Original Article

Study of Peptidyl Arginine Deiminases 4 (PAD 4) Antibodies in Rheumatoid Arthritis Patients

Darwish A. El-Hallous[1], Nevine Mohannad[2], Maher A. Kamel[3], El-Sayed H. Radwan[4]

Departments of Internal Medicine, Alexandria University[1], Alexandria University Hospitals[2], Damanhour Teaching Hospital[4], Medical Biochemistry[3], Alexandria University; Alexandria; Egypt

ABSTRACT

Rheumatoid arthritis (RA) is one of the most commonly occurring form of inflammatory polyarthritis. If untreated, 20%–30% of RA patients become debilitated within the first three years following initial diagnosis. The clinical examination often fail to identify patients with early RA partly due to heterogeneity of disease presentation and course. To meet the need for improved diagnostic and prognostic tests, various serum biomarkers are being assessed, including a wide range of autoantibodies. Autoantibodies against peptidyl arginine deiminase type 4 (PAD-4) have recently been described as a specific biomarker in subjects with clinically apparent RA. In this study, we tested the presence of anti-PAD4 antibodies in 40 Egyptian subjects with RA and their first degree relatives in order to determine whether these autoantibodies play a role in early disease evolution. In addition, we aimed to describe the Anti-PAD4 relation to anti-CCP autoantibodies, and potential associations with a more severe RA phenotype. The study was carried out on total of 100 subjects divided into; 40 RA patients, 40 of first degree relatives (sister or brother) of RA patients and 20 matched controls. **Results:** The results indicated that the serum level of Anti-PAD 4 is increased in Egyptian RA patients and their relatives and has a positive association with disease activity. Also its level together with Anti-CCP provides a good diagnostic tool of RA. **Conclusion:** The serum level of Anti-PAD4 may provide diagnostic information of RA and may be considered as an early marker of the disease among the first degree relatives of RA patients. **[Egypt J Rheumatology & Clinical Immunology, 2016; 4(1): 59-66]**

BACKGROUND

Rheumatoid arthritis (RA), one of the most commonly occurring form of inflammatory polyarthritis, is prevalent in approximately 0.8% of adults worldwide. If untreated, 20-30% of RA patients become severely debilitated within the first three years following initial diagnosis that they become permanently disabled.[1]

Within the last decade, treatment options for RA patients have improved dramatically. Treatment focus has shifted to early intervention with aggressive treatment aimed at suppressing inflammation and preventing further joint damage. Accumulating evidence indicates that early introduction of methotrexate (MTX) therapy in undifferentiated arthritis patients seropositive for anti-CCP delays progression to clinically overt RA and retards the development of joint destruction[2,3].

However, initiation of treatment without a confirmed diagnosis of RA is inappropriate for at least half of patients with undifferentiated arthritis, as therapies are potentially toxic and costly.[4] Therefore, serological diagnostic testing is of growing importance in the early detection and differentiation of rheumatoid arthritis. Apart from the traditional detection of the rheumatoid factor, new specific autoantibodies to citrullinated antigens have made a crucial contribution to the diagnosis of RA.[5]

For Correspondence: nmohannad@yahoo.com

Citrullination is a post-translation modification whereby the amino acid arginine is modified to the non-standard residue (citrulline). This is done by the enzyme peptidyl arginine deiminase (PAD) in the presence of calcium[6].

PAD4 is localized in the cytoplasm of monocyte, T and B cells, neutrophil, eosinophils and NK cells and can move to the nucleus upon cell activation. It plays a physiological role in gene regulation via citrullination of histones. In RA, PAD4 contributes to the generation of anti- citrullinated peptide antibodies (ACPA) specific substrates and is itself a target of autoantibodies[7].

Autoantibodies against PAD-4 have been described as a specific biomarker in subjects with clinically apparent RA[8]. This process is likely to be of significance in patients with RA given the established association of ACPAs with disease presence and activity.[9]

Several studies have identified an association between genetic polymorphisms of the PAD 4 gene and RA, although it has not been confirmed across all racial and ethnic groups[10-14]. Moreover, researchers demonstrated the presence of specific anti-PAD-4 antibodies in patients with RA, as well as association with disease activity[15].

In this study we tested the presence of anti-PAD 4 antibodies in 40 Egyptian subjects with RA and their relatives in order to determine the role it may play in early disease evolution. In addition, we aimed to describe the Anti-PAD4 relation to anti-CCP autoimmunity, and potential associations with a more severe RA phenotype.

SUBJECTS AND METHODS

A case-control study comprising early diagnosed RA patients (less than 2 years disease duration) was carried out in Alexandria, Egypt. The study was carried out on total of 100 subjects divided into; 40 RA patients, 40 of first degree relatives (sister or brother) of RA patients and 20 matched controls. Rheumatoid patients were recruited from rheumatology outpatient clinic of Alexandria main university hospital. All patients fulfilled the revised American College of Rheumatology (ACR) criteria for RA[16]. Criteria for exclusion from the study included history of renal, cardiac or liver dysfunction.

All subjects were exposed to full clinical examination including the tender joint count, swollen joint count and DAS 28 was calculated.

After overnight fasting venous blood sample was obtained for clinical investigations which include; Complete blood picture, Blood urea and serum creatinine, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), Rheumatoid Factor (RF), Anti-CCP and anti-PAD 4 antibody level.

Anti-PAD4 Autoantibody EIA Kit (Cayman, USA) is an immunometric assay which can be used to measure anti-PAD4 autoantibodies of any isotype (IgM, IgG, and IgA) in human plasma and serum without prior sample purification. Affinity-purified PAD4 autoantibody isolated from the plasma of a patient with RA is used as the standard. One unit is approximately equal to 1 ng of anti-PAD4 Ig protein.

Statistical analysis was done using the SPSS software package version 17.0 to obtain the mean, the standard deviation; the standard error for comparison between the different groups involved in this study using one way analysis of variance (ANOVA).

RESULTS

Regarding sex, age and BMI, the subjects in each group were matched (Table 1). There was a highly significant increase in ESR, RF and CRP in RA patients than control subjects (Table 1). Although ESR, RF and CRP were significantly increased in relative group than control group, yet, their values still within the normal clinical range. Other laboratory parameters as liver , renal functions and FBG showed no significant differences between the studied groups.

Table (2) summarize the different features of RA patients including DAS28, RF, ESR, CRP, duration of morning stiffness (min) and disease duration (months).

Figure (1) represents the results of serum level of Anti-PAD4. It appears that RA patients have a significantly higher level of Anti-PAD4 than control subjects. However , female relatives of RA patients have significantly higher level of Anti-PAD4 than control values, yet, male relatives showed no significant change. Although the level of Anti-PAD4 showed higher levels in females than males in RA patients and their relatives, these differences didn't reach the level of significance (Figure 1).

Figure (2) represents the results of serum level of Anti-CCP which showed significant elevated level in RA patients compared to control subjects. Female and male relatives of RA patients have no significant change in Anti-CCP level compared to control values.

The sensitivity and specificity of AntiPAD4 and Anti-CCP are presented in Tables (3) and (4), respectively. The sensitivity of Anti-CCP was 87.5%, and the anti-PAD4 was 60.0%, the sensitivity rose to be 97.5% for combined positivity of anti-PAD4 and Anti-CCP. The specificity of anti-CCP was 95.0% and for anti-PAD4 was also 95.0%, the specificity of both markers together reached 100%.

The RA patients were divided into two categories according to the positivity for Anti-PAD4 (Table 5). There was no significant difference between positive and negative patients regarding the DAS28, ESR, CRP and disease duration. The rheumatoid factor and Anti-CCP showed significant higher levels in positive anti-PAD4 patients than the negative patients. When the patients categorized according to the positivity for Anti-CCP (Table 6) no significant difference was observed between positive and negative patients regarding the DAS28, ESR, CRP, morning stiffness and disease duration. Rheumatoid factor showed significant higher level in positive anti-PAD4 patients than the negative ones ($p=0.001$). Also, Anti-CCP positive patients had significant longer duration of morning stiffness than negative patients ($p<0.05$).

Serum level of Anti-PAD4 in RA patients was positively correlated with DAS28 (Figure 3), Anti-CCP (Figure 4), and RF levels (Figure 5).

Table 1. Demographic and laboratory data of the studied population.

	Control (N=20)	RA patients (N=40)	Relatives (N=40)
Demographic Data			
Sex (M/F)	5/15	8/32	18/22
Age (Year)	29.7±0.98	31.0±0.82	28.3±0.93
BMI (Kg/m^2)	25.1±0.25	28.6±0.66	26.8±0.61
Laboratory Data			
ESR (mm/hour)	8.8±0.4	74.9±4.48*	16.1±1.6*
CRP (mg/dl)	3.2±0.27	43.0±2.64*	7.3±0.65*
FBS (mg/dl)	88.1±1.74	96.7±3.1	84.9±1.90
Urea (mg/dl)	33.0±1.32	34.7±0.76	33.6±0.83
Creatinine (mg/dl)	0.87±0.03	0.97±0.03	0.89±0.03
Uric Acid (mg/dl)	3.9±0.16	3.8±0.11	4.0±0.13
ALT (U/L)	33.0±1.5	30.9±0.85	32.0±0.89
AST (U/L)	30.4±0.83	35.8±0.85	32.5±0.76

Data presented as Mean±SE
*Significantly different from control group by t-test ($p<0.05$)

Table 2. Diagnostic features of rheumatoid arthritis patients.

	RA patients (N=40)
DAS28	6.1±0.11
Rheumatoid factor (U/mL) (n. up to 8)	102.9±8.8
Anti-CCP (U/ml) (n. up to 20)	86.8±20.99
ESR1(mm/hour)	74.9±5.59
CRP (mg/dl) (N. up to 3)	43.0±2.64
Morning stiffness (Minute)	57.4±2.37
Disease duration (Month)	16.3±0.74

Data presented as Mean ±SE

Table 3. Sensitivity of Anti-CCP and Anti-PAD4 for RA.

	Anti-CCP	Anti-PAD4	Both
Positive	35	24	39
Negative	5	16	1
Total No. of RA patients	40	40	40
Sensitivity (%)	87.5	60.0	97.5

Table 4. Specificity of Anti-CCP and Anti-PAD4 for control healthy subjects.

	Anti-CCP	Anti-PAD4	Both of them
Positive	1	1	0
Negative	19	19	20
Total No. of control	20	20	20
Specificity (%)	95	95	100

Table 5. The clinical data of RA positive and negative for Anti-PAD4.

	Anti-PAD4 negative (n=16)	Anti-PAD4 positive (n=24)
Age	29.3±1.17	34.1±0.94
Sex (M/F)	4/12	4/20
DAS28	6±0.15	6.2±0.16
Rheumatoid factor (U/ml)	81.8±9.38	117.1±12.64*
Anti-CCP (U/ml)	12.0±1.4	136.7±31.29*
ESR (mm/hour)	70.8±7.1	77.6±6.1
CRP (mg/dl)	42.7±4.4	43.2±3.33
Morning stiffness (Minute)	56.9±3.6	57.7±3.2
Disease duration (Month)	16.3±1.4	16.3±0.84

Data presented as Mean± SE
*Significantly different from Anti-PAD positive group by t-test ($p<0.05$)

Table 6. The clinical data of RA positive and negative for Anti-CCP.

	Anti-CCP negative (n=5)	Anti-CCP positive (n=35)
Age	32.8±1.03	32.1±0.93
Sex (M/F)	2/3	6/29
DAS28	5.4±0.27	6.2±0.11
Rheumatoid factor (U/ml)	77.4±19.5	106.6±9.6*
Anti-PAD4 (U/ml)	39.4±5.9	76.5±14.25*
ESR (mm/hour)	60.2±9.0	77.0±5.03
CRP (mg/dl)	37.8±5.8	43.7±2.9
Morning stiffness (Minute)	43.0±2.0	59.4±2.5*
Disease duration (Month)	18.8±1.97	15.9±0.79

Data presented as Mean ± SE
*Significantly different from Anti-CCP positive group by *t-test* ($p<0.05$)

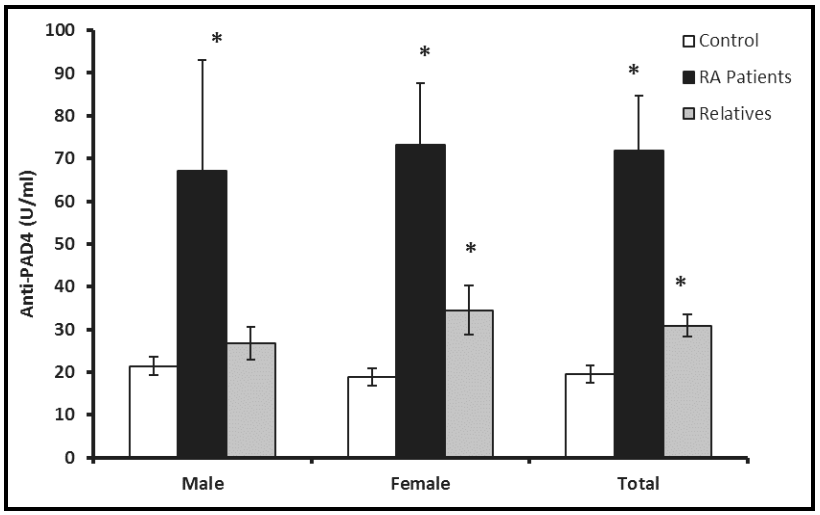

Figure 1. Serum level of Anti-PAD4 in healthy control subjects, RA patients and their relatives. Data presented as Mean±SE, * significantly different from control subjects by ANOVA ($p<0.05$).

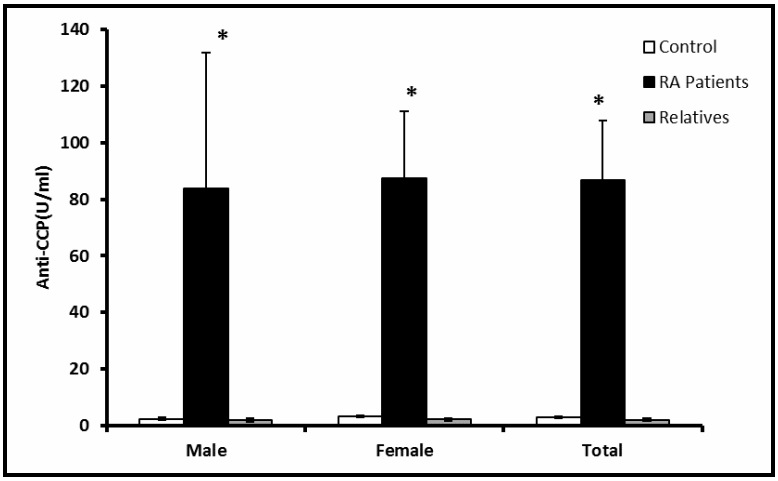

Figure 2. Serum level of Anti-CCP in healthy control subjects, RA patients and their relatives. Data presented as Mean±SE, * significantly different from control subjects by ANOVA ($p<0.05$).

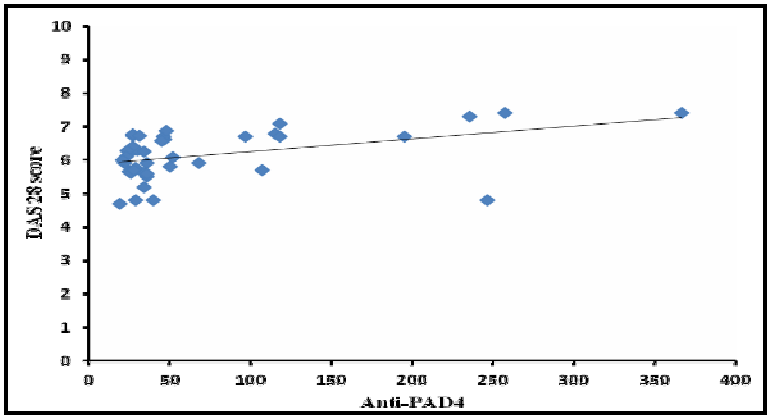

Figure 3. Correlation between Anti-PAD4 and DAS28 score in RA patients ($r=0.43$, $p=0.006$).

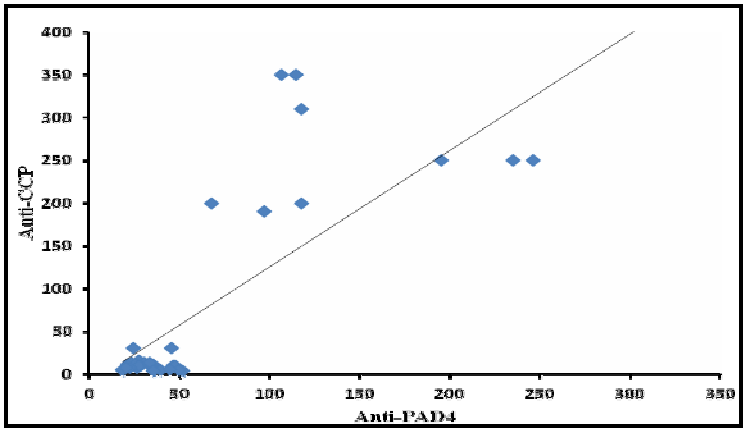

Figure 4. Correlation between Anti-PAD4 and Anti-CCP in RA patients ($r=0.81$, $p=0.00001$).

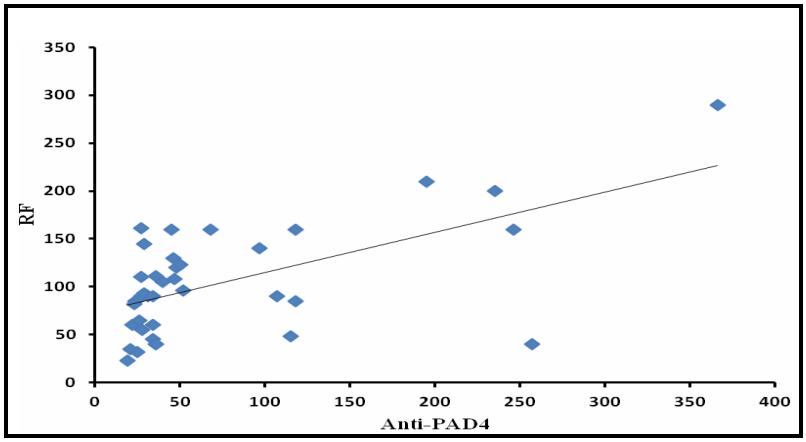

Figure 5. Correlation between Anti-PAD4 and RF in RA patients (r= 0.6, p=0.0001).

DISCUSSION

Our results clearly indicated that the serum level of anti-PAD4 antibodies is significantly elevated in RA patients; sixty percent are Anti-PAD4 positive while only five percent is positive in the control group. Anti-PAD4 shows sensitivity of 60% and specificity of 95% (Tables 5, 6 and 7). Also, the relatives of RA patients show a significantly higher level of Anti-PAD4 antibodies than control subjects which may imply that those relatives who apparently normal may develop RA with time or are at risk of progression to clinical overt RA by time.

The correlation studies of our data indicate a significantly positive correlation between the serum level of anti-PAD4 antibodies and the DAS 28 score as a measure of disease activity. Also, anti-PAD4 antibodies show a strong positive correlation with the conventional diagnostic markers; anti-CCP and RF.

In line with our results many studies have implicated anti-PAD4 antibodies in the progression of the RA disease. Kolfenbach et al.[17] documented that autoantibodies against the PAD-4 enzyme are present in the prediagnosis period and could be specific for the future development of RA. The same study also reported the presence of positive association between anti-PAD-4 antibody and anti-CCP antibody[17]. Anti-PAD-4 antibodies were evident as early as four years prior to clinical diagnosis, similar to findings reported in other studies for pre-clinical anti-CCP and RF[18-22].

The first association between PAD4 and RA was reported in a Japanese population[9] and similar results were found in some European and North American populations[12,23]. While, other studies using European populations were inconsistent[11,13,24-25].

Nissinen, et al.[26] showed that anti-PAD antibody had been detected in sera from patients with RA, SLE, and Primary Sjogren Syndrome, which lead them to suggest that PAD may be a novel autoantigen in inflammatory rheumatic diseases. Roth, et al.[27] also observed a significantly increased frequency of anti-PAD among RA patients (31%) compared to controls (3.4%), and found a correlation between anti-PAD and anti-CCP antibody levels[27]. Takizawa, et al found that, the prevalence and the titers of anti-PAD4 were significantly higher in RA patients than in other rheumatic disease patients and controls; 21 out of the 42 patients with RA (50%), 2 of 19 with SLE (10.5%), 1 of 23 with other collagen diseases (4.3%), and 1 of 40 healthy controls (2.5%) were seropositive for anti-PAD4. These indicate that PAD4 may act as an autoantigen in some patients with RA[28]. In line with these, a large cohort of Chinese RA patients by Zhao et al.[15] found the prevalence and titers of anti-PAD4 were higher in RA than in other rheumatic diseases and healthy individuals. Although the sensitivity of anti-PAD4 was only 45.0%, the specificity did reach 93.5% which were comparable to our results (95%). They also found positive correlation between Anti-PAD4 and DAS28, and Anti-CCP. Also, RA patients with anti-PAD4 showed more severe radiographic changes than patients without anti-PAD4 in a transversal radiographic assessment. All of these studies confirm our results of significantly elevated levels of Anti-CCP and RF in AntiPAD4 positive patients than negative patients.

The correlation between anti-PAD4 and anti-CCP antibody may indicate that both antibodies may play roles in the pathogenesis of RA. The mechanism of breakdown of the immunological tolerance to

PAD4 in RA remains unknown. One possible mechanism suggest that anti-PAD4 is formed after the production of anti-CCP antibody by epitope spreading. After the generation of immune response to citrullinated proteins, immune tolerance to the enzyme PAD4 can also be broken since they act as an enzyme and substrates. Another mechanism suggest that; PAD4 acts as an autoantigen separately. The overexpression of PAD4 in RA patients may leads to the breakdown of immunological tolerance to PAD4 and production of anti-PAD4. On the other hand, as more PAD4 enzymes are being produced, this leads to increased citrullination of proteins and an increased chance of developing anti-CCP antibodies. The mechanism of how the anti-PAD4 and anti-CCP antibodies contribute to perpetuation of the inflammation and the chronicity of RA remains to be explained[27].

In addition, we observed that 5 of 40 (12.5%) patients with RA are anti-CCP negative 4 of them are anti-PAD4-positive. These results indicated that anti-PAD4 may be helpful for the diagnosis of RA patients lacking anti-CCP. This suggestion is supported by our analysis, which indicated that the sensitivity of anti-PAD4 and anti-CCP alone are 60 and 87.5% respectively while if both tests are combined the sensitivity increased to be 97.5%.

The observed low sensitivity of anti-PAD4 may be due to autoantibody reversion over time to a seronegative status. It was documented that the percentage of subjects with anti-PAD-4 reversion was higher than either anti-CCP or RF[17]. Studies on RA patients have shown declining RF and anti-CCP antibody titers with good clinical response to ongoing therapy[29,30]. The loss of seropositivity during the post-diagnosis period may reflect improved disease control, which may be the case in our study as most RA patients enrolled in this study are chronic and all of them received methotrexate treatment.

There is a mutual influence of Anti-PAD4 autoantibody and PAD4 enzyme function. The binding of anti-PAD4 to PAD4 enzyme may have loss- or gain- of function. The loss-of-function could result in the enzyme inhibition and decreased levels of protein citrullination. On the other hand, antibody binding could lead to altered substrate specificities and increase protein citrullination which enhance the autoimmune activation and account for the association between PAD-4 antibody and increased disease activity, and advanced radiographic progression[15,31,32]. Auger et. al. has provided insight into this area by demonstrating that autoantibodies to PAD-4 can inhibit PAD-4 mediated citrullination[33]. These suggestions remain an area where additional research is necessary. Recently, many small PAD4 inhibitor was developed to treat RA and also cancer[34].

In summary, the serum level of Anti-PAD4 is increased in Egyptian RA patients and their relatives and has a positive association with disease activity. Also its level together with Anti-CCP provides a good diagnostic tool of RA. A large cohort study will be of great value to confirm these results and further studies of the molecular mechanism of anti-PAD4 production will be of great significance in understanding the pathogenesis of RA

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

REFERENCES

1. Rindfleisch JA, Muller D. Diagnosis and management of rheumatoid arthritis. Am Fam Physician 2005 ; 72: 6, 1037–47.
2. Cannella AC, O'Dell JR. Early rheumatoid arthritis: pitfalls in diagnosis and review of recent clinical trials. Drugs 2006; 66,1319–37.
3. Van Dongen H, Van Aken J, Lard LR. Efficacy of methotrexate treatment in patients with probable rheumatoid arthritis: a double-blind, randomized, placebo controlled trial. Arthritis Rheum 2007; 56,1424–32.
4. Orozco C, Olsen NJ. Identification of patients with early rheumatoid arthritis: challenges and future directions. Clin Dev Immunol 2006; 13, 295–307 .
5. Egerer K, Feist E , Burmester GR. The Serological Diagnosis of Rheumatoid Arthritis: Antibodies to Citrullinated Antigens. Dtsch Ärztebl 2009; 106(10), 159–63.
6. György B, Tóth E, Tarcsa E, et al. Citrullination: a post-translational modification in health and disease. Int J Biochem Cell Biol. 2006; 38(10),1662-77.
7. Anzilotti C, Pratesi F, Tommasi C, et al. Peptidylarginine deiminase 4 and citrullination in health and disease. Autoimmun Rev. 2010; 9(3):158-60.
8. Harris ML, Darrah E, Lam GK, et al. Association of autoimmunity to peptidyl arginine deiminase type 4 with genotype and disease severity in rheumatoid arthritis. Arthritis Rheum. 2008; 58,1958–67.
9. Suzuki A, Yamada R, Chang X, et al. Functional haplotypes of PADI4, encoding citrullinating enzyme peptidylarginine deiminase 4, are associated with rheumatoid arthritis. Nat Genet 2003; 34: 395-402.

10. Ikari K, Kuwahara M, Nakamura T, et al. Association betweenPADI4 and rheumatoid arthritis: a replication study. Arthritis Rheum. 2005; 52, 3054–7.
11. Iwamoto T, Ikari K, Nakamura T, et al. Association between PADI 4 and rheumatoid arthritis: a meta-analysis. Rheumatology (Oxford) 2006; 45, 804–7 .
12. Plenge RM, Padyukov L, Remmers EF, et al. Replication of putative candidate-gene associations with rheumatoid arthritis in >4,000 samples from North America and Sweden: association of susceptibility with PTPN22, CTLA4, and PADI4. Am J Hum Genet.2005; 77: 1044–60 .
13. Martinez A, Valdivia A, Pascual-Salcedo D, et al. PADI4 polymorphisms are not associated with rheumatoid arthritis in the Spanish population. Rheumatology. 2005; 44:1263–6 .
14. Burr ML, Naseem H, HinksAet al. PADI4 genotype is not associated with rheumatoid arthritis in a large UK Caucasian Population. Ann Rheum Dis. 2010; 69: 666-70 .
15. Zhao J, Zhao Y, He J. Prevalence and significance of anti-peptidylarginine-deiminase 4 antibodies in rheumatoid arthritis. J Rheumatol. 2008; 35: 969-74.
16. Arnett FC, Edworthy SM, Bloch DA, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988; 31:315–24 .
17. Kolfenbach JR, Deane KD, Derber LA, et al. Autoimmunity to peptidylarginine deiminase type 4 precedes clinical onset of rheumatoid arthritis. Artritis Rheum. 2010; 62(9):2633-9 .
18. Rantapaa-Dahlqvist S, de Jong BA, Berglin E, et al. Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. Arthritis Rheum. 2003; 48: 2741–9 .
19. Nielen MM, van Schaardenburg D, Reesink HW, et al. Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. Arthritis Rheum.2004;50:380–6 .
20. Majka DS, Deane KD, Parrish LA, et al. Duration of preclinical rheumatoid arthritis-related autoantibody positivity increases in subjects with older age at time of disease diagnosis. Ann Rheum Dis. 2008; 67,801–7.
21. Berglin E, Padyukov L, Sundin U, et al. A combination of autoantibodies to cyclic citrullinated peptide (CCP) and HLA-DRB1 locus antigens is strongly associated with future onset of rheumatoid arthritis. Arthritis Res Ther. 2004; 6:R 303–8.
22. VanGaalen FA, Linn-Rasker SP, van Venrooij WJ, et al. Autoantibodies to cyclic citrullinated peptides predict progression to rheumatoid arthritis in patients with undifferentiated arthritis: a prospective cohort study. Arthritis Rheum. 2004; 50: 709–15.
23. Hoppe B, Häupl T, Gruber R, et al. Detailed analysis of the variability of peptidylargininedeiminase type 4 in German patients with rheumatoid arthritis: a case-control study. Arthritis Res Ther 2006; 8: R 34-9.
24. Barton A, Bowes J, Eyre S, et al. A functional haplotype of the PADI4 gene associated with rheumatoid arthritis in a Japanese population is not associated in a United Kingdom population. Arthritis Rheum. 2004; 50:1117-21.
25. Caponi L, Petit-Teixeira E, Sebbag M, et al. A family based study shows no association between rheumatoid arthritis and the PADI4 gene in a white French population. Ann Rheum Dis.2005; 64,587-93 .
26. Nissinen R, Paimela L, Julkunen H, et al. Peptidylargininedeiminase, the arginine to citrulline converting enxyme, is frequently recognized by sera of patients with rheumatoid arthritis, lupus erythematosus and primary Sjögren syndrome. Scand J Rheumatol. 2003; 32: 340-5 .
27. Roth EB, Stenberg P, Book C, et al. Antibodies against transglutaminases, peptidylargininedeiminase and citrulline in rheumatoid arthritis — new pathways to epitope spreading. Clin Exp Rheumatol. 2006; 24:12-8 .
28. Takizawa Y, Sawada T, Suzuki A. Peptidylargininedeiminase4 (PADI4) identified as a conformation-dependent autoantigen in rheumatoid arthritis. Scand J Rheumatol.2005; 34:212–5.
29. Mikuls TR, O'Dell JR, Stoner JA, et al. Association of rheumatoid arthritis treatment response and disease duration with declines in serum levels of IgM rheumatoid factor and anti-cyclic citrullinated peptide antibody. Arthritis Rheum. 2004; 50: 3776–82.
30. Cuchacovich M, Catalan D, Wainstein E, et al. Basal anti-cyclic citrullinated peptide (anti-CCP) antibody levels and a decrease in anti-CCP titers are associated with clinical response to adalimumab in rheumatoid arthritis. Clin Exp Rheumatol. 2008; 26: 1067–73 .
31. Halvorsen EH, Pollmann S, Gilboe IM, et al. Serum IgG antibodies to peptidylarginine deiminase 4 in rheumatoid arthritis and association with disease severity. Ann Rheum Dis.2008; 67: 414-7 .
32. Auger I, Martin M, Balandraud N., Rheumatoid arthritis-specific autoantibodies to peptidyl arginine deiminase type 4 inhibit citrullination of fibrinogen. Arthritis Rheum. 2010; 62: 126–31 .
33. Halvorsen EH, Haavardsholm EA, Pollmann S, et al. Serum IgG antibodies to peptidylargininedeiminase 4 predict radiographic progression in patients with rheumatoid arthritis treated with tumour necrosis factor-alpha blocking agents. Ann Rheum Dis.2009; 68: 249–52.
34. Bozdag M, Dreker T, Henry C, et al. Novel small molecule protein arginine deiminase 4 (PAD4) inhibitors. Bioorg Med Chem Lett. 2013; 23, 715–9.