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# Rheumatoid arthritis markers: antibodies against citrullinated peptides

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**The objective** of this study is to assess the diagnostic value and to determine the frequency of the ACCP (antibodies against cyclic citrullinated peptides), AKA (antikeratin antibodies), RF-IgA (rheumatoid factor), RF-IgM serological markers in the diagnosis of early rheumatoid arthritis (RA), and to compare the association of these antibodies with disease activity, disease duration and erythrocyte sedimentation rate (ESR).

**Patients and methods**. The analysis involved 115 RA and 40 osteroarthritis patients. Sera from each patient were tested for ACCP and RF isotypes by enzyme linked immunosorbent assay (ELISA) and for AKA by indirect immunofluorescence (IIF).

**Results**. 91 (79.1%) sera from 115 patients with RA were ACCP-positive, 55 (47.8%) AKA-positive, 74 (64.3%) RF-IgA-positive and 103 (89.6%) RF-IgM-positive. We determined the diagnostic value of the serological markers for RA. Sensitivity for RA was the highest for the RF-IgM test (89%), followed by the ACCP (79%), RF-IgA (64%) and AKA (48%) tests. The AKA test was less sensitive than the other three tests. The best specificity was achieved with the ACCP test (97%). The specificity of ACCP was significantly higher than that obtained for the AKA (87%), RF-IgA (87%) and RF-IgM (80%) tests. Our study suggests that diagnostic efficacy is the highest when tests for ACCP and RF are used in combination. When used in combination, the ACCP and RF demonstrate a high specificity (100%) for RA. In this study, we found no statistically significant correlation between ACCP, AKA, RF-IgA, RF-IgM and the disease duration, the disease activity and ESR.

**Conclusions**. These results indicate that ACCP is the most specific serological marker of RA and therefore most useful for diagnosis but not for disease monitoring or as a disease activity marker.

**Key words**: rheumatoid arthritis, antikeratin antibodies, rheumatoid factor, antibodies against cyclic citrullinated peptides

## INTRODUCTION

Rheumatoid arthritis (RA) is a common chronic inflammatory autoimmune disease of unknown origin. It affects ~1% of the population and causes the irreversible functional and anatomical joint damage. Early diagnosis with the help of the new diagnostic tools is the main standpoint of modern rheumatology as it is absolutely necessary for early treatment with disease-modifying drugs (DMARD(s)).

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Rheumatoid factor (RF) is the only one immunological marker of RA included into RA classification criteria and has been known for more than fifty years. RF is found quite frequently in RA patients' sera (~80%), although its specificity is rather low. It was demonstrated in a number of studies to be present in the sera of patients with rheumatic and non-rheumatic diseases and even of healthy persons (1). Many efforts have been made for years to find out more specific diagnostic tools like anti-RA33 (2), Sa/anti-Sa system (3, 4), etc. However, their sensitivity seems not to be evidently higher than RF itself.

Today RA immunology focuses on a new generation of antibodies and particularly on antibodies against citrullinated peptides. The latter include the antiperinuclear factor (APF) (5) and antibodies against

keratin (AKA) (6) specifically targeting filaggrin (7). The amino acid citrulline apears to be the main component of filaggrin (8). The expression of citrulinated peptides results from inflammation when the radicals of arginin compose the peptide citrulline. The process is called citrullinization of peptides. For their identification, the ELISA method was worked out and citrullinated peptides of cyclic structure for well coating were derived. Their specificity was found to be in the range of 96–100% and the sensitivity somewhat lower – 45–80% (9). Therefore, the antibodies against cyclic citrullinated peptides (ACCP) became a new immunological marker in the diagnosis of RA, with an increasingly more interest paid to them.

The objective of this study was to assess the diagnostic value and to determine the frequency of the ACCP, AKA, RF-IgA, RF-IgM serological markers in the diagnosis of early rheumatoid arthritis and to compare the association of these antibodies with disease activity, disease duration and erythrocyte sedimentation rate (ESR).

## MATERIALS AND METHODS

115 patients suffering from RA and 40 from OA (osteoarthritis) (the control group) were enrolled into the study. All RA patients fulfilled the revised ARC'87 criteria for this disease and had less than 4 years of disease duration. The interview comprised socio-demographic, disease-related questions, and VAS (visual analogue scale) for patient's global disease assessment over the past 4 weeks. During the clinical examination, the 28 painful and 28 swollen joints were counted and the last value of erythrocyte sedimentation rate (ESR) was obtained from the records. From these data the disease activity score (DAS-28) was counted. The disease activity is considered lowest when DAS-28 $\leq$ 3.2 and the highest when DAS-28 > 5.1. The questionnaire for the control group contained only the demographical data. For all 155 patients immunological analyses were performed.

RF (rheumatoid factor) isotypes and ACCP were detected by the ELISA test (EUROIMMUN) incubating the diluted samples in the wells. To detect the bound antibodies, enzyme-labelled anti-human IgG was added, followed by a stop-solution terminating the reaction. RF-IgM and RF-IgA were considered positive when higher than 20 RU/ml and the ACCP test was considered positive when higher than 5RU/ml.

AKA were detected by an indirect immunofluorescence test. Frozen sections of Wistar rat oesophagus covering the slides were incubated with serum samples diluted 1:10. If a sample was positive, specific antibodies attached to the keratin layers were stained with fluorescein-labelled anti-human antobodies and made visible with a fluorescence microscope. The result of immunofluorescence was considered positive if staining was seen clearly and uninterrupted in *stratum corneum* in the mentioned dilution.

All study data were entered into the SPSS version 6.0 data file. Statistical analysis was performed using ANOVA for continuous variables and the chi-square method was used for proportions. The differences were considered significant when the p level was lower than 0.05.

## RESULTS

The demographic data on RA and OA patients are shown in Table 1. There were more females than males in both groups, but the patients from the control group were older.

Immunological data were analysed for all 155 study patients. Positive imunological test results between RA and OA patients are show in Table 2.

The most frequent positive result in both groups was RF-IgM. In the RA group, 79.1% were positive for ACCP, while only one patient had this test positive in the OA group. AKA demonstrated the lowest frequency in the RA group, but three patients from 40 in control had this test positive.

Table 1. **Clinical and demographic characteristics of the patients. Mean(SD) for continuous variables, numbers(%) for dichotomous variables**

| Characteristics | RA patients | OA patients |
|---|---|---|
| | n = 115 | n = 40 |
| Women | 83(72.2%) | 30(75%) |
| Age, years | 52.04 (13.24) | 73.45(12.01) |
| Disease duration, months | 21(15.0) | |
| Duration in month from first four symptoms of RA to sera collection for immunological analysis | 23.74(16.16) | |
| Patient's global disease assessment (VAS) | 5.35(2.02) | |
| 28 SJC | 9.68(7.13) | |
| 28 TJC | 13.05(9.56) | |
| Disease activity (DAS-28) | 5.2(1.19) | |
| ESR | 46.26(25.64) | |

Table 2. **Frequency of positive immunological tests in RA and OA patient groups**

| Laboratory test | RA patients | OA patients |
|---|---|---|
| | n = 115 (100%) | n = 40 (100%) |
| ACCP(+) | 91(79.1%) | 1(2.5%) |
| AKA(+) | 55(47.8%) | 3(7.5%) |
| RF-IgA(+) | 74(64.3%) | 5(12.5%) |
| RF-IgM(+) | 103(89,6%) | 8(20.0%) |

Table 3. **AKA, ACCP, RF-IgA , RF-IgM sensitivity and specificity**

| RA serological markers | Sensitivity (%) | Specificity (%) |
|---|---|---|
| ACCP | 79 | 97 |
| AKA | 48 | 92 |
| RF-IgA | 64 | 87 |
| RF-IgM | 89 | 80 |
| ACCP + RF | 76 | 100 |
| AKA + RF | 45 | 97 |
| ACCP + AKA | 39 | 100 |
| RF | 93 | 87 |

Table 4. **Frequency of immunological analysis in 115 RA patients depending on disease duration**

| Immunological tests | Disease duration, months | | | |
|---|---|---|---|---|
| | 1–12 N = 48 | 13–24 N = 24 | 25–36 N = 20 | 37–38 N = 23 |
| ACCP(+) | 36(75.0%) | 20(83.3%) | 16(80.0%) | 16(69.6%) |
| AKA(+) | 22(45.8%) | 114(58.3%) | 7(35.0%) | 12(52.2%) |
| RF-IgA(+) | 33(68.8%) | 18(75.0%) | 14(70.0%) | 11(47.8%) |
| RF-IgM(+) | 45(93.8%) | 22(91.7%) | 18(90.0%) | 17(73.9%) |

Table 5. **Frequency of immunological analysis in 115 RA patients depending on disease activity**

| Immunological tests | Disease activity | |
|---|---|---|
| | DAS I–II N = 49 | DAS III N = 66 |
| ACCP(+) | 40(83.3%) | 47(71.2%) |
| AKA(+) | 20(41.6%) | 34(51.5%) |
| RF-IgA(+) | 33(68.7%) | 42(63.6%) |
| RF-IgM(+) | 39(81.2%) | 62(93.2%) |

The majority of the patients were positive for ACCP and RF (both 76.5%), while RF as the single positive marker was found in 16.5% and ACCP as a single marker only in a small proportion of 2.6% (Figure).

Table 3 presents the specificity and sensitivity of RA immunological tests. In our study, the highest specificity (100%) was demonstrated both for ACCP and RF and ACCP and AKA. As the single test, the highest diagnostic specificity was shown for ACCP

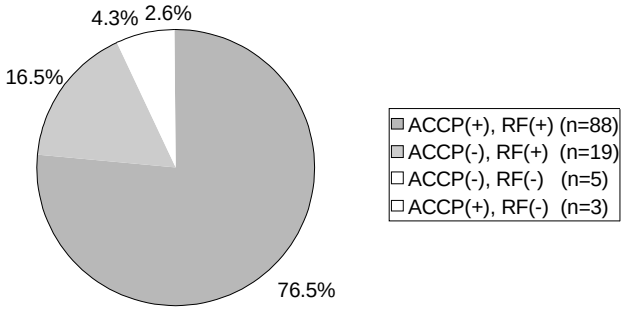


**Figure.** Frequency of ACCP and RF tests in 115 RA patients

(97%) and AKA (92%). The highest sensitivity was demonstrated by the RF test. Least sensitive was the AKA test (48%).

To assess the association between immunological test results and disease duration or disease activity, the patients were divided into four groups according to disease duration and into two groups discriminating between lower and higher disease activity. The data are presented in Tables 4 and 5. No associations were found between the disease variables or ESR and the immunological tests.

## DISCUSSION

The role of ACCP in the pathogenesis of RA remains elusive, but they appear in the serum many years before the onset of clinical disease, suggesting an early break in B cell tolerance resulting in defective apoptosis, although the genetic factors could not be ruled out (10). In line with many papers discussing the origin and role of ACCP, the present study contains a comparative information on their role derived from hospital-based rheumatological patients. The aim of this study was to try to find associations between immunological markers and the course and activity of disease.

115 RA and 40 OA patients were included in the study. The questionnaires for demographical and clinical data were filled in and serum samples were obtained from each patient. The following immunological markers were tested: ACCP, AKA, RF-IgA, RF-IgM. It is worth noting that all the patients were included in the early stage of their disease (up to four years of disease duration) what might be of special interest if the question of early diagnostic value is considered.

The specificity and sensitivity of ACCP was stated at 97% and 79%, respectively. The specificity is comparable with the data from other studies (91–98%) [11, 12] while the sensitivity is quoted in a wider but lower range of 41–68% (11–13). Our study reports a higher ACCP specificity. It migh be

speculated that the results vary depending on titration and cutoff level specificities.

A higher diagnostic sensitivity was exhibited by RF-IgM (89%), while RF-IgA sensitivity was evidently lower (64%). Humbel et al. quoted the sensitivy of RF-IgM up to 91% and RF-IgA 80%. The lowest sensitivity in our study (and in others as well) was adherent to AKA being as high as 48%. The same figures are quoted in the study of Vasiliauskiene et al. and were published internationally (14).

The specificity of all antibodies estimated in this study exhibited higher values including ACCP (97%), AKA (92%), RF-IgM (80%) and RF-IgA (87%).

It might be supposed that the information obtained from one particular immunological test is more valid if supported by other data employing other immunological tests. RF, being the internationally accepted golden standard for RA diagnosis, still remains the main standpoint around which other immunological tests are worked out.

The AKA test was the most questionable one in our study and similarly in other studies. Despite the efforts made by two independent researchers, the staining of the internal layer of rat oesophagus was doubted by both and the results remained vague. Therefore, this method is not for use in clinical practice, because it requires perfect optical conditions and a top experience.

ACCP remains the most specific and sensitive test for RA diagnosis even in very early stages. Notably, it doesn't change in the course of the disease and is not a tool for following it up. The recently published RA treatment demonstrated that the levels of ACCP do not change depending on anti-TNF treatment, in contrast to RF, C-reactive protein and ESR. It has been concluded that RA and ACCP are two different systems of antibodies reacting differently to the treatment prescribed (15). The patients included in this study were under the different regimes of treatment (disease modifying drugs, steroids, antiinflammatory drugs) and no parallels could be lined out.

We couldn't draw any conclusions as regards the duration of disease and the frequency of RF, although the data proving an increase of the percentage of RF-positive patients over time are presented in other studies. It is generally accepted that around 55% of patients among those with disease duration up to half a year tend to be RF-positive, with an increase up to 70% along with disease duration increasing to two years. Our data are inconsistent with it, and we aim to clarify this disagreement between our data and the results claimed by other studies in the nearest future.

RF is known to be an independent prognostic factor for radiological progression of disease. Data from other studies imply ACCP to be a factor of poor prognosis and rapid radiological progression, even more powerful than RF (13). Radiological progression is the most important linkage between new immunological tools and disease outcomes; unfortunately, radiological data were not included in this study. Contrary to Kastbom et al. who found an association between ACCP and ESR, our study failed to demonstrate any association between immunological markers and disease activity or ESR. Thus, ACCP appears to be an important instrument for diagnosis, but its role in disease activity or progression remains arguable.

## CONCLUSIONS

We conclude that ACCP is a new and valuable serological marker for RA diagnosis, and the sensitivity and specificity of the method are high enough to suggest it for routine clinical practice. Performed together with RF, it supports the early diagnosis at early stages and gives implications for early treatment. When tests for ACCP and RF are used in combination, they demonstrate the highest specificity for RA.

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**REUMATOIDINIO ARTRITO ÞYMENYS: ANTIKÛNAI PRIEÐ CITRULINIZUOTUS BALTYMUS**

S a n t r a u k a

**Ávadas**. Ðio tyrimo tikslas – nustatyti serologiniø rodikliø – ACCP (anticirulininiø peptidø), AKA (antikeratininiø antikûnø), RF-IgA (reumatoidinio veiksnio), RF-IgM, ankstyvo reumatoidinio artrito (RA) – daþnumà bei diagnostinæ vertæ, jø ryðá su ligos aktyvumu, trukme ir eritrocitø nusëdimo greièiu (ENG).

**Ligoniai ir metodai**. Á tyrimà buvo átraukta 115 RA ir 40 osteoartritu serganèiø ligoniø. Kiekvieno ligonio serumas buvo iðtirtas imunofermentiniu metodu (ELISA) dël ACCP ir RF bei netiesioginës imunofluorescencijos bûdu (NIF) dël AKA.

**Rezultatai**. 91 (79,1%) ligonis ið serganèiøjø RA turëjo ACCP, 55 (47,8%) – AKA, 74 (64,3%) – RF-IgA ir 103 (89,6%) – RF-IgM. Mes nustatëme ðiø serologiniø rodikliø diagnostinæ vertæ diagnozuodami RA. Didþiausiu jautrumu pasiþymëjo RF-IgM (89%), maþesniu – ACCP (79%), RF-IgA (64%) ir AKA (48%) imunologiniai rodikliai. AKA jautrumas buvo maþiausias. Specifiðkiausias buvo ACCP (97%). Ðio rodiklio specifiðkumas buvo kur kas didesnis nei kitø trijø – AKA (87%), RF-IgA (87%) ir RF-IgM (80%). Didþiausias specifiðkumo rodiklis buvo gautas tuomet, kai kartu buvo tiriami du tø paèiø ligoniø rodikliai – RF ir ACCP (100%). Tirti imunologiniai rodikliai nebuvo susijæ su ligos aktyvumu, trukme ir ENG.

**Išvados**. ACCP yra pats specifiðkiausias imunologinis rodiklis diagnozuojant RA, bet jis negali bûti nei ligos eigos, nei jos aktyvumo rodiklis.