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Autoantibodies and Wrist Synovitis in Early Rheumatoid Arthritis, Ultra-Sonographic Study

Authors
Ahmed Fathy Abogamal[1], Ahmed Fathy Abdalaziz [2], Yusuf Nassar[3], Maged A H Shoukeer[4]

[1]Assistant Professor of Rheumatology& Rehabilitation, Faculty of Medicine, AL-Azhar University Cairo- Egypt
[2]Assistant Professor of Clinical Pathology, Faculty of Medicine, Al-Azhar University, Cairo, Egypt
[3]Lecturer of Internal Medicine, Faculty of Medicine, Al-Azhar University, Cairo, Egypt
[4]Assistant Professor of Radiology, Faculty of Medicine, Dept of Radiology, Al-Azhar University, Cairo, Egypt

Abstract
Several autoantibodies has been linked to rheumatoid arthritis, diagnosis but its relation to joint synovitis needs to be evaluated.
Aim of the work: *To investigate the relation between rheumatoid factor, anti-cyclic citrullinated peptide, and anti-mutated citrullinated vimentin and ultrasound detected wrist synovitis in female patients with early rheumatoid arthritis.*
Patients and Methods: *80 female patients with early rheumatoid arthritis presenting at outpatient rheumatology clinics of AL-AZHAR University Hospitals Egypt through 2015-2016, were assessed for DAS28, rheumatoid factor, anti-cyclic citrullinated protiens (Anti CCP), anti-mutated citullinated vimentin (Anti MCV), and ultrasound quantification of wrist synovitis using gray scale and power Doppler.*
Results: *A significant positive correlation has been found between the Anti MCV and DAS28, where p< 0.05. A highly significant correlation found between Anti MCV, synovitis in gray scale and power Doppler were p< 0.01. Correlation study of Anti CCP, shows a highly significant correlation found between Anti CCP, DAS28, and synovitis in gray scale and power Doppler were p< 0.01. While rheumatoid factor fails to have any significant correlation with ultrasound parameters.*
Conclusion: *Anti CCP, and anti MCV are significantly correlated with wrist synovitis in female patients with early rheumatoid arthritis with superior correlation to anti CCP with the clinical activity.*
Keywords: *rheumatoid arthritis; rheumatoid factor; Anti CCP; anti MCV; ultrasound.*

Introduction

Rheumatoid arthritis (RA) is a complex rheuma-tological disorder that represent the harmful effect of autoimmunity on synovial joints. Synovial membrane is the primary site of pathology in RA and synovial proliferation, pannus formation with synovitis is the hallmark of RA lesions in synovial joints, including small joints of the hands and wrist joints which are the main joints affected initially by RA. [(1,2)]

From the old history up to date RA was classified according to presence and absence of auto-

antibodies into seropositive and seronegative. Rheumatoid factor (RF) which is the antibody directed against the Fc portion of the human IgG, typically considered as the first autoantibody discovered in RA and upon it the first classify-cation of seropositive and seronegative has been done. [1,2]

Till today it is the most popular test for RA and till now still included in any diagnostic or classification criteria for RA. RF in spite of being much known as a marker for RA but its deficient specificity and sensitivity for RA diagnosis also has been reported and established in many studies since many years.[3,4]

More recently a new family of autoantibodies has been shown to be linked to RA pathogenesis with efficient specificity, that antibodies targeting the citrullinated proteins, the anti citrullinated proteins antibodies (ACPA). [5,6]

The anti-cyclic citrullinated protiens (Anti CCP), was the first to appear among these group of autoantibodies, and extensively investigated in RA patients, and in spite of being with high specificity but shows low sensitivity, and doesn't close the gap of seronegative RA. Then RA reclassified again to seropositive and seronegative but this time according to absence and presence of Anti CCP. [5-7]

The most recent member of the antibodies against the citrullinated proteins is the anti-mutated citullinated vimentin (Anti MCV). Several studies have addressed the diagnostic performance of the anti MCV assay in RA, to be with efficient specificity but still with low sensitivity as the other anti citrullinated proteins. [8,9]

Away from the diagnostic value of autoantibodies, the ACPA are highly expressed in the RA joint, its presence were linked to joint inflammation, and there was an evident relationship between ACPA titers and oxidative stress in RA. Several publications have reported that ACPAs not only diagnostic but also were responsible for many pathogenic mechanisms of RA. [9,10].

In the last decade with the major development of ultrasound (US), and its introduction in the field of rheumatology. US confirmed to have a reliable, effective, and sensitive, ability to detect and quantify many articular lesions in RA. [11]

In the current study we assessed and quantified the primary lesion in RA which is the synovitis depending on US. And investigate the relation between quantified synovitis and autoantibodies including RF, Anti CCP, and Anti MCV in early RA patients.

Aim of the Work

To investigate the relation between rheumatoid factor, anti-cyclic citrullinated peptide, and anti-mutated citrullinated vimentin and ultrasound detected wrist synovitis in female patients with early rheumatoid arthritis.

Patients and Methods

Study Design & Setting: The current study was based on a cross sectional hospital-based survey, conducted among female patients with rheumatoid arthritis presenting at outpatient rheumatology clinics of AL-AZHAR University Hospitals. Egypt through 2015-2016.

Inclusion Criteria

- Female Patients
- Rheumatoid arthritis diagnosed according to: American College of Rheumatology Criteria 2010.[4]
- On regular treatment by conventional DMARDs, low dose corticosteroids 5mg.
- Active rheumatoid arthritis, with as DAS28 score more than 3.2.
- Disease duration not more than three months.

Exclusion Criteria

- Recent wrist joint trauma or intra-articular procedures.
- Previous wrist surgery.
- Patient's on biological therapy.

Data Collection

The data was collected through an interview questionnaire from all participants. Field survey was conducted after obtaining approval from hospital authority and Research Ethical Committee. The data was collected through 2015-2016. The field work took 2 days/week in the rheumatology clinic. The average number of female patient interviewed per day was 7-10 and total of 864 RA patients assessed and only 80 patients had me our research criteria.

- All patients were subjected to clinical history taking and examination.
- DAS score assessment: The DAS28 based on joint counts of 28 joint. The DAS has proven prognostic utility with respect to both function and radiographic progression. The 28-joint score is valid, reproducible and consumes little time. Four measures have been monitored for all participants:
  - Number of tender joints (TEN28): out of fourteen possible areas: right or left PIPs, MCPs, wrist, elbow, knee, ankle, MTPs joints.
  - Number of swollen joints (SW28): out of fourteen possible areas: right or left PIPs, MCPs, wrist, elbow, knee, ankle, MTPs joints.
  - Erythrocyte Sedimentation Rate (ESR) after one hour in mm.
  - General health or patient's global assessment of disease activity.

    Patients assessed their disease activity in the last seven days. Patients gave a score out of 10 (0 indicates the lowest value and 10 indicate the highest value).

The collected scores were calculated using the following formula: [0.56 × √ (TEN 28)] + [0.28 × √ (SW 28)] + [0.70 × (ESR after 1 hour in mm)] + [0.014× (patient's assessment in mm)] = DAS 28 score. [12].

- **Ultrasonographic assessment** of both wrist joints for detection of synovitis performed using a scanner with a multi frequency 12L linear array transducer (General electric Systems; LOGIQU-E) in B mode and power Doppler. Wrist synovitis was assessed by dorsal longitudinal scan according to EULAR standards, synovitis defined as intraarticular hypoechoic lesion that is non displaceable and poorly compressible. Grading for synovitis in gray scale as normal, mild, moderate and sever. Power Doppler graded as: Grade 0: Absence of abnormal vessel dots, Grade 1, mild synovitis: Single vessel dots, Grade 2, moderate synovitis: Several vessel dots, partially confluent covering less than half the area of synovium, Grade 3, sever synovitis: Confluent vessel dots covering more than half the area of synovium. [13,14] After assessment of both sides the side with highest grade of synovitis in gray scale and power Doppler was recorded and taken as the patient grade of synovitis.
- **Laboratory Assessment:**

The following laboratory investigations were done:

Blood Sampling

7 ml venous blood samples were obtained from each subject in the study one mL was drawn in EDTA tube for CBC; 1.6 mL was drawn into a tube containing trisodium citrate for ESR. The rest was delivered into a plain tube and left to be clotted, then centrifuged, and the separated serum was divided in 2 aliquots one used for routine biochemical laboratory investigations (liver, kidney, and random blood glucose) and CRP while the second aliquot was stored freezed at -20 C until used for determination of Anti CCP, RF and Anti MCV.

The following laboratory investigations were performed to all patients participating in this study:

1. Erythrocyte sedimentation rate by Westergren method.
2. Semi quantitative measurement of C-Reactive protein by Slide agglutination methods using Kits from Spin react*
3. Urine analysis.
4. Liver, kidney function tests and Random blood sugar. (all were analzeyd by by fully automated clinical chemistry auto-analyzer cobas c 311 (Roche diagnostics).
5. Complete Blood Picture by fully automated cell counter Sysmex Kx-21 N (Roche diagnostics).
6. Semi quantitative determination of Rheumatoid factor by Slide agglutination method using Kits from Spinreact (SPINREACT,S.A./S.A.U Ctra. Santa Coloma, 7 E-17176 SANT ESTEVE DE BAS (GI) SPAIN Tel. +34 972 69 08 00 Fax +34 972 69 00 99 e-mail: spinreact@spinreact.com)

Principle of the Method: The RF-latex is a slide agglutination test for the qualitative and semi quantitative detection of RF in human serum. Latex particles coated with human gammaglobulin are agglutinated when mixed with samples containing RF.

7. Anti CCP antibodies (by enzyme linked immunosorbent assay for quantitative determination of Anti cyclic citrullinated peptide antibody

Kits: ORGENTEC Diagnostika GmbH.
Analyzer: Stat fax -2600 (ChroMate)

The principle of assay of anti-CCP antibody
The anti-CCP antibody Kit is based on an ELISA method. The test utilizes microtitre plate wells coated with citrullinated synthetic peptides (antigen).
Diluted patients sample serum is applied to the wells and incubated. If specific antibodies are present, they will bind to the antigen in the wells. Unbound materials are washed away and any bound antibodies are detected by adding horse radish peroxidase (HRP) labeled anti-human IgG, followed by a second washing step and incubation with substrate. The presence of reacting antibodies will result in the development of colour, which is proportional to the quality of bound antibody, and this is determined photometrically.

8. Anti MCV antibodies (by enzyme linked immunosorbent assay for quantitative determination of Anti Mutated citrullinated Vimentin

Kits: ORGENTEC Diagnostika GmbH, (ORGE-NTEC Diagnostika GmbH Visitors' address: Carl-Zeiss-Straße 49-51 55129 Mainz, Deutschland Postal address: P.O. Box 100352 D-55134 Mainz Tel: +49 (0) 6131 / 9258-0 Fax: +49 ... 14 April 2010).
Analyzer: Stat fax -2600 (ChroMate, Awareness Technology, Inc. Offices USA1935 S.W. Martin Hwy. Palm City, FL 34990 USA Phone: 772-283-6540 Fax: 772-283-8020)
The principle of assay of anti-MCV antibody: similar to that of Anti-CCP antibodies

Data Analysis: Data were entered, organized, tabulated and analyzed using the standard computer program SPSS version 18. Quantitative data were expressed as Mean±SD, while qualitative data were expressed as frequency and percent. Student t-test was used to measure the difference between means of two quantitative groups, with the significant level set at 0.05, and highly significant at 0.01.

Results
Descriptive analysis of the RA patients, shows that age was 38.11±3.41, DAS28 4.65±1.95, rheumatoid factor 124.24±120.61, ANTIMCV 234.35±450.87, and ANTICCP 136.28±227.29. Table (1)
Analysis of ultrasound results shows that: on gray scalenormal synovium found only in one patient (1.3%), mild synovitis in 25 (31.3%) patients, moderate synovitis in 27 (33.8%) patients, and sever synovitis in 27 (33.8%) patients. Table (2), figure (1), figure (2).

While in Power Doppler was negative in 21(26.3%) patients, mild synovitis in 23 (28.8%) patients, moderate synovitis in 13 (16.3%) patients, and sever synovitis in 23 (28.8%) patients. Table (2), figure (3), figure (4).

As regard the correlation study, a significant positive correlation has been found between the Anti MCV and DAS28, where $p< 0.05$. A highly significant correlation found between Anti MCV, synovitis in gray scale and power Doppler were $p< 0.01$. Table (3), figure (5).

Correlation study of Anti CCP, shows a highly significant correlation found between Anti CCP, DAS28, and synovitis in gray scale and power Doppler were $p< 0.01$. Table (3), figure (6).

Table (1): shows descriptive analysis of the results.

	Mean	Std. Deviation
AGE	38.11	3.41
DAS28	4.65	1.95
RF	124.24	120.61
ANTIMCV	234.35	450.87
ANTICCP	136.28	227.29

(RF: rheumatoid factor, AntiMCV: anti-mutated citrullinated vimentin, AntiCCP: anti-cyclic citrullinated peptide)

Table (2): shows ultrasound results.

	Normal		Mild synovitis		Moderate synovitis		sever synovitis	
	frequency	**%**	**frequency**	**%**	**frequency**	**%**	**frequency**	**%**
Gary Scale	1	1.3	25	31.3	27	33.8	27	33.8
Power Doppler	21	26.3	23	28.8	13	16.3	23	28.8

Table (3): shows ultrasound results.

		DAS28	GERY	PD
RF	r	.039	.074	-.187
	p	.787	.604	.190
ANTIMCV	r	.257*	.469**	.392**
	p	.022	.000	.000
ANTICCP	r	.340**	.463**	.371**
	p	.002	.000	.001

(RF: rheumatoid factor, AntiMCV: anti-mutated citrullinated vimentin, AntiCCP: anti-cyclic citrullinated peptide)

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Figure (1): ultrasound results of gray scale ultrasound.

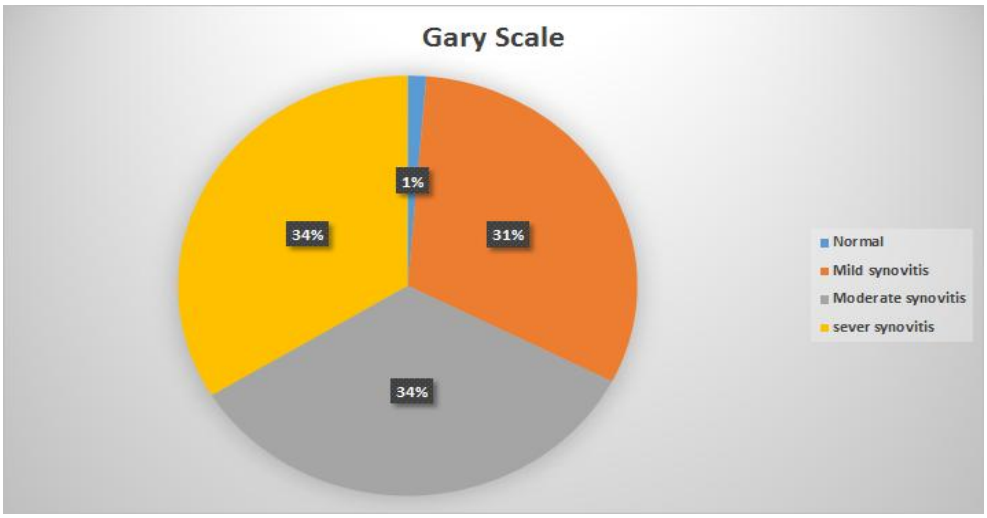
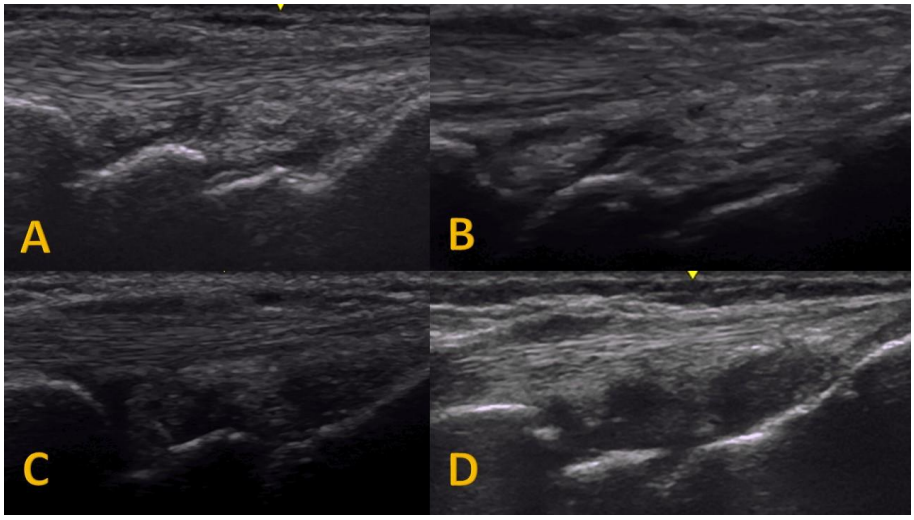


Figure (2): Gray scale ultrasound grading of synovitis.



(A: normal, B: mild, C: moderate, D: sever synovitis)

Figure (3): ultrasound results of power Doppler ultrasound.

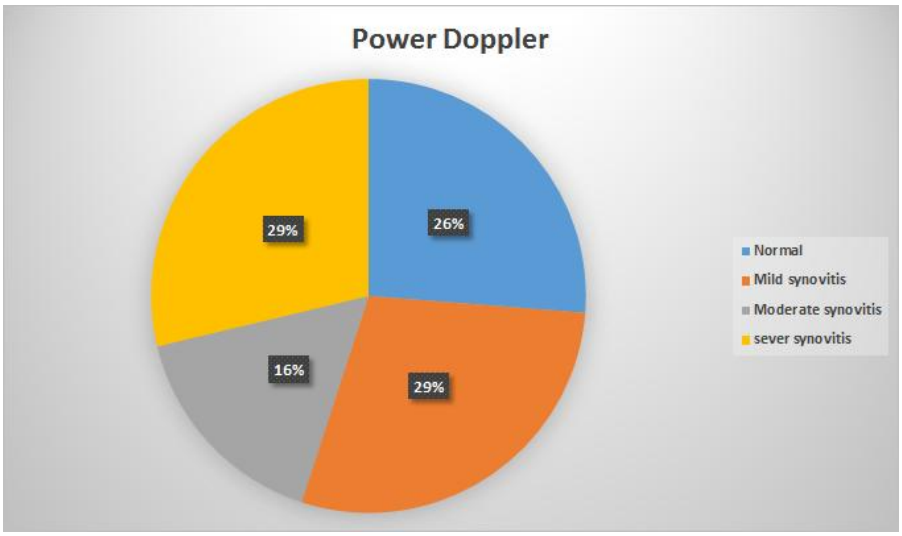
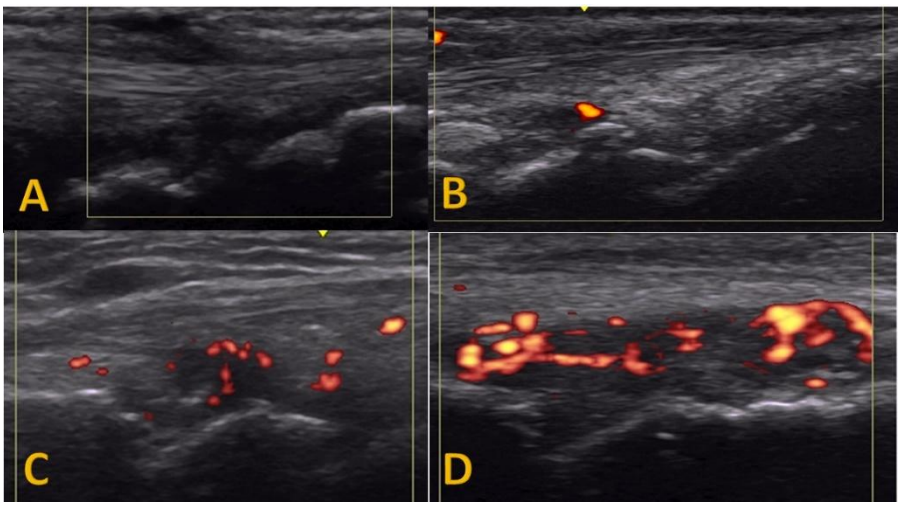


Figure (4): Power Doppler ultrasound grading of synovitis.



(A: normal, B: mild, C: moderate, D: sever synovitis)

Figure (5): Anti MCV correlation with ultrasound parameters and DAS28.

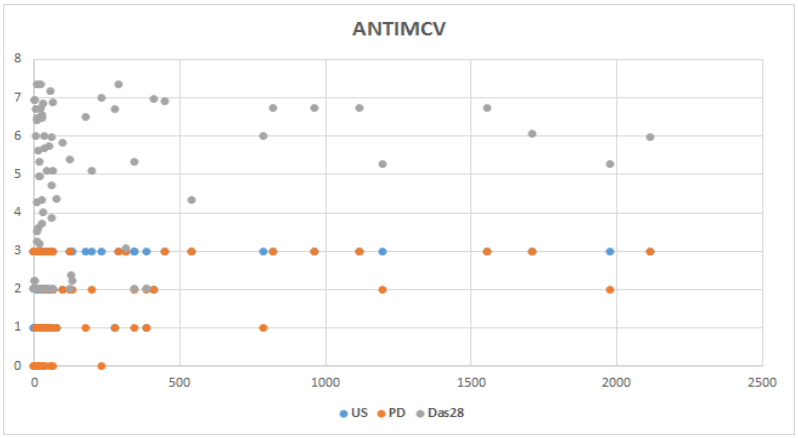
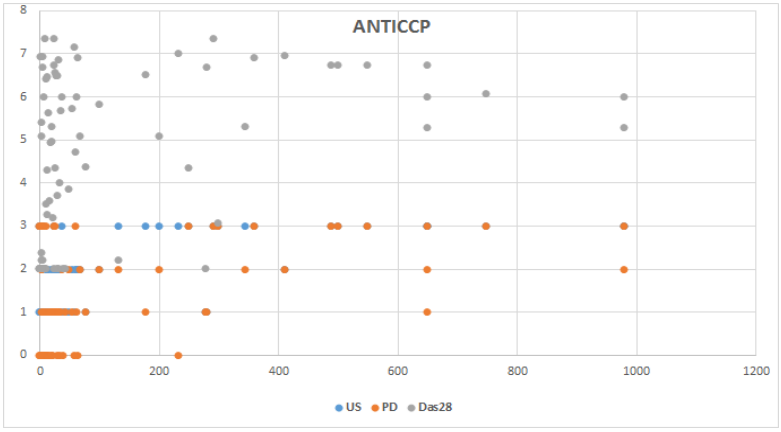


Figure (6): Anti CCP correlation with ultrasound parameters and DAS28.



Discussion

Rheumatoid arthritis is a common autoimmune disease characterized by chronic inflammation of the synovial joints, with initial synovial membrane inflammation, proliferation, and pannus formation, which can ultimately lead to cartilage and bone destruction. [1, 2]

Beyond the diagnostic value of autoantibodies in RA, the value of its presence has been linked to the development and progression of articular lesions in RA with differential attribution between RF and ACPA.

In the current study we quantified the primary RA lesion inside the wrist joints, as an objective impression for intra articular activity of RA. This primary lesion was the synovitis which was quantified depending on high frequency US in both gray scale and power Doppler.

The study group was a homogenous group of RA patients characterized by; early onset RA to quantify the actual synovitis before the effect of DMARDs; high DAS28 score to ensure that remission was not yet achieved; and female sex to have similar functional performance and anatomical articular properties.

We studied the relation between the quantified synovitis and different group of RA autoantibodies; including the RF, anti CCP, and anti MCV.

In our results RF fails to have any positive correlation with the quantified synovitis, either in gray scale or in power Doppler; which express a limited role for RF in the amount of synovitis in RA patients.

On the contrary ACPA by its two types the anti CCP and anti MCV shows a highly significant association with the quantified synovitis in both gray scale and power Doppler which represent a highly important role for ACPA in the development and progression of synovitis in RA joint.

Several follow up studies have reported the contribution of ACPA in the pathogenesis of RA

synovitis. A significant study on a cohort of 238 patients with RA was followed for 10 years with the collection of clinical data, serum samples for ACPA, IgA-RF and IgM-RF and scoring of their hand radiographs. Presence of ACPA was the strongest independent predictor of theirradiogr-aphic progression which support our results. [15]

Another supportive results from a study investigated the value of ACPA and RFin191 patients with early RA; were followed up prospectively for five years and the total increase in Sharp score of their hand radiographs after five years was significantly greater among patients with ACPA but not with rheumatoid factor which express the significant involvement of ACPA in articular lesions of RA. [16]

Snir and his colleagues in 2010, investigated the serum and synovial ACPA in 290 RA patients, and reported that antibodies to several citrullinated antigens are enriched in the joints of rheumatoid arthritis patients which could explain the link between synovitis and ACPA in our results. [17]

ACPA presence and its level in the serum of RA patients significantly correlated with the enhanced aberrant NETosis, and with the subsequent induction of inflammatory process including cytokines TNF alpha, interleukins 17, 6,8, chemokines, and adhesion molecules. [18,19]

So according to our results ACPA are much more than diagnostic autoantibodies as in comparison to RF its presence has been linked to joint synovitis and radiographic progression which extend their value from diagnosis to prognosis as its presence and titer should be considered during setup to management plan. [15-18]

More in depth analysis within the ACPA results shows that both anti CCP and anti MCV significantly correlated with DAS28 with higher significance towards the anti CCP, Several studies have addressed the diagnostic ability of the MCV assay to be stratified at lower sensitivity and specificity, than the anti CCP. [19, 20].

Al-Shukaili and his colleagues in their study also reported anti-CCP antibodies to have a higher sensitivity for the prediction of RA development and activity , in comparison to anti-MCV which come in agreement with our results and indicating that anti CCP may be a better indicator than the anti MCV for detection of disease activity. [20].

Our study limitation includes lack of use of magnetic resonance imaging (MRI) for actual quantitative assessment of synovial tissue but due to the high expense of MRI in comparison to US, plus the proven evident reliability and efficiency of US were lead us to depend on US.

We recommend further studies that involve investigate the synovial fluid and tissue for autoantibodies with longitudinal follow up for disease course and articular progression.

Conclusion

Anti CCP, and anti MCV are significantly correlated with wrist synovitis in female patients with early rheumatoid arthritis with superior correlation to anti CCP with the clinical activity.

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