

Use and utility of serologic tests for rheumatoid arthritis in primary care

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ABSTRACT

INTRODUCTION: In this retrospective, register-based population study, we evaluated if anti-citrullinated protein antibodies (ACPA) is a better choice than immunoglobulin M rheumatoid factor (IgM RF) in primary care when rheumatoid arthritis (RA) is suspected, as it determines predictive values in real-life settings. Furthermore, the study described ordering patterns to investigate the benefit of repeated testing.

METHODS: Test result, requisitioning unit, test date and the patient's social security number were collected from the Department of Clinical Immunology at Odense University Hospital in 2007-2016 and merged with patient diagnoses from the Danish National Patient Registry.

RESULTS: Overall, 5% were diagnosed with RA. IgM RF remained the preferred test during the entire period. Test sensitivity was 61% for IgM RF and 54% for ACPA. The test specificity was 88% for IgM RF and 96% for ACPA. PPV was higher for ACPA than for IgM RF (30% versus 12%) and NPV was equal (99%) in primary care. Few individuals seroconverted from negative to positive (ACPA 2% and IgM RF 5%) and positive to negative (ACPA 3% and IgM RF 6%).

CONCLUSIONS: ACPA has a higher PPV for RA than IgM RF, whereas their NPV is identical. ACPA is the better choice when testing for RA in primary care. Seroconversion is rare, and it is only rarely relevant to retest.

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TRIAL REGISTRATION: not relevant.

In Denmark, the majority of first patient contacts are provided in primary care practices that serve as gatekeepers for tertiary care. Diagnosis and treatment of rheumatoid arthritis (RA) are done by rheumatologists in private practices or at hospital wards. RA is a chronic autoimmune joint disease characterised by pain, inflammation and joint destruction [1]. In Denmark, the estimated prevalence is 0.7%, and about 1,500 patients were diagnosed with RA in 2016, which is equivalent to an annual incidence of approximately 25 per 100,000 individuals [2, 3]. In primary care, musculoskeletal complaints are common, but only few patients will suffer from arthritis. The RA diagnosis is based on anamnesis, clinical examination, imaging modalities and blood testing. The final diagnosis is always made by an

expert clinical physician. Serological markers are of importance and therefore included in the American College of Rheumatology/European League Against Rheumatism 2010 classification criteria for RA. The classification criteria are useful for standardisation of clinical trials and comparison of study populations, but they are not diagnostic criteria [1].

It has been shown that clinicians tend to overestimate the utility of a positive test result when the diagnostic accuracy is reported as sensitivity and specificity [4]. In a study by Vermeersch et al, clinicians were given information on disease prevalence, test sensitivity and specificity and 81% responded that the probability for disease was approximately 90% when the correct answer was 20% [4]. As opposed to positive and negative predictive values (PPV and NPV), sensitivity and specificity are not related to disease prevalence. PPV is an estimate of the proportion of patients with a positive test result who develop a given disease, in this case RA. NPV represents the proportion of patients with a negative test result who do not develop the disease. To determine the PPV and NPV, the study population needs to match the population in which the test is used [5].

Immunoglobulin M rheumatoid factor (IgM RF) is found in 70-80% of patients with confirmed RA and has been included in different sets of classification criteria for many decades [6, 7]. However, IgM RF has a known low specificity and is found in patients with infections, other autoimmune disease and occasionally even in healthy subjects [8]. Anti-citrullinated protein antibodies (ACPA) are found in 63-76% of patients with confirmed RA, and are considered more specific for RA than IgM RF [9]. ACPA are included in the most recent 2010 classification criteria for RA [1]. Anti-nuclear antibodies (ANA) are often included as a part of the diagnostic workup for connective diseases. However, ANA do not contribute to the diagnosis or classification of RA [10]. Despite this, ACPA, IgM RF and ANA are often ordered as a triad. IgM RF and ACPA have been described as relatively stable, and hence seroconversion is rare. Even so, we suspect they are frequently retested at our laboratory [11].

In a retrospective register-based population study, we evaluated if ACPA is a better predictor than IgM RF

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when RA is suspected in primary care. Furthermore, we examined the sensitivity and specificity of ACPA and IgM RF and calculated their PPV and NPV in real-life settings, both in primary care and at rheumatologic departments. Finally, we described ordering patterns of ACPA, IgM RF and concomitant ANA and investigated the benefit of repeated testing.

METHODS

Population

The Department of Clinical Immunology is the only laboratory performing ACPA, IgM RF and ANA on the island of Funen, which has almost 500,000 inhabitants. We used the laboratory information system employed at the Department of Clinical Immunology to extract information regarding test results (ACPA, IgM RF and concomitant ANA) analysed during the 2007-2016 period, date of testing, ordering unit and patient social security number. We excluded individuals under 18 years of age at the time of testing. Data were enriched with patient diagnosis data obtained from the Danish National Patient Registry (DNPR) [12]. We included diagnoses from both the inpatient and the outpatient ward in 1995-2016. The diagnoses were encoded by physicians according to the World Health Organization's (WHO) International Classification of Diseases tenth revision (ICD-10). Diagnoses from private practice physicians are not included in the register. Therefore, patients who had a test ordered from a private practicing

rheumatologist were excluded. To enhance the validity of the RA diagnosis, it had to be registered two times within 18 months for the patient to be classified as an RA patient. To avoid bias due to lack of follow-up, we excluded test results after 1 August 2016 before merging these data with patient diagnoses. All test results were kept for analyses unrelated to diagnosis, such as test variability and ordering pattern.

The following ICD-10 diagnoses were included for the diagnosis of RA: M009, M051, M052, M053, M058, M059, M060, M068 and M069.

The following ICD-10 codes were included for the diagnosis of connective tissue disease: M320, M321, M328, M329, M330, M331, M332, M339, M340, M341, M342, M348, M349 and M350.

Detection of antibodies

All analyses were performed using validated standard methods at the Department of Clinical Immunology, Odense University Hospital, in a laboratory accredited by the Danish Accreditation Fund (DANAK) according to the ISO/EN 15189 standard.

Determination of ACPA was performed by a second-generation ELISA assay (Immunoscan CCPlus, Euro Diagnostica, Malmo, Sweden) according to the manufacturer's instruction. The lower cut-off was set to 25 U/ml and the upper cut-off to 1,600 U/ml. Samples with values > 1,600 U/ml were not titrated. IgM RF was determined by ELISA (AESKULISA IgM RF, AESKU.GROUP, Wendelsheim, Germany) according to the manufacturer's instruction. The lower cut-off was 15 U/mL and the upper cut-off was 300 U/ml. Samples above > 300 U/ml were not titrated. Both assays have undergone few minor changes during the study period, but they have remained biochemically unaltered.

Screening for ANA was done by indirect immunofluorescence using HEp2 cells as substrate at a 1:160 dilution (AESKU Diagnostics, Wendelsheim, Germany).

Statistics

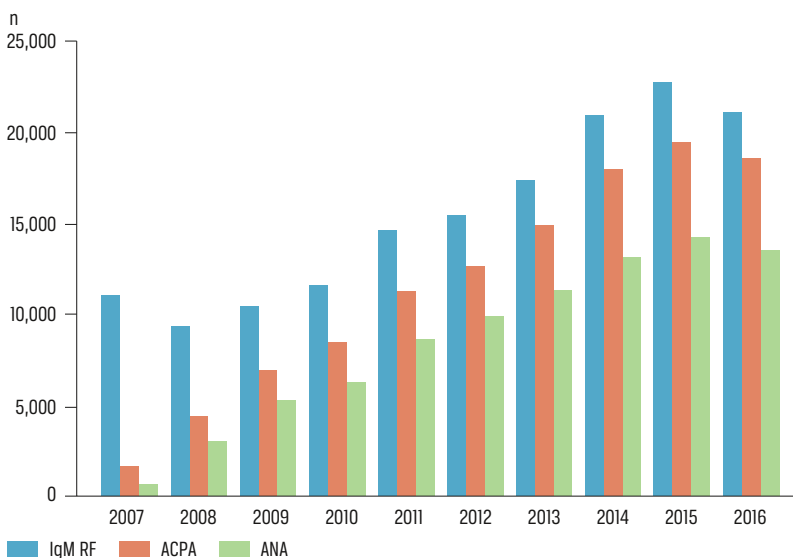
We used STATA 15 for data processing and calculated the sensitivity, specificity, PPV and NPV manually. In case of multiple tests per individual, data were collapsed into one result per test per individual before merging with the diagnosis. If there was a positive and a negative test result in one patient, the positive result was kept.

Ethics

This study was conducted in accordance with the Declaration of Helsinki. Due to the size of the population (n = 69,114) and the study design (retrospective laboratory and registry data with full anonymisation of all data), individual consent was not required.

The Danish Patient Safety Authority approved the

FIGURE 1 / Since 2007, there has been a steady increase in the number of tests ordered. Data were censored after 1 August 2016, which explains the apparent decrease in 2016. During the study period, immunoglobulin M rheumatoid factor (IgM RF) remained the preferred test, even though anti-citrullinated protein antibodies (ACPA) was considered more specific. Anti-nuclear antibodies (ANA) is often ordered; it is, however, rarely relevant when suspecting rheumatoid arthritis.



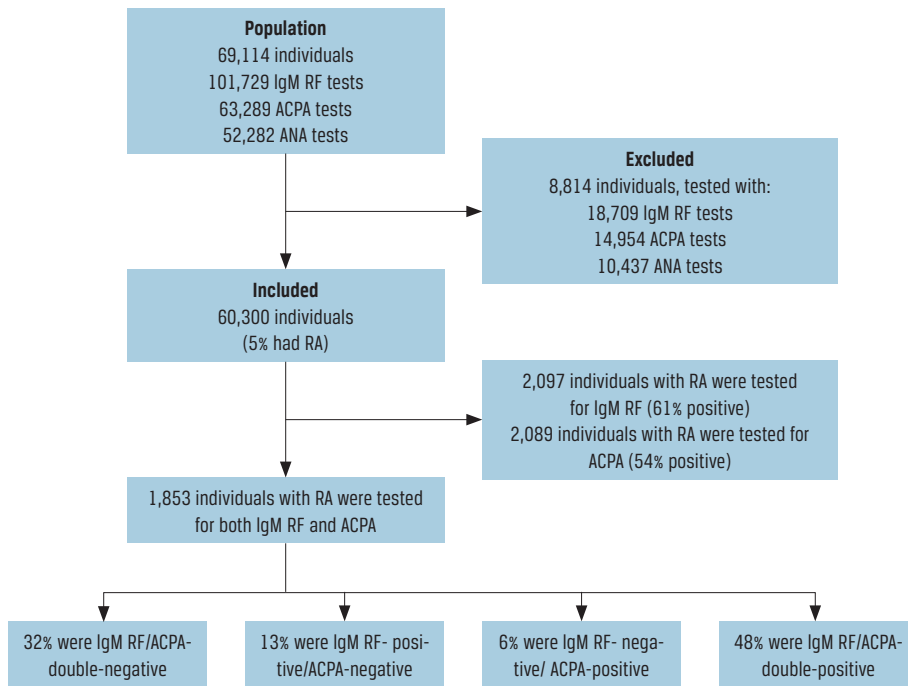


FIGURE 2 / Flow chart showing the number of patients excluded and included and the distribution of tests and test results in the subgroup of patients diagnosed with rheumatoid arthritis (RA), which required two registrations within 18 months. Patients were excluded if under 18 years of age at the time of testing, if tests were ordered from a private practicing rheumatologist and if test results were obtained after 1 August 2016 due to lack of follow-up. Among patients diagnosed with RA, 67% were tested for both anti-citrullinated protein antibodies (ACPA) and immunoglobulin M rheumatoid factor (IgM RF). In 48%, both tests were positive, 13% had a positive IgM RF but a negative ACPA, 6% had a positive ACPA in combination with a negative IgM RF and 32% were double seronegative.

ANA = antinuclear antibodies.

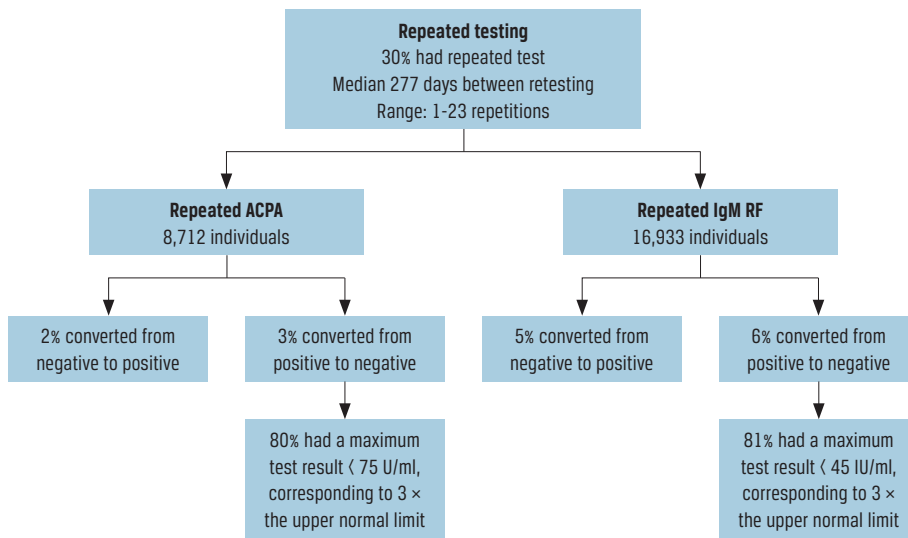


FIGURE 3 / Repeated testing was seen frequently, as 30% of the individuals were tested several times. Seroconversion was rare. In individuals converting from a positive to negative anti-citrullinated protein antibodies (ACPA), 80% were initially low positive, defined as < 3 × the upper normal limit. For immunoglobulin M rheumatoid factor (IgM RF), 81% of those who seroconverted from positive to negative were low positives.

IU = international unit.

study protocol (3-3013-1266/2), and the Danish Data Protection Agency approved the study (17/34008). Data were processed in accordance with the European Union General Data Protection Regulations (EU GDPR). Approval by a regional research ethics committee was not required under Danish law [13].

Trial registration: not relevant.

RESULTS

Distribution of test results

The total number of tests increased (IgM RF, ACPA,

ANA) from 13,440 in 2007 to 53,276 in 2016 (exact numbers are not shown) (Figure 1). During the observation period 2007-2016, a total of 190,300 test results (ACPA, IgM RF and concomitant ANA) from 69,114 individuals were analysed (tests per individual ranged 1-24) (Figure 2 and Figure 3) and 32% of the population had ANA tested. In the cohort, 5% had a diagnosis of RA (Figure 2). Among patients diagnosed with RA, 67% were tested for both ACPA and IgM RF. In 48% of these patients, both tests were positive, 13% had a positive IgM RF but a negative ACPA, and 6% had a positive ACPA in combination with a negative

TABLE 1 / Performance of the anti-citrullinated protein auto-antibody (ACPA) and the immunoglobulin M rheumatoid factor (IgM RF) tests depends on the clinical setting. Overall, ACPA has a better specificity but a lower sensitivity than IgM RF. The positive predictive values (PPV) for both ACPA and IgM RF were higher at rheumatologic departments than in primary care. Test variability was low, and very few individuals seroconverted.

	ACPA, %	IgM RF, %
Sensitivity	54	61
Specificity	96	88
<i>Test variability^a</i>		
Negative to positive	1	3
Positive to negative	2	4
<i>Individuals who seroconvert^a</i>		
Negative to positive	2	5
Positive to negative	3	6
<i>Primary care vs rheumatologic department</i>		
PPV	30 vs 60	12 vs 34
NPV	99 vs 92	99 vs 93

NPV = negative predictive value.

a) "Test variability" is the % of tests differing from the previous test on the same individual, as opposed to "individuals who seroconvert", which is the % of individuals who seroconvert.

The difference between the 2 is due to the fact, that some individuals have had several tests.

IgM RF. Finally, 32% were double seronegative (Figure 2).

As seen in Figure 1, IgM RF was the preferred test during the entire study period, and there was an increase in concomitant ANA orders (the exact percentage of concomitant ANA orders was 6% in 2007 and 61% in 2016).

Determination of predictive values

Since the ordering institutions were known, we were able to determine the predictive values in different clinical settings - at the general practitioner (primary care) and at rheumatologic departments (tertiary care). As seen in Table 1, the PPV for the ACPA test in a tertiary care setting, i.e. a rheumatologic department, was 60%, and the NPV was 92%. The PPV of the IgM RF test at rheumatologic departments was 34% and the NPV was 93%. In a primary care setting, i.e. a general practitioner, the PPV for the ACPA test was 30% and the NPV was 99%, compared to a PPV for the IgM RF test of 12% and a NPV of 99%. We calculated sensitivities and specificities for both tests and found that, overall, IgM RF has a slightly higher sensitivity than ACPA (61% and 54%, respectively), but ACPA has a higher specificity (96% compared with 88% for IgM RF) (Table 1). 75% of the patients with RA in our cohort were diagnosed within 143 days after testing, and 0.3% of the individuals were diagnosed with both RA and a simultaneous connective tissue disease.

Repeated testing and seroconversion

In our study population, 30% of the individuals were tested repeatedly (range: 1-24). The median interval between tests was 277 days. ACPA converted from negative to positive in 2% of the individuals, and from positive to negative in 3% of the individuals. IgM RF converted from negative to positive in 5% of the individuals, and from positive to negative in 6% of the individuals (Figure 3 and Table 1). Among patients who seroconverted from positive to negative for both tests, 80% were low positives, defined as < 3 × upper limit of normal.

DISCUSSION

In this population-based retrospective register study, we found that the PPV of the ACPA test is superior to the IgM RF test in primary care, while the NPVs for both tests were equal. Furthermore, we found that both tests were often repeated several times, although very few seroconverted. Lastly, we noted that ANA often, and increasingly, is ordered in combination with ACPA and/or IgM RF.

In Denmark, we have a unique opportunity to evaluate test performance in a real-life setting owing to mandatory registration of diagnoses at public hospitals in the DNPR [12]. The DNPR diagnosis of RA has been validated and was found to be over-reported with a PPV of 75-79% [14, 15]. To increase the validity of the DNPR diagnosis, two registrations within 18 months were required for inclusion in the RA population. As most patients are followed in the outpatient ward at least once annually, we find it reasonable to assume that the patients in our cohort are categorised correctly. The inclusion period (1995-2016) for the diagnosis is long in order to avoid false negatives if a patient already had the diagnosis prior to testing. The follow-up time of six months after testing should be sufficient, as 75% of the RA individuals in our cohort had been diagnosed 143 days after testing. The classification criteria for RA were last updated in 2010. The updating was performed in order to heighten the sensitivity and correctly to classify patients earlier in the disease course [1]. However, with increased sensitivity comes a loss of specificity. We can only speculate how this change has affected the PPVs and NPVs for ACPA and IgM RF. Most likely, it has contributed to a higher PPV for ACPA, as some ACPA-positive patients would not have been classified as RA with the previous criteria, but were, indeed, classified as such with the inclusion of ACPA in the 2010 criteria.

We have shown that IgM RF and ACPA are increasingly ordered and often repeated several times (Figure 1 and Figure 3). A great strength of this study is the large population, which consists of 60,300 individuals, of whom 5% had RA (Figure 2).

RA has a low prevalence in primary care, and due to the low specificity of IgM RF, about nine out of ten positive tests are false positives compared to seven out of ten for ACPA, as the PPV is 12% and 30%, respectively (Table 1). The NPV is equal at 99% for ACPA and IgM RF alike, despite the slightly higher sensitivity of IgM RF (Table 1). Predictive values depend on the prevalence of the disease in the tested population. Due to a higher prevalence of RA at rheumatologic departments compared to primary care, the PPVs for both tests are higher at rheumatologic departments. NPVs are lower in rheumatologic departments owing to the fact that 32% of the individuals with RA were seronegatives (both ACPA and IgM RF-negative) (Table 1, Figure 2). A nested case control study with age and sex-matched controls performed on RA pre-patients in a tertiary care setting found that the PPV was 82% for the ACPA test and 52% for IgM RF test [16]. However, this was a case control study, which does not represent a routine setting, and the results are therefore not immediately applicable to everyday practice. Other studies have examined the PPV for ACPA and IgM RF in cohorts focusing on early RA and report similar findings [17]; but to the best of our knowledge, this is the first paper describing and comparing predictive values in primary care and rheumatologic departments in a real-life setting.

Few patients converted from negative to positive (IgM RF 5%; ACPA 2%) or from positive to negative (IgM RF 6%; ACPA 3%) (Figure 3, Table 1). Similar results have been found for ACPA in a Swedish cohort study [18]. The low percentage of seroconversions from negative to positive indicates that these biomarkers are present at the time of initial testing with few exceptions. Hence, the value of retesting is marginal, which should be kept in mind before repeating a test in primary care. The low percentage of seroconversions from positive to negative indicates that seropositive individuals remain positive. Among patients who seroconverted from positive to negative, 80% were low positives. Low levels of IgM RF and ACPA are less specific for RA than high concentrations [19]. Serology is only useful during the diagnostic workup; when a RA diagnosis has been reached, there is no reason to repeat the tests [1].

In primary care, ANA was ordered concomitantly with ACPA and/or IgM RF in 32% of the individuals. Testing for ANA is not recommended when suspecting RA but may be useful when suspecting a connective tissue disease [10]. Despite frequent testing, only 0.3% of the individuals were diagnosed with both RA and a simultaneous connective tissue disease. In primary care, the population is unselected, and most positive results will be false positives, yet an increasing number of concomitant ANA tests are ordered (Figure 1) [10]. RA and connective tissue diseases usually have different

clinical presentations and overlap syndromes are rare. Concomitant testing of ANA with IgM RF and/or ACPA to the extent seen in 2016 seems excessive (Figure 1).

The incidence of RA in Denmark is not increasing. However, during the study period, we observed an increase in tests ordered (Figure 1) [20]. There are several potential explanations for this; one is increased awareness of the disease and others are increased demand from patients and a more defensive diagnostic approach from physicians. A thorough medical history and examination is the best screening for rheumatoid diseases and cannot be replaced by serology [10]. When interpreting test results, it is important to note the high amount of false positive tests in primary care as well as the low sensitivity of both tests, which must not delay referral of patients with positive objective findings. When ordering tests in primary care on suspicion of RA, it is worth considering that ACPA outperforms IgM RF owing to markedly higher PPVs and similar NPVs (Table 1).

CONCLUSIONS

Our findings suggest that the ACPA test is a better predictor and should be preferred when suspecting RA in primary care as it has a similar NPV but a better PPV than IgM RF. Seroconversion is rare and although often practiced, it is only rarely relevant to retest. ANA testing is not recommended when suspecting RA.

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