High frequency ultrasonography of the hand versus anti-RA33 evaluation in early rheumatoid arthritis – a pilot study

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Abstract

Aim: Accurate diagnosis and early treatment in rheumatoid arthritis (RA) can lead to a good outcome and a correct management of the disease. We aimed to investigate the prognostic value of anti-RA33 antibodies, by evaluating the relationship with ultrasonographic (US) findings in patients with early RA. **Material and methods:** We performed a prospective study which included 29 patients, diagnosed with early RA according to the ACR/EULAR 2010 criteria and 21 sex and age-matched control subjects. All patients underwent clinical and biological assessment, followed by US examination in grayscale (GS) and power Doppler (PD) at baseline and after 12 months [from the second to the fifth metacarpophalangeal (MCP) and proximal interphalangeal (PIP) joints and wrists (RCC), in dorsal aspect]. The second and fifth MCP joints were scanned also in lateral aspects. **Results:** The initial GS evaluation revealed the presence of synovitis in all 29 patients; PD found at least one joint with a PD grade higher than 1 in 23 patients, higher than 2 in 20 patients, and grade 3 in 6 patients; at 12 months, we revealed the presence of GSUS synovitis in 25 patients and PDUS examination found active synovitis in 12 subjects. In those patients, the anti-RA33 titre was significantly lower compared to those without PDUS active synovitis ($p=0.031$), with a moderately negative correlation ($r=-0.519$, $p=0.0039$). **Conclusions:** The current study shows that anti-RA33 antibodies might constitute an additional tool for diagnosing early RA patients and can help identify patients with mild disease and a low level of active synovitis.

Keywords: early rheumatoid arthritis; anti-RA33 antibodies; ultrasonography; synovitis.

Introduction

In rheumatoid arthritis (RA) an early diagnosis is essential for an optimal management of the disease [1], with a real impact on the patients' quality of life [2]. An accurate diagnosis encloses to identify antibodies with

high specificity and sensitivity for both diagnostic and prognostic purpose [2]. Besides anti-cyclic citrullinated peptide (anti-CCP) and rheumatoid factor (RF), already included in 2010 ACR/EULAR diagnostic criteria [3], new serological markers were tested in order to increase the diagnostic specificity. Among these, anti-RA33 antibodies have emerged as a promising tool, due to their absence or rare presence in non-autoimmune rheumatologic conditions or other autoimmune diseases [4]. Besides the utility in the early diagnostic, the anti-RA33 antibodies improve the real-time assessment of disease activity and help to identify different clinical and pathogenic subsets of RA [5]. Thus, by recognizing the patients with unfavourable prognostic factors, the treatment

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can be optimized using a customized therapeutic agenda [6,7]. There are many unanswered questions on how new immunological markers change during the evolution and how they impact on disease course, but it has been observed that anti-RA33 antibodies are associated with a relatively mild disease.

New imaging techniques have emerged as sensitive and reproducible tools for diagnosis, monitoring and assessing the prognosis in different area of research, and their good performance has been proved by several studies [8-11]. Ultrasonography (US), an imaging method with several advantages that has made it lately indispensable in daily medical practice, has an unquestionable value in RA, and offered new opportunities by defining and outlining certain pathologic aspects regarding synovial inflammation, the characteristic hallmark of this disease [12-14]. ACR/EULAR 2010 classification criteria acknowledged that US findings can be used for the confirmation of joint involvement, as an essential and accurate tool for assessing the presence of synovitis. Besides its diagnostic utility, US assessment has an important role in monitoring the disease, in which case, US findings may constitute a predictor for the therapeutic response. In patients in clinical remission, US can reveal persistent synovitis, which can be predictive for a relapse and future joint damage. Therefore, in early RA, assessing a real remission is the major objective for every rheumatologist and thus, developing an algorithm for the diagnosis and monitoring, enclosing both US and serological markers, can provide benefit for optimal management.

There is emerging data about US role both in evaluating RA disease activity and the disease progression risk [15] and in the same time, there is evidence that anti-CCP antibodies have a high prognostic value. However, a complex immunologic pattern could help predict treatment response. Thus, we aimed to investigate a possible prognostic value of anti-RA33 antibodies by evaluating the relationship with US findings and to identify a cut-off value for the serum titre in a cohort of patients with early RA.

Material and methods

We performed a longitudinal, prospective study, which included a cohort of 29 patients, diagnosed with early RA, according to the ACR/EULAR 2010 criteria [4] that presented in the Rheumatology Department of Emergency County Hospital Craiova, between March 2014-September 2015, with a duration of the symptoms under 12 months. The control group included 21 sex and age-matched subjects, without acute or chronic inflammatory disease, history of connective tissue, or other autoimmune diseases.

The study was performed in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the University of Medicine and Pharmacy of Craiova. All patients provided their written informed consent, after receiving a standard form for US and biologic samples, which mentioned that the results would be used for research purposes.

Patients assessment

The patients' evaluation included clinical examination, laboratory tests and US evaluation performed at 0 and 12 months.

Laboratory tests were performed according to the manufacturer's kit indications. In order to determine anti-RA33 antibodies, we used a Human anti-RA33 Assay kit; venous blood samples were centrifuged at 9700 rotations/minute, for 15 minutes, and the serum obtained was stored at -80 °C until analyze. Anti-CCP antibodies were determined using fluorescence immunoassay, RF by latex method, C reactive protein (CRP) by immunoturbidimetry, and erythrocyte sedimentation rate (ESR) by Westergreen.

Functional status was evaluated using the Health Assessment Questionnaire (HAQ) [16] and disease activity by calculating the 28-Disease Activity Score (DAS) [17], with 4 variables.

Synthetic disease-modifying antirheumatic drugs (sDMARDs) were initiated (Methotrexate for 21 patients, Leflunomide for 4 patients, one patient with Sulfasalazine, and 2 with Hydroxychloroquine), along with variable doses of glucocorticoids (between 8 and 32 mg methylprednisolone) in the RA group.

US evaluation

US was performed from the second to the fifth metacarpophalangeal (MCP) and proximal interphalangeal (PIP) joints and wrists (RC) joints, in dorsal side of both hands. Moreover, the second and fifth MCP joints were scanned also in lateral aspects.

The examiner used a MyLab 25 machine (EsaoteSpA Genoa, Italy) with a 10-18 MHz frequency linear probe, according to EULAR guidelines [18] and noted synovitis both in grayscale (GS) and Power Doppler (PD). PD examinations were carried out using a Doppler frequency of 8.0 MHz and a pulse repetition frequency of 750 Hz [19]. All examinations were performed by an expert sonographer, blinded for clinical and laboratory data, according to the OMERACT-EULAR consent and OMERACT preliminary definitions [20,21]. The scoring systems for joint inflammation considered both synovial fluid and synovial proliferation, indicative of joint inflammation [15]. MCP, PIP, and RC joint effusion and synovitis were subjectively scored in GS from 0 to 3 (0=absence; 1=mild; 2=moderate; 3=marked) (fig 1). The

Fig 1. Dorsal longitudinal GS scan showing scoring of the MCP joint synovitis: Grade 0 – absence of synovitis; B. Grade 1 = mild synovitis; C. Grade 2 = moderate synovitis; D. Grade 3 = marked synovitis. m = metacarpal head; p = proximal phalanx.

intra-articular PD signal was subjectively graded using a semiquantitative scoring system ranging from 0 to 3 (0=absence, no intra-articular PD signal; 1=mild, PD signal due to a single vessel; 2=moderate, PD signal due to confluent vessels; 3=marked, PD signals in more than half of the intra-articular area).

Statistical Analysis

Statistical analysis was performed using GraphPad Prism 5.5. Results are presented as mean $\pm$ SD and data were analyzed using t-test and One-way ANOVA for comparing groups, and Pearson/Spearman's coefficient for evaluating correlations. We considered a level of $p < 0.05$ statistically significant.

Results

The initial clinical and biological findings of the 29 patients included in the study are presented in table I.

RF was positive in 14 patients (48.27%), with a mean value of 86.65 ± 105.7 UI/ml, anti-CCP antibodies were positive in 17 patients (58.62%), with a mean value of 254.0 ± 531.3 UI/ml. For anti-RA33 antibodies, we found a mean value of 0.129 ± 0.130 ng/ml in the RA group and 0.099 ± 0.05 ng/ml in controls ($p = 0.0103$). We considered the threshold for positivity the mean value calculated for the control group (0.099 ± 0.05 ng/ml), after cutting out the minimum and maximum values. Thus, 14 patients (48.27%) were considered to be positive for anti-RA33 antibodies; hence, the diagnostic sensitivity was 40%, the specificity 90%, with a positive predictive value of 0.875, and a negative predictive value of 0.475. The frequency of anti-RA33 antibodies between patients with RF positive, was 21.42% (4 cases), while anti-CCP antibodies were present in 9 cases (64.42%). We found a moderately positive correlation between the RF and anti-CCP antibodies titres ($r = 0.47$, $p = 0.0097$), while for anti-RA33, we found no statistical significant relationship with the presence of RF or anti-CCP antibodies.

Table I. Baseline characteristics of the patients

Characteristics	
Age (years)	41.72 $\pm$ 9.38
Sex (women)	28 (96.55)
Disease duration (months)	6.72 $\pm$ 2.55
ESR (mm/h)	48.76 $\pm$ 37.27
CRP (mg/l)	12.37 $\pm$ 1.85
anti-RA33 (ng/ml)	0.139 $\pm$ 0.138
RF (UI/ml)	86.65 $\pm$ 105.7
RF positive	14 (48.27)
anti-CCP (UI/ml)	254.0 $\pm$ 531.3
anti-CCP positive	17 (58.62)
TJC	8.24 $\pm$ 1.88
SJC	3.75 $\pm$ 1.52
VASp	66.20 $\pm$ 1.65
VASm	59.31 $\pm$ 7.22
DAS28 (4v)	4.61 $\pm$ 0.76
HAQ	1.31 $\pm$ 0.53

The results are expressed as a percentage (n%) or mean $\pm$ standard deviation. ESR-erythrocyte sedimentation rate; CRP- C reactive protein; RF-rheumatoid factor; anti-CCP anti cyclic citrullinated peptide; TJC-tender joint count; SJC-swollen joint count; VASp-visual analogue scale patient; VASm- physician's visual analogue scale; DAS28_(4v)-disease activity index with 28 joints; HAQ-health assessment questionnaire.

At the moment of diagnosis, we evaluated the disease activity, using the DAS28_(4v) (CRP) scoring and we reckoned a mean value of 4.61 ± 0.76 (min 2.99; max 6.29) in the RA patients group; most of the patients (24; 82.7%) had a moderate disease activity, while 4 of them (13.79%) registered a value corresponding to a high disease activity and only one was in low disease activity.

The initial GSUS evaluation revealed the presence of at least one joint with a synovitis score higher than 1 in all 29 patients; using the PDUS score, we found 23 patients with at least one joint with a score higher than 1, 20 patients with score higher than 2, and 6 patients with grade 3.

After 12 months, using DAS28_(4v) (CRP), 17 patients (58.62%) registered a low disease activity, 8 (27.58%) were in remission and 3 (10.34%) had a moderate disease activity. Anti-RA33 titre was significantly different between the three groups (0.143 ± 0.178 ng/ml; 0.153 ± 0.073 ng/ml; respectively 0.087 ± 0.094 ng/ml, $p = 0.044$). Analyzing the response, we established a medium delta DAS of 1.79 ± 0.86 ; therefore, 7 patients (24.13%) registered a moderate EULAR response, 2 (6.89%) had no response and 20 (68.96%) a good response. We found a moderate positive correlation ($r = 0.4566$, $p = 0.0128$) between the initial titre of anti-RA33 antibodies and delta DAS (fig 2).

US examination at 12 months revealed the presence of GS synovitis in 25 patients with a mean number of interested joints in the whole study group of 1.58 ± 1.27

(limits 0-8); PDUS examination found active synovitis for 12 subjects, with a mean PDUS score of 2.24 ± 3.02 ; all scores higher than 2 were found in patients with an anti-RA33 titre lower than 0.99 ng/ml.

Analysing the anti-RA33 titre, depending on the GSUS findings, we found a slightly higher value (0.144 ± 0.151 ng/ml), but without statistical significance ($p=0.377$) in patients with synovitis (GSUS+) in comparison to patients without synovitis (GSUS-) (0.105 ± 0.026 ng/ml). At the same time, anti-RA33 titre was 0.107 ± 0.056 ng/ml for patients with active synovitis, significantly lower compared to the second group, without PDUS synovitis (0.159 ± 0.177 ng/ml, $p=0.031$) (table II, fig 3). Also between the 14 patients with an anti-RA33 titre higher than 0.099 ng/ml, only 3 had active synovitis.

When evaluating the disease activity in relation to the anti-RA33 antibodies titre, we found the same tendency for negative correlation between the antibodies titre,

DAS28, and PDUS scores, with a higher difference for the last one.

Anti-CCP antibodies had different values in relationship to the presence/absence of GSUS synovitis (703.3 ± 1226 UI/ml vs. 139.4 ± 212.7 UI/ml) but with no statistical significance ($p=0.490$). PDUS active synovitis revealed a titre of anti-CCP antibodies of 961 ± 1179 UI/ml vs. 125.6 ± 191.7 UI/ml for patients without PDUS active synovitis ($p=0.0051$).

We found a moderately negative correlation ($r=-0.519$, $p=0.0039$), between the baseline titre of anti-RA33 antibodies and the presence of US active synovitis after 12 months of treatment. Also, for anti-CCP antibodies and RF we established a moderately positive correlation coefficient ($r=0.412$ and $r=0.472$, respectively).

Patients that needed therapy with two conventional sDMARDs (6; 20.68%) at 12 months, had a baseline titre of anti-RA33 antibodies significantly lower compared to the subjects that needed only one sDMARD (23; 79.31%) (0.088 ± 0.011 ng/ml vs 0.151 ± 0.156 ng/ml, $p=0.013$).

Discussions

Setting an antibody profile, highly specific and sensitive, with a diagnostic and prognostic value, could be essential for assessing remission [1,22-26]. Besides anti-CCP antibodies, present very early in the course of the disease, anti-RA33 antibodies can constitute an additional marker, not only for improving diagnosis, but also for assessing the prognosis and therapeutic response [5,27,28].

The reported percentage of positive cases for anti-RA33 antibodies varies between studies [3,5,29-31]. In our cohort almost half of the patients were positive. Consistent to our data, a recent meta-analysis, which included studies performed between 2000-2015 concerning the diagnostic value of anti-RA33 antibodies in RA, showed similar results [5]. It is also noteworthy that for positive RF patients, our results showed a low percentage of anti-RA33 positivity, while more than half of the anti-CCP positive subjects were anti-RA33 positive. Similar data were reported by Nell et al [6], in a cohort of 102 early RA subjects, and also by Al-Mughales et al [29].

Fig 2. Anti-RA33 titre (ng/ml) depending on a) disease activity and b) EULAR response

Fig 3. Anti-RA33 antibodies (ng/ml) depending on the presence/absence of GSUS/PDUS synovitis

Table II. Anti-RA33 and anti-CCP titre depending on the presence/absence of synovitis in grey scale and power Doppler ultrasonography.

	GSUS +	GSUS -	PDUS +	PDUS -
Anti-RA33 (ng/ml)	0.105 ± 0.151	0.144 ± 0.026	0.107 ± 0.056	0.159 ± 0.177
p	0.377		0.031	
Ac anti-CCP (UI/ml)	703.3 ± 1226	139.4 ± 212.7	961 ± 117	125.6 ± 191.7
p	0.490		0.005	

GSUS-grey scale ultrasonography, PDUS- power Doppler ultrasonography, + present, - absent.

This finding underlines the necessity and benefit of determining anti-RA33 antibodies in RF seronegative RA patients.

Analysing disease activity over the 12 months, our results revealed a highly divergent, statistically significant, anti-RA33 titre between patients with low disease activity, moderate activity, and remission. Moreover, when assessing the improvement of DAS28_(4v) score and anti-RA33 titre, we found a moderate positive correlation, statistically significant between the two variables. The data are consistent with other studies which concluded that the presence of anti-RA33 characterises patients with mild disease [6].

Persistent synovitis, especially in patients with early and very early RA, was proven to be present in patients being considered in clinical remission and was associated with a more unfavourable prognosis [15,22,26,32]. Analysing anti-RA33 titre, depending on US findings, we established statistically significant differences, between patients with/without active synovitis; thus, we found active synovitis only in 3 of the 15 patients with an anti-RA33 titre over the threshold value. A moderate, negative correlation between the two variables was established.

In agreement with these findings that underline the protective role of higher anti-RA33 antibodies titre, we noticed that for patients who received two conventional sDMARDs, the titre of anti-RA33 antibodies was significantly lower, compared to the subjects receiving one sDMARD.

Regarding anti-CCP antibodies, they have been proven to be directly related to a more erosive and progressive disease, with an important impact on future articular damage and, consequently on the evolution and prognosis. Analysing US findings, we found a moderately positive correlation, but not statistically significant, between the initial titre of anti-CCP antibodies and the presence of active synovitis, after 12 months of treatment. A similar observation was noted by Harman et al [1].

Both GSUS and PDUS are important in order to discriminate between the clinical remission with US sub-clinical inflammation and the real one, with no sign of US active synovitis, and can constitute the base of a prediction model that integrates several outcome measures. As it was shown by our results, patients with high titres of anti-RA33 antibodies had a milder evolution, with a low degree of US inflammation. These results led to the idea that remission can be predicted by a combination of clinical and immunological items and confirmed by US.

The main limitations of the study are as follows: firstly, the low number of patients, with the prevalence of younger female patients, a fact that might restrict some-

how the practical applicability of the results in a general RA population, without further extension of the group; in addition, the number of patients in which we established the cut-off value is low. Secondly, we acknowledge the fact that the US evaluation was performed by a single examiner, even if expert, using a machine with some technical limitations, especially on PDUS sensitivity and also that we had not performed other imaging techniques, such as MRI, to confirm our findings. Finally, the study design permits the different types of treatment in patients, which could influence the disease outcome.

Conclusions

The current preliminary study shows that anti-RA33 antibodies might constitute an additional tool for diagnosing early RA patients, with a particular contribution in RF seronegative cases. Also, in our cohort, the presence of anti-RA33 antibodies identified patients with a mild disease and with a very low level of active synovitis assessed by PDUS. Despite the relatively low number of subjects included in the study, the results are in agreement with other recently published papers and calls for its extension, with multicentre contribution and larger cohort of patients with early and very early RA. Determining additional antibodies, specific for a disease, with an important impact on the evolution of the patients, and integrating the results with ultrasound findings, not only improves the diagnosis, but can also help quantify the future articular damage and outcome of the patients.

Conflict of interest: none

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