Use of Highly Sensitive C-Reactive Protein for Followup of Wegener's Granulomatosis

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ABSTRACT. ***Objective.*** Since Wegener's granulomatosis (WG) represents a relapsing disease, efforts have been made to reliably predict relapses using blood tests. Followup measures such as conventionally determined C-reactive protein (CRP), antineutrophil cytoplasmic antibody (C-ANCA) titer, and proteinase-3 (PR3) ELISA are applied. We evaluated whether during remission elevated highly sensitive CRP (hsCRP) precedes relapse as a marker of subclinical inflammation and thus might improve clinical assessment.

Methods. We investigated 227 sera of 57 patients with WG: 74 sera collected from patients in remission who subsequently relapsed (before relapse), 30 sera collected during relapse, and 123 sera from patients in remission without relapse. We also distinguished between major and minor relapse. hsCRP, conventionally determined CRP (CRP), C-ANCA, PR3-ELISA, and erythrocyte sedimentation rate (ESR) were measured using commercial kits, and levels were correlated to clinical status.

Results. Only hsCRP and ANCA titer, but not CRP levels, were higher in sera from patients who subsequently relapsed versus those who did not, indicating patients at risk. Levels of hsCRP, CRP, and ESR were higher in sera collected during relapse than in the sera before relapse. hsCRP, conventional CRP, and ESR were also higher in samples collected during major relapse than before major relapse. Looking at the levels just before relapse compared to previous levels during remission, none of these measures rose directly before the clinical manifestation of the relapse.

Conclusion. Our study provides evidence for an additional value of hsCRP in the clinical assessment of patients with WG. (First Release August 15 2010; J Rheumatol 2010;37:2319–25; doi:10.3899/jrheum.100302)

Key Indexing Terms:
C-REACTIVE PROTEIN
RELAPSE
WEGENER'S GRANULOMATOSIS
ANTINEUTROPHIL CYTOPLASMIC ANTIBODIES

Wegener's granulomatosis (WG) belongs to the antineutrophil cytoplasmic antibody (ANCA) associated systemic vasculitides and is, according to the Chapel Hill Classification, characterized by necrotizing inflammation of small blood vessels, particularly of the respiratory tract, nerves, and skin, and by pauciimmune crescentic glomerulonephritis. Detection of anti-proteinase-3 (PR3) antibodies by ELISA and indirect immunofluorescence (IIF) techniques is a well established diagnostic test for evaluating patients suspected of having these diseases and is used for clinical followup[1].

Since WG is a relapsing disease, much effort has been made to predict relapse and thereby distinguish patients who need strong immunosuppressive treatment from those in stable remission who should be protected from medication-related morbidity and mortality. But a rise in ANCA titer, either in PR3 ANCA ELISA or in IIF is unreliable in predicting clinical relapse with sufficient sensitivity and specificity[2,3,4]. Therefore, diagnosis of relapse is still a clinical decision based on various clinical and serological measures.

C-reactive protein (CRP) is an acute-phase protein of the innate immune system acting via activation of the classical complement pathway[5]. Elevations of CRP may be due to different stimuli such as infection, inflammation, autoimmune diseases, tumor, and injury. In recent years, assays used to quantify CRP became more and more sensitive. On using highly sensitive CRP assays (hsCRP), it became clear that CRP levels even below the threshold of detection of conventional assays might be associated with an inflammatory response. For example, an association between hsCRP and arteriosclerotic diseases has been described[6,7,8]. Elevated CRP levels have also been described for various

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Accepted for publication June 30, 2010.

autoimmune diseases such as systemic lupus erythematosus (SLE)[9] and rheumatoid arthritis (RA)[10].

The aim of our study was to evaluate whether elevated hsCRP during remission can predict subsequent relapse of WG, as a marker of subclinical inflammation. Therefore, we compared hsCRP with the classical followup measures cytoplasmic (C)-ANCA and PR3 ELISA and with the established markers of inflammation erythrocyte sedimentation rate (ESR) and CRP using a conventional assay. Our retrospective study analyzed 227 sera of 57 patients with WG: patients without relapse, patients before relapse, and patients during relapse. Additionally, we investigated possible differences in the elevation of these markers between major and minor relapse.

MATERIALS AND METHODS

Patients and sera. From 57 consecutive patients with WG who initially presented in complete clinical remission, we investigated 227 consecutive sera collected during at least 2 in- and outpatient visits between January 1996 and January 2003 in the Klinikum Bad Bramstedt, University of Lübeck, Germany. In all patients, diagnosis of WG was established in accord with the Chapel Hill nomenclature. Our study was approved by the local ethics committee, and all patients gave informed consent. Twenty-nine patients were male and 28 were female. The mean age was 55.2 years (range 27–79). Disease activity was evaluated using clinical and radiological measures according to the Birmingham Vasculitis Activity Score (BVAS). Remission was defined as the absence of pathological findings in variables attributable to active vasculitis, irrespective of ANCA titers, as summarized by Merkel, *et al*[11]. Absence of clinical disease activity was indicated by a BVAS of zero. The definition of relapse required recurrence of active disease threatening organ function. Major relapse was defined by involvement of organs of vital importance (heart, lung, central nervous system, kidney) and minor relapse was defined by involvement of other organs or organ systems [e.g., arthritis, otorhinolaryngologic disease (ENT)]. Immunosuppressive treatment of patients at the time of serum collection is shown in Table 1.

Patients with an infectious disease, advanced artherosclerosis, or neoplasm at the time of blood collection were excluded from the study since all these conditions can cause elevated hsCRP.

Patient series. A series of sera was analyzed in all included patients. A series was defined as all consecutive sera from one patient collected between onset of remission until occurrence of relapse or until end of study for patients without relapse during the study period. The series comprised a minimum of 2 and a maximum of 7 sera. For 9 patients, 2 series were included in the study.

For statistical analysis, series were divided into 2 groups: 30 series consisted of sera in remission followed by relapse (before relapse, n = 74) and sera collected during the subsequent relapse (during relapse, n = 30) and 36 series consisted of sera in remission without subsequent relapse (without relapse, n = 123). The sera during relapse were divided into those during major relapse (n = 24) and corresponding sera before major relapse (n = 54); and those during minor relapse (n = 6) and before a minor relapse (n = 20). Sera were collected during outpatient visits, which took place every 3 to 9 months, depending on the clinical condition of each patient. In exceptional cases of long-lasting stable remission the interval between 2 blood samples was 12 months. All sera were stored at –20°C.

Blood tests. HsCRP was measured with the N High Sensitivity CRP kit (sensitivity < 0.175 mg/l; Dade Behring, Marburg, Germany) using particle-amplified immunonephelometry. Conventional CRP was determined by immunoturbidimetry (Roche Diagnostics, Mannheim, Germany). PR3 ANCA were detected by the direct Wielisa Kit Anti-PR3 ANCA (Wieslab, Lund, Sweden).

Statistical analysis. Data are expressed as mean ± standard deviation. Normal distribution was assessed by Kolmogorov-Smirnov test. Differences in continuous variables were compared by Student's t-test for normal distribution, or otherwise by Mann-Whitney test. A 2-sided $p < 0.05$ was considered to indicate statistical significance.

RESULTS

Clinical followup. For patients with relapse, the median time from remission to relapse was 17.3 months (range 2–72). Patients without relapse were in remission at the end of the study for a median of 28.6 months (range 5–130). Relapse included the following organ systems: ENT in 12 series, lung in 5 series, kidney in 10 series, rheumatic symptoms in 15 series, skin in 3 series, eyes in 4 series, and constitutional symptoms in 7 series.

HsCRP. HsCRP was significantly higher in sera of patients during relapse than in those before or without relapse, and in sera before relapse than in those without relapse (mean 19.84 mg/l during relapse, 7.01 mg/l before relapse, and 3.34 mg/l without relapse; Figure 1). Comparing minor and major relapses, hsCRP was significantly higher during major relapse than before major relapse (before 6.47 mg/l,

Table 1. Treatment at the time of blood collection.

Therapy	Without Relapse, n = 123	Before Relapse, n = 75	During Relapse, n = 30	p
Glucocorticosteroids, % (n)	49.6 (61)	40 (30)	20 (6)	0.036* 0.001**
Glucocorticosteroids, mg, mean ± SD	2.38 ± 2.90	1.72 ± 2.37	1.03 ± 2.41	0.011**
Cyclophosphamide, % (n)	8.94 (11)	13.33 (10)	3.33 (1)	NS
Mycophenolate mofetil, % (n)	0 (0)	0 (0)	0 (0)	NS
Azathioprine, % (n)	14.63 (18)	0 (0)	3.33 (1)	< 0.001† 0.016**
Methotrexate, % (n)	34.15 (42)	48 (36)	43.33 (13)	NS
Trimethoprim/sulfamethoxazol, % (n)	9.76 (12)	12 (9)	20 (6)	NS
Leflunomide, % (n)	10.57 (13)	13.33 (10)	13.33 (4)	NS

* Before vs during; ** without vs during; † without vs before. NS: nonsignificant.

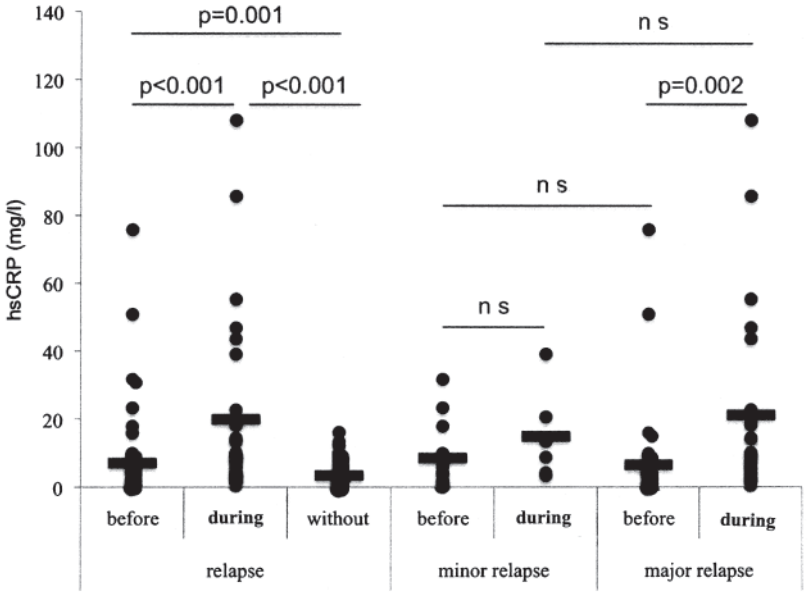

Figure 1. hsCRP was measured in sera of patients before relapse, during relapse, and without relapse. Additionally, hsCRP was compared between sera before and during major relapse and before and during minor relapse. Comparisons were made by Student's t-test. ns: nonsignificant.

during 21.09 mg/l). For minor relapse there was no significant difference between hsCRP before (8.47 mg/l) and during relapse (14.8 mg/l), but we could test only a small number of sera during minor relapse (n = 6). Levels of hsCRP were not significantly different between sera collected before minor and before major relapse and between sera collected during major and during minor relapse (all Figure 1).

Conventional CRP and ESR. In results similar to hsCRP, we measured a significantly higher CRP in patients during relapse versus sera from patients before relapse (CRP before relapse 0.76 mg/l vs 2.42 mg/l during relapse; Figure 2a). But in contrast to hsCRP, we found no difference in levels of conventional CRP in sera without relapse versus during relapse or between sera of patients before relapse versus without relapse. Also, no difference was detected in CRP levels between sera of major and minor relapse, neither before nor during relapse. Only CRP during major relapse was significantly higher than CRP before major relapse (before relapse 0.68 mg/l; during relapse 2.94 mg/l; $p = 0.001$; Figure 2a).

ESR was significantly elevated during relapse as compared with ESR before relapse and in patients without relapse. In contrast, no difference in ESR was detected between samples before relapse and samples without relapse (Figure 2b). Similar to conventional CRP, ESR was higher during versus before major relapse (before relapse 21.9 mm/h, during relapse 41.0 mm/h; $p < 0.001$). For the other conditions tested, no differences were observed (Figure 2b).

ANCA immunofluorescence and PR3 ELISA. ANCA titers measured by IIF were significantly higher in patients experiencing relapse (both before and during relapse) than in those patients without relapse ($p < 0.001$ for both comparisons). But there was no difference between ANCA titer before and during relapse, neither for the whole group, nor for patients with major or those with minor relapse (Figure 3a).

Using PR3 ELISA, there was a trend for higher values during relapse than in patients without relapse ($p = 0.056$). For all other groups no differences were detected (Figure 3b).

Predictive value of measures. To test whether the examined measures are useful to predict relapse, we compared the value of each measure in sera collected during remission with values from sera collected just before relapse and during relapse. In all measures tested, there was no significant difference between sera in remission and those immediately before relapse. HsCRP, CRP, and ESR were significantly higher in sera collected during relapse versus sera collected during remission and immediately before relapse, whereas for ANCA and PR3 ELISA, there were no differences detected (Figure 4).

DISCUSSION

We describe the association between serological markers and clinical disease activity in patients with WG. HsCRP, ESR, and ANCA were higher in sera during relapse than in those without relapse. HsCRP, conventional CRP, and ESR were also higher during relapse than in sera before relapse, but only hsCRP and ANCA titer were higher in sera before relapse versus those without relapse, indicating which patients are at risk for relapse. HsCRP, conventional CRP, and ESR were also higher during major relapse than before

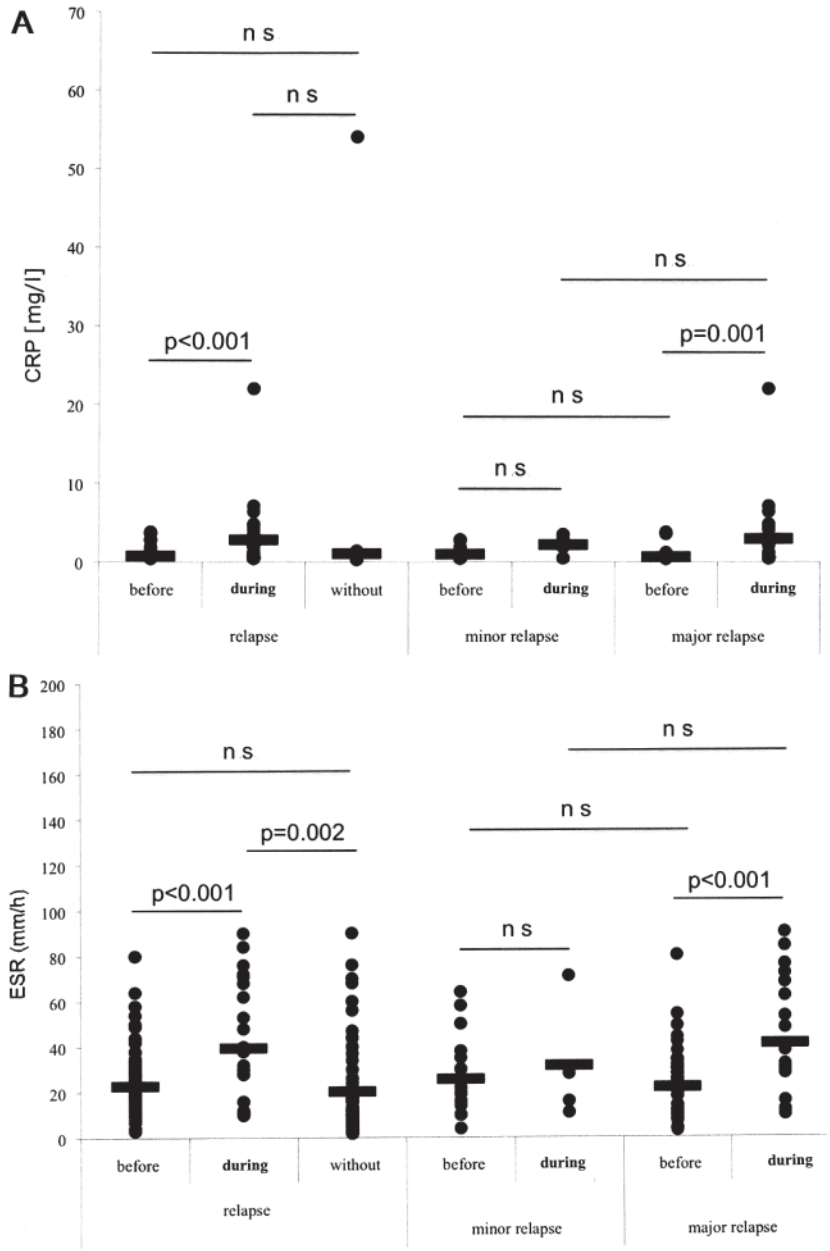

Figure 2. CRP (A) and ESR (B) were measured in the same sera groups as in Figure 1. CRP levels were missing in 6 sera during relapse, in one serum before relapse, and in 18 sera without relapse. ESR results were missing in 6 sera during relapse, in 4 sera before relapse, and in 21 sera without relapse. ns: nonsignificant.

major relapse. Looking at the course of the rise, we compared sera of patients who later relapsed during the period of remission, both immediately before relapse and during that relapse. Among the measures studied, we found no significant increase in the values during remission versus those immediately before relapse. Therefore, in our study, none of the tested measures increased immediately before relapse. However, hsCRP, CRP, and ESR were significantly higher in sera collected during relapse versus those collected immediately before relapse and during remission; this was not the case for the classical followup measures ANCA titer and PR3 ELISA.

Our results are in line with observations of Gronhagen-Riska *et al*, who studied alpha-1-antitrypsin, CRP, and interleukin 6 serum levels and compared the association with clinical activity and with ANCA titer[12]. They found that both alpha-1-antitrypsin and CRP fluctuations indicated changes in activity that could not always be detected by ANCA[12]. Similarly, Hind, *et al* found the correlation between CRP levels and disease activity to be closer

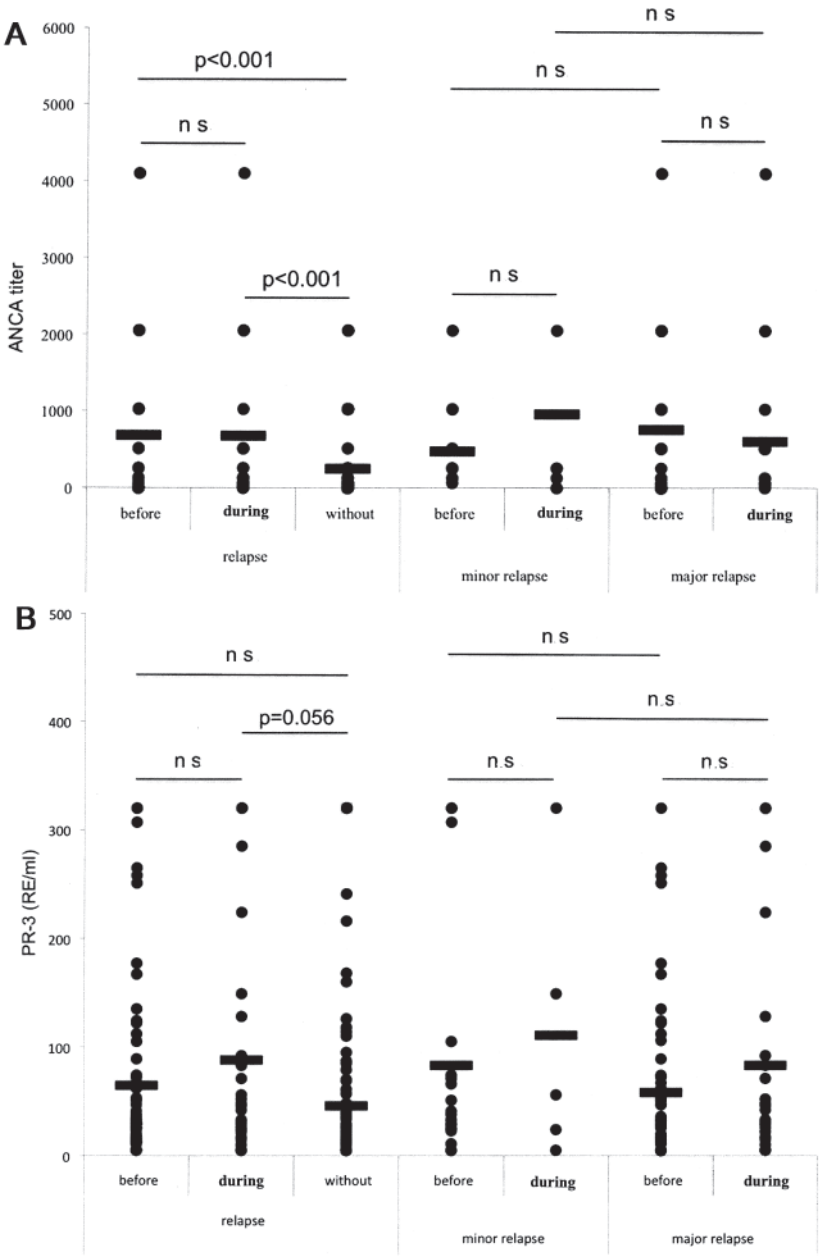

Figure 3. ANCA IIF titer (A) and PR3 ANCA ELISA (B) were measured in the same sera groups as in Figure 1. PR3 ANCA ELISA results were missing in 2 sera without relapse and in 1 serum before relapse.

than that between ESR and disease activity[13]. In contrast, Jayne, *et al* assessed the value of sequential monitoring of ANCA, CRP, and ESR levels and identified ANCA monitoring to be superior to measurement of CRP or ESR in the prediction and diagnosis of relapse[14].

As hsCRP was more elevated in patients during remission who experienced a relapse than in those with stable remission, an elevated hsCRP might indicate a higher risk for relapse. A similar observation was made by Cantini, *et al* for polymyalgia rheumatica, where an increased CRP and ESR were predictors of subsequent relapse[15]. Recently, higher levels of CRP were shown to be associated with a more severe course of Crohn's disease[16]. CRP is a nonspecific marker that is elevated not only during inflammatory conditions like infections but also in a proinflammatory environment such as in obese people[17] and smokers[18]. But although the interindividual differences in CRP levels seem to be broad, the intraindividual spread of CRP, which is important for individual clinical followup, has been shown to be rather narrow[19,20].

To date, the best-studied blood tests for clinical followup of WG patients are ANCA titer and PR3 ELISA, but with

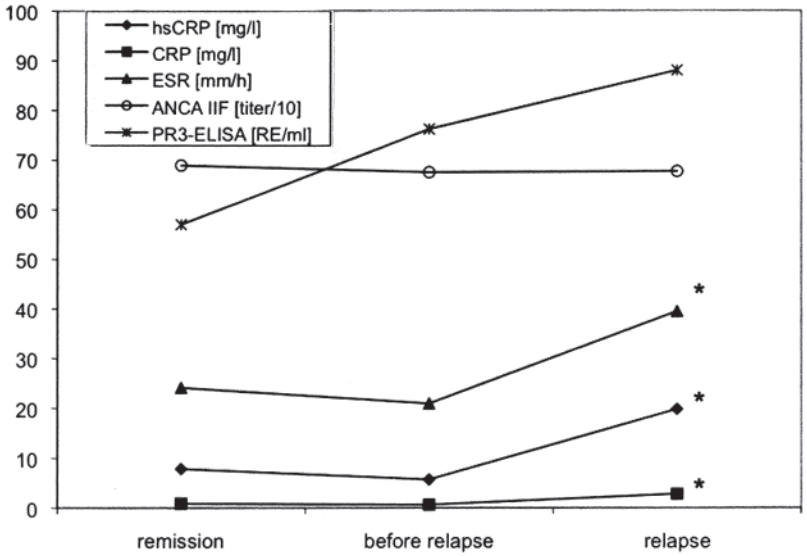

Figure 4. hsCRP, CRP, ESR, ANCA IIF titer, and PR3 ANCA ELISA were measured in sera during remission, immediately before relapse, and during relapse (relapse). Thus, we investigated whether an increase in any measure immediately preceded relapse. No significant difference was detected for any of the measures between remission and before relapse. *Significant difference in sera during relapse versus sera before relapse.

conflicting results[2]. Finkielman, *et al* concluded in their study that ANCA levels cannot be used to manage immunosuppressive treatment[3]. Also, Kerr, *et al* found that an increase in C-ANCA titer preceded the clinical exacerbation of disease in only 24% of patients with WG[21]. In contrast, Tervaert, *et al*[22] and Boomsma, *et al*[23] recommend a serial measurement of ANCA levels for early prediction of relapse. In the latter study, 92% of patients showed a rise in ANCA levels before relapse, but in 43% of patients a rise in titer was not followed by relapse[23]. The advantage of hsCRP compared to ANCA levels and PR3 ELISA in our study is that hsCRP was increased in patients before a relapse compared to those in stable remission — similar to ANCA titer; but in contrast to ANCA titer, hsCRP was also significantly higher during relapse than before relapse.

Are elevated hsCRP and CRP levels just an epiphenomenon of inflammation for use as diagnostic tools to differentiate between patients in remission and those at risk for a relapse, or does the increase in inflammatory molecules also have a pathophysiological relevance? CRP and hsCRP levels are associated with arteriosclerotic diseases, and increased risk for atherosclerosis has also been described for systemic vasculitides independent of known risk factors and treatment[24]. A direct effect of CRP has been demonstrated *ex vivo*, where CRP can affect monocyte activation, resulting in an altered recruitment to endothelial cells[25]. Although CRP activates the complement cascade, antiinflammatory effects of CRP were demonstrated in animal models of SLE (as summarized[26]). Therefore, a clinically significant direct proinflammatory role of CRP remains questionable.

In patients with WG, levels of hsCRP were more closely associated with disease activity than ANCA titer, PR3 ELISA, and conventional CRP and ESR, which are routinely used as markers of inflammation. Although a relapse was not directly preceded by a rise in any of the measures tested, our study provides the first evidence that hsCRP is also useful in the clinical assessment of patients with WG.

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