There is no benefit in routinely monitoring ANCA titres in patients with granulomatosis with polyangiitis

B. Verstockt¹, X. Bossuyt², S. Vanderschueren³, D. Blockmans³

ABSTRACT

Objective. To analyse the link between antineutrophil cytoplasmic antibody (ANCA) levels and risk of relapse in patients with granulomatosis with polyangiitis (GPA), as the clinical benefit of monitoring ANCA levels is uncertain.

Methods. A retrospective analysis was made of all charts available from 43 patients diagnosed with GPA, fulfilling The American College of Rheumatology (ACR) criteria, and followed between 1994 and 2012 at a general internal medicine department of a university hospital. Clinical and biochemical data (i.e. anti-proteinase 3 (PR3) levels) were collected and correlated.

Results. 43 relapses occurred in 25 patients (58.1% of 43 patients). When blood samples are routinely taken at a follow-up visit (i.e. low pre-test probability, 5.5%) in the GPA-population, a 75%-increase in the PR3-level or its reappearance has only limited positive predictive value (PPV 15.0% and 22.5% respectively) for predicting relapse. Adversely, when clinical suspicion of relapse is high (i.e. high pre-test probability, for example 50%), an increase of 75% or reappearance of PR3 makes relapse even more likely (PPV 77.5%, 81.6% respectively). Conversely, a high negative predictive value (NPV) of 99.3% and a negative likelihood ratio (LR-) of 0.12 suggest that, in the absence of PR3, relapse is unlikely if patients had detectable ANCAs at diagnosis.

Conclusion. Routine ANCA monitoring in patients diagnosed with GPA has limited value. However, targeted determination of ANCA levels may be useful if a relapse is clinically suspected (i.e. high pre-test probability).

Introduction

Antineutrophil cytoplasmic antibodies (ANCAs) are autoantibodies directed against several antigens of the azurophilic granules of neutrophils and monocytes. ANCAs are predominantly seen in patients with GPA, whereas MPO ANCAs are predominantly seen in patients with MPO, but is also seen with antibodies against PR3, whereas the p-ANCA pattern is associated with antibodies against MPO. ANCAs are autoantibodies directed against several antigens of the azurophilic granules of neutrophils and monocytes. ANCAs are predominantly seen in patients with GPA, whereas MPO ANCAs are predominantly seen in patients with MPO. PR3 ANCAs are predominantly seen in patients with GPA, whereas MPO ANCAs predominate in patients with GPA. PR3 ANCAs are seen in 70–80% and MPO ANCAs in 10% of GPA patients with systemic disease. In limited GPA, confined to the upper airways, ANCAs are detected in only 60% of the cases (13, 14). p-ANCA staining without specific recognition of PR3 and MPO is associated with diseases such as ulcerative colitis, sclerosing cholangitis, autoimmune hepatitis, and rheumatoid arthritis (15, 16).

Competing of interests: none declared.

¹University Hospitals Leuven, Leuven, Belgium;
²Department of Microbiology and Immunology, University Hospitals Leuven, Leuven, Belgium;
³Clinical Department of General Internal Medicine Department, Research Department of Microbiology and Immunology, Laboratory of Clinical Infectious and Inflammatory Disorders, University Hospitals Leuven, Leuven, Belgium.

Bram Verstockt, MD
Xavier Bossuyt, MD, PhD
Steven Vanderschueren, MD, PhD
Daniël Blockmans, MD, PhD

Please address correspondence to: Bram Verstockt, University Hospitals Leuven, Herestraat 49, B-3000 Leuven, Belgium.
E-mail: bram.verstockt@student.kuleuven.be

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Although the diagnostic value of these antibodies is well established in AAV, the usefulness of serial ANCA determinations in predicting recurrent disease in patients with AAV has remained controversial for the last twenty years (11, 12, 17-19). Birck et al. performed a systematic review and concluded that, due to a methodological heterogeneity in the current literature, no firm conclusion can be drawn concerning the clinical value of serial ANCA determinations for monitoring AAV (17).

Since uncertainty remains about this subject, this retrospective study examined the relationship between evolutions in PR3-levels and the prediction of relapse.

Materials and methods

Patients

Sixty-eight patients diagnosed with GPA, corresponding to The American College of Rheumatology (ARC) 1990 criteria (20) and under regular follow-up at the University Hospitals Leuven (Leuven, Belgium), were enrolled in this retrospective study. Charts from all these patients, observed between January 1994 and December 2012, were reviewed. Twenty-five patients were excluded: 4 had a follow-up period of less than one year, 1 had insufficient data, in 7 patients the diagnosis was in doubt because of repeatedly negative ANCs and negative biopsies, 3 were MPO-positive, 9 never had detectable ANCs although their diagnosis was histologically confirmed and 1 patient never got in complete remission for several years.

Most patients with systemic disease were initially treated with a combination of corticosteroids and cyclophosphamide orally or intravenously in combination with trimethoprim-sulfamethoxazole. After induction of clinical remission, therapy was tapered. In almost all cases, azathioprine was started as a maintenance therapy together with a low dose of corticosteroids. In some cases, methotrexate or mycophenolate mofetil was chosen because of different reasons related to disease extent, comorbidities, adverse effects, drug intolerance, insufficient efficacy of other products or in the context of a study protocol.

ANCA detection methods

Throughout the study period, PR3 was determined by ELISA (Enzyme-Linked ImmunoSorbent Assay) (QUANTA Lite PR3 kit, INOVA Diagnostics, Inc. (San Diego) till April 2009. Afterwards, they were determined by FEIA (FluoroEnzyme ImmunoAssay) (EliA™ 1 (Phadia, Uppsala, Sweden). Assays were performed according to the instructions of the manufacturer. Cut-off values proposed by the manufacturer were used (20 U/ml for ELISA and 7 U/mL for FEIA). As the detection of increases in PR3/ANCA by FEIA gives results comparable to ELISA (21), no subgroup-analysis of the individual data acquired using respectively ELISA and FEIA, was made.

Definition of PR3

For the detection of increases in PR3-level, the level in each sample was compared to that in the previously obtained sample, using the same assay. Time window between two visits (or PR3-measurements) varies between patients or within one patient during the long follow-up period (range: 3–9 months). Only reappearances and increases in PR3/ANCA level, which preceded a clinical relapse for a maximum of 6 months, were considered as being predictive.

According to the literature an increase of 75%, as measured by ELISA, was defined as a significant rise in the PR3-level (3, 22). Furthermore, reappearances of PR3 (i.e. detectable PR3 above cut-off values proposed by the manufacturer) were evaluated too.

Relapse/remission

In accordance with the European League Against Rheumatism (EULAR) recommendations, relapse was defined as the re-occurrence or new onset of disease activity attributable to active inflammation. Remission was defined as the absence of disease activity, with or without taking immunosuppressive therapy (23). Diagnosis of relapse was made based on clinical (e.g., bloody nasal crusts, weight loss, fever, cutaneous lesions, haemoptysis, ...) and biochemical findings (proteinuria, renal insufficiency, ...), included in the Birmingham Vasculitis Activity Score Modified for GPA (24).

In our tertiary centre, the experience of treating patients with GPA is well established. Experienced physicians could, based on all these clinical and biochemical findings, estimate if the risk of relapse is high or rather low. In cases of high suspicion of relapse, a pre-test probability of at least 50% is reasonable. Using the found positive and negative likelihood ratios (LR+ and LR-), predictive values could mathematically be deduced, as was done in this retrospective analysis.

Statistical analysis

Sensitivity, specificity, LR+, LR- and predictive values were calculated (25).

All statistical analyses were performed with SPSS 20.0 and Microsoft Excell.

Results

Patient characteristics

Relevant features of the 43 included patients (20 women and 23 men, mean age 57.6 years) are given in Table I. During the follow-up period from January 1994 till December 2012, five patients died (11.6%). The data cover a total follow-up period of 3265 patient months (on average 75.9 months per patient, range 12–226 months).

Initially, 6 patients were found to have a limited GPA disease. Three of them evolved to a systemic disease during their first relapse. In the other three patients, GPA remained in a limited form (mean follow-up of 18.6 months).

Relapse

Forty-three clinical relapses occurred in 25 patients (mean number of relapse 1.72 each, standard deviation (SD) 1.12). The number of relapses in single patients varied between 0 and 6. Mean time to first relapse was 63.8 months (±67.2 SD) during the study period.

Of the 43 patients studied – all with at least once detectable PR3 – 25 relapsed (58.1%). Twenty-two of them (88.0%) always had detectable PR3-levels at relapse.

Prediction of relapse

Fifty-one per cent of the PR3 positive patients ever demonstrated a 75%
increase with ELISA/FEIA during the follow-up period. Though, many times relapse was not associated with a clear 75% increase in PR3. Only 6 times relapse was preceded by at least a 75%-increase in PR3, resulting in a low sensitivity of 25.0% (Table II). Nevertheless specificity is high (92.7%). Reappearance of PR3 was found to have a sensitivity of 88.9% and specificity of 79.9% (Table II). Presence of PR3 seemed to be even more sensitive (95.4%), although with loss of specificity (38.5%).

As shown in Table II, a 75%-rise in PR3 has a positive likelihood ratio (LR+) of 3.43, resulting in positive predictive value (PPV) of 15.0% (Table III).

In situations where relapse is clinically more likely (i.e. high pre-test probability), the PPV of a 75%-increase will reach 77.5% (Table III). Similarly, due to a LR+ of 4.43, the PPV of reappearance of PR3 will rise up to 81.6% in conditions where relapse is suspected (Table II, III). A negative likelihood ratio (LR-) of 0.12 explains the negative predictive value (NPV) for predicting relapse of 96.0%.

III. In our cohort with more than 800 samples, the overall prevalence of relapse approximates 5.5%, resulting in a general low pre-test probability for relapse. However, when relapse is clinically suspected, the pre-test probability of the individual patient is much higher, rising to 50% or even higher. In this instance, a positive test-result (i.e. a 75%-increase in PR3-level) will have a highly increased predictive value, approaching 77.5% instead of 15.0% (Table III). As Thai et al. recently published (30), ANCA reappearance seems to correspond more closely with relapse than PR3 persistence, as was reflected by a higher PPV (Table III). We agree with previous studies that patients, in whom PR3 reappear and relapse is clinically presumed, should be clinically followed with increased scrutiny (30-32).

In contrast, when this reappearance is noticed in a routinely taken sample at a regular follow-up visit, the risk of relapse is rather low (i.e. 22.5%, Table III). Therefore, reappearance should warn the physician but it cannot be the only argument to change therapy in absence of any clinical change (29, 33, 34). In contrast, when clinical findings suggest relapse, reappearance of PR3 can be an additional argument to confirm the clinical suspicion (PPV 81.6%, Table III).

As a consequence, we advise against routine determination of ANCAs in clinically stable GPA-patients. Analysis will not influence therapeutic approach, as in these circumstances low PPV can not predict relapse. On the other hand, if patients present with complaints compatible with a relapse, ANCA evaluation should be performed. Indeed, the absence of ANCAs at that moment strongly argues against a relapse (NPV 89.2%, Table III).

As this is a retrospective study, time windows between samples vary greatly

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<th>Table I. Baseline characteristics of all included patients.</th>
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<td>Baseline characteristics of the 46 patients with GPA</td>
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<tr>
<td>Epidemiological features</td>
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<td>Sex, no of female : male</td>
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<td>Age, mean (SD) years</td>
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<td>Number of relapse in one patient</td>
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<td>0</td>
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<td>≥ 1</td>
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<td>Death</td>
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<td>GPA: granulomatosis with polyangitis.</td>
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<th>Table II. Prediction of relapse using ELISA/FEIA.</th>
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<td>Increase of at least 75%</td>
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<td>Sensitivity</td>
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<td>PR3 reappearance</td>
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<td>LR+</td>
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<td>PR3 Presence</td>
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| ELISA: Enzyme-Linked ImmunoSorbent Assay; FEIA: FluoroEnzyme ImmunoAssay; LR+: positive likelihood ratio; LR-: negative likelihood ratio. |

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<th>Table III. Influence of pre-test probability on predictive values.</th>
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<td>Pre-test probability around 0.05 = Low suspicion of relapse</td>
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<td>Pre-test probability 0.5 = High suspicion of relapse</td>
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<td>75% increase PR3 PR3 reappearance PR3 presence</td>
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<td>75% increase PR3 PR3 reappearance PR3 presence</td>
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<td>PPV</td>
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Key message
Routine ANCA monitoring in patients diagnosed with GPA has limited value. Targeted determination of ANCA levels may be useful if a relapse is clinically suspected.

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istics of a laboratory test. A practical approach in the autoimmune laboratory. Auto-

29. Kyndt D, Reumaux D, Brijdoux F et al.: with no regular (e.g. three-monthly) measurement of the ANCA levels. This might explain why only few relapses were preceded by a clear increase of the PR3 level. Since the ANCA level was part of the clinical decision to decide whether or not a patient had relapsed, a bias could be present, possibly inducing a circular reasoning. However, we conclude that given the moderate LR+ found based on all data, this bias should be limited. If not, a much higher LR+ would be expected. The different intervals of clinical and laboratory follow-up are another weak point of this study, but they reflect daily practice. Due to these different intervals and the retrospective character of this study, a solid methodological study to correlate the kinetics of increase in ANCA titre with clinical relapse was not possible. A prospective observational study is warranted to confirm these data. As immunosuppressive therapy has a lot of adverse events, tapering and discontinuation of therapy in vasculitis is an import issue (35). Therefore we are interested in the results of a randomised comparative study, hold by the French Vasculitis Study Group (Clintrial NCT01731561), concerning the benefit of determining ANCA levels to adjust therapy. In this ongoing trial, they compare the efficacy of systematic rituximab infusions (conventional therapy) to rituximab infusion based on a rate of ANCA and CD19-lymphocytes in patients with systemic ANCA-associated vasculities in remission. Furthermore new promising developments in methodology and techniques for ANCA testing have been proposed but studies comparing the performances of the different assays are scarce (36). In conclusion, we disprove the benefit of routine ANCA monitoring in clinically stable patients with GPA. Determination of ANCA levels is useful if patients present in a clinical context compatible with a relapse or at the moment of diagnosis. Absence of PR3 virtually rules out a GPA relapse. Rising PR3-levels or their reappearance alone do not justify intensification of therapy but should be interpreted together with clinical and laboratory parameters.


