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Serological Biomarkers of Joint Tissue Turnover Predict Tocilizumab Response at Baseline

Anne C. Bay-Jensen, PhD, MSc, Inger Byrjalsen, PhD,* Anne Sofie Siebuhr, MSc,* Claus Christiansen, MD,* Adam Platt, PhD,† and Morten A. Karsdal, PhD, MSc**

Rheumatoid arthritis (RA) is an autoimmune disease characterized by polyarticular joint inflammation resulting in massive tissue turnover. The turnover is partly mediated by an up-regulation of proteolytic enzymes, such as matrix metalloproteinases (MMPs).[1] Matrix metalloproteinase 3 is 1 of the MMPs responsible for the degradation of the extracellular matrix (ECM).[2] The MMP-mediated degradation of the main joint ECM proteins (eg, types I and III collagen)[3] results in the release of specific biomarkers such as the connective tissue biomarkers C1M and C3M,[4,5] known as protein fingerprints. These biomarkers are direct measures of changes to the tissue affected by the disease, in contrast to measurement of acute reactants such as C-reactive protein (CRP) or interleukin 6 (IL-6), which are upstream of tissue changes.[6] Thus, protein fingerprint biomarkers may be more sensitive tools for measuring disease changes or changes caused by intervention. Protein fingerprint biomarkers have been associated with disease progression and response to therapy.[7,8] CRPM is a protein fingerprint formed through degradation of CRP. In response to IL-6, CRP is secreted by the liver as an acute phase reactant.[9] C-reactive protein accumulates in inflamed tissue, where it is degraded by MMPs, resulting in the release of CRPM.[10] The ratio of C3M to CRPM may depict MMP3 is 1 of the MMPs responsible for the degradation of the ECM; its expression is highly elevated in the affected joint and may therefore be a relevant marker of proteolytic activity.[2]

Tocilizumab (TCZ) is approved in 2 doses for intravenous infusion: 4 and 8 mg/kg. Although both doses provide structural progression and symptomatic relief, 8 mg/kg generally affords a higher level of response.[11] Composite quantifiable measures depending on CRP were more reduced in 8 mg/kg compared with 4 mg/kg.[12,13] As there are more adverse events in the higher dose,[14] identification of those patients who respond most optimally to 4 mg/kg would significantly improve the benefit-to-risk assessment. The aim of present study was to identify responders to 4 mg/kg TCZ by measuring protein fingerprints at baseline.

METHODS

Study Design and Serum Samples

Fasting RA patients' serum samples (n = 200) from the 4 mg/kg TCZ treatment arm of the LITHE phase III study[11] were analyzed for the following protein fingerprint biomarkers: C1M,[4] C3M,[15] and CRPM[10] were measured by manual competitive enzyme-linked immunosorbent assays (Nordic Bioscience, Herlev, Denmark), and serum total MMP-3 was measured by a 2-site enzyme-linked immunosorbent assay (Quantikine; R&D Systems, Lille, France). Five additional markers were measured; however, these showed no discriminative power when plotted (area under the receiver operating characteristic curve). Treatment response was recorded according to the American College of Rheumatology criteria for 50% improvement (ACR50) at week 52. Baseline patient characteristics are summarized in the Table. The study was approved by the ethics committee at each recruiting institution[11] and was conducted according to the Principles of Good Clinical Practice and according to the Declaration of Helsinki.

Statistical Analysis

Biomarker data were log transformed to reach normal distribution. The biomarker data were plotted separating ACR50 responders and nonresponders, and cutoffs were determined by areas under the receiver operating characteristic curves. Primary cutoffs were selected at sensitivity of a minimum of 70% (Sen70% bootstrapping). Secondary cutoffs were selected for MMP-3 and C1M at sensitivity of a minimum of 60% (Sen60% bootstrapping). The same was done for the ratio between C3M and CRPM; however, these were also plotted as a scatterplot (responder vs nonresponder, not shown), where distribution patterns were investigated to identify a threshold range including must responders (minimum 70%). The odds ratio (OR) for likelihood of being an ACR50 responder with a biomarker level at baseline above/below the set cutoff levels was determined by 2 × 2 tables. A decision tree was used to segregate ACR50 responders and nonresponders.

RESULTS

Determination of Cutoffs to Be Used in the Decision Tree for Predicting ACR50 Response

The MMP3 and C1M scatterplot showed that the variances were similar for the responder and nonresponder groups, whereas the variance of C3M/CRPM was markedly lower for responder group compared with the nonresponder group (Fig. 1). High level of MMP3 was significantly associated with ACR50 response with ORs of 3.1 for both Sen70% and Sen60% ($P < 0.001$), and cutoffs were set as 1.57 and 1.64 (Fig. 1A). There were trends toward a lower level of C1M in the responder group; however, these were

From the *Nordic Bioscience, Herlev Hovedgade, Herlev, Denmark; and †Roche Products Ltd, Welwyn Garden City, United Kingdom.

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Correspondence: Anne C. Bay-Jensen, PhD, MSc, Nordic Bioscience A/S, Herlev Hovedgade 207, DK-2730 Herlev, Denmark. E-mail: acbj@nordicbioscience.com.

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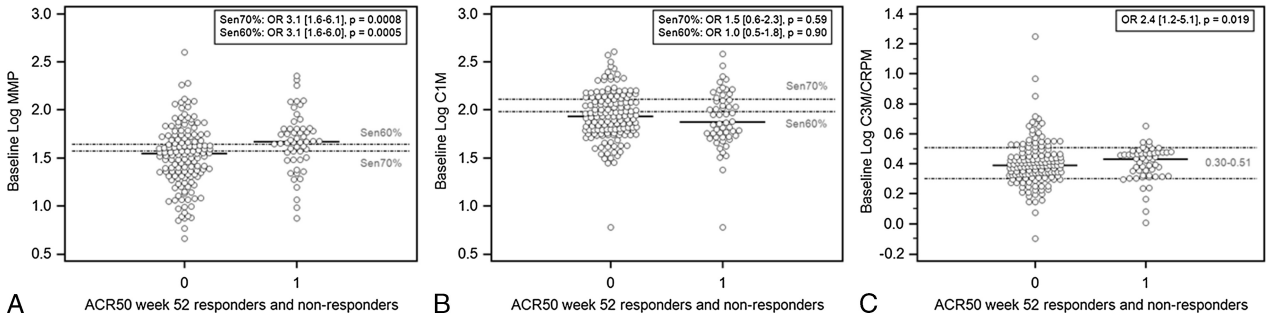

FIGURE 1. Determination of cutoffs to be used in the decision tree for predicting ACR50 response. The cutoffs were set for the biomarkers at sensitivity levels 70% and 60% (Sen70% and Sen60%) for MMP3 (A) and C1M (B) and at Sen70% for C3M/CRPM (C), shown by the dotted lines. Odds ratios for being a responder for the sensitivity levels are shown in the top right corner of each graph.

not significant (Fig. 1B). Cutoffs were set at 2.04 and 2.00 for Sen70% and Sen60%, respectively (Fig. 1B). The scatterplot of the C3M/CRPM showed that 70% of responders felt in the range between 0.30 and 0.51 (Fig. 1C) with an OR for response of 2.4 ($P = 0.019$).

Segregation ACR50 (Week 52) Responders and Nonresponders

The cohort's overall ACR50 response rate was 27% (Fig. 2). As serum MMP3 was the strongest predictor of ACR50 response by logistic regression, it was entered into the first level of CART followed by C3M/CRPM and C1M. In the first decision tree (Fig. 2A), 22% of the population was selected; response rate increased to 54%. In the tree, 86% of the nonresponders were identified as nonresponders, whereas 43% of the responders were identified as responders, giving an OR of 4.7 for prediction of response.

In the second tree (Fig. 2B), the secondary cutoff value for MMP3 was used; 15% of the population was selected; response rate increased to 63%. In addition, 93% of the nonresponders had a negative test, whereas 35% of the responders had a positive test, giving an OR of 6.7 for prediction of response.

In the third tree (Fig. 2C), the secondary cutoff for C1M was used; 10% of the population was selected with a response rate of

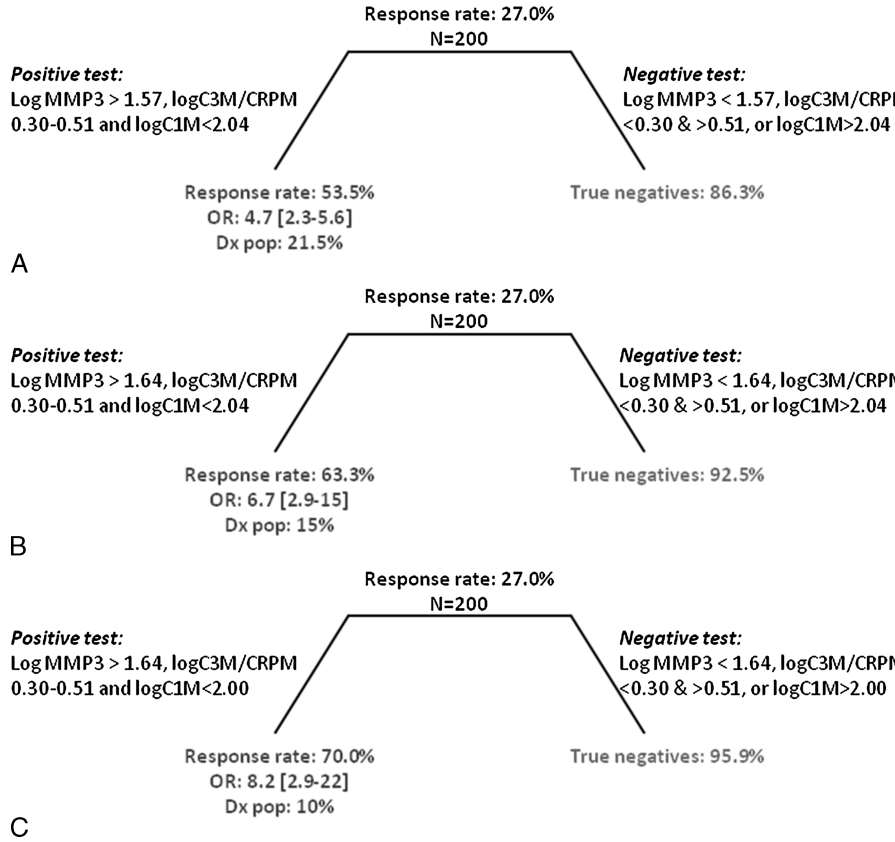

FIGURE 2. Segregation of ACR50 (week 52) responders and nonresponders by measurement of baseline biomarkers. A positive test was set for selection of those patients who were most likely to respond to TCZ and a negative test for identification of those patients who are most likely to be nonresponders. Dx pop indicates the percentage of patients with a positive test. OR, the OR of being a responder; true negative, the rate of nonresponders with a negative test.

TABLE 1. Baseline Description of the Study Biomarker Study Population

	TCZ4 + Methotrexate
N	200
ACR50 responders at week 52, %	27
Males, %	34
Age, mean (SD), y	50.9 (12.7)
Disease duration, mean (SD), y	9.9 (7.9)
DAS28, mean (SD	6.5 (0.9)
HAQ score, mean (SD)	1.5 (0.7)
SHP, mean (SD)	29.1 (28.8)
ESR, mean (SD), mm/h	17.3 (16.0)
JSN, mean (SD), mm	11.7 (13.9)
CRP, mean (SD), mg/dL	1.9 (2.4)
CRPM, geometric mean (95% CI), nmol/L	15.3 (14.5–16.3)
C1M, geometric mean (95% CI), nmol/L	85.3 (78.3–93.0)
C3M, geometric mean (95% CI), nmol/L	38.9 (36.3–41.6)
MMP3, geometric mean (95% CI), nmol/L	36.2 (32.6–40.3)

ESR indicates erythrocyte sedimentation rate; HAQ, health assessment questionnaire; JSN, joint space narrowing; SHP, sharp score.

70%. Ninety-six percent of the nonresponders were deselected, and 26% of the responders were selected, with an OR of 8.2 for prediction of response.

DISCUSSION

Biologic RA therapies provide on average 20% to 45% ACR50 response rates in phase III clinical studies,[16] demonstrating that a significant number of patients derive insufficient benefit from therapeutic intervention. In this small cohort of a phase III clinical study, significantly improved response rates were achieved through analysis of 4 protein fingerprint biomarkers measured at baseline. In the first decision tree, 86% of the ACR50 nonresponders were positively identified by the negative test (low MMP, high/low C3M/CRPM, and high C1M at baseline). Thus, the biomarkers may provide means for deselection of patients who may not respond sufficiently. In addition, 22% of the patients had a positive test; patients with a positive test had 4.7 time chance of benefit from treatment with TCZ. It seemed that by measuring the level of biomarkers at baseline, it may enable selection of a treatment population where the response rate can be increased from 27% to 54%. These percentages could be further refined to increase response rates to 70%, albeit on the expense of selection of a smaller subpopulation.

The model was also tested for predictability for Disease Activity Score in 28 Joints (DAS28) remission rate at week 52. Of the 200 patients, only 112 had their DAS28 recorded at week 52, resulting in low power; thus, data were not shown. However, the model could indeed somewhat predict who experienced remission after treatment with 4 mg/kg TCZ with an OR of 2.5 ($P = 0.057$). This needs to be validated in a larger cohort.

The biomarkers measured in current cohort are protein fingerprints, which are direct measures of connective tissue degradation and inflammation that are downstream of the proinflammatory pathways, for example, the IL-6 or tumor necrosis factor α signaling pathways.[6] As these are downstream biomarkers, it may be speculated that this class of biomarkers will show less fluctuation than cytokine markers or acute phase proteins, such as CRP. This hypothesis needs to be tested further. Current work presents an example of how biomarkers potentially can be used for personalized medicine, which should be validated in another TCZ trial before finally concluding its validity. The limitation of the cohort is that it is from a phase III clinical trial, thus not representing the general heterogeneous RA population.

This exploratory analysis was undertaken on a relatively small, but well-characterized, patient cohort and thus requires validation in an independent second cohort, which will also allow us to determine if this response profile is specific for anti–IL-6 receptor intervention or may be generalized to other populations. However, the perspective is that an intervention-predictive model would potentially be of value for both the industry and physicians and hopefully in the end for the patients. The RA field is in need of personalized medicine, and as the choice of treatment is becoming more complex and there is a demand for higher response rates, there is a home for easily assessable tools.

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