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Superior specificity of anti-citrullinated peptide antibodies in patients with chronic lymphocytic leukaemia and arthritis

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ABSTRACT

Objective. *Sera from patients with lymphoid neoplasias contain rheumatoid factors (RF) so often that RF are of limited use for diagnosing arthritis in lymphoma patients. Antibodies against citrullinated peptides (ACPA) might be helpful in distinguishing between true RA and rheumatoid factor-positive conditions with arthritis. We compared the specificity of RF and of ACPA for the diagnosis of RA in patients with B-cell chronic lymphocytic leukemia (CLL).*

Methods. *One hundred and seven patients with CLL without any clinical signs of arthritis and five patients with RA and concomitant CLL were included in the investigation. Serum samples were tested for RF-isotypes IgM, IgG and IgA. ACPA were determined with an ELISA that detects anti-cyclic citrullinated peptide (aCCP) antibodies.*

Results. *RF well beyond the cut-off levels were detected in 50% of the CLL-patients without RA. The isotype distribution was 41% IgM-RF, 20% IgG-RF and 3% IgA-RF. None of the 107 CLL patients without arthritis had aCCP antibodies. Within the whole cohort of CLL patients the specificity for the diagnosis of RA was 100% for aCCP antibodies and 59% for IgM-RF.*

Conclusion. *Only aCCP antibodies but not IgM-, IgG- or IgA-RF are useful for the diagnosis of RA in patients with CLL.*

Introduction

In chronic lymphocytic leukaemia (CLL) up to 25% of patients develop autoimmune disorders during the course of their disease. In addition, rheumatoid factors or other auto-antibodies are frequently detectable, but without clinical relevance (1). Thus, the confirmative value of RF for the diagnosis of RA in a patient with CLL is low. On the other hand, differentiating RA from the rare entity of paraneoplastic arthritis is relevant for the choice of treatment.

Over the past few years, antibodies against citrullinated peptide (ACPA) has become a diagnostic tool for the diagnosis of early rheumatoid arthritis (RA) with specificities superior to rheumatoid factors (RF). The presence of ACPA is a good predictor for the development of erosions and associated with significantly higher radiologic damage (2). ACPA have been detected in a minor proportion of patients with systemic sclerosis, Sjögren's syndrome, psoriatic arthritis or systemic lupus erythematodes (3-6), whereas in patients with acute viral polyarthritis no significant ACPA titers could be found (7). In addition, the presence of aCCP antibodies in liver diseases was associated with concomitant RA in these patients (8). The aim of the study was to compare the specificity of RF and of anti-CCP antibodies for the diagnosis of RA in patients with B-cell chronic lymphocytic leukaemia (CLL).

Patients and methods

Patients

All patients enrolled in this study had been referred to the Department of Internal Medicine, Division of Haematology, Medical University, Graz, Austria. One hundred and seven consecutive CLL-patients without any signs of paraneoplastic phenomena were included in the study. None of them complained of arthralgias or arthritis, and rheumatologic examination did not reveal any swollen or tender joints (detailed demographic data shown in Table I). In addition, none of the patients had a history of sicca symptoms, such as dry eyes or mouth. Seventy (64%) were male, 37 (36%) were female, the median age was 67 years (range 39 to 84), median disease duration was 5 years (0-18). Forty-six patients had been treated with cytotoxic agents including fludarabine, 18 of the patients exhibited autoimmune haemolytic anemia (AIHA).

As a control group for the prevalence of RA-specific autoantibodies we used sera from 48 consecutive patients fulfilling the revised 1987 ACR criteria for RA (9).

Furthermore, sera of five patients (four female, one male) who suffered from erosive rheumatoid arthritis and CLL concomitantly were examined (Table I). Four of them were diagnosed with RA long before they developed leukaemia, one was newly diagnosed in the course of our investigation when she developed erosive rheumatoid arthritis during chemotherapy with fludarabine.

Table I. Demographic characteristics of patients.

	CLL (n=107)	RA (n=48)	RA and CLL (n=5)
Male (%)	70 (64)	4 (8,4)	1 (20)
Female (%)	37 (36)	44 (91,6)	4 (80)
Age, years, (range)	67 (39-84)	56 (22-82)	66 (56-83)
Disease duration, years, (range)	5 (0-18)	10 (1-32)	
CLL, years, (range)			4 (2-9)
RA, years, (range)			10 (1-40)

Table II. Distribution of autoantibodies in CLL with or without arthritis and RA patients.

Autoantibody	Number (%) observed		
	CLL without arthritis (n=107)	CLL with arthritis (n=5)	RA (n=48)
RF			
positive	54 (50.4)	5 (100.0)	40 (83.3)
RF-IgM	44 (41.1)	5 (100.0)	34 (70.8)
RF-IgG	21 (19.6)	1 (20.0)	27 (56.2)
RF-IgA	3 (2.8)	2 (40.0)	14 (29.2)
aCCP antibodies	0 (0.0)	4 (80.0)	30 (62.5)
RF combination			
IgM + IgG	11 (10.3)	0 (0.0)	1 (2.1)
IgM + IgA	2 (1.9)	0 (0.0)	0 (0.0)
IgM + IgG + IgA	1 (0.9)	0 (0.0)	1 (2.1)
aCCP antibodies and RF			
aCCPA + IgM	0 (0.0)	2 (40.0)	7 (14.6)
aCCPA + IgM + IgG	0 (0.0)	1 (20.0)	9 (18.7)
aCCPA + IgM + IgA	0 (0.0)	1 (20.0)	2 (4.2)
aCCPA + IgM + IgG + IgA	0 (0.0)	0 (0.0)	10 (20.8)

Informed consent for drawing an extra quantity of blood at the time of routine venipuncture was obtained from all patients. Sera were stored at -20°C until further analysis. The study was approved by the Ethics Committee of the Medical University Graz.

Laboratory tests

Rheumatoid factor-isotypes were measured by ELISA (Autostat II RF-IgG, IgA and IgM; Hycor Biomedical, Kassel, Germany). According to the manufacturer's recommendations RF-IgM >16U/ml, RF-IgA>20U/ml and RF-IgG >30U/ml were considered positive. Anti-cyclic citrullinated peptide (aCCP) antibodies were detected in serum using the Immunoscan RA (Mark 2) from Euro-Diagnostica (Arnhem, The Netherlands) with the standard cut-off-level of 25Units/ml.

Statistical analysis

Distributions of laboratory test results are described as the percentage per category for qualitative items and as mean (±SD, SE) or median (and range) for quantitative items as stated. ROC curve analysis was performed to test whether changing the cut-off values for the different RF-isotypes would increase their diagnostic value.

Differences in RF and aCCP antibody levels between the evaluated groups were tested using a non-parametric Mann-Whitney U-test. Results were accepted as statistically significant when $p<0.05$. All statistical analyses were performed using Prism 5, Version 5.0c (GraphPad Software, Inc.).

Results

Auto-antibodies in CLL-patients without arthritis

In this cohort no aCCP antibodies were found. In contrast, RF well beyond the cut-off levels have been detected in 54 arthritis-negative CLL-patients (50%). Among those, RF-IgM was most frequently expressed (44/107; 41%), followed by RF-IgG (21/107; 20%) and RF-IgA (3/107; 3%). Eleven patients were positive for RF-IgG+IgM, two for RF IgA+IgM and one individual had elevated levels of all three RF isotypes (Table II).

Interestingly, the mean values ± standard error of RF was significantly lower in the CLL-group compared to RA (RF-IgM 29±9IU/ml *vs*. 90±22 IU/ml, $p<0,0001$; RF-IgG 32±9 IU/ml *vs*. 67±16 IU/ml, $p<0,0001$; RF-IgA IU/ml 4±2 *vs*. 22±3 IU/ml, $p<0,0001$; respectively). No significant differences could be found when the distribution of RF-isotypes was investigated referring to the haematological stage of disease (data not shown).

Auto-antibodies in RA-patients

In the RA-patients (Table II), the frequency of autoantibodies was as expected: 40 (83%) of the RA patients showed positivity for at least one RF-isotype. Thirty-four out of forty-eight patients (71%) were positive for RF-IgM, 27/48 (56%) for RF-IgG, and 14/48 (29%) for RF-IgA. Anti-CCP antibodies were detected in 30/48 patients (63%). Combinations of RF isotypes and/or anti-CCP could be found in 30/48 RA-patients (63%) as well. The most common combinations were aCCP with all RF-isotypes (10/48; 21%) and aCCP with RF-IgG and -IgM (9/48; 19%). Positive aCCP antibody levels combined with RF-IgM were found in 7/48 sera (15%). In 6/48 (13%) patients with RA all of the tested parameters were in the normal range, and 2 patients (4%) showed positivity for aCCP antibodies with normal RF-values (data not shown). Two others were positive for aCCP antibodies, RF-IgM and RF-IgA. Two RA patients who were negative for aCCP antibodies tested either positive for all RF isotypes or RF-IgG and -IgM, (2%, respectively).

Auto-antibodies in patients with CLL and RA

In the group of patients who suffered from both CLL and RA, all patients were positive for RF-IgM and 4 (80%) were also positive for aCCP antibodies. One patient was positive for RF-IgM only (data not shown), whereas two showed combined elevations of aCCP

antibodies and RF-IgM. Combinations of aCCP antibodies with RF-IgM and -IgA or RF-IgM and -IgG were found in one patient each (Table II).

Specificity, sensitivity, positive and negative predictive value of RA-specific auto-antibodies

In our analysis aCCP antibodies had the highest specificity for the diagnosis of RA (100%), followed by RF-IgA (97%), RF-IgG (80%) and RF-IgM (59%). The highest sensitivity was reached by RF-IgM (74%). The sensitivity of aCCP antibodies was 64%. RF-IgG and RF-IgA reached a sensitivity of 53% and 28%, respectively.

Determining the positive predictive values, aCCP antibodies reached 100%, followed by RF-IgA (83%). The best performance in determining the negative predictive value for developing RA was also seen for aCCP antibodies (85%) and for RF-IgM (82%).

Using ROC curve analysis the likelihood ratio (LHR) for RA in CLL patients with a positive RF-IgM is 1.66. Setting the cut-off at >65U/ml would increase the LHR to 7.13 with a sensitivity of 33.3 (95%CI: 20.4–48.1) and a specificity of 95.3 (95%CI: 89.4 –98.5). Using RF-IgA the LHR is 11.8 with a sensitivity (95%CI) of 33.3 (20.4–48.0) and a specificity of 97.2 (92.0–99.0). Using a cut-off value of 30 U/ml the LHR would be 26.8 (sensitivity 25.0; 95%CI 13.6–39.0 and specificity 99.1; 94.9–99.0).

Discussion

B-Cell chronic lymphocytic leukemia (CLL) is the most common form of leukemia found in adults in the Western World, accounting for about a third of all cases (10). It is a monoclonal disorder, representing a malignancy of CD5 positive B-cells, that are known for the production of polyreactive natural auto-antibodies (11). Duek *et al*. postulated, that the B cells in CLL might disclose an activated phenotypic pattern that is leading to production of auto-antibodies (12). The majority of leukemic cells from patients with CLL express IgM autoantibodies, most notably rheumatoid factors (RF). RF of the IgM-isotype appear in up to 58% of CLL patients (1), although, in a recent multi-centre study by Barcellini *et al*., the frequency of RF was only 10% (13). Unfortunately, the authors did not indicate the isotype or the titre of the RF measured.

One of the features of advanced CLL is paraleukemic arthritis. This entity can be polyarticular, and sometimes symmetric and even rheumatoid-like nodules have been reported (14). On the other hand, there is clear evidence of an increased risk of developing lymphomas in patients with longstanding RA (15, 16) A number of case reports of RA patients developing CLL or vice versa has been published (17-19). When CLL patients develop arthritis and no radiographic changes occur yet, it is often very hard to tell clinically whether the arthritis is paraleukemic or the expression of concomitant RA. However, a definite diagnosis is desirable, since the therapeutic strategies for both entities differ considerably. While paraneoplastic arthritis is normally treated symptomatically, RA patients need a sufficient therapy to prevent erosions and aggressive disease. As already mentioned, determination of RF-IgM is of little help, since the majority of all CLL-patients are positive for it.

Interestingly, RF-IgG and IgA can be present in CLL-patients who do not show any signs of arthritis, even though their average levels are right below the levels in RA. Considering the high specificity of RF-IgA for the diagnosis of RA (20, 21), it could be speculated that the numerous chemotherapies CLL-patients normally receive might mask the symptoms of RA.

As we were able to show, aCCP antibodies seem to be very specific for rheumatoid arthritis, since they were not present in the sera of arthritis-free CLL-patients, although half of the patients tested positive for RF. Increasing the cut-off level for the different RF-isotypes seems not to be practical since there was a dramatic decrease in specificity, *i.e*. the number of CLL patients with RA correctly identified as positive by the test. This makes testing for aCCP antibodies the only useful laboratory tool when RA is suspected in patients with CLL or other lymphomas.

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