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Laboratory characteristics of a cohort of patients with early rheumatoid arthritis

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ABSTRACT

Introduction/Objective: To characterize a population of patients with early rheumatoid arthritis (RA) according to laboratory aspects, comparing it with other similar cohorts. **Methods**: Data presented are part of a prospective incident cohort study that evaluated 65 patients with early RA, followed for 36 months from the diagnosis at Early Rheumatoid Arthritis Clinic of *Hospital Universitário de Brasília (HUB)*. We recorded demographics, clinical, and laboratory data relevant to the cohort initial assessment, including red blood cells, evidence of inflammatory activity, and presence of autoantibodies (rheumatoid factor (RF)), cyclic citrullinated peptide antibodies (anti-CCP), and antivimentin citrullinated (anti-Sa). **Results**: There was a preponderance of female (86%) with mean age of 45.6 years. Twelve patients (18.46%) had laboratory diagnosis of anemia (hemoglobin < 12 g / dL). Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) were above the reference value for 51 (78.46%) and 46 (70.76%) patients, respectively. Thirty-two patients (49.23%) were positive for at least one of the RF isotypes, and 28 patients (43.07%) were positive for IgA RF, 19 (29.23%) for IgG, and 32 (49.23%) for IgM RF, respectively; 34 patients (52.30%) were positive for at least one of the techniques used in investigation of anti-CCP (CCP2, or CCP3, or CCP3.1), while 9 (13,85%) were positive for anti-Sa. **Conclusions**: The laboratory characteristics of patients enrolled in this Brazilian cohort are similar in many respects to those of North-American, European, and Latin-American cohorts previously published.

Keywords: early rheumatoid arthritis, RF, anti-CCP, anti-Sa, cohort, Brazilian population, early arthritis.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic condition with irreversible potential for bone and cartilage damage, which, despite recent advances regarding its management, remains costly for the affected individual and society.[1]

Although it is well known that RA has varying characteristics according to the population affected, most available information, especially with regard to early RA, comes from Europe and the United States, with few studies in Latin American populations.[2-4]

There is no Brazilian cohort study involving patients with early RA. Thus, the laboratory features of early RA in the Brazilian population are unknown, as well as if there is difference from other populations previously studied.

The aim of this study was to characterize a population of patients with early RA according to laboratory tests, including hemoglobin levels; evidence of inflammatory activity by erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP); presence of autoantibody rheumatoid factor (RF), cyclic citrullinated peptide (CCP) antibody, and antivimentin citrullinated (anti-Sa).

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PATIENTS AND METHODS

Data presented are part of a prospective incident cohort study, which evaluated consecutive patients with early RA diagnosis, regularly followed-up for 36 months since diagnosis performed at Early Rheumatoid Arthritis Clinic of *Hospital Universitário de Brasília (HUB)*.

Early RA was defined as the occurrence of joint symptoms compatible with the disease (pain and swelling joints of inflammatory pattern, with or without morning stiffness or other manifestations suggestive of inflammatory joint disease, as assessed by a single observer) and lasting more than six weeks and less than 12 months, regardless of meeting the qualifying criteria of the American College of Rheumatology (ACR).[5]

We recorded demographic and clinical data, as well as laboratory parameters relevant to the cohort initial assessment. Complete blood count and evidence of inflammatory activity (ESR and CRP) were performed at the Laboratory of Clinical Pathology of *Hospital Universitário de Brasília.*

Measurement of RF (IgG, IgM and IgA) was performed at INOVA Diagnostics, Inc., San Diego, California, United States, using "Quanta Lite™ RF IgA ELISA", "Quanta LiteTM RF IgG ELISA", and "Quanta Lite™ RF IgM ELISA" assays (Inova Diagnostics, CA, USA), according to manufacturer protocol. We considered as cutoff points for positivity values higher than 15 IU/mL (IgM and IgA) and 20 UI/mL (FR IgG).

Anti-CCP was studied using "Quanta Lite™ CCP IgG ELISA", "Quanta Lite™ CCP3 IgG ELISA" and "Quanta Lite™ CCP3.1 IgG/IgA ELISA" (Inova Diagnostics CA, USA), according to manufacturer protocol. The serum of each patient was initially diluted at 1:100 in sample diluent. If the result of a sample had an optical density greater than 2.5, it was retested with dilutions at 1:500 and 1:2500, and the unit resulting value multiplied by the dilution factor. Results were expressed in units (U), with < 20 U considered negative, 20-39 U weak positive, 40-59 U moderate positive, and ≥ 60 U strong positive, for all tests.

The test for detection of anti-Sa was performed on the original plates developed by McGill University Autoimmune Research Laboratory.[6] The results were calculated and expressed in units: < 20 U negative, 21-79 U doubtful, and ≥ 80 U positive. The samples were processed for this purpose in the Division of Rheumatology, McGill University Health Center, Quebec, Canada.

Patients received standard treatment regimen used in the service, including the traditional disease modifying anti-rheumatic drugs (DMARDs) or biological response modifier therapy, according to necessity.

Descriptive statistics was performed for all assessed variables. To detect differences between two means, the Student's t-test or paired t-test for samples of normal distribution was used, considering the average values and standard deviation.

For cases in which normality was rejected, we applied the nonparametric Wilcoxon test or Mann-Whitney test taking into account the median value and the interquartile range.

The study was approved by the Research Ethics Committee of *Faculdade de Medicina da Universidade de Brasília (CEP FM-UnB)*. Project registration: CEP-FM 028/2007.

RESULTS

Characteristics of study population

Initially, we evaluated 65 patients diagnosed with early RA, mean age of 45-64 years (± 14-51), ranging from 26 to 71 years. There was a predominance of female (56 patients, 86.15%) and white ethnic group (31 patients, 47.69%). The duration of joint symptoms before diagnosis was on average 32 weeks (± 15.41). Although the ACR criteria have not been considered for early RA definition in this study, all 65 patients met at least four criteria in the first assessment. Table 1 summarizes these population demographic and clinical characteristics.

Red cell counts

In the initial evaluation, the mean hemoglobin value of 65 patients was 12.73 g/dL (± 1.06). Twelve patients (18.46%) had laboratory diagnosis of anemia (hemoglobin < 12 g/dL), with mean hemoglobin value of 10.91 g/dL (± 1.21).

Evidence of inflammatory activity

As for VHS, 51 patients (78.46%) had values above those of reference test, with the average value of 40.43 mm in the first hour (± 16.97). The level of CRP showed higher values than the reference test (1.0 mg/dL) in 46 patients (70.76%), and the average value found was 2.46 mg/dL (± 1.72).

Autoantibodies

Rheumatoid factor

In the first assessment, among the 65 patients, 32 subjects (49.23%) were positive for at least one of the RF isotypes, and 28 patients (43.07%) were positive for RF IgA, 19 (29.23%) for IgG, and 32 (49.23%) for IgM RF, respectively. Among

Table 1
Patients with early rheumatoid arthritis assessed at *Hospital Universitário de Brasília* according to their general characteristics (baseline assessment, n: 65)

Characteristics		n (±) or n (%)
Age (years)		45.64 (± 14.51)
Sex	Male	9 (13.80%)
	Female	56 (86.15%)
Ethnic group	White	31 (47.69%)
	White/black	18 (27.69%)
	White/native	13 (20%)
	Black	1 (1.53%)
	Black/native	2 (3.07%)
Education (years)		8.3 (± 4.95)
Duration of disease (weeks)		32 (± 15.41)
Current or previous smoking		7 (10.76%)
DAS 28		6.79 (± 1.11)
HAQ		1.87 (± 0.81)
Radiographic erosion		31 (47.69%)

Variables are represented in mean absolute value (± standard deviation) or n (%).
DAS 28: disease activity index; HAQ: Health Assessment Questionnaire.

those with positive serology for RF, the mean titers of IgA at baseline were 76 IU/dL (± 56.17), RF IgG 71 IU/mL (± 51.21), and RF IgM 105 IU/mL (± 73.13).

Twenty-eight patients (43.07% of the total sample and 87.50% of those positive for at least one of the RF serotypes) were positive for more than one serotype. Seventeen patients (26.15% of the total sample and 53.12% of those positive for at least one of the RF serotypes) were positive for all three RF serotypes. Two patients (3.07% of the total sample and 6.25% were positive for at least one of the RF serotypes) were positive only for IgA RF, and six patients (9.23% of the total sample and 18.75 % of those positive for at least one of the RF serotypes) were positive only for IgM and negative for the other serotypes. No patient tested positive only for IgG. Five patients (7.69% of total sample and 15.62% of those positive for at least one RF serotype) were positive for RF IgA, IgM and negative for IgG, while two (3.07% of the total sample and 6.25% of those positive for at least one of the RF serotypes) were positive for IgG and IgM but negative for IgA RF. No patient was positive for IgA and IgG or negative for IgM.

Cyclic citrullinated peptide antibodies (anti-CCP)

As for anti-CCP antibodies, 34 patients (52.30% of the total) were positive for at least one of the techniques used in screening (CCP2, CCP3, or CCP3.1). With the use of ELISA 2 (CCP2) technique, 33 patients (50.77% of the total population tested) were negative, five (7.69%) were weak positive, and 27 (41.54%) were strong positive. With ELISA 3 (CCP3) technique, 30 patients (46.15%) were negative, five (7.69%) were weak positive, two (3.08%) were moderate positive, and 28 (43.08%) were strong positive. With ELISA 3.1 (CCP3.1) technique, 31 patients (47.69%) were negative, two (3.08%) were weak positive, three (4.62%) were moderate positive, and 29 (44.62%) were strong positive.

Among those with positive serology for anti-CCP, the average values obtained by CCP2 technique at baseline were 568 IU/dL (± 833.28); by CCP3, 1,148 IU/mL (± 1,584.15); and by CCP3.1, 1,272 IU/mL (± 1,721.97). Titles obtained by the third generation techniques were not significantly higher than those obtained by the second generation technique ($P > 0.05$).

Thirty-two patients (49.23% of the total population and 94.11% of those with positive results for at least one of the techniques) were positive for anti-CCP in more than one technique, while 29 patients (44.61% of total population and 85.29% of those positive for at least one of the techniques) were positive for all three techniques.

Three patients (4.62% of the total population and 8.82% among the positives) were positive for anti-CCP3 and CCP3.1 and negative for anti-CCP2, and one patient (1.53% of total population and 2.94% among positives) was positive for CCP3 and negative for CCP2 and CCP3.1 (in all cases, positive results were weak positive). Two patients (3.08% of total population and 5.88% of the total number of positives) were positive for anti-CCP 3.1 and CCP 2 and negative for CCP3 (in both cases the result by CCP3.1 technique was weak positive). There was no statistical difference between positivity for anti-CCP analyzed by different techniques – CCP 2, CCP 3, and CCP 3.1 ($P > 0.05$).

Antivimentin citrullinated (anti-Sa)

At baseline assessment, of the 65 patients evaluated, 52 (80%) were anti-Sa negative, four (6.15%) had an uncertain outcome, and nine (13.85%) were positive. Among those with anti-Sa positive serology, the mean values obtained at baseline was 370.2 IU/dL (± 263.80). Table 2 summarizes the profile of positivity for RF, anti-CCP, and anti-Sa in 65 patients initially evaluated.

DISCUSSION

The interaction between multiethnic origins, colonial heritage, and immigration patterns in Latin America resulted in rather complex demographic characteristics and in a highly mixed population, varying between different countries in the region, with a wide variability of gene expression. [2-4]

Data on incidence, prevalence, and characteristics of RA in populations of Latin American countries are scarce.[7] In analyzing the results of studies on AR performed in developing countries, one should bear in mind that the disease characteristics can be affected by socioeconomic, demographic, and health systems of these countries.[8]

The characteristics of patients in our cohort were compared with data from other cohorts, American and European, and with preliminary information from GLADAR,[9] prospective, multicenter observational cohort study that evaluated 1,059 patients with early RA, allocated in 46 centers of 14 Latin American countries.[10,11] The Rheumatology Service of HUB/UnB participated in the GLADAR study with 30 patients other than those evaluated in this study.

Hemocytometer

Anemia is a relatively frequent extra-articular manifestation in early RA (6-25%), and seems to correlate with worse joint prognosis,[12] functional disability, need for orthopedic intervention,[13] and mortality.[14] Decreased RBC count is more frequent among male, smokers, patients with high levels of evidence of inflammatory activity, presence of RF, ANA and shared epitope.[14]

Nikolaisen *et al*.[15] investigated the prevalence of anemia in a cohort of 111 consecutive patients with early RA during 74 months of follow-up and found reduced levels of hemoglobin in 25% of patients during the first year of follow-up. In this study, the presence of anemia was associated with higher levels of ESR, CRP, and IL-6, but not the more aggressive joint disease or mortality.

Table 2
Baseline serum characteristics of patients with early rheumatoid arthritis (n: 65)

Autoantibody	n (%)/title (IU/dL) - mean (± SD)
RFs (any isotype)	32 (49.23%)
RF IgM	32 (49.23%)/ 105 (± 73.13)
RF IgG	19 (29.23%) / 71 (± 51.21)
RF IGA	28 (43.07%) / 76 (± 56.17)
RF IgM+ IgG + IgA+	17 (26.15%)
RF IgA+ IgM+IgG-	5 (7.69%)
RF IgM + IgG- IgA-	6 (9.23%)
RF IgA +IgM- IgG-	2 (3.07%)
RF IgM+ IgG+ IgA -	2 (3.07%)
Anti-CCP (any technique)	34 (52.3%)
CCP2	32 (49.23%) / 568 (± 833.28)
CCP3	35 (53.85%) / 1,148 (± 1,584.15)
CCP3.1	34 (52.31%) / 1,272 UI/mL (± 1,721.97)
CCP2 + CCP3 + CCP3.1 +	29 (44.61%)
CCP2 - CCP3 + CCP3.1 +	3 (4.62%)
CCP2 - CCP3 + CCP3.1 -	1 (1.53%)
CCP2 - CCP3 - CCP3.1 +	2 (3.08%)
Anti-Sa	9 (13.85%) / 370.2 (± 263.8)

Variables are represented in mean absolute value (± standard deviation) or n (%).
RF: rheumatoid factor; CCP: cyclic citrullinated peptide antibodies;
anti-Sa: antivimentin citrullinated antibodies.

Evidence of inflammatory activity

More than two thirds of patients in our cohort showed increased evidence of inflammatory activity (ESR and CRP) tested at baseline.

The evidence of inflammatory activity at baseline does not seem to discriminate RA from other early arthritis, and do not predict persistent disease (erosive).[16-18] Tunn and Bacon reported that, among patients of an early RA clinic who developed persistent arthritis, levels significantly higher of ESR was found. However, in this study, ESR had low discriminatory power and poor contribution to patient's long-term prognosis.[19]

Autoantibodies

Rheumatoid factor

At baseline evaluation, about 50% of patients in our cohort were positive for at least one of the RF serotypes, a percentage lower than that reported for GLADAR cohort (76%).[20] It is important to highlight the different methods employed for RF research by GLADAR (Waaler-Rose, nephelometry, ELISA). RF positivity in our study was similar to other studies that used ELISA,[21-22] including the results of Nishimura *et al*. meta-analysis.

In our study, we chose to research RF isotypes IgA, IgG, and IgM. The validity of the RF isotypes research in the assessment of early RA remains questionable. For example, the existence of correlation between titers of different isotypes of RF and the diagnosis of RA is not defined, as well as the relation between the presence of some specific serotype (or more than one) and a worst radiologic prognostic, or the behavior of different RF isotypes over time.[23]

In our population, we observed IgM RF in 50%, IgA in 42% and IgG in 30% of patients diagnosed with RA and symptoms lasting less than 12 months, similar rates to those

referred in other works, such as the work by Vittecoq *et al*., [24] which described the presence of IgM RF in 51%, IgA RF in 36%, and IgG RF in 32% of patients diagnosed with RA with less than two years duration.

IgM RF is a useful marker to discriminate patients with polyarthritis that will evolve or not to RA.[25-30] In contrast, the diagnostic properties of RF IgA and IgG are questionable.[30-32] In our study, the research of RF serotypes IgA and IgG did not increase the frequency of RF positivity and, therefore, does not contribute to RA diagnosis.

Some published studies have evaluated, as in our cohort, the average titles of different RF serotypes in early RA, and our results are similar to those reported by other authors.[23,33]

Cyclic citrullinated peptide antibodies (anti-CCP)

The percentage of positivity for anti-CCP in our study was similar to that reported by several other studies involving patients with early RA. Fifty percent of patients in our cohort were positive for at least one of the techniques used in the assessment (CCP2, CCP3, or CCP3.1), and most were strong positive by the all three techniques. In a systematic literature review, the combined analysis of publications referring to more than 2,000 patients with early undifferentiated arthritis showed a 23% prevalence of anti-CCP (ELISA 2nd generation). This prevalence increased to 51% in over 1,000 patients who met criteria for RA after a mean follow-up of 18 months.[34]

In our cohort, the prevalence of RF and anti-CCP was approximately the same (considering CCP positive for any of the three techniques examined), which was similar to other studies on the subject-matter.[35-36] As reported by several authors, CCP2 appears to be as sensitive as – and more specific than – IgM RF, but their advantage would be the detection of antibodies in approximately 15% of patients with RA who are negative for RF. [37-45] Nishimura *et al*.,[23] in their meta-analysis of published studies on accuracy of anti-CCP and RF for RA, have concluded that positivity for anti-CCP alone is more specific than the isolated positivity for IgM RF in RA diagnosis.

In our cohort, there was no difference between the techniques considered for detection of anti-CCP (CCP2, CCP3, and CCP3.1), and the prevalence of anti-CCP antibodies was almost the same by all three techniques (40%). The difference in sensitivity, specificity, and cost-effective among the three techniques for detection of anti-CCP is still controversial in literature, and studies in different populations are needed.[46]

In 2005, a third generation of anti-CCP (CCP3) became available for laboratory diagnosis of RA. It was reported that these tests would recognize additional citrullinated epitopes, which would not be identified by the second generation test (CCP2), with sensitivity 5% greater than CCP2, maintaining specificity.[47]

CCP3 test was evaluated Santiago *et al*.[48] and Wu *et al*.[49] and found to be more sensitive than the CCP2 while maintaining specificity. Anjos *et al*.[46] reported in a population of 70 patients with RA in southern Brazil that both CCP2 and CCP3 showed good diagnostic performance, with CCP3 4.3% more sensitive than CCP2, while maintaining specificity. However, other authors have reported very similar diagnostic performance between CCP2 and CCP3 tests.[50-51]

The CCP3.1 evaluated in our study (INOVA) uses a conjugate that detects antibodies IgA, as well as the usual IgG antibodies, which in theory would improve the method sensitivity, since some patients with RA have IgA antibodies against CCP3, in the absence of IgG antibodies.[52] Bizzaro *et al*.,[53] however, comparing 11 different laboratory techniques for CCP detection, noted a slight difference in results between CCP2 and CCP3 INOVA (sensitivity 64% and 67%, respectively) and no difference between CCP3 and CCP3.1, suggesting that the combination of IgA and IgG would not improve test performance, similar to what was observed in our cohort.

In our cohort, the titles of CCP obtained by three different techniques were similar. Titles of CCP2 on average tended to be lower than the third generation techniques. We observed high values (on average > 500 IU/dL) with the three techniques, higher than those reported in publications by Lee *et al*.[54] and Papadopoulos *et al*.,[55] two of the few studies on absolute titles of anti-CCP and its correlation with disease progression.

Antivimentin citrullinated (anti-Sa)

Less than 15% of our patients' cohort presented with anti-Sa antibodies in the initial evaluation; this value is less than that reported by Boire *et al*.[6] (28% of their cohort of 165 patients with early polyarthritis) and Vossenaar *et al*.[56] (40% of 87 sera from patients with established RA).

The mean titers of anti-Sa found in our cohort varied from 200 to 300 IU/dL, values similar to those found by other authors,[6,57] although there are few publications on the subject.

CONCLUSIONS

Although the demographic and clinical characteristics of patients enrolled in this Brazilian cohort differ in several aspects from those of North American, European, and Latin American cohorts previously published, our laboratory

findings, including the initial prevalence of autoantibodies, are similar to other populations.

The prevalence of RF and anti-CCP (50%) was similar to that reported in other cohorts of early RA. As the initial positivity for both autoantibodies was similar in our cohort, we infer that, in our specific population, anti-CCP did not aggregate value for the diagnosis of RA in its initial phase.

Additionally, there was no difference between the techniques considered for detection of anti-CCP (CCP2, CCP3, and CCP3.1), suggesting that the third-generation tests did not bring contribution to the diagnosis of early RA. Moreover, the research of anti-Sa was not useful for diagnosis of early RA, compared to RF and anti-CCP.

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