Antineutrophil cytoplasmic antibodies (ANCA) in rheumatoid arthritis: a prospective study

Abstract  To investigate a possible relationship between the presence of antineutrophil cytoplasmic antibodies (ANCA), rheumatoid factor (RF), antinuclear antibodies (ANA), complement, disease activity and disease severity, 111 clinically well-documented RA patients were studied prospectively for ANCA, RF, ANA, C-reactive protein (CRP), total haemolytic complement (CH50) and complement split product C3d. Disease activity and severity were also assessed clinically, as well as anamnestically, using the Hannover Activity of Daily Living Questionnaire, the functional Steinbrocker grades, and numeric and verbal rating scales. At a serum dilution of 1:50, 20% of the 111 sera showed predominantly an atypical perinuclear staining pattern. There was no correlation between ANCA positivity and serological markers, disease activity and disease severity. Regarding previous therapies with disease-modifying antirheumatic drugs, ANCA+ patients took sulphasalazine significantly more often than ANCA− patients.

Key words  Antineutrophil cytoplasmic antibodies (ANCA) · Rheumatoid arthritis

Introduction

Antineutrophil cytoplasmic antibodies (ANCAs) are predominantly directed against lysosomal enzymes of human neutrophils and monocytes. On ethanol-fixed granulocytes two major immunofluorescent staining patterns can be distinguished. The diffuse, granular cytoplasmic staining (cANCA) with specificity in ELISAs for the neutral proteinase 3 (PR3) [1] is highly specific for Wegener’s granulomatosis (WG) [2–4]. The perinuclear staining pattern (pANCA) with specificity for myeloperoxidase (MPO) is more common in microscopic polyarteritis (mPAN) and rapidly progressive segmental necrotizing and crescentic glomerulonephritis (RPGN) [5–7].

More recently, ANCAs have also been described in a variety of diseases other than primary systemic vasculitis [8–14]. In rheumatoid arthritis (RA), a fine granular, atypical fluorescent pattern with perinuclear accentuation has been reported in 17–40% of patients [15–22] and correlates probably to the previously described granulocyte-specific antinuclear antibodies (ANAs) [23, 24]. The target antigen(s) of ANCA in RA is not clearly defined. In ELISAs the patients’ sera often react with multiple lysosomal enzymes such as cathepsin G, lactoferrin, lysozyme, elastase, β-glucuronidase and others [18, 21, 25–30].

Retrospective studies have demonstrated that the ANCA titre does not correlate with the activity of RA, but that the presence of this antibody is associated with a more progressive course of the disease [15, 31]. The present study was intended to confirm this result prospectively. Additional measurements for the activity and severity of the disease were included, and the serological results of ANCA+ patients of earlier investigations were verified. For the first time ANCA positivity in RA patients was correlated to previous treatment with disease-modifying drugs (DMARDs).
Patients and methods

Patients

Sera from 111 consecutive RA patients (four or more ACR criteria [32]) admitted for diagnosis and treatment to the division of rheumatology and clinical rheumatology at the University of Freiburg Medical Centre were divided into an ANCA+ (related to neutrophil granulocytes fixed in ethanol) and an ANCA- group. In addition to rheumatoid factor (RF), ANA, C-reactive protein (CRP), total haemolytic complement (CH50) and complement split product C3d, the medication administered within the previous 12 months and earlier was documented. The current activity of the disease, the severity of the RA as compared to other patients and the functional Steinbrocker grades were assessed by a physician. In a self-administered questionnaire, the patients assessed their intensity of pain, their disabilities within the 7 days and their global health status. The activity of daily living was assessed by an additional questionnaire.

Disease activity

The current activity of the disease was assessed by the examining physician on a modified rating scale ranging from 0, “inactive” to 10 “highly active” [33, 34]. “How severe is the disease as compared to other patients with the same diagnosis?” This question was answered by the patient on a rating scale: (0) asymptomatic, (1) mild, (2) moderate, (3) severe and (4) very severe.

Clinical grading

Clinical grading was done according to the criteria described by Steinbrocker [35]. The coding was as follows: (1) I, (2) I–II, (3) II, (4) II–III, (5) III, (6) III–IV and (7) IV. “How would you rate the intensity of your pain within the last 7 days? How severely were you disabled in performing your activities of daily living?”

These questions were answered by the patient on a modified rating scale ranging from 0 to 10, where 0 indicates “no pain” or “not disabled” and 10, “unbearable pain” or “completely disabled”. “How would you describe your current global health status?” This question was answered by the patient on a rating scale: (0) very well, (1) well, (2) satisfactory, (3) fair and (4) poor.

Hannover Activity of Daily Living Questionnaire

The Hannover Activity of Daily Living Questionnaire (FFbH) measures the limitations in functional capacity caused by rheumatic diseases. The FFbH includes one multi-item scale that assesses four health concepts: mobility; personal hygiene; dressing and undressing; grasp and moving objects [36, 37]. In our study the combined version of FFbH-P and -R with 18 questions was used.

The FFbH-P is designed especially for polyarticular joint diseases and contains questions concerning complex movements and limitations of the hands. The FFbH-R contains questions concerning complex movements that can be limited in patients with back pain. The FFbH is a standardized, self-administered questionnaire. The items in the form of questions represent daily activities in the dimensions mentioned. The time window is 7 days (“referring to the average level of difficulty during the past 7 days”). The functional capacity is given as a percentage. To each question three possible answers are given: “can do without difficulty” (= 2 points), “can do but with some difficulty” (= 1 point) and “either unable to do or able only with help” (= 0 points).

Items in the FFbH Questionnaire

- Can you spread butter on a sandwich?
- Can you turn on and off taps?
- Can you spread butter on a sandwich?
- Can you write? (minimum one postcard)
- Can you sit on a hard chair for 1 h?
- Can you sit up in bed from a lying position?
- Can you put on and take off socks or similar garments?
- Can you bend sideways from a seated position to pick up a small object on the floor just beside your chair?
- Can you lift a box containing six 1-l bottles of liquid and put them on to a table?
- Can you put on and take off a wintercoat?
- Can you run 100 m without stopping in order to catch a bus?
- Can you use public transport (for example bus, train)?
- Can you lift a full suitcase and carry it for 10 m?
- Can you reach up and get for example a book from a high shelf or cupboard?
- Can you wash and dry yourself from head to toe?
- Can you bend forward to pick up a small lightweight object from the floor?
- Can you wash your hair over a washbasin?
- Can you spread butter on a sandwich?
- Can you sit on a hard chair for 1 h?
- Can you sit up in bed from a lying position?
- Can you put on and take off socks or similar garments?
- Can you bend sideways from a seated position to pick up a small object on the floor just beside your chair?
- Can you lift a box containing six 1-l bottles of liquid and put them on to a table?
- Can you put on and take off a wintercoat?
- Can you run 100 m without stopping in order to catch a bus?
- Can you use public transport (for example bus, train)?

ANCA testing by immunofluorescence

ANCA were detected by the standard method described by the First International Workshop on ANCA [38]. Briefly, human neutrophil granulocytes from healthy donors (blood group 0 positive) were separated by Percoll gradient centrifugation and washed twice in medium RPMI 1640. Using Shandon Elliot cytocentrifuge, 2 x 10^5 cells were centrifuged onto precleansed slides. The cells were then fixed in fresh 96% ethanol for 5 min at −20°C and incubated with 1:10 diluted patients’ sera for 30 min at room temperature. Following extensive washing in phosphate-buffered saline (PBS), pH 7.2, the cells were stained with fluorescein-conjugated rabbit anti-human IgG antibodies (Dakopatts Lab.,), washed again and viewed under a fluorescent microscope (Zeiss Axioskop) using oil immersion and a 100 x 10 magnification. Two fluorescent staining patterns were distinguished. Most sera produced a fine granular, atypical pANCA, while a few sera yielded a cANCA pattern similar to that seen in Wegener’s granulomatosis (Fig. 1a, b). Microphotographs were taken by means of the microscope camera Zeiss MC 100. All sera were tested as coded samples, and evaluated independently by two investigators.

The ANCA screening was done with 1:10 diluted patient serum. Only definitely positive sera were titrated to a dilution of 1:400. Sera were considered ANCA positive if they showed at least a weakly positive (+) fluorescence on ethanol-fixed granulocytes at a dilution of 1:50. Sera with a weakly positive (+) fluorescence at a dilution of 1:10 were disregarded and not considered ANCA negative.

The interpretation of the pANCA fluorescent staining pattern was rendered more difficult by the presence of ANA in the same sera. Antibodies of this specificity can cause similar or superimposed fluorescent staining patterns. Most of the sera being pANCA positive on ethanol-fixed neutrophil granulocytes were also examined on paraformaldehyde (PFA) fixed neutrophil granulocytes. Additionally, 25 normal control sera and 40 ANCA-RA sera were examined.

The preparation of the granulocytes was performed as described above. The cells were fixed with 2% PFA in PBS, pH = 7.4, for 10 min at room temperature and incubated for 5 min in 96% ethanol/5% PBS at 4°C [39]. The slides were then dried for 15 min. The examination was performed with 1:10 diluted patient serum. In every assay, known cANCA- and pANCA-positive vasculitis sera (as determined on ethanol-fixed granulocytes) one pANCA-positive SLE serum and serum from a healthy person were included as controls. A weakly positive fluorescence (+) at a dilution of 1:10 was considered as positive. Titres were not determined on PFA-fixed granulocyte preparations.

A cANCA fluorescence on ethanol-fixed granulocytes did not change its pattern on PFA-fixed granulocytes. However, a pANCA or fine granular, atypical pANCA pattern on ethanol-fixed neutrophil granulocytes changed to a cytoplasmic coarse granular fluorescence (cANCA) on PFA-fixed neutrophil granulocytes if no ANA was present. The perinuclear/nuclear ANCA pattern on ethanol-fixed granulocytes was unchanged on PFA-fixed granulocytes if no ANCA was present but ANAs were highly positive.