Genetics, autoantibodies and clinical features in understanding and predicting rheumatoid arthritis

Helm-van Mil, A.H.M. van der

Chapter 17

SUMMARY AND DISCUSSION

Rheumatoid arthritis is a chronic and self-amplifying autoimmune process that ultimately leads to the destruction of cartilage and bone. The pathophysiologic development of inflammation in RA is complex and involves both the adaptive and the innate arms of the immune system. The events triggering the autoimmune mechanisms are thought to be a combination of genetic and environmental factors and the process is best described by a multiple hit model.

The most important genetic risk factor for RA, the HLA-DRB1 alleles encoding for the shared epitope motif, was recognized three decades ago (1). In this thesis the shared epitope alleles are investigated in the perspective of the presence and absence of another important risk factor, the autoantibodies. The shared epitope alleles appeared to predispose only to RA-patients that carry anti-CCP antibodies and not to anti-CCP negative RA (**chapter 7**). This finding led to the hypothesis that the shared epitope alleles confer risk to anti-CCP antibodies, rather than to (anti-CCP positive) RA. To investigate this assumption, the progression from recent-onset UA to RA was studied in relation with the HLA-DRB1 alleles and autoantibodies (**chapter 10**). In patients that presented with UA, the shared epitope alleles were associated with anti-CCP antibodies, but after correction for the presence of anti-CCP antibodies the share epitope alleles were not an independent risk factor for progression to RA. Furthermore, the shared epitope alleles were primarily associated with the anti-CCP antibodies and not with RF. Although no formal conclusions on causality can be drawn from this association study, the findings presented in this chapter suggest that the shared epitope alleles confer risk to anti-CCP antibodies and that these antibodies mediate the association between the shared epitope alleles and RA.

Next, the best-known environmental risk factor for RA, smoking, was studied. The presence of both shared-epitope alleles and smoking resulted in a gene-environmental interaction for the presence of anti-CCP antibodies (**chapter 9**). The data of this study indicate that the gene-environmental interaction is more pronounced for the development of anti-CCP antibodies than for the development of RF. Further analysis revealed that tobacco exposure in the presence of shared epitope alleles did not significantly increase the risk for anti-CCP antibodies among patients with after one-year follow-up persistent undifferentiated arthritis (UA). This might indicate that the gene-environmental interaction is specific for RA. On the other hand, the gene-environmental interaction seems specific for anti-CCP antibodies and, in that case, the interaction should not be depended on a clinical diagnosis. Considering the small number of (anti-CCP positive) patients with persistent undifferentiated arthritis in this study, the power of the present study was probably inadequate to conclude definitely on the presence/absence of a gene-environmental interaction in UA.

The finding that the shared epitope alleles primarily associate with RA in the presence of anti-CCP antibodies led us to address the question whether anti-CCP negative RA is

associated with other, non-shared epitope encoding, HLA-DRB1 alleles. Analysis revealed that HLA-DR3 was more frequently present in the anti-CCP-negative RA and anti-CCP negative UA patients compared to controls, indicating that HLA-DR3 is associated with anti-CCP negative arthritis (**Chapter 8**).

The observation that anti-CCP positive and negative RA have different genetic risk factors indicates that different pathogenetic mechanisms underlie anti-CCP-positive and anti-CCP-negative disease. Therefore, it is conceivable that anti-CCP positive and negative RA are different disease entities and thus have different phenotypical properties. An extensive comparison of clinical characteristics at first presentation between anti-CCP positive and negative RA patients (evaluating among others morning stiffness, type and distribution of early symptoms, C-reactive protein level, tender and swollen joint count) revealed no significant differences. Nevertheless, during the disease course RA patients with anti-CCP antibodies had more swollen joints and more radiological joint destruction compared to RA patients without anti-CCP antibodies (**chapter 11**). Thus, although different risk factors are associated with anti-CCP positive and negative RA, the presence or absence of anti-CCP antibodies is not associated with a distinguishable clinical phenotype at the first presentation of the disease.

Considering the results of the above mentioned association studies, and the findings that citrullinated antigens are found in inflamed joints, anti-CCP antibodies are specific for RA and anti-CCP antibodies are generally present before the onset of clinical symptoms, it seems likely that the anti-CCP antibodies are important in the pathogenesis of RA. So far, there little evidence demonstrating that anti-CCP antibodies induce or mediate the development of chronic joint inflammation. Kuhn and collegues injected monoclonal antibodies reactive to citrullinated fibrinogen in mice and observed an exacerbation of arthritis. They also showed in a collagen-induced arthritis model that tolerization of CCP-specific immune responses resulted in a strong inhibition of arthritis severity (24). As these results are not yet replicated, the issue whether anti-CCP antibodies are involved in RA pathogenesis or are a byproduct of a more general immunological reaction is not clarified.

The Shared Epitope hypothesis postulates that the shared epitope motif is directly involved in the pathogenesis of RA. Data providing evidence that this hypothesis is correct is lacking. Since its initial description, the Shared Epitope hypothesis has been proven robust because of its consistent association with RA susceptibility and severity among varied ethnic populations, but so far a specific athritogenic peptide that binds to the HLA-DR proteins and induces a T cell response has not been found.

Since the identification of citrullinated proteins and anti-CCP antibodies it has been proposed that in genetically predisposed (shared epitope positive) subjects and an inflammatory environment citrullinated proteins break immunologic tolerance and initiate an

HLA-class II restricted T cell response (2,3). Subsequently, the help provided by T cells might allow maturation and switching of B cells, resulting in further maturation of the anti-CCP immunoglobulin response (2). With this hypothesis in mind, it is interesting to note that the data presented in chapter 7 show that carrying two copies of shared epitope alleles confers a significant higher risk on anti-CCP positive RA than the presence of one shared epitope allele (odds ratio 12 and 4 respectively). Compare these findings with the data presented in chapter 10. This chapter reveals that the presence of two shared epitope alleles is not associated with higher anti-CCP antibody levels compared to the presence of one shared epitope allele (the shared epitope alleles act like the previously described immune response genes). At first sight these observations seem contradicting, but this might be explained as follows. In the presence of more shared epitope alleles/ more HLA molecules, more antigen can be presented and immune tolerance is broken more easily, leading to a dose-response relation between the shared epitope alleles and the presence of anti-CCP antibodies. Conversely, antigen presentation might not be the limiting factor that determines the antibody level and the level of autoantibodies might be influenced by other factors. Nonetheless, it must be realized that at present no convincing experimental data exist showing that in shared epitope positive humans the presence of citrullinated peptides induces T cell proliferation. Currently, there are two studies that assess the effect of citrullination on T cell activation in relation with MHC molecules. Hill et al showed in mice transgenic for MHC-DRB1*0401 a vimentin peptide citrullinated in a region with contact to SE-binding site harboured a higher affinity for the MHC-DRB1*0401 molecule resulting in a better T cell acitivation compared to its noncitrullinated counterpart (4). Ireland et al used hen egg-white lysozyme as a model antigen and demonstrated the occurrence of a specific T cell response after citrullination (5). Both studies were performed in mice and the immunopathologic role of citrullinated peptides in humans needs further investigation.

This thesis considered not only the shared epitope alleles but also four other genetic risk factors for RA. The first is also located on the HLA class II alleles and is positioned on the same locus. It concerns the alleles encoding for the amino acids D^{70}ERAA. From previous (smaller) studies it was not clear whether the presence of DERAA was really protective or whether the observed effect was the result of the absence of predisposing shared epitope alleles. By comparing subgroups of RA-patients and controls with an equal amount of shared epitope alleles, we were able to show that the presence of the DERAA-encoding alleles had a protective effect on RA that was independent from the presence/absence of shared epitope alleles (**Chapter 2**). The presence of DERAA was associated with both a lower risk to develop RA and a less severe disease course. The protective effect of DERAA on susceptibility was not confined to anti-CCP positive RA, but seemed stronger for anti-CCP positive RA than for anti-CCP negative RA (odds ratio's of 0.3 and 0.7 respectively).

Considering the severity of RA, the protective effect of DERAA was most pronounced in the patients that were shared epitope positive, smoked or had anti-CCP antibodies. We realize now that these risk factors all influence the same pathway. Thus, the protective influence of DERAA can be detected best in patients that are prone to severe disease (Chapter 2). It is remarkable that although the presence of DERAA was evidently associated with less severe joint destruction and the (small group of) RA patients that were homozygous for DERAA had a non-destructive disease course, the presence of DERAA was not associated with a higher rate of clinical remission. Speculatively, the pathways associated with the expression of DERAA-encoding HLA-alleles are able to dampen the pathways underlying bone and cartilage destruction, but do not affect the principal pathway that drives chronicity. It has been demonstrated that peptides carrying the DERAA motif are naturally processed by human antigen-presenting cells (6). As discussed in chapter 2 the protective effect of DERAA might be due to the activation of regulatory T cells. These T cells are believed to play a key role in mediating transplantation tolerance and inhibiting the induction of tumor immunity; and might likewise suppress the immune response in RA. There are however other presumptions on the pathways underlying the effects of DERAA. The amino acid sequence DERAA is present in several microorganisms, such as mycobacteria, Haemophilus influenzae, Salmonella, and is also present in vinculin, a protein localized in the cell's cytoplasma. It can be speculated that patients that carry DERAA-encoding alleles are immunotolerant for DERAA when encountered during infections, but that DERAA-negative individuals develop an immune reaction against DERAA. During apoptosis vinculin, a part of the cytoskeleton, will be degraded, possibly leading to its presentation in the context of MHC-molecules. Indeed, it has been described that apoptotic cells overexpress vinculin and after ingestion by dendritic cells can be presented toward T cells under certain pathophysiological conditions (8). It is conceivable that DERAA-negative patients harbouring a DERAA-directed T cell response against all sorts of pathogens experience an accelerated disease progression as a consequence of T cell cross reactivity with vinculin-DERAA that is perhaps chronically presented in the inflamed joint in RA. According to this hypothesis, the presence of DERAA does not result in immunosuppression/protection but the absence of DERAA enforces the immune reaction and thereby disease progression. Currently, laboratory experiments are being undertaken to investigate this hypothesis.

The findings presented in chapter 2 lead to the question whether the presence of DERAA is helpful in predicting the development of RA in patients with UA. Analysis (not described in this thesis) revealed that in univariate analysis the presence of DERAA-encoding alleles in patients with recent-onset UA was significantly associated with a lower rate of progression towards RA. In multivariate analysis the absence of DERAA-encoding alleles appeared not to be an independent risk factor for the development of RA. Further analysis showed

that the presence of DERAA was associated with a lower number of tender and swollen joints, a lower level of C-reactive protein and a lower frequency of anti-CCP and RF positivity. This implies that the DERAA-encoding alleles associate with a milder phenotype and that the clinical and serological factors are stronger predictors for the disease course in UA-patients than the DERAA-encoding alleles. The determination of the DERAA-encoding alleles therefore does not add to the discriminative ability of the prediction model described in chapter 16.

Non-HLA genetic risk factors that are investigated in this thesis are single nucleotide polymophisms in the genes encoding for PTPN22, TNFR2 and RAGE.

The C1858T SNP in the *PTPN22* gene encoding for a lymphoid tyrosine phosphatase (Lyp) is associated with RA susceptibility, which was first identified by Begovich et al (9). This intracellular Lyp binds a Csk kinase and Csk-Lyp inhibits T-cell-receptor signaling. *In vitro* experiments have shown that in T-allele carriers Lyp binds less efficiently to Csk suggesting that T cells expressing the T-allele might be hyperresponsive (9). Knocking out the murine homologue of *PTPN22* resulted in lower thresholds for T-cell-receptor signaling (25). A recent study revealed a more active phosphatase in T-allele carriers and the authors suggested that the increased efficacy to inhibit T-cell-receptor signaling may lead to insufficient activity of regulatory T cells (26). The study described in **chapter 4** replicates the association between *PTPN22* and RA, but shows that compared to healthy controls the 1858 T-allele confers risk to only anti-CCP antibody positive RA and not to anti-CCP negative RA. A similar finding has been described for rheumatoid factor (10, chapter 4). In addition, the present study observed no correlation between *PTPN22* and the degree of radiological joint destruction in RA. This seems unsuspected regarding the finding that *PTPN22* associates with anti-CCP positive RA and anti-CCP antibodies are associated with severe disease. The most likely clarification for this finding is mathematical: 48 of 76 patients (63%) carrying the genotype CT or TT had anti-CCP antibodies compared to 149 of 274 (54%) patients with genotype CC. Thus, the percentages anti-CCP positivity were in the same range (and not significantly different), making it comprehensible that no significant difference in the degree of joint destruction was observed between these two groups of RA-patients. It is not excluded that, considering the small ORs of the effect of *PTPN22,* the number of RA-patients was too small to discern an effect of *PTPN22* on RA severity. Finally, this study showed that *PTPN22* is not only a risk factor for RA but also for (persistent) UA. This is in line with reports showing that the 1858 T-allele is associated with multiple autoimmune diseases as SLE, type 1 diabetes and Graves disease (11-14).

The investigated SNPs in the *TNFR2* and *RAGE* genes were not associated with RA. In **chapter 3** the *TNFR2 196M/R* genotypes of the patients with the most severe joint destruction and with clinical remission were similar. An effect of this SNP on RA severity, if present, was likely to be found by the comparison of the extremes of the phenotypes.

The absence of an association between *TNFR2 196 M/R* and RA severity is replicated by the French (15). Interestingly, one of the two reports that initially observed an association between this *TNFR2* SNP and susceptibility to familial RA was recently corrected, as the described finding appeared to be due to an error in genotyping (16).

There are findings that suggest a role for receptor for advanced glycosylation end products (RAGE) signaling in the pathogenesis of RA. RAGE is upregulated in synovial tissue macrophages in active RA (17) and activation of RAGE may lead to upregulation of adhesion molecules and expression of pro-inflammatory cytokines (18). Although initial findings suggested an association between the G82S SNP and RA susceptibility, this association is depended on the strong linkage disequilibrium between RAGE and the HLA-DR4 allele (**chapter 5**).

In conclusion, the genetic risk factors that associate with RA susceptibility and are described in this thesis are the shared epitope HLA-DRB1 alleles and the C1858T *PTPN22* SNP (predisposing effects) and the DERAA encoding HLA-DRB1 alleles (protective effects). Considering that the heritability of RA is estimated to be 50-60% (19) and that the identified genetic factors account for at most half of this percentage, more genetic risk factors for RA susceptibility are likely to be identified.

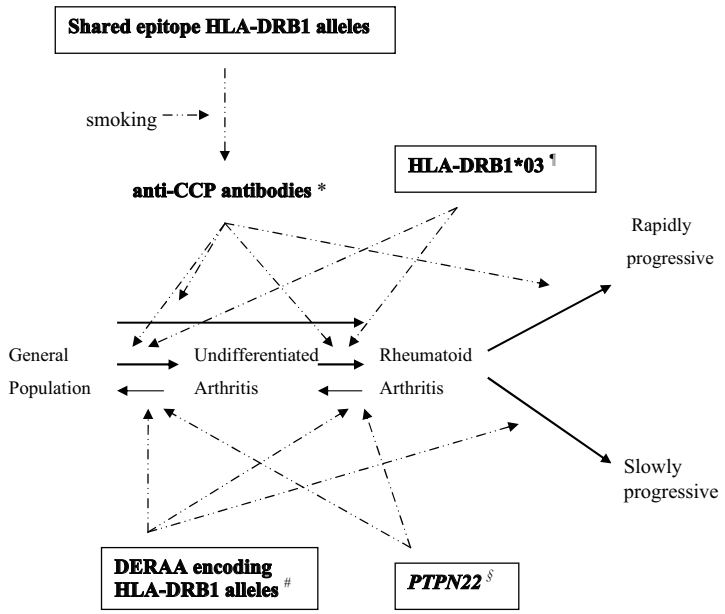
The genetic factors that associate with RA severity and are identified so far are the HLA-DRB1 alleles that encode for the shared epitope motif (effect via anti-CCP antibodies) or encode for DERAA. The heritability of RA severity is not known. The observation of an increase in variation of radiological joint destruction comparing respectively monozygotic twins, dizygotic twins and unrelated pairs of RA patients (after correction for differences in disease duration and autoantibody status) indicates that genetic factors are important for RA severity (**chapter 13**). More extensive twin studies are needed to quantify the genetic contribution to RA severity.

The level of joint destruction in RA patients correlated with the degree of *in vitro* measured invasiveness of fibroblast-like synoviocytes in a matrigel matrix (**chapter 12**). This study provides the first evidence that the *in vitro* measured invasiveness of fibroblast-like synoviocytes might be of relevance for the pathophysiology of RA.

The described genetic and environmental risk factors for the development of both UA and RA and for RA severity can be positioned in a multiple hit model, see Figure 1.

From the patients' and clinicians' perspective, an increased understanding of the pathogenesis of RA is particularly relevant when it has implications for (the treatment of) individual patients. Both UA and RA are diseases with a heterogeneous course. Achieving individualized treatment decisions in these patients is an important challenge for the nearby future. This signifies that for individual patients the predicted prognosis is

weighted against the possible desired and toxic effects of various treatments. For early UA, recent evidence indicates that treatment with methotrexate hampers progression to RA (20). However, from several inception cohorts it is known that only about one third of the patients that present with UA progress to RA and 40-50% remits spontaneously (20-23); these patients are preferably not treated with methotrexate. This underscores the need for a model that identifies the UA-patients with a high risk to progress to RA. The development of such a model is described in **chapter 16**. The derived model predicts in UA-patients the risk to develop RA using nine clinical variables that are all commonly assessed during daily clinical practice (gender, age, localisation of joints symptoms, morning stiffness, counts for tender and swollen joints, CRP, RF and anti-CCP antibodies). The resulting prediction score (a value between 0 and 14) corresponds with a chance on progression to RA. The positive and negative predictive values depended on the chosen cut-off values. If the upper and lower cut-off value were 8.0 and 6.0, the corresponding positive and negative predictive values were 84% and 91%. Twenty-five percent of the patients had a score between 6.0 and 8.0; in these patients no adequate prediction was made. Clinical characteristics are apparently insufficient to predict the disease outcome for these patients and it would be of interest to assess whether the addition of genetic factors increases the predictive ability. The known genetic risk factors for RA susceptibility, HLA-DRB1 alleles and *PTPN22*, do not have additive predictive value in this model, as the shared epitope encoding alleles correspond with the presence of anti-CCP antibodies that are already included in the prediction model and *PTPN22* confers risk both to UA and RA. Overall, the discriminative ability of the model was excellent with an area under the receiver operator curve of 0.87 after internal validation and 0.97 for external validation. As the cohort used for external validation was relatively small, the developed prediction rule needs to be validated in other cohorts of patients with UA. Since the developed prediction rule is accurate and easily assessed in clinical practice, the present model might prove to be an important step forward in achieving individualised decision-making in patients with UA. For the future it is hoped and expected that early treatment of the UA-patients with a high risk to develop RA will hamper the progression to RA. As the social impact of RA is nowadays still considerable (see introduction), the goal of such a therapeutic strategy is to diminish the impact of arthritis on patients' daily life.



* Predisposing effect on susceptibility to RA (chapter 7) and UA and development from UA to RA (chapter 10,16). Association with destructive disease (chapter 11). Interaction with smoking (chapter 9).

¶ Predisposing effect on anti-CCP negative RA (chapter 8).

Protective effect on susceptibility to UA (discussion), susceptibility to RA (chapter 2) and severity of RA (chapter 2).

§ Predisposing effect on both UA and RA (chapter 4).

Figure 1. Multiple hit model for the development of RA.

REFERENCES

1. Stastny P. Mixed lymphocyte cultures in rheumatoid arthritis. J Clin Invest. 1976 May;57(5):1148-57.
2. van Gaalen FA, Ioan-Facsinay A, Huizinga TWJ, Toes REM. The devil in the details: the emerging role of anticitrulline autoimmunity in rheumatoid arthritis. J Immunol 2006, in press.
3. Lundberg K, Nijenhuis S, Vossenaar ER, Palmblad K, van Venrooij WJ, Klareskog L, et al. Citrullinated proteins have increased immunogenicity and arthritogenicity and their presence in arthritic joints correlates with disease severity. Arthritis Res Ther. 2005;7(3): R458-67.
4. Hill JA, Southwood S, Sette A, Jevnikar AM, Bell DA, Cairns E. Cutting edge: the conversion of arginine to citrulline allows for a high-affinity peptide interaction with the rheumatoid arthritis-associated HLA-DRB1*0401 MHC class II molecule. J Immunol. 2003;15;171(2):538-41.
5. Ireland J, Herzog J, Unanue ER. Unique T cells that recognize citrullinated peptides are a feature of protein immunization. J Immunol 2006;177:1421-25.
6. Snijders A, Elferink DG, Geluk A, van Der Zanden AL, Vos K, Schreuder GM, et al. An HLA-DRB1-derived peptide associated with protection against rheumatoid arthritis is naturally processed by human APCs. J Immunol. 2001;166(8):4987-93.
7. Christen U, Edelmann KH, McGavern DB, Wolfe T, Coon B, Teague MK, et al. A viral epitope that mimics a self antigen can accelerate but not initiate autoimmune diabetes. J Clin Invest. 2004;114(9):1290-8.
8. Propato A, Cutrona G, Francavilla V, Ulivi M, Schiaffella E, Landt O, et al. Apoptotic cells overexpress vinculin and induce vinculin-specific cytotoxic T-cell cross-priming. Nat Med. 2001 Jul;7(7):807-13.
9. Begovich AB, Carlton VE, Honigberg LA, Schrodi SJ, Chokkalingam AP, Alexander HC, et al. A missense single-nucleotide polymorphism in a gene encoding a protein tyrosine phosphatase (PTPN22) is associated with rheumatoid arthritis. Am J Hum Genet. 2004;75(2):330-7.
10. Dieude P, Garnier S, Michou L, Petit-Teixeira E, Glikmans E, Pierlot C, et al. Rheumatoid arthritis seropositive for the rheumatoid factor is linked to the protein tyrosine phosphatase nonreceptor 22-620W allele. Arthritis Res Ther. 2005;7(6):R1200-7.
11. Wu H, Cantor RM, Graham DS, Lingren CM, Farwell L, Jager PL, et al. Association analysis of the R620W polymorphism of protein tyrosine phosphatase PTPN22 in systemic lupus erythematosus families: increased T allele frequency in systemic lupus erythematosus patients with autoimmune thyroid disease. Arthritis Rheum. 2005;52(8):2396-402.
12. Skorka A, Bednarczuk T, Bar-Andziak E, Nauman J, Ploski R. Lymphoid tyrosine phosphatase (PTPN22/LYP) variant and Graves' disease in a Polish population: association and gene dose-dependent correlation with age of onset. Clin Endocrinol (Oxf). 2005;62(6):679-82.
13. Zheng W, She JX. Genetic association between a lymphoid tyrosine phosphatase (PTPN22) and type 1 diabetes. Diabetes. 2005;54(3):906-8.
14. Criswell LA, Pfeiffer KA, Lum RF, Gonzales B, Novitzke J, Kern M, et al. Analysis of families in the multiple autoimmune disease genetics consortium (MADGC) collection: the PTPN22 620W allele associates with multiple autoimmune phenotypes. Am J Hum Genet. 2005;76(4):561-71.
15. Goeb V, Dieude P, Vittecoq O, Mejjad O, Menard JF, Thomas M, et al. Association between the TNFRII 196R allele and diagnosis of rheumatoid arthritis. Arthritis Res Ther. 2005;7(5):R1056-62.

16. Potter C, Worthington J, Silman A, Barton A. TNFR2 is not associated with rheumatoid arthritis susceptibility in a Caucasian population. Arthritis Rheum. 2005;53:2579-80.
17. Sunahori K, Yamamura M, Yamana J, Takasugi K, Kawashima M, Makino H. Increased expression of receptor for advanced glycation end products by synovial tissue macrophages in rheumatoid arthritis. Arthritis Rheum. 2006;54(1):97-104.
18. Moser B, Herold, KC Schmidt AM. Receptor for advanced glycation end products and its ligands: Initiators or amplifiers of joint inflammation - A bit of both? Arthritis Rheum. 2006;54(1):14-18.
19. MacGregor AJ, Snieder H, Rigby AS, Koskenvuo M, Kaprio J, Aho K, et al. Characterizing the quantitative genetic contribution to rheumatoid arthritis using data from twins. Arthritis Rheum 2000;43:30-7.
20. van Dongen H, van Aken J, Lard LR, et al. Treatment of Patients with Undifferentiated Arthritis with Methotrexate: a Double-Blind, Placebo-Controlled, Randomized Clinical Trial to Prevent Evolvement into RA. ACR 2005, abstract (479)L4
21. van Aken J, van Dongen H, le Cessie S, Allaart CF, Breedveld FC, Huizinga TW. Comparison of long term outcome of patients with rheumatoid arthritis presenting with undifferentiated arthritis or with rheumatoid arthritis: an observational cohort study. Ann Rheum Dis. 2006;65(1):20-5.
22. Tunn EJ, Bacon PA. Differentiating persistent from self-limiting symmetrical synovitis in an early arthritis clinic. Br J Rheumatol. 1993;32(2):97-103.
23. Harrison BJ, Symmons DP, Brennan P, Barrett EM, Silman AJ. Natural remission in inflammatory polyarthritis: issues of definition and prediction. Br J Rheumatol. 1996;35(11):1096-100.
24. Kuhn KA, Kulik L, Tomoooka B, Braschler KJ, Arend WP, Robinson WH, Holers VM. Antibodies against citrullinated proteins enhance tissue injury in experimental autoimmune arthritis. J Clin Invest. 2006;116:961-73.
25. Hasegawa K, Martin F, Huang G, Tumas D, Diehl L, Chan AC. PEST domain-enriched tyrosine phosphatase (PEP) regulation of effector/memory T cells. Science. 2004;303: 685-89.
26. Vang T, Congia M, Macis MD, Musumeci L, Orru V, Zavattari, et al. Autoimmune-associated lymphoid tyrosine phosphatase is a gain-of-function variant. Nat Genet, 2005;37:1317-19.