. The limited value of serial ANCA measurements for predicting relapses has been found for ANCA measured by IIF [13], as well as for the first generations

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Table 5. BVAS2, ANCA by IIF, complete IgG, and IgG3 ELISA for Pr3 or MPO in patients 9–19 in whom follow-up started in remission

Patient	Diagnosis/ANCA specificity	BVAS2	IIF	Anti Pr3/MPO IgG (U)	Anti Pr3/MPO IgG3 (U)
9	MPA/P-ANCA	0	0	4.4	2.6
10	WG/C-ANCA	2	1:64	30.1	1.2
11	WG/C-ANCA	4	1:128	19.5	13.5
12	WG C-ANCA	10	1:128	38.7	9.3
13	WG/C-ANCA	6	1:64	16.2	0
14	WG/C-ANCA	1	0	4.1	1.1
15	WG/C-ANCA	7	1:64	22. 4	16.3
16	WG/C-ANCA	2	0	7.1	1
17	WG-/C-ANCA	2	1:64	13.9	7.5
18	WG/C-ANCA	4	0	5.1	0.4

of antigen-specific ELISAs [19] and this result can be extended from WG to MPA, Churg–Strauss syndrome, and idiopathic rapidly progressive glomerulonephritis, although this has been studied less well. ANCA in follow-up of AASV patients can help to distinguish infective complications from relapses of WG [17] and they are better indicators of flares of the disease than ESR and C-reactive protein [18], and an ANCA rise clearly justifies closer clinical monitoring [19,20]. However, no hard data with the exception of a single study [16] exist, that justify a therapeutic decision based solely on the ANCA titre.

Increased sensitivity and specificity for relapses was expected from monitoring of ANCA in the IgG subclasses. Several studies have shown that ANCA, although predominantly present in the IgG1 and IgG4 subclasses, is increasingly found as IgG3 in times of active disease [5,6,21]. ANCA-IgG3 seems to be pathogenically more important than ANCA in the other IgG subclasses, and selective measurement of ANCA-IgG3 could therefore indicate disease activity more reliably.

In the present study, increases in ANCA-IgG3 did not indicate the observed relapses with sufficient sensitivity or specificity to be useful for monitoring. To our knowledge, this is the first study to have systematically looked for serial ANCA-IgG3 in relation to disease activity. ANCA-IgG3 was indeed relatively increased in WG-patients with ANCA persistence and multiple organ involvement, and there seems to be a correlation with grumbling disease activity in those patients (see Table 5). Finally, a new capture-ELISA for Pr 3 was tested because it was reported to have more sensitivity to indicate relapses [10]. The capture-ELISA was developed to overcome problems with existing solid-phase ELISAs for detection of ANCA. During the purification of Pr 3 and the coating onto the solid phase, the protein could be destroyed and antibody-binding sites hidden from detection. The capture-ELISA avoids this problem by binding of Pr 3 to a monoclonal antibody precoated onto the plate. In our samples there was a good correlation between conventional and capture-ELISA. In some patients with decreasing ANCA-titres during induction treatment, the capture-ELISA became negative later than the conventional ELISA. The capture-ELISA therefore appears to be a very sensitive instrument for measurements of antibodies against Pr 3. The fact that the test was not useful to predict relapses should therefore not point to a problem with the test, but it could mean that the level of ANCA, measured by whichever methodology, is not linked to ongoing or following relapses.

Soluble CD14 has not been studied in AASV so far, but has recently been proposed as a marker of inflammatory systemic disease [8] and *in vitro* data show that ANCA-IgG is able to upregulate CD14 on monocytes [22]. Soluble CD14 was elevated in every single serum of the present study. There was no difference between sCD14 in sera from active as compared to inactive disease and there was no difference between MPA and WG. The ELISA had been checked with nine healthy subjects in the same laboratory and had yielded normal values. Elevated sCD14 appears to be useless in monitoring patients with AASV. Whether it could be another diagnostic parameter remains to be investigated. Since there was no tendency for the elevated sCD14 levels to normalize within the observation time, the parameter could be a long-term memory of disease.

In conclusion, this retrospective study provides no support for serial measurements of ANCA parameters in AASV. These parameters should only be used in conjunction with clinical information to change therapy.

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