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Original article

Prediction of response to disease modifying antirheumatic drugs in rheumatoid arthritis

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ABSTRACT

Aim: To investigate potential predictors of response to conventional DMARDs in RA.
Methods: Study design – 6-month follow-up prospective study.
Participants: RA patients with active disease.
Intervention and follow-up: Introduction of one DMARD. Response to treatment evaluated at 6 months (ACR20 criteria).
Analysis: Potential predictors of response, patients' demographics, disease activity, percentages of PBMC subsets expressing P-gp, serum IL-1β, IL-6, IL-8, IL-10, IL-12, TNF-α levels, were evaluated using univariate and multivariate logistic regression analysis. ROC curve analyses were performed in order to obtain thresholds allowing the prediction of response.
Results: Forty-two patients (mean age = 57 ± 13 years, mean disease duration = 5.4 ± 7.2 years) were included. MTX was given to 30. The response to therapy was predicted by the baseline serum level of TNF-α (mean = 30.2 pg/ml ± 18 in non-responders vs. 11.9 pg/ml ± 11.2 in responders). The threshold, which predicted with the best accuracy the response to treatment, was 20.1 pg/ml (sensitivity, specificity, positive and negative predictive values of 75, 78.9, 83.3, and 69.2%, respectively; AUC = 80.3%, 95% CI = 62.8–97.7%). Similar results were obtained in the subgroups of patients treated with MTX and patients with early RA of less than 3 years duration.
Conclusion: In the present work, the serum concentration of TNF-α was related to further response to DMARDs. Other works are needed for confirmation and to assess whether such biomarker could be used to predict the response to DMARDs at the individual level.

Abbreviations: ACR, American College of Rheumatology; CRP, C-reactive protein; DAS, disease activity score; DMARDs, disease modifying antirheumatic drugs; ESR, erythrocyte sedimentation rate; EULAR, European League Against Rheumatism; HAQ, health asessment questionnaire; IL, interleukine; IL-1β, interleukin one beta; MAb, monoclonal antibody; MDR, multidrug resistance; MRP, multidrug resistance-associated proteins; MTX, methotrexate; PBMC, peripheral blood mononuclear cells; P-gp, P glycoprotein; PE, phycoerythrin; RA, rheumatoid arthritis; RF, rheumatoid factor; ROC, receiver operating characteristic; TNF, tumor necrosis factor.

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It is widely accepted that DMARDs should be introduced as early as possible in the course of RA. Moreover, several studies have suggested that an aggressive therapy, such as combination therapy, and possibly biologics, might be useful in the early stages of the disease, and might influence the long-term prognosis, leading to the concept of the "window of opportunity" [1–9]. However, RA is a heterogeneous disease, which has a benign course in many patients, in whom a prolonged low-disease activity state or remission can be achieved using a single conventional DMARD [10]. Consequently, it might be unreasonable to offer primary aggressive therapy to all patients since such therapy could be considered possibly harmful in patients who would have responded to treatment with a single DMARD. Thus, it is important to obtain prognostic criteria to distinguish between patients who should be treated with an aggressive treatment and those who should not. In addition, predicting the efficacy of DMARDs at the individual level would also be useful in all RA patients, whatever the duration of the disease, as it would help the physician to choose between different treatment options, and particularly in the choice of classical DMARD or biologics.

Many patient or disease-related predictive factors have been described, in early as well as in late RA [11–23], but their usefulness in guiding the choice of treatment at the individual level remain unclear. In particular, the response to initial DMARD treatment is a powerful prognostic factor, at least for subsequent remission in patients with early RA [11,12]. Therefore, it has been proposed that most patients should be given classical DMARD therapy, with close monitoring, and regular reevaluation. Then, if the primary objective of treatment, e.g. remission or EULAR/ACR response is not achieved, DMARD therapy may be modified by switching to combination therapy or biologics [12,24]. However, it takes several months for the initial response to a first-line DMARD to be obtained and as a result some patients may miss the "window of opportunity". Thus, identifying a factor that allows doctors to predict the efficacy of DMARD should help them choose the first-line treatment. For RA of a longer duration, long disease duration and the previous use of a range of DMARDs have been reported to be associated with a poor response to future treatment, but the usefulness of such factors in clinical practice is doubtful.

The aims of this prospective study were:

1. to investigate demographic and disease-specific characteristics, as well as serum concentrations of cytokines and MDR PBMC expression as predictive factors of response to DMARDs;
2. to obtain thresholds that would allow such factors to be used in clinical practice.

1. Methods

1.1. Study design

Six-month prospective follow-up study. The design of the study was in compliance with the declaration of Helsinki, and was approved by the local ethics committee. All patients gave written informed consent.

1.2. Participants

RA patients (ACR criteria) with active disease, defined by at least three of the following four criteria: $\geq$ 6/44 tender joints, $\geq$ 3/44 swollen joints, $\geq$ 45 min morning stiffness, ESR $\geq$ 28 mm/h, requiring DMARD treatment according to the patient's rheumatologist. The choice of DMARD was left to the decision of each patient's rheumatologist.

1.3. Non inclusion criteria

Non inclusion criteria were treatment with another DMARD during the previous month, any change in the daily dose of oral corticosteroid during the previous month, intra-articular corticosteroid injection during the previous 6 weeks.

1.4. Evaluation

1.4.1. Evaluation of clinical and usual biochemical variables

The patients' demographics and disease's characteristics were obtained at baseline. The following parameters of disease activity were obtained at baseline and at the 6-month evaluation: 44 tender joint count, 44 swollen joint count, patient's evaluation of pain (100 mm visual analog scale), patient's and physician's global evaluation of disease activity (100 mm visual analog scale), functional impairment (HAQ), ESR, CRP serum level, DAS44 score.

At the 6-month evaluation, the response to therapy was evaluated using the ACR20 criteria for improvement. Patients who stopped therapy or changed treatment dose before the 6-month evaluation, but at least 3 months after inclusion were evaluated at the time of treatment withdrawal. Patients who stopped treatment during the first 3 months for adverse events were excluded. During the first 3 months, withdrawal or a change in treatment dosage for inefficacy was not allowed.

1.4.2. MDR resistance peripheral blood mononuclear cell expression and serum cytokine profile

Blood samples were shipped on ice within 1 day to one place (laboratory of haematology, CHU Dijon) in which the analysis was performed. The blood mononuclear cell expression was analysed as soon as received, while samples were stored ($-70\,^{\circ}C$) until they were analyzed for cytokine profile.

1.4.3. MDR resistance peripheral blood mononuclear cell expression

The percentage of PBMC subsets expressing P-gp was determined using a two-step procedure. PBMC were separated by density gradient centrifugation ($d = 1.077$) (Eurobio, France) and washed once in Hank's buffer containing 1% bovine serum albumin (Hank's-BSA) (Sigma). The cells were incubated for 30 min at $4\,^{\circ}C$ with directly conjugated MAb associated as follows: CD3-PECy5 /CD4-PE, CD3-PECy5 /CD8-PE, CD19-PECy5/CD14-PE (Beckman Coulter, France). Subsequently, the cells were washed once in 1% Hank's-BSA in order to eliminate the excess of MAb, and incubated for 30 min at $4\,^{\circ}C$ with UIC-2, a MAb specifically directed against P-gp (2.5 mg of UIC-2 MAb/500 000 cells) in 200 ml of phosphate-buffer saline without Ca^{2+} and Mg^{2+} (PBS) (Eurobio, France). After two washes, the cells were incubated with a goat anti-mouse FITC conjugated polyclonal F(ab)'2 fraction (Silenius, Australia) for 30 min at $4\,^{\circ}C$. They were washed again twice and kept in the dark until flow cytometry analysis. An isotypic control was performed using a purified mouse monoclonal IgG2a Ab (Beckman Coulter, France). Results are expressed as percentages of UIC-2 positive cells (P-gp expressing) within CD3+/CD4+, CD3+/CD8+, CD19+ and CD14+ cells.

1.4.4. Serum cytokine profile

Serum IL-1β, IL-6, IL-8, IL-10 and IL-12, as well as TNF-α concentrations were determined at baseline. The cytokine concentrations were simultaneously quantified on 50 μl of sera by means of a suspension array technology based on a two-color flow cytometric analysis performed on a GALAXY flow cytometer (Partec) using the BD^{TM} Cytokine Bead Array (BD Biosciences Pharmingen, San Diego, CA, USA) according to the manufacturer's procedure. To this purpose, fluorescent polystyrene beads (diameter: 7.5 μm; excitation