

Original article

The diagnostic and monitoring value of serum anti-mutated citrullinated vimentin antibodies (anti-MCV) in juvenile rheumatoid arthritis

Background: JRA is currently diagnosed using the American College of Rheumatology (ACR) 1987 revised criteria that are primarily based on clinical parameters. The criteria may be insufficient for the diagnosis of early RA as they are based upon measurements of disease classification predominately featuring manifestations typical of later-stage disease. Measurement of serum anti-mutated citrullinated vimentin antibodies (MCV) has been shown to be a better marker for early adult RA, and it correlates well with the disease activity score (DAS). **Objectives:** The aim of this work is to evaluate the role of anti-mutated citrullinated vimentin antibodies (anti-MCV) in the diagnosis, and in monitoring disease activity in juvenile rheumatoid arthritis. **Methods:** The study included 40 children with JRA fulfilling the American College of Rheumatology criteria for diagnosis of JRA: 4 children with oligoarticular JRA, 12 with polyarticular JRA and 24 children with systemic onset JRA. Fifty healthy children, matching the patients in age and sex served as a control group. The studied children with JRA were subjected to laboratory tests including CBC, ESR, CRP, ANA and rheumatoid factor (RF). Serum samples from both patients and controls were assayed for anti-MCV levels using an ELISA technique. **Results:** The study showed high mean serum anti-MCV antibodies level in JRA patients when compared to controls ($P= 0.00$). In addition, there were no significant correlations between anti-MCV antibody levels and parameters of disease activity, namely, number of swollen joints, number of tender joints, ESR and CRP. The receiver operating characteristic (ROC) curve was drawn and it showed that the area under the curve (AUC) was (0.896). At a cutoff level ≥ 17 u/mL, anti-MCV antibodies had diagnostic specificity of 88%, diagnostic sensitivity of 87.5%, negative and positive predictive values of 89.8% and 85.4%, respectively and diagnostic efficacy of 87.8%. We also reported 3/40 of JRA patients to be positive for RF and 2/40 of JRA patients to be positive for ANA. **Conclusion:** Measurement of serum anti MCV antibody level holds promise as a diagnostic tool in JRA. However, they failed to show a significant efficacy in determining disease activity.

Keywords: Juvenile rheumatoid arthritis, MCV.

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INTRODUCTION

Juvenile rheumatoid arthritis (JRA) is a common rheumatic disease of children that begins before the age of 16 years. It is a major cause of chronic disability characterized by synovitis of the peripheral joints and associated with soft tissue swelling and effusion resulting in loss of joint functions and morbidity¹. JRA is currently diagnosed using the American College of Rheumatology (ACR) 1987 revised criteria that are primarily based on clinical parameters, the only serological criteria being IgM rheumatoid factor (RF). Rheumatoid factors sensitivities and specificities for discrimination of RA from non-RA vary in different studies with a report of a mean specificity of only 79%, and sensitivity of 60%².

Moreover, several studies have shown that only 5% to 25% of children with juvenile rheumatoid arthritis have positive latex agglutination tests for RF. So the diagnostic importance of RF in a child with possible chronic arthritis is of little utility³.

Identification of autoantigens containing the amino acid citrulline in adult RA is considered one of the most important serological discoveries in rheumatology in recent years, as they can be detected years before the onset of RA and reach a specificity of more than 95%⁴. This family of autoantibodies is an overlapping group of antibodies dependent on the citrullination of arginine residue. It includes antiperinuclear factor (APF), antikeratin antibody (AKA), antifilaggrin antibodies (AFA), anti-Sa (vimentin), and anti-cyclic citrullinated peptide (CCP) antibodies⁵.

Vimentin, one of the intermediate filaments, is a 58 kD protein. Vimentin is a Latin word which means arrays of flexible rods. Immunohistochemical staining revealed vimentin filaments as part of a wavy network of filaments in the cytoplasm of fibroblasts, associated with both nuclear and plasma membranes⁶. Vimentin isoforms were identified in which arginine residues were replaced by glycine, which they named mutated citrullinated vimentin. So, vimentin is usually not in a citrullinated state, but deimination of this protein occurs in macrophages undergoing apoptosis by Peptidylarginine deiminase enzyme (PAD)⁷. In addition, vimentin was found to represent a predominant substrate of PAD enzymes family, especially in activated and dying macrophages in patients with RA⁸.

Some studies showed that anti-MCV antibody is more sensitive compared to other antibodies against citrulline-containing epitopes for RA diagnosis⁹, and that anti-MCV antibody was present even earlier in the course of RA than anti-CCP, and therefore was a better marker of early RA¹⁰. Besides the higher sensitivity it has been shown that anti-MCV antibody is a better marker for early RA, and it correlates well with the disease activity score (DAS). The presence of anti-MCV antibodies at disease onset is associated with a more severe disease course, measured as higher level of inflammatory activity compared with anti-CCP⁷.

This work was aimed to evaluate the role of anti-mutated citrullinated vimentin antibodies (anti-MCV) in diagnosis and for monitoring disease activity in juvenile rheumatoid arthritis.

METHODS

This case control study was carried out at the Pediatric Allergy and Immunology unit, Children's Hospital, Ain Shams University. An informed consent was obtained from the parents or caregivers of patients and controls before enrollment. The study protocol was approved by the ethics committee of the Department of Pediatrics, Ain Shams University.

A) Patients' Group: It comprised 40 children with JRA fulfilling the American College of Rheumatology criteria for diagnosis of JRA¹¹. Four children had oligoarticular, 12 had polyarticular and 24 had systemic onset type. They were 18 males and 22 females. Their ages ranged from 2-18 years with a median of 9.5 years and their disease duration ranged from 5 months to 15 years with a median of 2.6 years.

B) Control group: Fifty healthy age and sex matched to the patients children comprised the

control group. They were 22 males and 28 females; their ages ranged from 1-16 years with a median of 8.3 years.

Clinical Methods:

The studied children with JRA were subjected to medical history taking, laying stress on age of onset, onset type, disease duration, symptoms of active disease and current medications. Clinical examination was done with record of number of tender and/or swollen joints and current activity of systemic manifestations (fever or skin rashes)¹².

Laboratory methods:

Eight mL of peripheral venous blood were withdrawn aseptically from each patient and 3 mL from each control subject. Three mL blood were left to clot for 15 minutes then centrifuged and sera were put into aliquots and stored at -20°C until assayed for anti-MCV antibodies for both patients and controls. The remaining 5 ml were used for other investigations done to patients:

- i. Complete blood picture using coulter counter.
- ii. Measurement of ESR by the Westergren method.
- iii. Measurement of CRP by latex agglutination test.
- iv. Measurement of serum rheumatoid factor by latex agglutination test.
- v. Measurement of ANA by indirect immunofluorescence technique.

Measurement of serum anti-MCV antibodies:

This was done using an indirect solid phase enzyme immunoassay (ELISA) kit for the quantitative measurement of IgG class autoantibodies against mutated citrullinated vimentin (MCV) in human serum (The kit was provided by ORGENTEC Diagnostika GmbH Carl-Zeiss-Straße 4955129 Mainz-Germany). (<http://www.hcmed.net/orgentec-diagnostika-gmbh>).

Statistical Methods:

Statistical analysis was done using SPSS software package, version 15.0, 2006, Ecosoft corporation, USA. Data was expressed descriptively as median and interquartile range for quantitative skewed data and qualitative data were expressed as percentage. Comparison between groups was done using Wilcoxon's rank sum test and Chi square test. Correlation study between the different analyzed parameters was done using Spearman's rank correlation coefficient test. Furthermore, the diagnostic performance of anti-MCV antibodies was evaluated using receiver operating characteristic curve (ROC) analysis. The best cutoff value (the point nearest to the left upper corner of the curve) was determined. For all tests a probability (P) less than 0.5 was considered significant.

RESULTS

Serum anti-MCV levels of JRA patients ranged from 5 to 1000 u/mL (Median = 26.5 u/mL). These values were significantly higher than the corresponding values of the control group in whom the level ranged from 2 to 40 (Median = 10 u/ml) as shown in figure (1).

Serum levels of anti-MCV correlated positively yet insignificantly with age, onset of JRA, duration of the disease, number of tender joints, hemoglobin concentration and ESR. (rs = 0.259, 0.136, 0.201, 0.143, 0.135, 0.019 respectively P>0.05). They correlated negatively with the number of swollen joints, platelets, WBCS and CRP (rs = -0.043, -0.127, -0.093, -0.074 respectively P>0.05) in JRA patients .

The receiver operating characteristic (ROC) curve, plotted to assess the diagnostic performance of serum anti-MCV antibody in JRA patients revealed that the area under the curve (AUC) was (0.896) and the optimum cutoff level was (>17 u/mL) (Figure 2). The analysis revealed that serum anti-MCV antibody level had diagnostic specificity of 88%, diagnostic sensitivity of (87.5%), both negative and positive predictive values of (89.8% and 85.4%, respectively) and diagnostic efficacy of (87.8%) (Table 1).Based on this cut off level the frequency of elevated anti-MCV antibody level was 90% in patients compared to 16% in controls.

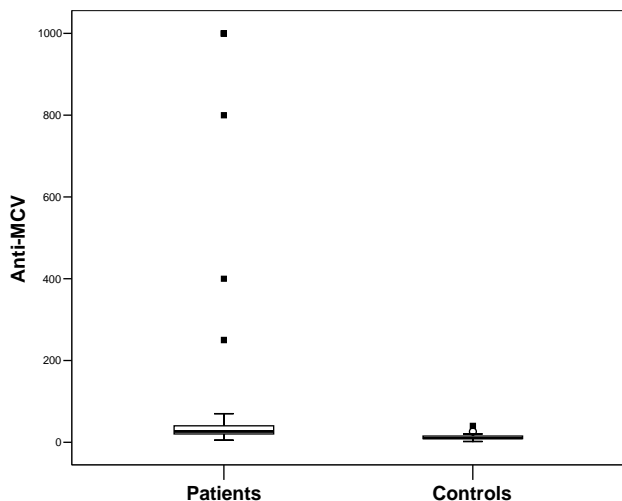


Figure 1. Serum anti-MCV antibody levels (U/mL) in JRA patients versus controls.

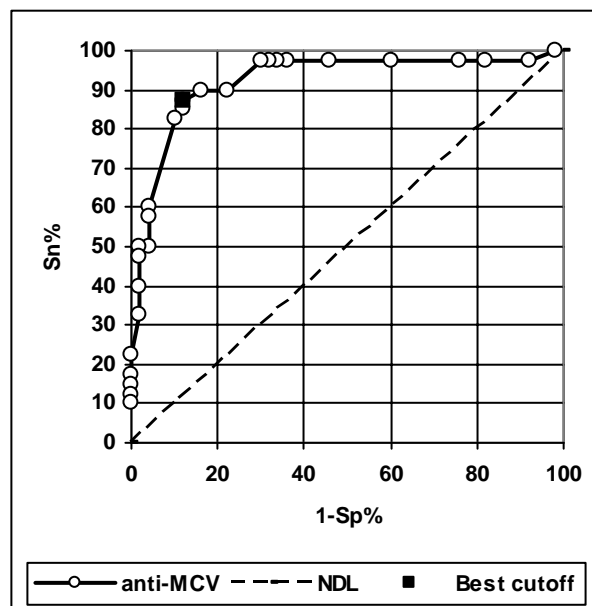
With respect to disease onset type, we found that serum anti-MCV antibody was elevated in 20/24 (83.3%) of those with systemic onset type, in 9/12 (75%) of those with the polyarticular onset

type and in 4/4 (100%) of those with the pauciarticular onset type. The differences were statistically insignificant (p=0.51).

We found that among patients with elevated antibody level, 2/33 (6.1%) patients were RF positive in comparison to 1/7 (14.3%) in the group of patients with normal antibody level. As regards ANA the frequency was 2/33 (6.1%) and 0/7 (0.0%) respectively. However, there was no significant variation in anti-MCV level with RF and ANA negativity or positivity.

No significant differences were observed in anti MCV antibody positivity among JRA patients with respect to corticosteroid therapy, as the percentage of those on steroid therapy who were anti MCV positive was comparable to those who were not using corticosteroids (p = 0.268). Similar findings were found as regards non-steroidal anti-inflammatory drugs and methotrexate.

Similarly, clinical and laboratory markers of disease activity (namely, number of swollen joints, number of tender joints, CRP and ESR) were comparable in patients with elevated and those with normal serum anti MCV antibody level. However, positive correlations were observed between serum anti- MCV antibody and patient’s age, duration of illness, number of tender joints, and ESR, although they were statistically non-significant.



AUC = 0.896

Figure 2. ROC curve analysis showing the diagnostic performance of anti-MCV antibody.

Table 1. Diagnostic performance of serum anti-MCV antibodies in JRA patients versus healthy control group.

Parameter	ROC cut off	Diagnostic sensitivity (%)	Diagnostic specificity (%)	Negative Predictive value (%)	Positive Predictive value (%)	Diagnostic efficacy (%)
Anti-MCV antibody (u/mL)	≥ 17	87.5	88.0	89.8	85.4	87.8

DISCUSSION

As limited numbers of serological laboratory tests are helpful for the classification, diagnosis and for evaluating the clinical status of JRA, continuing efforts are made to find more sensitive and specific markers for diagnosis of JRA. Autoantibodies against citrullinated peptide antigens (ACPA) have been described in RA diagnosis. The newest member of this autoantibody family is anti-mutated citrullinated vimentin (MCV)⁵.

The present study revealed a significantly higher serum anti-MCV antibody level in JRA patients when compared to healthy controls (mean of 158.43 versus 11.98 u/ml respectively). This can be explained by the hypothesis that vimentin might trigger the initial immune response in RA¹³. It activates T-lymphocytes by binding on HLA-DR4 on the surface of antigen presenting cells and may contribute to certain pathways in the pathogenesis of RA. This comes in accordance with many previous studies^{5,7,8,15} all of which reported a significant elevation in serum anti-MCV in RA patients versus controls.

However other researches^{16,17,18} failed to find this significant difference, a finding which can be explained by the fact that vimentin contains 43 arginine residues with 10 citrullination sites experimentally confirmed and anti-MCV antibodies are considered a heterogeneous group of antibodies directed against different epitopes on the citrulline molecule⁷.

In a trial to assess the diagnostic performance of serum anti-MCV antibodies in JRA, the ROC curve was plotted. We found that at a cut off value of 17 u/ml serum anti-MCV antibody had a specificity and a sensitivity of 88% and 87.5% respectively and the negative and positive predictive values were 89.8% and 85.4% respectively, with a diagnostic efficacy of 87.8%. At a cut of value of 20 u/ml, Poulson and Charles¹⁵ found a specificity and sensitivity of 87% and 84% respectively, while Dejaco and associates¹⁹ reported a result of 90.8% and 69.5% respectively. However, at a cut off value of 25 u/ml, Bang and colleagues⁷ reported the specificity and sensitivity to be 88% and 82% respectively.

Variation in the sensitivity and specificity can be attributed to the fact that some of the studies included patients with enthesitis-related arthritis,

undifferentiated arthritis and psoriatic arthritis depending on the new classification of RA in children, the International League of Associations For Rheumatology (ILAR) classification of JIA which represents an umbrella term for a group of heterogeneous, chronic inflammatory diseases of unknown etiology²⁰.

Our study could not demonstrate a significant value for serum anti-MCV antibody in diagnosis of active disease status in JRA as there was no significant difference between patients with elevated and normal antibody level as regards number of swollen and tender joints, ESR and CRP. This was in spite the presence of positive correlations between the antibody level, ESR, duration of illness and number of tender joints. The small sample size as well as the facts that serum anti-MCV antibody was not measured at diagnosis, but during routine follow-up visits in the specialized clinic and were not compared in the same patient at different statuses of the disease offer explanation for the lack of significant relation to disease activity. This finding was supported by previous studies^{5,10}.

On the other hand, multiple researches^{7,8,14,21}, succeeded to show a significant elevation in clinical and laboratory disease parameters in patients with elevated serum anti-MCV antibody. Innala and associates²² reported that in patients with early RA, persistent inflammatory activity, measured by the DAS28, ESR, CRP and swollen joint count were identified best by anti-MCV antibody compared to other ACPA. A significant decrease in anti-MCV level concentration corresponded to the therapeutic response.

In our study, the type of disease onset had no impact on serum anti-MCV antibody as there was no significant difference in the antibody level among patients with polyarticular, oligoarticular or systemic disease onset (P 0.515). However, Morbach and associates¹⁸ found significantly increased frequencies of anti-MCV positive patients among polyarticular RA with increased specificity as compared to other rheumatoid subgroups.

The results of the present study provide preliminary evidence that there was no association between serum anti-MCV antibody and the presence of positive RF or ANA based on the findings in our patients. Several studies²³ have

shown that only 5% to 25% of children with JRA have positive latex agglutination for RF. Children with high titers of RF likely represent a subgroup distinct from the larger number of children with seronegative disease³. Although positive ANA tests are sometimes seen in children with JRA, they are not distinguishing feature of the disease and they reach their highest prevalence (65%-85%) in children with oligoarthritis and uveitis³.

Strange enough, we could not elicit in our study a significant link between serum anti-MCV antibody and drug therapy. This comes in agreement with Poulson and Charles¹⁵ who reported that there was no statistically significant change in the antibody level in patients treated with methotrexate alone or a combination of methotrexate and interferon over a longitudinal study covering four time points (pretreatment, 6, 18 and 52 weeks). On the other hand, Mathsson and associates⁸ found that serum anti-MCV antibody level declined significantly with treatment during the first study year.

Serum anti-MCV antibody could be a promising tool in diagnosing JRA, however it failed to show a significant link with disease activity or the type of disease onset. Serum anti-MCV antibody should be studied in a larger sample including different entities of chronic arthritis in childhood and at different time points of the disease comparing its efficacy in early diagnosis of these arthritides.

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