Autoantibodies in early rheumatoid arthritis – Brasília cohort – results of a three-year serial analysis

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ABSTRACT

The diagnostic and prognostic value of the serial measurement of antibodies, such as rheumatoid factor (RF), anti-cyclic citrullinated peptide (anti-CCP), and anti-citrullinated vimentin (anti-Sa) antibodies, has not been defined in early rheumatoid arthritis (ERA). **Objectives:** To prospectively assess the presence of RF, anti-CCP, and anti-Sa in ERA patients. **Patients and methods:** Forty ERA (less than 12 months) patients of the Brasília cohort were evaluated and followed up for three years. Both clinical and demographic data were recorded, in addition to the results (ELISA) of RF (IgM, IgG, and IgA), anti-CCP (CCP2, CCP3, and CCP3.1), and anti-Sa at the baseline assessment and after 3, 6, 12, 18, 24 and 36 months of follow-up. The results were compared by use of Student *t* test and paired *t* test. **Results:** The patients' mean age was 45 years, and 90% of them were female. At the time of diagnosis, RF was identified in 50% of the patients (RF IgA, 42%; RF IgG, 30%; and RF IgM, 50%), anti-CCP in 52.5% (no difference between CCP2, CCP3, and CCP3.1), and anti-Sa in 10%. After three years, no difference was observed in RF and anti-CCP prevalence, but anti-Sa increased to 17.5% (P = 0.001). **Conclusion:** Repeated RF and anti-CCP measurement, including different isotypes, during three years of follow-up showed no significant changes. The third generation of anti-CCP assays did not increase the diagnostic value of the second-generation assays.

Keywords: rheumatoid arthritis, rheumatoid factor, citrulline, vimentin.

INTRODUCTION

Rheumatoid arthritis (RA) still remains a chronic disease with potential for irreversible bone and cartilage damages, resulting in high costs to both the individual affected and society.

The generalization of the "initial or early RA" (ERA) concept and the existence of a "therapeutic window of opportunity", period in which the institution of the adequate therapy for disease determines a better clinical outcome, have defined the idea that early diagnosis and treatment may change the course of the disease.[1]

Until now, the value of the serial analysis of serologic markers, such as rheumatoid factor (RF), anti-cyclic citrullinated peptide antibodies (anti-CCP), and anti-citrullinated vimentin (anti-Sa) antibodies, has not been established in the assessment of patients diagnosed with ERA. It is very important to establish the behavior of serologic markers over time, both individually and in combination, because it can validate

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the need for their routine measurement, including repeated measurements during follow-up.

This study aimed at prospectively evaluating the behavior of the RF, anti-CCP and anti-Sa serologic markers during the three-year follow-up of a cohort of ERA patients (symptoms for less than 12 months), the Brasília cohort.

PATIENTS AND METHODS

Data shown are part of the Brasília cohort, a prospective incident cohort study, in which 40 consecutive patients diagnosed with ERA were evaluated. The patients were regularly followed up for 36 months from diagnosis, which was established at the Early Rheumatoid Arthritis Clinic of the Hospital Universitário de Brasília, at Brasília, DF, Brazil.

ERA was defined as the occurrence of joint symptoms compatible with the disease (inflammatory joint pain and edema, followed or not by morning stiffness or other manifestations suggesting inflammatory joint disease, according to the evaluation of a single observer), lasting more than six weeks and less than 12 months, regardless of meeting the American College of Rheumatology (ACR) classification criteria[2] – although, as shown in the results, all our patients met the ACR classification criteria.

The titration of serologic markers was performed at the baseline assessment and then serially for 36 months (assessments at 3, 6, 12, 18, 24, and 36 months).

The RF (IgG, IgM, and IgA) was measured by using the Quanta Lite™ RF IgA ELISA, Quanta Lite™ RF IgG ELISA, and Quanta Lite™ RF IgM ELISA assays (INOVA Diagnostics, CA, USA), according to the producer's protocol. Values over 15 UI/mL (RF IgM and IgA) and 20 UI/mL (RF IgG) were considered positive cut-off points.

The anti-CCP was measured by using the Quanta Lite™ CCP IgG ELISA, Quanta Lite™ CCP3 IgG ELISA, and Quanta Lite™ CCP3.1 IgG/IgA ELISA assays (INOVA Diagnostics, CA, USA), according to the producer's protocol. The serum of each patient was initially diluted to 1:100 in a diluent sample. If the result of a sample was greater than 2.5 optical density (OD), it was retested with 1:500 and 1:2,500 dilutions, and the resulting value unit was multiplied by the dilution factor. The results of all assays were expressed in units (U) and were as follows: negative, < 20 U; weakly positive, 20–39 U; moderately positive, 40–59 U; and strongly positive, ≥ 60 U.

The assay to detect anti-Sa was conducted on original plates developed by the McGill University Autoimmune Research Laboratory [bovine myelin basic protein (MBP) ELISA assay].[3] The results, calculated and expressed in units, were as follows: negative, < 20 U; questionable, 21–79 U; and positive, ≥ 80 U.

During all follow-up, patients received the standard type of treatment used in the service, including traditional disease-modifying antirheumatic drugs (DMARDs) and/or biological-response modifiers, as needed, but always within a standardized sequence.

To detect differences between two means, Student *t* test or paired *t* test were used for normal distribution samples, considering mean and standard deviation values. To nonparametric variables, Wilcoxon or Mann-Whitney tests were used, considering the mean value and interquartile range. The significance level of 5% was adopted.

This study was approved by the Ethics Committee in Research of the Medical School of the Universidade de Brasília.

RESULTS

Studied population's characteristics

Both demographic and clinical characteristics of the Brasília cohort have already been published.[4]

In this subgroup of 40 patients diagnosed with ERA and followed up in the Brasília cohort, the female gender (36 patients, 90%) and the white ethnic group (14 patients, 35%) prevailed. The mean age was 45.3 years (21–71). The mean duration of the joint symptoms at the time of diagnosis was 27 weeks

Table 1
General characteristics of ERA patients at the baseline assessment (n = 40)

Characteristic	n
Age (years)	45.37 (± 12.01)
Gender	
Male	4 (10%)
Female	36 (90%)
Ethnic group	
White	14 (35%)
White/black	13 (32.5%)
White/Indian	11 (27.5%)
Black	1 (2.5%)
Black/Indian	1 (2.5%)
Education (years)	7.65 (± 5.02)
Disease duration (weeks)	27 (± 15.6)
Current or previous tobacco smoking	5 (12.5%)
DAS 28	6.86 (± 1.07)
HAQ	1.89 (± 0.78)
Radiographic erosion	21 (52.5%)

DAS 28: 28 Joint Disease Activity Score; HAQ: Health Assessment Questionnaire.

(± 15.6), and 13 patients (32.5%) had symptoms for less than 12 weeks at the diagnosis. Most patients (34, representing 85%) had not undergone previous treatment for RA by the time of the baseline assessment. All patients met the American College of Rheumatology classification criteria at the initial assessment. The general characteristics are summarized in Table 1.

Brasília cohort patients were followed up at a public hospital, with all medications given free of charge. There was no loss to follow-up in three years of study.

Autoantibodies

The baseline laboratory characteristics of the Brasília cohort have been previously published.[5] Tables 2 and 3 summarize the frequency of autoantibodies measured at baseline and during the three-year follow-up.

Table 2
Baseline serologic characteristics of ERA patients (n = 40)

Serology	n (%)/titer (UI/dL) mean (± SD)
RF (any isotype)	21 (52.5%)
RF IgM	20 (50%)/95 (± 73.2)
RF IgG	12 (30%)/69.1 (± 41.1)
RF IgA	17 (42.5%)/70 (± 54.8)
RF IgM+ IgG+ IgA+	10 (25%)
RF IgA+ IgM+ IgG–	6 (15%)
RF IgM+ IgG– IgA–	3 (7.5%)
RF IgA+ IgM– IgG–	2 (5%)
Anti-CCP (any technique)	21 (52.5%)
CCP2	19 (47.5%)/533 (± 1,014.7)
CCP3	21 (52.5%)/1,065 (± 1,769.7)
CCP3.1	21 (52.5%)/1,209 (± 1,991.3)
Anti-Sa	4 (10%)/209.16 (± 206.54)

Rheumatoid factor

At the baseline assessment, out of the 40 patients, 21 (52.5%) were positive for at least one RF serotype, 17 patients (42.5%) were positive for RF IgA, 12 (30%) for RF IgG, and 20 (50%) for RF IgM.

Among those positive for RF, the mean titers at the baseline assessment were as follows: RF IgA, 70 UI/dL (± 54.81); RF IgG, 69.1 UI/mL (± 41.09); and RF IgM, 95 UI/mL (± 73.22).

Sixteen patients (40% of the total sample and 76.19% of those positive for at least one RF serotype) were positive for more than one serotype. Ten patients (25% of the total sample and 47.61% of those positive for at least one RF serotype) were positive for the three RF serotypes. Two patients (5% of the total sample and 9.52% of those positive for at least one RF serotype) were positive only for RF IgA. No patient was positive only for RF IgG.

After a three-year follow-up, no significant change in the positivity profile for RF was observed among the 40 patients prospectively analyzed. Twenty patients (50%) remained positive for at least one RF serotype, 15 (37.5%) were positive for RF IgA, 12 (30%) for RF IgG, and 17 (42.5%) for RF IgM ($P > 0.05$ for all, *t* test, regarding the baseline assessment).

Among those with positive serology for RF, the mean titers after the three-year follow-up were as follows: RF IgA, 108.86 UI/dL (± 78.54); RF IgG, 62.91 UI/mL (± 55.09); and RF IgM, 114.29 UI/mL (± 67.93). The RF IgA and RF IgM titers were significantly higher after the three-year follow-up as compared with those of the baseline assessment ($P = 0.002$ for RF IgA and $P = 0.003$ for RF IgM, paired *t* test). No significant change was observed in the RF IgG titers ($P > 0.05$, paired *t* test).

Thirteen patients (32.5% of the total sample and 65% of those positive for at least one RF serotype) were positive

Table 3
Serial measurements of RF, anti-CCP, and anti-Sa titers at baseline assessment and during the three-year follow-up

	RF IgM	RF IgG	RF IgA	CCP2	CCP3	CCP3.1	Anti-Sa
Baseline	20 (50%)/96	12 (30%)/69.1	17 (42.5%)/70	19 (47.5%)/533	21 (52.5%)/1,065	21 (52.5%)/1,209	4 (10%)/209.16
3 m	19 (45%)/94.6	9 (22.5%)/62.4	17 (42.5%)/66.5	19 (45%)/567.68	21 (52.5%)/1,093.33	21 (52.5%)/1,153.47	3 (7.5%)/319
6 m	17 (42.5%)/98.9	8 (20%)/66.25	16 (40%)/73.56	20 (50%)/637.9	21 (52.5%)/1,233	22 (55%)/1,308.31	5 (12.5%)/197.4
12 m	18 (45%)/104.5	9 (22.5%)/72.44	15 (37.5%)/65.26	18 (45%)/721.5	20 (50%)/1,393.75	21 (52.55)/1,436.9	6 (15%)/242.8
18 m	17 (42.5%)/101.94	9 (22.5%)/63.44	15 (37.5%)/100	15 (37.5%)/559.73	20 (50%)/1,029.4	20 (50%)/1,109.8	4 (10%)/358.5
24 m	17 (42.5%)/120.9	13 (32.5%)/60.53	17 (42.5%)/86.05	16 (40%)/649.25	19 (47.5%)/1,165.73	18 (45%)/1,593.9	4 (10%)/359
36 m	17 (42.5%)/114.29	12 (30%)/62.91	15 (37.5%)/108.86	18 (45%)/583.72	20 (50%)/1,207.63	20 (50%)/1,413.2	7 (17.5%)/274.14
Paired *t* test (baseline vs. 36 m)	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P = 0.01

for more than one serotype. Eleven patients (27.5% of the total sample and 55% of those positive for at least one RF serotype) were positive for the three RF serotypes. Three patients (7.5% of the total sample and 15% of those positive for at least one RF serotype) were positive only for RF IgA, and four patients (10% of the total sample and 20% of those positive for at least one RF serotype) were positive only for RF IgM and negative for the other serotypes. No patient was positive only for RF IgG.

One patient (2.5% of the total sample and 5% of those positive for at least one RF serotype) was positive for RF IgG and IgM, but negative for RF IgA, and another patient was positive for RF IgA and IgM and negative for RF IgG. No patient was positive for RF IgA and IgG and negative for IgM.

Regarding the changes in the positivity profile for the different serotypes during the three-year follow-up, four patients who were positive for RF IgA became negative, while two others who were negative became positive. A patient who was positive for RF IgG became negative, and three others who were negative became positive after the three-year follow-up. Three patients who were positive for RF IgM became negative, while one who was negative became positive.

Anti-cyclic citrullinated peptide antibodies (anti-CCP)

Regarding anti-CCP antibodies, at the baseline assessment, 21 of the 40 patients (52.5%) were positive for at least one technique used (CCP2, CCP3 or CCP3.1). By using ELISA 2 technique (CCP2), 21 patients (52.5%) were negative, four (10%) were weakly positive, and 15 (37.5%) were strongly positive. When ELISA 3 technique (CCP3) was used, 19 patients (47.5%) were negative, two (5%) were weakly positive, three (7.5%) were moderately positive, and 16 (40%) were strongly positive. By using ELISA 3.1 technique (CCP3.1), 19 patients (47.5%) were negative, two (5%) were weakly positive, two (5%) were moderately positive, and 17 (42.5%) were strongly positive.

Among those with positive serology for anti-CCP, the mean values obtained at the baseline assessment were as follows: with the CCP2 technique, 533 UI/dL (± 1,014.67); with the CCP3, 1,065 UI/mL (± 1,769.73); and with the CCP3.1, 1,209 UI/mL (± 1,991.28) ($P > 0.05$).

At the baseline assessment, 20 patients were positive for anti-CCP by use of more than one technique, while 18 patients (45% of the total and 90% of the positive ones) were positive by use of three techniques. Two patients (5% of the total and 10% of the positive ones) were positive for anti-CCP3 and anti-CCP3.1, and negative for CCP2 (weakly positive for CCP3 and CCP3.1). After the three-year follow-up, no significant change was observed in the anti-CCP positivity profile. Twenty-one patients (52.5%) remained positive by use of at least one technique. By use of the CCP2 technique, 22 patients (55% of the total) were negative, two (5%) were weakly positive, one (2.5%) moderately positive, and 15 (37.5%) were strongly positive. When CCP3 technique was used, 20 patients (50%) were negative, one (2.5%) was weakly positive, three (7.5%) were moderately positive, and 16 (40%) were strongly positive. By use of CCP3.1 technique, 20 patients (50%) were negative, one (2.5%) was weakly positive, two (5%) were moderately positive, and 17 (42.5%) were strongly positive.

Among the patients with positive serology for anti-CCP, the mean values obtained after the three-year follow-up were as follows: by use of the CCP2 technique, 583.72 UI/dL (± 717.68); by use of the CCP3 technique, 1,207.63 UI/mL (± 1,768.31); and by use of the CCP3.1 technique, 1,413.2 UI/mL (± 2,156.69). No significant difference in the three techniques was observed in the anti-CCP titers ($P > 0.05$, paired *t* test).

After the three-year follow-up, 21 patients were positive for anti-CCP by use of more than one technique, while 17 patients (42.5% of the total and 80.95% of the positive ones) were positive by use of the three techniques. Three patients (7.5% of the total and 14.28% of the positive ones) were positive for anti-CCP3 and anti-CCP3.1, and negative for CCP2 (weakly positive for CCP3 and CCP3.1). One patient (2.5% of the total and 4.76% of the positive ones) was positive for CCP2 and CCP3.1 (low titers), and negative for CCP3.

The changes in the positivity profile for different serotypes during the three-year follow-up by using the CCP2 technique were as follows: one patient with negative serology became weakly positive; two positive patients (one weakly positive and another strongly positive) became negative; one weakly positive patient became moderately positive; and one weakly positive patient became strongly positive. Changes in the positivity profile by using the CCP3 technique were as follows: two patients with weakly positive titers became negative; one patient with weakly positive titers and another with moderately positive titers became strongly positive; and two patients with strongly positive titers became moderately and weakly positive. Changes in the positivity profile by using the CCP3.1 technique were as follows: one patient who was initially negative became moderately positive; two patients (one weakly positive and one moderately positive) became negative; one weakly positive patient became strongly positive; and another with strongly positive serology at baseline became weakly positive after the three-year follow-up.

Anti-citrullinated vimentin (anti-Sa)

Regarding anti-Sa antibodies, at the baseline assessment, of the 40 patients prospectively followed up in the Brasília cohort, 34 (85%) were negative for anti-Sa, four (10%) were positive, and two (5%) had a questionable result.

Among those with positive serology, the mean titer obtained at baseline was 209.16 UI/dL (± 206.54).

After the three-year follow-up, 32 individuals (80%) were negative for anti-Sa, one (2.5%) had a questionable result, and seven (17.5%) were positive. The anti-Sa positivity after three years was significantly higher as compared with that at baseline ($P = 0.01$, paired *t* test).

Among those with positive serology after a three-year follow-up, the mean anti-Sa value was 274.14 UI/dL (± 215.57). No significant difference was observed as compared with the baseline assessment ($P > 0.05$, paired *t* test).

Changes in the positivity profile for anti-Sa after the three-year follow-up were as follows: three patients with negative serology became positive; one positive patient became negative; and one patient with a questionable serology became positive.

All positive patients for anti-Sa were also positive for anti-CCP or RF.

DISCUSSION

This is an important study showing that the simultaneous and serial measurement of various autoantibodies and their different isotypes in early arthritis does not alter significantly after a three-year follow-up in a population with considerable ethnic variety and low tobacco smoking rate.

At the baseline assessment, 50% of the patients of our cohort were positive for at least one RF serotype, similarly to other studies that used ELISA,[6,7] including the results of the meta-analysis by Nishimura et al.[8]

Although controversial, it has been suggested that both RF IgM and RF IgA and IgG are significantly associated with the diagnosis of RA.[9] In our study, RF IgM was found in around 50% of the patients diagnosed with RA with symptoms lasting less than 12 months, IgA in 42%, and IgG in 30%, a rate similar to those mentioned in other studies, such as that by Vittecoq et al.[10] Those authors have described the presence of RF IgM in 51%, RF IgA in 36%, and RF IgG in 32% of the patients diagnosed with RA lasting less than two years. The isotypes positivity seems to vary according to the population studied.[10,11]

The RF IgM is an useful marker to differentiate patients with polyarthritis who will progress to RA.[10,12–17] But the diagnostic properties of RF IgA and IgG are questionable.[10,17,18] In our study, the search for RF IgA and RF IgG serotypes did not increase the positivity frequency of RF, and, thus, did not contribute to the diagnosis of RA.

After the three-year follow-up, positivity for the three RF serotypes assessed, as well as their titers, remained similar to the initial values, which is in accordance with the findings of other studies,[8,19] confirming the small importance of repeating those tests.

Brasília cohort had 50% of patients positive by use of at least one technique (CCP2, CCP3 or CCP3.1), and most of them were strongly positive with the three techniques. The positivity percentage for anti-CCP in our study was similar to that reported by many other studies involving ERA patients. In a systematic literature review, the combined analysis of publications regarding over 2,000 patients with undifferentiated early arthritis showed a 23% prevalence of anti-CCP (ELISA second generation). This prevalence increased to 51% in over 1,000 patients who met the RA classification criteria after a mean 18-month follow-up.[20]

In our cohort, the anti-CCP prevalence was approximately the same (considering positive CCP by use of any of the three analyzed techniques) of that of RF, which was similar to that of other studies published on the subject.[21,22] According to some authors, CCP2 seems to be as sensitive as RF IgM and more specific. Its advantage is to detect antibodies in approximately 15% of RA patients who are negative for RF.[23–31] However, Nishimura et al.,[8] in their meta-analysis about anti-CCP and RF accuracy for RA, have concluded that isolated positivity for anti-CCP is more specific than isolated positivity for RF IgM in the RA diagnosis.

Nevertheless, it is important to emphasize that when each technique was tested in isolation, the anti-CCP prevalence was approximately the same by use of any of the three techniques (40%, increasing to 50% when the three techniques were used together). Therefore, in isolation, CCP2, CCP3 and CCP3.1 showed, in our study, anti-CCP prevalence below that of RF IgM and similar to that of RF IgA, which differs from many studies previously reported.[23–31] The sensitivity, specificity and cost-benefit difference between the three techniques to detect anti-CCP is still a controversial subject in the literature, requiring further studies in different populations.[32]

In 2005, a third generation of anti-CCP (CCP3) was made available for RA laboratory diagnosis. Such assay was then claimed to recognize additional citrullinated epitopes that could not be identified by second-generation assays (CCP2), with sensitivity 5% greater than that of CCP2 and same specificity.[33] The CCP3 assay has been assessed by Santiago et al.[34] and Wu et al.,[35] being considered more sensitive than CCP2,

and with the same specificity. Anjos et al.[32] have reported in a population of 70 RA patients from Southern Brazil that both CCP2 and CCP3 had a good diagnostic performance and same specificity, but CCP3 was 4.3% more sensitive than CCP2. Nevertheless, other authors have reported a very similar diagnostic performance of the CCP2 and CCP3 assays.[36,37]

The CCP3.1 assay used in our study (INOVA) is a combination that detects IgA antibodies, in addition to the usual IgG antibodies. That would theoretically improve the sensitivity of the method, because some RA patients, who lack IgG antibodies, have IgA antibodies against CCP3.[38] However, Bizzaro et al.,[39] comparing 11 different laboratory techniques for detecting anti-CCP, have reported a slight difference of results between INOVA's CCP2 and CCP3 (sensitivity of 64% and 67%, respectively) and no difference between CCP3 and CCP3.1. Thus, those authors have suggested that the combination of IgA and IgG antibodies do not improve the performance of the test, similarly to that which was observed in our cohort.

Chibnik et al.[40] have reported that anti-CCP titers and their oscillation are important in the pre-development phase of RA – the greater the titers, the shorter the time interval for disease onset. The anti-CCP titers have gradually increased until the onset of typical RA symptoms, and then stabilized. Rantapää-Dahlqvist et al.[41] had already suggested that anti-CCP titers increase before disease onset. Bos et al.,[42] in their cohort of 188 consecutive patients diagnosed with RA and treated with adalimumab, have assessed the relative changes in anti-CCP levels and have not observed substantial modifications between initial and final anti-CCP positivities, similarly to that which was observed in our cohort. In accordance with those authors, our data indicate that anti-CCP antibodies can be qualitatively stable RA markers, not associated with disease activity.[42]

In our cohort, less than 15% of the patients had anti-Sa antibodies at the baseline assessment, a value lower than that reported by Boire et al.[3] (28% of their cohort of 165 patients with early polyarthritis) and by Vossenaar et al.[43] (40% of 87 sera of patients with established RA). However, the positivity percentage for anti-Sa increased from 10% to 18% by the end of the follow-up, a statistically significant difference that may be associated with more established disease.

The mean anti-Sa titers found in our cohort ranged from 200 to 300 UI/dL, values similar to those reported by other authors,[3,44] despite the low number of publications about that topic. Variations in the anti-Sa titers have been evidenced in the studies by Innala et al.[44] and Ménard[45] according to disease activity and response to treatment, while in our cohort they remained stable during the three-year follow-up.

CONCLUSIONS

In conclusion, the measurement of different RF isotypes does not increase the positivity frequency of RF in early arthritis, and, thus, that measurement does not contribute to diagnosis.

The RF stability observed over time does not justify repeated RF measurements during the ERA progression. The percentage of patients who had positive anti-CCP, as well as their titers, remained stable over time, which also does not justify the request for serial anti-CCP measurements. The techniques used to detect anti-CCP (CCP2, CCP3 and CCP3.1) did not differ, suggesting that third-generation assays have contributed to neither ERA diagnosis nor ERA follow-up. The anti-Sa measurement was not useful in diagnosing ERA as compared with that of RF and anti-CCP.

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