Association between anti-citrullinated alpha enolase antibodies and clinical features in a cohort of patients with rheumatoid arthritis: a pilot study

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SUMMARY

In recent years several antibodies against citrullinated peptides (ACPAs) have been identified in patients with rheumatoid arthritis (RA) and their pathogenic, diagnostic and prognostic significance is under intense investigation. Among ACPAs, those targeting citrullinated alpha enolase (anti-CEP1) have been identified in RA but data about their ability to predict the development of erosive disease are conflicting. Furthermore, no data are currently available concerning their possible association with extra-articular manifestations (EAMs) in RA. The aim of this study was to investigate the prevalence and significance of anti-CEP1 from a prognostic point of view. In this pilot study we confirmed that anti-CEP1 Abs are associated with higher prevalence of bone erosions, but we also provided the first evidence of an association between anti-CEP1 Abs and RA interstitial lung disease (ILD). These results provide the basis to investigate the association between anti-CEP1 Abs and EAMs in larger cohorts of RA patients to possibly confirm its role as biomarker for RA-ILD.

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■ INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterised by synovitis and leading to bone erosion, joint damage and disability (1, 2). In a subgroup of patients, the clinical spectrum is heterogeneous and includes extra-articular manifestations (EAMs) (3). EAMs worsen RA prognosis, since cardiovascular disease and pulmonary involvement are the two most frequent causes of death in RA (4). The identification of predictive biomarkers of erosive disease and EAM development is under intense investigation, but none of them is reliable enough to ensure the exact identification and thorough follow-up of all the patients at higher risk (5). For instance, although rheumatoid factor (RF) and anti-cyclic citrullinated peptide (anti-CCP) antibodies (Abs) have been clearly linked to a

more severe articular disease and a higher risk of EAMs (6), these manifestations can nevertheless be detected also in seronegative patients, suggesting that other biomarkers are needed to identify this subgroup of high risk subjects. In this regard, the field of Abs anti-citrullinated peptides (ACPA) is rapidly expanding with several target antigens identified over the last decade. The diagnostic and prognostic role of different ACPAs in RA is debated and conflicting results have been obtained in different cohorts (7, 8). The immunodominant citrullinated alfa-enolase peptide 1 (CEP1) is one of these newly identified targets of ACPA and anti-CEP1 Abs were first described in RA synovial fluid (SF) in 2008 (9) and in RA serum in 2009 (10). According to different studies, serum Anti-CEP1 Abs are detectable in 24-80% of RA patients (10-13) and are associated with genetic fac-

tors that increase RA susceptibility such as HLA-DRB1 and PTPN22 (12-14). The association between anti-CEP1 Abs and bone erosions is unclear, as the available studies yield conflicting results (12, 13). In addition, no evidence regarding an association between anti-CEP1 and EAMs is available. The aim of our study was to investigate the prevalence of bone erosions and EAMs in RA patients displaying anti-CEP1 compared to anti-CEP-1 negative patients.

■ MATERIALS AND METHODS

Patient cohort

One-hundred patients with RA according to the 1987 American College of Rheumatology Classification criteria (15), referring to the Rheumatology Unit of the University of Perugia and the Clinical Immunology and Allergy Unit of the University of Pisa, were enrolled. Fifty sex- and age-matched healthy donors (HD) acted as controls. Demographic, clinical (including all possible EAMs), radiological and serological records were collected retrospectively through the revision of patient charts. The study was approved by the local Ethical Committee and all enrolled subjects provided written informed consent according to the Declaration of Helsinki.

Antibody detection

Anti-CCP IgG antibodies were assessed in serum samples with second generation ELISA kits (EliA - Thermo Scientific). Anti-CEP-1 IgG antibodies were assessed with a commercially available ELISA kit (Euroimmun) according to the manufacturer's instructions.

Statistical analysis

Data were processed with SPSS 21.0. Chi square test, Mann-Whitney U test and Spearman correlation coefficient were calculated as needed.

■ RESULTS

Anti-CEP1 positivity distribution in RA and HD

As depicted in Figure 1, anti-CEP1 antibodies resulted significantly more prevalent in the RA cohort compared to HD. In fact, 45 out of 100 RA patients (45%) and 1 out of 50 HD (2%) displayed these antibodies (p<0.0001). RA patients also displayed higher antibody titers (p<0.0001). With regard to the co-existence of anti-CEP1 and anti-CCP Abs, we observed that 38 out of 100 RA patients (38%) displayed both anti-CEP1 and anti-CCP Abs, 28 patients (28%) displayed neither anti-CEP1 nor anti-CCP Abs, 28 patients (28%) displayed only anti-CCP Abs and 7 patients (7%) displayed only anti-CEP1 Abs. In addition anti-CEP1 and anti-CCP titers were strongly correlated (Spearman's rho=0.56, p<0.0001).

Anti-CEP1 antibodies are associated with erosive disease and ILD

We subsequently divided patients according to their serological status (both Abs negative, both Abs positive, only one Abs positive). No difference was observed regarding gender, age, age at diagnosis and disease duration. However, a different prevalence of erosive disease and interstitial lung disease (ILD) was detected among the groups (Table I). We did not observe any difference in the prevalence of any other EAM among the 4 groups using the Chi-square test (data not shown). In our cohort, the prevalence of erosive disease and ILD
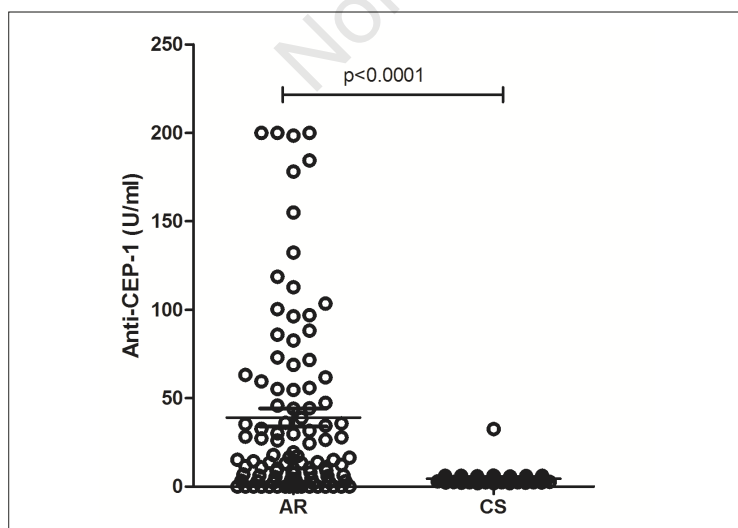
Figure 1 - Distribution of anti-CEP1 antibodies in patients with rheumatoid arthritis (RA) and healthy donors (HD).

was similar in current/former smokers and non-smokers. Interestingly, however, distribution and titer of anti-CEP1 and CCP in smokers/non-smokers with ILD were different. The majority of smokers with ILD (75%) displayed either anti-CCP alone or anti-CCP plus anti-CEP-1. Conversely, 80% of non-smokers with ILD displayed either anti-CEP1 alone or anti-CCP plus anti CEP-1 (Table II).

■ DISCUSSION AND CONCLUSIONS

In the last decades, the diagnostic and prognostic roles of ACPA in RA have been extensively investigated (16). As previously reported (12), we confirmed in this pilot study that anti-CEP1 Abs are associated with higher prevalence of bone erosions, but we also provided the first evidence of an association between anti-CEP1 Abs and RA-ILD.

Bone erosions represent a hallmark of RA, being detectable in more than half of RA patients and leading to irreversible joint damage and loss of function (17). Erosions develop as consequence of persistent chronic inflammation through a multistep process. Several cells and soluble mediators belonging to the immune system are involved in this process, including autoAbs, the latter being able to activate osteoclasts either directly or through their Fc (18). In addition, it is known that ACPA may occur years before the diagnosis of RA and that some patients already display erosions at the time of diagnosis. These observations may further strengthen the role of ACPA in the development of bone erosions (19-22). With regard to the association between anti-CEP1 and ILD, it is interesting to note that 70% of patients with ILD display either anti-CEP1 alone or in association with anti-CCP, while only a small proportion of anti-CCP+ anti-CEP1- patients display ILD. This evidence is partially in contrast with previous literature reporting a higher

Table I - Demographic and clinical features of patients with rheumatoid arthritis according to the anti-CCP and anti-CEP1 serological status.

	All	anti-CCP- anti-CEP1-	anti-CCP+ anti-CEP-	anti-CCP- anti-CEP+	anti-CCP+ anti-CEP+	P
Patients (number)	100	28	28	7	37	-
Age (years)	62±2	63±2	63±2	60±1	61±2	ns
Age at diagnosis (years)	50±2	52±1	52±2	51±3	50±2	ns
Disease duration (years)	12±3	12±2	13±1	11±2	13±2	ns
Erosions number (percentage)	60 (60%)	10 (36%)	19 (68%)	7 (100%)	25 (67%)	0.004
RA-ILD number (percentage)	21 (21%)	3 (11%)	3 (11%)	5 (70%)	10 (27%)	0.002

Table II - Smoking habit and interstitial lung disease prevalence in patients with rheumatoid arthritis the anti-CCP and anti-CEP1 serological status (all values are shown as number (percentage) of patients).

	anti-CCP- anti-CEP1-	anti-CCP+ anti-CEP-	anti-CCP- anti-CEP+	anti-CCP+ anti-CEP+
Patients	28	28	7	37
Non-smokers No ILD	21 (75%)	20 (72%)	0 (0%)	14 (38%)
Non-smokers ILD	1 (3%)	2 (7%)	5 (72%)	5 (13%)
Smokers	5 (18%)	5 (18%)	1 (14%)	13 (36%)
No ILD				
Smokers ILD	1 (3%)	1 (3%)	1 (14%)	5 (13%)

prevalence of ILD in anti-CCP+ patients (23). In fact, if we cumulate data from all anti-CCP+ patients independently of anti-CEP1, we observe ILD in 20% of patients. Conversely, if we cumulate data from all anti-CEP+ patients independently of anti-CCP1, we observe ILD in 34% of patients. This clearly demonstrated that, in patients with double positivity, the association with ILD is mainly dependent on anti-CEP1, rather than on anti-CCP. In this regard, it is important to note that previous studies assessed only anti-CCP Abs. Therefore, the contribution of other ACPA subtypes to the association of anti-CCP and ILD cannot be ruled out.

The distribution of autoAbs between smokers and non-smokers in our RA cohort also deserves some consideration. It is now well established that cigarette smoke increases the activity of the peptidyl amino deaminase (PAD) enzyme, which is the mediator of protein citrullination. Furthermore, smoking is strongly associated with the development of ILD. Therefore, smokers are more likely to be seropositive and more often develop RA-ILD (23, 24). However, it is conceivable that other mechanisms could be responsible for aberrant peptide citrullination, otherwise the presence of ACPA in non-smokers could not be explained. In our cohort, among smokers and non-smokers with ILD, only one patient per group is negative for both anti-CEP1 and anti-CCP. It is of interest that all patients with ILD who were positive for anti-CEP1, but not anti-CCP, had never smoked. An explanation may be the possible coexistence of a *Porphyromonas* (*P.*) *gingivalis*-induced periodontitis, as this bacterium is equipped with the PAD enzyme (25). Furthermore, CEP-1 displays 82% homology with enolase from *P. gingivalis*, the levels of anti-CEP-1 correlate with the levels of Abs to the bacterial peptide (26) and anti-*P. gingivalis* Ab titer is higher in RA patients who had never smoked compared to patients who had regularly smoked and among non-smokers (27). The association between *P. gingivalis* and anti-CEP1 represents an intriguing issue deserving further investigation in the future.

In conclusion, our pilot study provides the basis to investigate the association between anti-CEP1 Abs and EAMs in larger cohorts of RA patients to possibly confirm its role as biomarker for RA-ILD.

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