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Original article

Soluble vascular endothelial (VE) cadherin and autoantibodies to VE-cadherin in rheumatoid arthritis patients treated with etanercept or adalimumab

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ABSTRACT

Objectives: The aim of this study was to investigate the clinical value of sVE and anti-vascular endothelial-cadherin antibodies (AAVE) in RA treated with etanercept or adalimumab combined with methotrexate.
Methods: This was an 18-month prospective multicenter study in which patients had active RA, requiring TNF antagonist. sVE rates and AAVE titers were measured respectively by dot blot and ELISA. The relationship of these biomarkers with parameters reflecting articular or systemic disease activity, progression of structural damage, and response or remission to treatment was analyzed.
Results: Forty-eight patients received TNF blocking agents. Variation of sVE rates were significantly correlated with that of C-reactive protein (CRP) levels at weeks 6, 12, 26 and 52. A significant decrease in sVE levels was observed in the group of patients exhibiting a decrease in CRP levels as compared to the patient group with unmodified CRP. AAVE at baseline were correlated with rheumatoid factor. Kinetics analysis of sVE levels and AAVE titers showed that their level were not associated with disease activity score and to methotrexate/adalimumab or etanercept response.
Conclusions: sVE is a biomarker associated with systemic RA activity under anti-TNF. AAVE are related to autoantibodies usually associated to RA.

1. Introduction

Rheumatoid arthritis (RA) is the most common form of chronic inflammatory arthritis with prevalence between 0.3 and 1% in the general population [1]. The primary cause of death is due to cardiovascular involvement. The initial connection between chronic inflammation and cardiovascular disease is related to endothelial dysfunction promoted by inflammatory cytokines such as tumor necrosing factor alpha (TNF-α), which in turn are responsible for loss of endothelial barrier and its anticoagulant function [2]. Thereafter, there is an infiltration of the arterial wall by monocytes and formation and progression of atherosclerotic plaques over time [3]. The stability of the endothelium is maintained by a precise control of endothelial cells adherens junctions. Structural modifications of VE-cadherin by phosphorylation of tyrosine residues in its intracellular domain or by cleavage of its extracellular domain (sVE) are involved in TNFα mediated-microvascular permeability [4]. Interestingly, sVE can be detected in blood stream from RA patients. Endothelial junctions breakdown has also been observed as the result of the action of antibodies directed against VE-cadherin

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extracellular domain that can induce vascular damage in mice [5]. We have previously demonstrated the existence of autoantibodies to VE-cadherin (AAVE) in human including in very early RA. Because synthetic monoclonal anti-VE-cadherin antibodies can also induce vascular damage in mice, these AAVEs might be biomarkers of endothelial injury [6,7].

Anti-TNF biotherapies have been widely used for the treatment of RA, as these compounds can improve endothelial dysfunction, especially when disease activity is controlled [8]. However, according to American College of Rheumatology criteria (ACR), around one-third of RA patients treated with theses therapies fail to respond [9]. The use of predictive biomarkers to identify responders to treatment may provide guidance in clinical care. We previously showed that sVE level in patients at the very early stages of RA was correlated to disease activity score while AAVE have never been investigated as potential markers of disease activity [4]. The aim of this study was to analyze the variations of sVE and AAVE in RA patients treated by TNF blocking agents to determine whether these endothelial biomarkers are of clinical interest in this disease. More precisely, analysis of the relationship between sVE and AAVE levels over time and parameters associated with articular or systemic disease activity, progression of ultrasonography erosive score (US) and response to treatment were performed.

2. Methods

2.1. Patient cohort

This study was a prospective and longitudinal study that enrolled patients from the SATRAPE cohort. Recruitment of patients was conducted over 18 months in four departments of rheumatology located in tertiary referral centers in the northwest of France. Inclusion criterias were RA according to the 1987 ACR classification criteria with active disease defined as disease activity score in 28 joints based on erythrocyte sedimentation rate (DAS 28 ESR) > 5.1 at baseline and an inadequate response to at least one traditional disease-modifying antirheumatic drug (DMARD) but naive of TNF antagonist. To be enrolled, patients had to have received background therapy with methotrexate (MTX) for at least 3 months with a stable dose for 4 weeks. Corticosteroids were authorized at a dose ≤ 10 mg/day of prednisone that was stable since 14 days. Patients gave written informed consent prior to participating in the present study. Exclusion criteria were those usually retained for biological agents [9] and other related to the study procedures, including: anemia with hemoglobin < 11 g/100 mL (justified by the volume of blood removed for biological analysis) and other rheumatic diseases (osteoarthritis, fibromyalgia. . .) likely to interfere with analysis. Patients were randomized in the group receiving etanercept or adalimumab, according to stratification by center. Clinical, biological and US data were collected at baseline (visit V0), at 6 weeks (visit V1), at 12 weeks (visit V2), at 26 weeks (visit V3) and 52 weeks (visit V4). Demographic and baseline collected parameters were age, sex, duration of rheumatism, calculation of health assessment questionnaire (HAQ), rheumatoid factor (RF) titers by Latex fixation test and Waaler Rose reaction, anti-cyclic citrullinated peptide (anti-CCP) titers (cut-off at 7 UA/mL), structural damage (presence of erosions or pinching) on hands and feet X-rays and use of corticosteroid or non-steroidal anti-inflammatory drugs (NSAIDs). Parameters reflecting disease activity were assessed at the different time points and included tender joint counts of 28 joints (TJC/28) and of 68 joints (TJC/68), swollen joint counts of 28 joints (SJC/28) and of 66 joints (SJC/66), patient's global assessment (PGA), global assessment by visual analogue scale by physician (MD-Global), erythrocyte sedimentation rate (ESR) and C-reactive-protein (CRP) (n < 5 mg/L) and disease activity score in 28 joints with sedimentation rate (DAS 28 VS) and with CRP (DAS 28 CRP). Ultrasonography parameters aimed to detect synovitis, doppler activity and bone erosions across the semi-quantitative scoring system by Szkudlarek were collected at V0, V2, V3 and V4 [10]. US study was focused on the 10 metacarpophalangeal joints and quantitative results were presented as a total score equal to the sum of these 10 joints for each patient and at each time point. To analyze the response to anti-TNF treatment, we used EULAR response criteria and remission status (DAS 28 ESR < 2.6) [11,12]. Patients were divided in two subsets according to EULAR response, one group of good responders and a second including moderate and non-responders. All patients self-administered a subcutaneous injection of adalimumab (Abbott Laboratory©) 40 mg every other week or etanercept (Pfizer Laboratory©) 50 mg weekly. Sera were collected from patients included in the SATRAPE study (nº 2005/06; ClinicalTrials.gov identifier: NCT00234234) and another study (nº 2004/120). This study was approved by the regional ethics committee ("comité de protection des personnes du Nord-Ouest 1, France") and all participants gave written informed consent at the time of enrollment. There was no funding from the pharmaceutical industry. This cohort was sponsored by academic grants.

2.2. Assays for measurement of sVE and AAVE levels

Longitudinally collected serum samples (week 0, 12, 26, 52) available from 48 RA patients were analyzed for sVE and AAVE content. The follow-up of sVE in RA patients was derived from our previously described method using [4]. As only one immunoreactive band of sVE was detected (90 kDa), in the present study, we used Dot blotting. All the samples from the same patient were then analyzed on the same nitrocellulose membrane. After incubation with sVE primary antibody BV9, and then exposure to secondary antibody α-M HRP, electro-chemiluminescence was then quantified using a ChemiDoc apparatus and Image Lab software. The results were presented as a variation range of sVE levels between a given time and V0 initial time.

AAVE were measured by enzyme immunoassay enzyme-linked immunosorbent assay (ELISA) as previously described [6]. Briefly, the recombinant fragment of human VE-cadherin (5 μg/mL in 0.1 M sodium carbonate buffer, pH 9.6) was coated onto 96-well ELISA plates (NUNC MaxiSorp) overnight at 4 °C. Plates were washed three times with 0.05% tween-containing TBS and blocked for 1 h at room temperature (RT) with Pierce protein buffer (Sigma) and then 100 μl of diluted serum 1:100 in TBS-BSA 1.5% (bovine serum albumin) was applied and incubated for 2 hours at RT. Binding was revealed by incubating for 60 min at RT with a biotin-conjugated goat-anti-human IgG (Sigma) (1:2000 dilution) followed by streptavidin-conjugated alkaline phosphatase (1:500 dilution, Sigma) for 30 min at RT. Finally, para-nitrophenyl phosphate (Sigma) was applied for 30 min in the dark at 25 °C, and absorbance was measured at 405 nm after addition of NaOH 3 M to stop the reaction. For each point, the average of two optical density (OD) values was calculated. A blank value was subtracted for each plate, and a positive control serum was included to establish the inter-assay coefficient of variation. For each sample, the AAVE value was calculated as followed: OD sample/(highest OD of the plate-lowest OD of the plate) × 100 [13]. Intra- and inter-assay coefficient of variation were < 5%.

2.3. Criteria of judgment

The three endpoints to assess the clinical potential of sVE and AAVE were: disease activity defined by articular or systemic parameters, evolution of structural damage defined by the progression of

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