Serum Biomarkers for Prediction of Response to Methotrexate Monotherapy in Early Rheumatoid Arthritis: Results from the SWEFOT Trial

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ABSTRACT. *Objective.* To investigate baseline levels of 12 serum biomarkers that constitute a multibiomarker disease activity test, as predictors of response to methotrexate (MTX) in patients with early rheumatoid arthritis (eRA).

Methods. In 298 patients from the Swedish Pharmacotherapy (SWEFOT) clinical trial, baseline serum levels of 12 proteins were analyzed for association with disease activity based on the 28-joint count Disease Activity Score (DAS28) after 3 months of MTX monotherapy using uni-/multivariate logistic regression. Primary outcome was low disease activity (LDA; DAS28 ≤ 3.2).

Results. Of 298 patients, 104 achieved LDA after 3 months on MTX. Four of the 12 biomarkers [C-reactive protein (CRP), leptin, tumor necrosis factor receptor I (TNF-RI), and vascular cell adhesion molecule 1 (VCAM-1)] significantly predicted LDA based on stepwise logistic regression analysis. Dichotomization of patients using receiver-operating characteristic curve analysis-based cutoffs for these biomarkers showed significantly higher proportions with LDA among patients with lower versus higher levels of CRP or leptin (40% vs 23%, p = 0.004, and 40% vs 25%, p = 0.011, respectively), as well as among those with higher versus lower levels of TNF-RI or VCAM-1 (43% vs 27%, p = 0.004, and 41% vs 25%, p = 0.004, respectively). Combined score based on these biomarkers, adjusted for known predictors of LDA (smoking, sex, and age), associated with decreased chance of LDA (adjusted OR 0.45, 95% CI 0.32–0.62).

Conclusion. Low baseline levels of CRP and leptin, and high baseline levels of TNF-RI and VCAM-1 were associated with LDA after 3 months of MTX therapy in patients with eRA. Combination of these 4 biomarkers increased accuracy of prediction. [Trial registration number: NCT00764725] (J Rheumatol First Release February 1 2019; doi:10.3899/jrheum.180537)

Key Indexing Terms:

RHEUMATOID ARTHRITIS   BIOMARKERS   METHOTREXATE
PREDICTION   RESPONSE

Methotrexate (MTX) is recommended as the first-line treatment of early rheumatoid arthritis (eRA)[1,2]. However, about 60–70% of patients do not achieve low disease activity[3] [MTX-incomplete responders (MTX-IR)] and need further intensification of their treatment. Identification of patients who would or would not benefit from MTX monotherapy could contribute to a personalized approach and optimal therapy choice at baseline.

Several factors have been shown to be associated with higher or lower effectiveness of MTX therapy. For example, male sex and nonsmoking status were predictive of response[4–16]. Other identified predictors are short symptom duration before diagnosis and lower disease activity at baseline[4,5,6,9,10,11,17,18,19]. Regarding molecular biomarkers, Maillefert, *et al* demonstrated in 42 patients with RA (of whom 19 had eRA) an association between low levels of

serum tumor necrosis factor-α (TNF-α) at baseline and response to MTX according to American College of Rheumatology 20 (ACR20) criteria at 6 months. No association was detected for interleukin 1β (IL-1β), IL-6, IL-8, IL-10, or IL-12[20]. A similar association with serum TNF-α was found by another research group[21]. In a Chinese study of patients with eRA, it was shown that high serum levels of haptoglobin were associated with response to MTX therapy[22]. A study of treatment-naive eRA patients in India demonstrated that serum baseline levels of myeloid-related protein 8/14 were significantly higher in responders versus nonresponders to 4 months of MTX monotherapy, according to European League Against Rheumatism (EULAR) criteria[23]. Despite many years of research, no biomarker has been validated or accepted as a routine clinical predictor for MTX treatment response.

The multibiomarker disease activity (MBDA) score is a commercially available test that is based on the serum levels of the following 12 protein molecules: vascular cell adhesion molecule 1 (VCAM-1), epidermal growth factor (EGF), vascular endothelial growth factor, IL-6, TNF receptor I (TNF-RI), matrix metalloproteinases 1 and 3 (MMP-1 and MMP-3), bone glycoprotein 39, leptin, resistin, serum amyloid A, and C-reactive protein (CRP). Previously, we showed predictive ability of the MBDA score for radiographic progression of joint damage in patients from the Swedish Pharmacotherapy (SWEFOT) clinical trial[24,25]. We also illustrated predictive ability of lower and higher levels of the MBDA score measured 3 months after MTX treatment initiation, for response to nonbiological and biological second-line therapy, respectively, in patients with MTX-IR[26]. In our present study, we investigated the associations of the individual components of the MBDA score at baseline with subsequent response to first-line treatment with MTX.

MATERIALS AND METHODS

Study population and outcomes. The SWEFOT trial (registration no. NCT00764725) enrolled 487 patients with eRA (symptom duration ≤ 1 yr), age ≥ 18 years, and moderate/high disease activity score based on examination of 28 joints and erythrocyte sedimentation rate [28-joint count Disease Activity Score (DAS28) > 3.2][3]. Patients started MTX monotherapy for the first 3 months, and those who achieved a low disease activity (LDA; DAS28 ≤ 3.2) remained on the same therapy afterward. Patients with MTX-IR (DAS28 > 3.2) were randomized at Month 3 to either of 2 add-on intensified therapy groups: conventional triple therapy or anti-TNF (infliximab). In our study we evaluated associations of baseline biomarkers that constitute the MBDA score with primary (LDA) and secondary (EULAR good response)[27] outcomes at 3 months in 298 patients, selected based on available data.

The study was approved by the regional ethics committees of all participating units [Karolinska Institutet 02-211, Örebro 2002/202 500:16, Umeå 2002, Linköping 02-186, Uppsala Ups 02-241, Göteborg Gbg Ö 282-01, M 088-02, Lund LU 398-01, and Stockholm Central Ethical Review Board (EPN) 2005/1361, 2006/248-3]. All patients received and signed written informed consent about the use of their materials in future research.

MBDA score and component biomarkers. The MBDA score (Vectra DA) was developed by Crescendo Bioscience as a clinical disease activity monitoring tool in patients with RA. It was designed to correlate with

DAS28-CRP and it has a scale between 1 and 100, with higher values indicating more active disease. The serum samples were shipped to the company (Crescendo Bioscience) for analysis of the 12-component protein biomarkers and subsequently the MBDA score was calculated. The biomarkers were measured by electrochemiluminescence-based multiplex immunoassays on the Meso Scale Discovery Multi-Array platform[28].

Statistical analyses. Baseline demographics and characteristics (including the MBDA score and the 12 comprising biomarkers) were compared between patients who achieved and who did not achieve LDA at 3 months. For continuous variables, Mann-Whitney U test was used and for categorical variables, chi-square or Fisher's exact test. Of the 12 biomarkers, those with p values < 0.2 (VCAM-1, TNF-RI, IL-6, CRP, MMP-1, MMP-3, and leptin) were included in a multivariate logistic regression model to calculate OR with 95% CI for the achievement of LDA at 3 months. Receiver-operating characteristic (ROC) curve analysis was thereafter performed on 4 biomarkers that remained significantly predictive in the stepwise multivariate model (VCAM-1, TNF-RI, CRP, and leptin), calculating areas under the curve (AUC) and defining cutoffs for high versus low values, based on the largest sum of sensitivity and specificity. After dichotomization of patients according to the generated cutoffs, proportions of patients achieving LDA or EULAR good response at 3 months were compared, using the chi-square test. The combined effect of the identified biomarkers as predictors of achievement of LDA or EULAR good response at 3 months was also studied by the generation of a combined biomarker score (see below). Thereafter, this combined biomarker score was included with previously known predictors [sex, age, smoking status, and Health Assessment Questionnaire (HAQ) score] of EULAR good response[11] and with anticyclic citrullinated peptide antibodies (anti-CCP) status into a multivariate logistic regression model to assess its independence in association with primary and secondary outcomes. Finally, the combined biomarker score was analyzed with other predictors in a matrix model (using chi-square test), to demonstrate its additive value for predicting achievement of the primary and secondary outcome. In the matrix model, patients were dichotomized according to age based on ROC curve analysis (≤ 59 and > 59 yrs), which was very close to a cutoff used by Saevarsdottir, *et al*, (≤ 60 and > 60 yrs) based on the highest tertile[11]. The statistical analyses were done by IBM SPSS Statistics (version 24).

Combined biomarker score. The combined score was based on dichotomized levels of CRP, leptin, TNF-RI, and VCAM-1 (cutoffs between low and high categories were based on the ROC curve analysis described above) and had a range from 0 to 4. Score 0 corresponded to patients with favorable category for all 4 biomarkers [CRP (low), leptin (low), TNF-RI (high), VCAM-1 (high); patients at lowest risk of nonresponse], score 1 corresponded to patients having 1 of 4 variables with unfavorable category (patients at low risk of nonresponse), score 2 corresponded to patients with 2 of 4 variables with unfavorable category (patients at moderate risk of nonresponse), score 3 corresponded to patients with 3 of 4 variables with unfavorable category (patients at high risk of nonresponse), and score 4 corresponded to patients with unfavorable category for all 4 biomarkers [CRP (high), leptin (high), TNF-RI (low), VCAM-1 (low); patients at highest risk of nonresponse].

In addition, a combined score was generated based on continuous data, in which contribution of each of the 4 biomarkers was accounted for according to their weights for association with treatment outcome.

RESULTS

Baseline characteristics. Baseline characteristics and demographic data are presented in Table 1. As shown previously, baseline variables and demographic data of patients with available MBDA data did not differ from those of all patients enrolled in the SWEFOT trial[24]. Of 298 disease-modifying antirheumatic drug (DMARD)–naive eRA patients, 104 achieved LDA after 3 months of MTX monotherapy (Table 1), of whom 101 had a EULAR good response. The analysis of

Table 1. Baseline characteristics of patients with early RA from the SWEFOT trial.

Variables	Stratified by Disease Activity (DAS28) at 3 Months of Followup		
	DAS28 ≤ 3.2, n = 104[a]	DAS28 > 3.2, n = 194[b]	p
Baseline characteristics			
Female, n (%)	60 (58)	154 (79)	< 0.001
Age, yrs	61 (51–69)	55 (44–62)	0.001
Smoking, n (%)	19 (24)	47 (26)	0.780
Symptom duration, mos	5 (3–8)	5 (4–8)	0.283
Anti-CCP–positive, n (%)	63 (64)	107 (58)	0.292
RF-positive, n (%)	73 (71)	124 (65)	0.274
Prednisolone use, n (%)	15 (14)	20 (10)	0.293
28–swollen joint count	9 (7–13)	11 (7–15)	0.087
28–tender joint count	6 (4–10)	10 (6–15)	< 0.001
ESR, mm/h	28 (15–42)	36 (24–62)	0.001
CRP, mg/l	15 (9–33)	20 (9–53)	0.033
PtGA (VAS 0–100 mm)	50 (29–67)	63 (43–77)	0.001
Pain (VAS 0–100 mm)	49 (33–64)	62 (46–74)	< 0.001
HAQ	1.0 (0.50–1.38)	1.25 (0.88–1.75)	< 0.001
DAS28	5.2 (4.6–5.9)	5.9 (5.3–6.4)	< 0.001
Baseline protein biomarkers and MBDA score			
VCAM-1, mg/l	0.70 (0.60–0.86)	0.64 (0.56–0.77)	0.005
TNF-RI, μg/l	1.9 (1.6–2.4)	1.7 (1.4–2.2)	0.005
IL-6, ng/l	49 (22–97)	67 (29–150)	0.044
CRP, mg/l	20 (8–43)	27 (10–110)	0.045
MMP-1, μg/l	9.35 (5.87–15)	11 (6.48–18)	0.067
MMP-3, μg/l	48 (28–82)	56 (29–123)	0.105
Leptin, μg/l	9.25 (3.58–14)	10.50 (4.60–20.25)	0.108
VEGF, ng/l	405 (265–600)	390 (250–590)	0.719
Resistin, μg/l	6.60 (5.35–8.78)	6.80 (5.10–8.68)	0.857
YKL-40, μg/l	85 (54–120)	79 (57–130)	0.862
EGF, ng/l	160 (103–258)	170 (100–253)	0.903
SAA, mg/l	20 (7–46)	17 (4–73)	0.990
MBDA score	59 (47–66)	59 (49–74)	0.143

Data are expressed in median (IQR) unless otherwise specified. [a] Missing patients from DAS28 ≤ 3.2 column: smoking (n = 25), anti-CCP (n = 6), and RF (n = 1). [b] Missing patients from DAS28 > 3.2 column: smoking (n = 11), anti-CCP (n = 9), RF, PtGA, pain (n = 2), HAQ (n = 4), and DAS28 (n = 3). RA: rheumatoid arthritis; IQR: interquartile range; anti-CCP: anticyclic citrullinated peptide; RF: rheumatoid factor; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; PtGA: patient's global assessment of disease activity score; VAS: visual analog scale; HAQ: Health Assessment Questionnaire; DAS28: 28-joint count Disease Activity Score; MBDA: multibiomarker disease activity; VCAM-1: vascular cell adhesion molecule 1; TNF-RI: tumor necrosis factor receptor I; IL-6: interleukin 6; MMP: matrix metalloproteinase; VEGF: vascular endothelial growth factor; YKL-40: human cartilage glycoprotein-39; EGF: epidermal growth factor; SAA: serum amyloid A; SWEFOT: Swedish Pharmacotherapy trial.

baseline characteristics between EULAR responders and nonresponders to MTX was previously published based on a larger subset (n = 405) from the SWEFOT trial[11].

As shown previously[29], the MBDA score at baseline was not significantly different between patients achieving and not achieving LDA at 3 months (Table 1). Of the 12 component proteins at baseline, CRP and IL-6 were significantly lower, and TNF-RI and VCAM-1 were significantly higher among patients who achieved LDA at 3 months compared with MTX-IR (Table 1).

Molecular predictors of achievement of low DAS28. Individual biomarkers that had p < 0.2 were included in the multivariate logistic regression analysis (Table 2). After stepwise multivariate analysis, higher levels of CRP and leptin were independently associated with lower likelihood of achieving LDA (OR 0.99, 95% CI 0.98–0.99, and OR 0.97, 95% CI 0.95–0.99, respectively), and higher levels of TNF-RI or VCAM-1 were associated with increased likelihood of achieving LDA at 3 months (OR 2.5, 95% CI 1.4–4.3, and OR 8.2, 95% CI 1.5–45.6, respectively; Table 2).

Similar results were observed for the secondary outcome measure (Supplementary Table 1, available from the authors on request).

ROC curve analysis and dichotomization of CRP, leptin, TNF-RI, and VCAM-1. The ROC curve analyses of CRP, leptin, TNF-RI, and VCAM-1 yielded AUC of 0.56–0.60 (Figure 1). The 95% CI of the AUC for leptin included 0.5 (p = 0.108; Figure 1A), while the AUC for CRP, TNF-RI, and

Table 2. Multivariate logistic regression of protein biomarkers at baseline for prediction of low DAS28 at 3 months.

Biomarkers	OR	95% CI
VCAM-1 (per mg/l increase)	**6.70**	1.17–38.62
TNF-RI (per µg/l increase)	**3.10**	1.70–5.71
IL-6 (per ng/l increase)	0.99	0.99–1.00
CRP (per mg/l increase)	**0.99**	0.98–0.99
MMP-1 (per µg/l increase)	0.98	0.94–1.01
MMP-3 (per µg/l increase)	1.00	0.99–1.00
Leptin (per µg/l increase)	**0.97**	0.95–0.99
Results after stepwise logistic regression analysis		
VCAM-1 (per mg/l increase)	**8.19**	1.47–45.65
TNF-RI (per µg/l increase)	**2.48**	1.43–4.31
CRP (per mg/l increase)	**0.99**	0.98–0.99
Leptin (per µg/l increase)	**0.97**	0.95–0.99

Values in bold face are statistically significant. DAS28: 28-joint count Disease Activity Score; VCAM-1: vascular cell adhesion molecule 1; TNF-RI: tumor necrosis factor receptor I; IL-6: interleukin 6; CRP: C-reactive protein; MMP: matrix metalloproteinase.

VCAM-1 were statistically significant ($p = 0.045$, 0.005, and 0.005, respectively; Figure 1A and 1B). These 4 biomarkers were dichotomized to low and high levels, based on the highest sum of sensitivity and specificity and resulted in the following cutoffs: for CRP ≤ or > 51.5 mg/l, for leptin ≤ or > 14.5 µg/l, for TNF-RI ≤ or > 1.75 µg/l, and for VCAM-1 ≤ or > 0.605 mg/l.

Proportions of patients who achieved low DAS28 at 3 months among those with low versus high levels of biomarkers. Of patients with low CRP at baseline (n = 203), 40% reached LDA after 3 months of MTX monotherapy, while of those with high levels of CRP (n = 95) the proportion was 23% ($p = 0.004$; Figure 2A). Similar proportions of patients achieving LDA were observed when comparing patients with low versus high leptin levels (40% vs 25%, respectively, $p = 0.011$; Figure 2B). The reverse relationship was seen when patients were dichotomized according to TNF-RI or VCAM-1: 27% vs 43% ($p = 0.004$), and 25% vs 41% ($p = 0.004$), respectively (Figure 2C and 2D).

As a secondary outcome we used EULAR good versus moderate/no response with similar findings (Supplementary Figure 1, available from the authors on request).

Prediction of low DAS28 at 3 months using a combined biomarker score at baseline. Based on dichotomized categories of the 4 biomarkers indicated above, we calculated a combined biomarker score ranging from 0 (patients with favorable categories for all 4 biomarkers, therefore associated with highest chance of response to MTX) to 4 (patients with unfavorable categories for all 4 biomarkers, therefore associated with highest risk of nonresponse). The combined biomarker score at baseline was negatively associated with treatment outcomes at 3 months (Figure 3). Patients in the lowest risk group for nonresponse (score = 0) had the highest
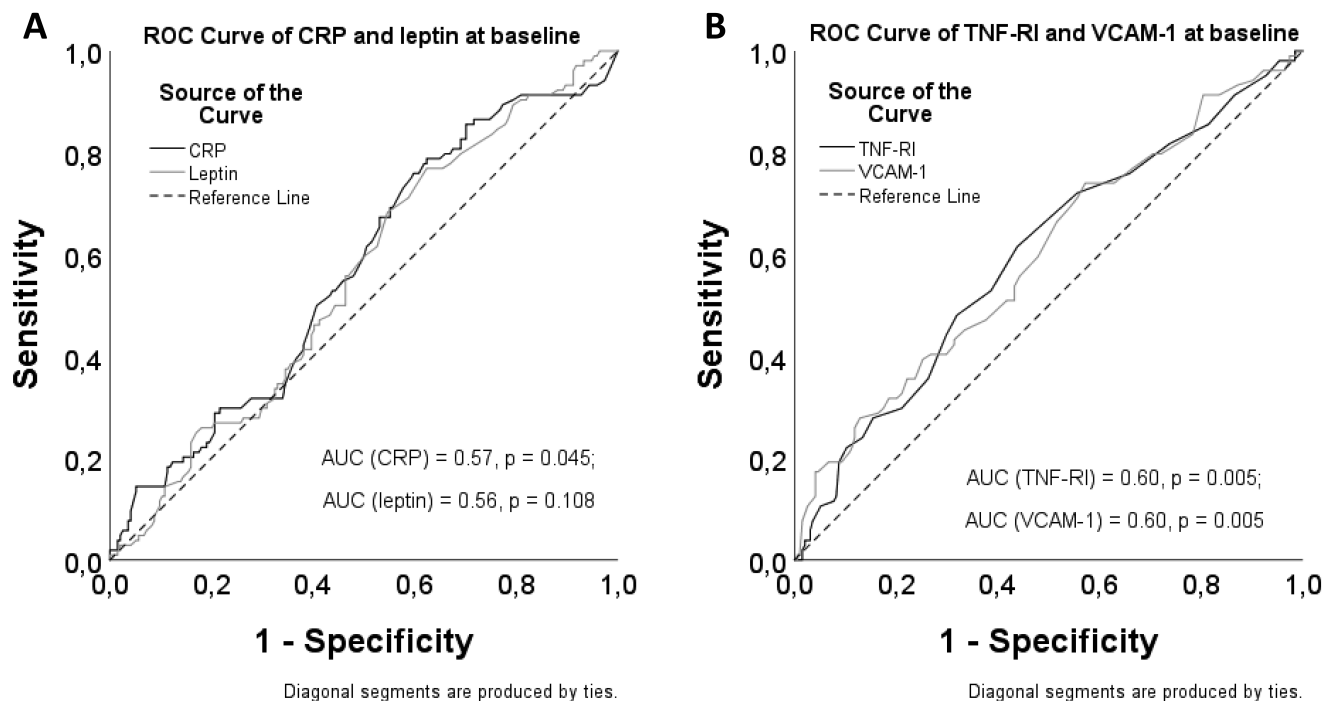
Figure 1. ROC curve analysis of serum biomarkers at baseline using low DAS28 at 3 months as an outcome. ROC curve and AUC of (A) CRP (black line) and leptin (grey line), and (B) TNF-RI (black line) and VCAM-1 (grey line). AUC: area under the curve; CRP: C-reactive protein; DAS28: 28-joint count Disease Activity Score; TNF-RI: tumor necrosis factor receptor I; VCAM-1: vascular cell adhesion molecule 1; ROC: receiver-operating characteristic.
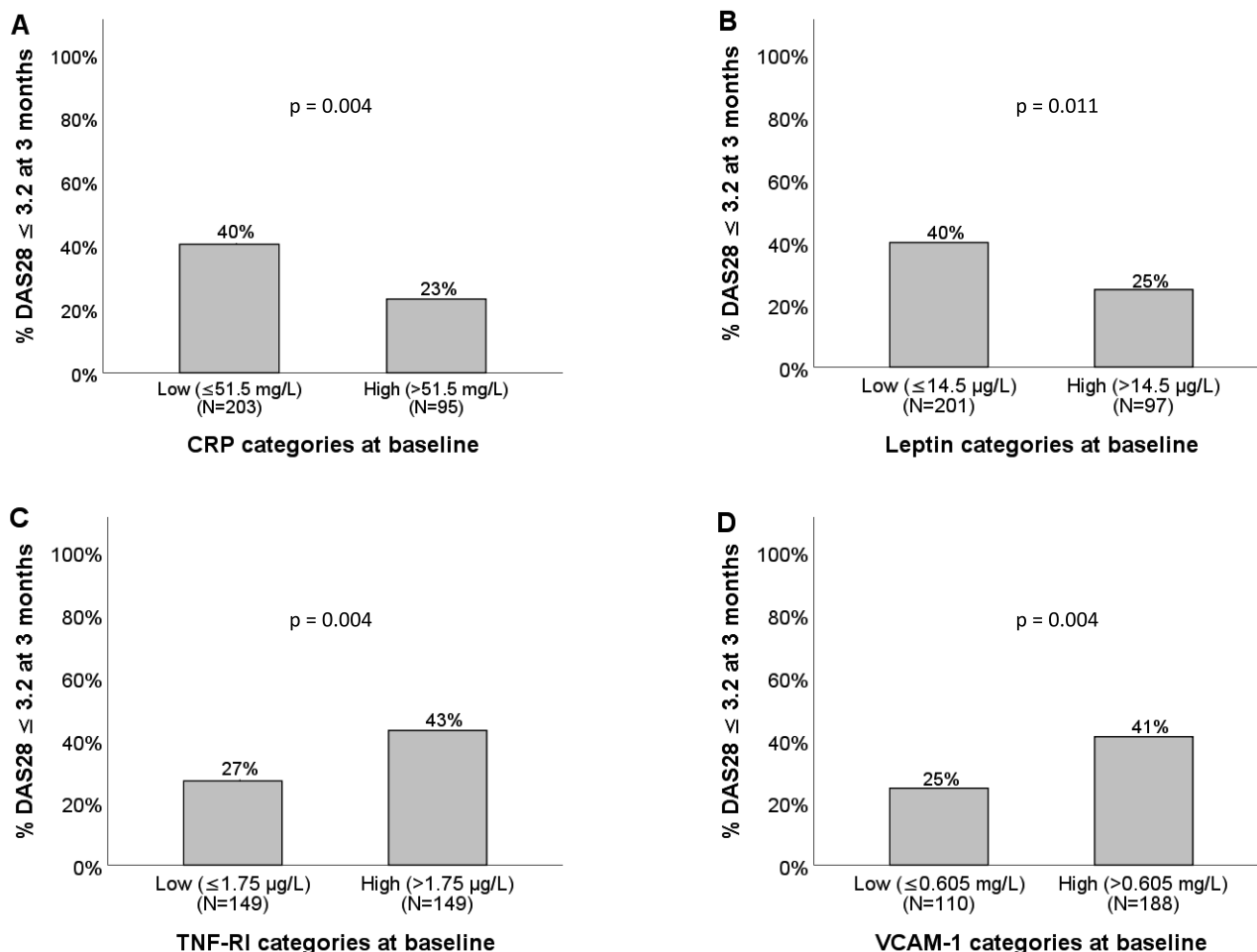
Figure 2. Proportions of patients with early rheumatoid arthritis achieving low disease activity after 3 months of MTX monotherapy, stratified for different biomarkers. Proportions of patients with low DAS28 in patients dichotomized according to (A) CRP, (B) leptin, (C) TNF-RI, and (D) VCAM-1. DAS28: 28-joint count Disease Activity Score; CRP: C-reactive protein; MTX: methotrexate; TNF-RI: tumor necrosis factor receptor I; VCAM-1: vascular cell adhesion molecule 1.

proportion of LDA achievement at 3 months (74%), while for those with score = 1 (n = 109), the proportion was 38% (p < 0.0001); for score = 2, 27% (p < 0.0001); for those with score = 3, 13% (p < 0.0001); and in the highest score group (score = 4), 0% (p = 0.003; Figure 3A).

Similar results were also obtained when using the secondary outcome measure of EULAR good response at 3 months, instead of LDA (Figure 3B).

Additional value of the combined biomarker score to previously described predictors for achievement of low DAS28 and EULAR good response. In univariate logistic regression analysis of the combined biomarker score and baseline characteristics [rheumatoid factor (RF), anti-CCP, sex, age, smoking status, and HAQ], sex, age, HAQ, and the combined biomarker score were significantly associated with treatment outcomes at 3 months (Table 3, and Supplementary Table 2, available from the authors on request). After including all these variables (except for RF, because of its correlation with

anti-CCP) in a multivariate logistic regression model, age, HAQ, and combined biomarker score were independently associated with treatment outcome (Table 3, and Supplementary Table 2, available from the authors on request). We did not adjust for baseline DAS28 in the multivariate model because the outcome measure was based on that, but when stratified for baseline DAS28 into high (> 5.1) and moderate (> 3.2–5.1), there was a similar pattern in both subgroups (data not shown). A sensitivity analysis using stepwise regression yielded the same independent predictors (Table 3, and Supplementary Table 2, available from the authors on request).

Comparison within each category of age and HAQ score (using categories from a previous publication[11]) still showed higher probability of LDA and EULAR good response in patients with lower compared with higher combined biomarker score; in some subgroups, the difference between patients with score = 0 and score = 1, 2, or 3 was statistically
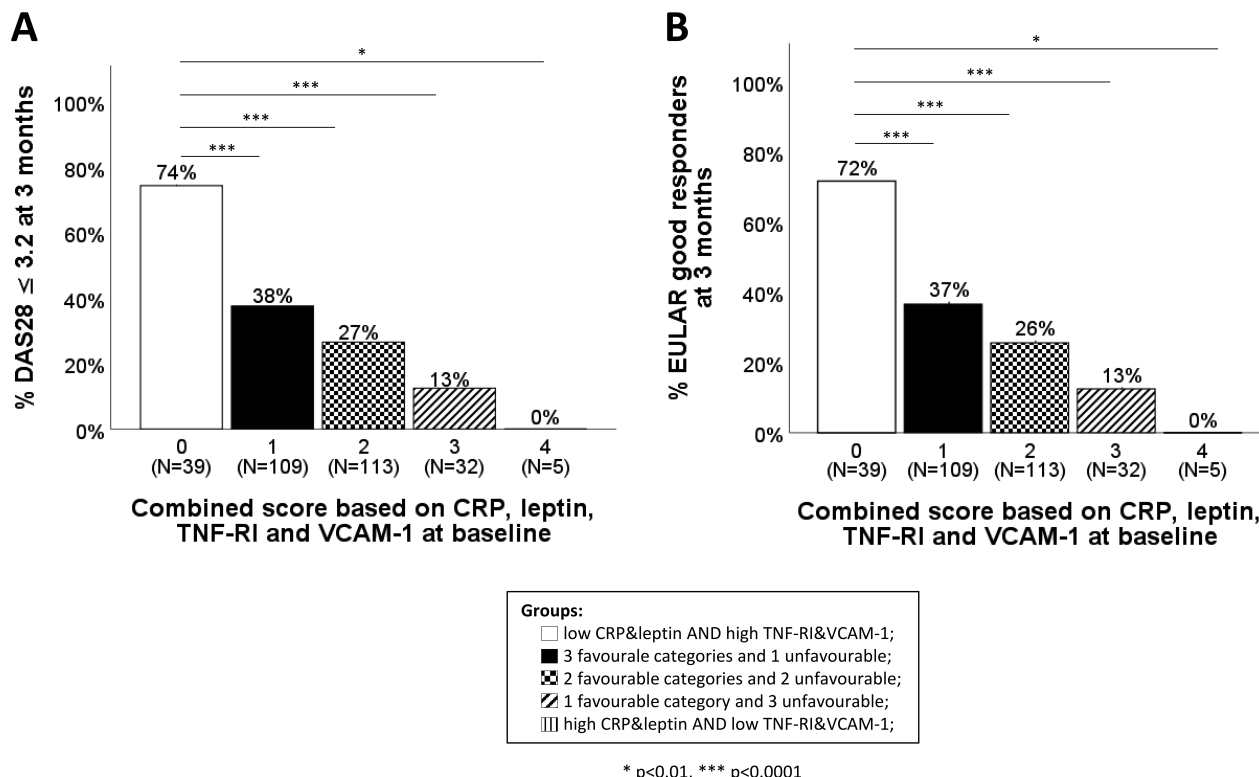
Groups:
☐ low CRP&leptin AND high TNF-RI&VCAM-1;
■ 3 favourale categories and 1 unfavourable;
▦ 2 favourable categories and 2 unfavourable;
▨ 1 favourable category and 3 unfavourable;
▥ high CRP&leptin AND low TNF-RI&VCAM-1;

* p<0.01, *** p<0.0001

Figure 3. Proportions of patients with early rheumatoid arthritis achieving low DAS28 or EULAR good response after 3 months of MTX monotherapy, stratified for the combined biomarker score. Proportions of patients with (A) low DAS28 or (B) EULAR good response within subsets based on the combined biomarker score: combined score = 0 (white bars), combined score = 1 (black bars), combined score = 2 (bars with checkered pattern), combined scores = 3 (bars with diagonal lines), and combined score = 4 (bars with vertical lines). DAS28: 28-joint count Disease Activity Score; EULAR: European League Against Rheumatism; CRP: C-reactive protein; MTX: methotrexate; TNF-RI: tumor necrosis factor receptor I; VCAM-1: vascular cell adhesion molecule 1.

Table 3. Univariate and multivariate logistic regression of combined biomarker score and some baseline predictors of low DAS28 at 3 months.

Variables	Univariate		Multivariate	
	OR	95% CI	OR	95% CI
RF-positive[a]	1.33	0.79–2.24	1.35	0.71–2.57
Anti-CCP–positive	1.31	0.79–2.18	1.65	0.87–3.14
Sex, male	**2.82**	1.67–4.76	1.76	0.88–3.50
Age (per yr increase)	**1.03**	1.01–1.05	**1.03**	1.01–1.05
Current smoking (yes)	0.92	0.50–1.69	0.78	0.38–1.64
HAQ (per unit increase)	**0.36**	0.23–0.57	**0.38**	0.22–0.65
Combined biomarker score (per unit increase)[b]	**0.40**	0.29–0.55	**0.43**	0.29–0.65
Results after stepwise logistic regression analysis				
Sex, male			1.64	0.90–2.98
Age (per yr increase)			**1.03**	1.01–1.05
HAQ (per unit increase)			**0.35**	0.22–0.58
Combined biomarker score (per unit increase)			**0.44**	0.31–0.63

Values in bold face are statistically significant. [a] RF was excluded from multivariate model because of its collinearity with anti-CCP. [b] OR for the combined biomarker score in univariate analysis indicates that for each unit increase, the odds of achievement of LDA decrease by 60%. Similarly, in multivariate model, after considering for other predictors (anti-CCP, sex, age and smoking), the odds of LDA decrease by 56% for each unit increase in the combined biomarker score. RF: rheumatoid factor; anti-CCP: anticyclic citrullinated peptide; HAQ: Health Assessment Questionnaire; DAS28: 28-joint count Disease Activity Score; LDA: low disease activity.

significant among patients within the highest category of HAQ score (Supplementary Figure 2, available from the authors on request).

DISCUSSION

In our current study, we evaluated baseline levels of all the 12 individual biomarkers included in a commercially

available blood multibiomarker test (MBDA score) as potential predictors of subsequent response to 3 months of MTX monotherapy in DMARD-naive eRA patients from the SWEFOT trial. Multivariate logistic regression assessment of biomarkers that had the strongest univariate associations with treatment outcome revealed 4 independent predictors: low levels of CRP and leptin as well as high levels of TNF-RI and VCAM-1 were associated with achievement of low DAS28 and EULAR good response.

The MBDA score was developed and validated for assessment and monitoring of clinical disease activity, but not prediction of later disease activity, in patients with RA. It has a range from 1 to 100 and can be categorized as follows: low (< 30), moderate (30–44), and high (> 44). It has been shown in different trials (including SWEFOT) that the MBDA score is predictive for radiographic progression[24,30,31]. In MTX-IR from the SWEFOT trial, we also showed that the MBDA score analyzed at 3 months — just before treatment escalation to triple therapy or infliximab — was differentially associated with clinical response to each of the second-line therapies[26]. At baseline, however, the MBDA score was not significantly associated with response to 3 months of MTX monotherapy[29] (Table 1). Regarding data from other studies, in the Nested-1 cohort it was shown that changes in the MBDA score already at the second week of MTX or anti-TNF therapy (pooled data) were associated with clinical response (EULAR good response criteria based on DAS28-CRP or ACR50 response) at weeks 6 or 12[32]. However, because of lack in statistical power, the analyses could not be done separately for each of the therapy arms. In the CAMERA trial, in which patients were mainly on MTX with allowance of nonsteroidal antiinflammatory drugs and intraarticular injection of glucocorticoids (when necessary), the MBDA score was able to discriminate patients in remission/LDA (DAS28-CRP ≤ 2.7) versus those with moderate/high disease activity[33]. All biomarkers except for EGF, leptin, and VCAM-1 were significantly correlated with DAS28-CRP or DAS28.

In our present study, we found baseline levels of CRP, leptin, TNF-RI, and VCAM-1 to be independently associated with clinical response to MTX at 3 months. However, data from other studies of these individual biomarkers are inconsistent, some showing poor or no association of baseline CRP with clinical response[5,9,23,34] and others illustrating predictive ability[35]. It is worth commenting on the method used to measure CRP. In the SWEFOT trial, CRP was measured in clinical routine laboratories during inclusion and followup. The CRP for the MBDA score was measured separately by a more sensitive method. In our study, CRP data from both sources showed similar associations with treatment outcome (compare Table 1 upper and lower parts).

While CRP is used as an inflammatory marker in routine care, leptin is not studied as much. Being a lipid hormone, leptin is produced mainly by white adipose tissue and regulates appetite by inhibition of neuropeptide Y secretion[36,37]. A study on 16 female patients with established RA and 16 healthy women did not reveal any difference in plasma leptin levels between these 2 groups[38], and no correlations were observed of leptin with CRP, ESR, or DAS28 before therapy. Similar nonlinearity of leptin with CRP among 75 patients with RA (of which 35 had DAS28 ≤ 3.2) was observed by Iranian researchers[39]. In our study, leptin had a weak inverse correlation with CRP, which may be because our patients were DMARD-naive as opposed to the 2 examples mentioned above, in which patients were receiving treatment leading to significant decreases of CRP but not leptin levels. Even though lower levels of both leptin and CRP at baseline in our study were significantly associated with subsequent response to MTX, the multivariate analysis still illustrated their independence from each other. However, sex should be considered because leptin is significantly higher in females compared with males, and female sex has been shown to be associated with poorer response. Therefore, we adjusted for sex and presented our findings stratified for sex in the risk matrices (Supplementary Figure 2, available from the authors on request).

TNF-α, one of the key players in the pathology of RA, realizes its proinflammatory function through 2 types of cell surface–bound receptors: TNF-RI and TNF-RII. Both may be released from the cell surface and circulate in the blood during inflammation, reducing the intracellular signal transduction and regulating inflammation. Xanthoulea, et al, for example, have demonstrated on a knockout mice model that inability to cleave the membrane TNF receptors led to better antibacterial defense, which, however, was accompanied by a shift in equilibrium of the immune system toward pathological inflammatory conditions[40]. Taking into consideration that an increased level of soluble TNF-RI might be an indicator of increased inflammation and/or TNF levels, as well as that MTX therapy may reduce levels of TNF[41,42,43,44], it becomes logical to assume that higher levels of TNF-RI in serum will be associated with higher likelihood of response to MTX, compared with those patients who have active disease but lower levels of TNF-RI.

VCAM-1 expression in endothelial cells is upregulated during inflammation. Its role is to facilitate adhesion of leukocytes and their migration from the bloodstream to the affected tissue. We observed a higher likelihood of achievement of good clinical outcome after 3 months of MTX monotherapy in patients with higher, as compared to lower, baseline serum levels of VCAM-1. Because all these patients had eRA and were DMARD-naive, we assume that those with higher levels of VCAM-1 had different mechanisms driving their disease, where VCAM-1 plays a more important role compared with those with lower levels. Subsequently, if the treatment decreases the levels of VCAM-1, most likely patients with higher levels of the biomarker would benefit more from such therapy. There are

several studies showing that MTX decreases serum levels of VCAM-1[45,46,47]. And in support of our hypothesis, Kraan and colleagues have noticed that patients who received MTX treatment and achieved ACR20 response had greater reduction of expression of VCAM-1 in synovial tissue compared with nonresponders[46]. In our study, we similarly observed a significantly greater reduction of soluble VCAM-1 at 3 months in responders compared to non-responders (data not shown).

Because these 4 biomarkers did not show collinearity, we combined them after dichotomization into a single score, ranging from 0 to 4 and indicating patients at lowest to highest risk of not responding to MTX, respectively. An analysis of a combined score based on continuous data demonstrated similar results for association with treatment outcome (Supplementary Figure 3, available from the authors on request). After adjusting for previously shown baseline predictors[11], the combined biomarker score remained an independent predictor of both LDA and EULAR good response at 3 months. Because leptin has a strong collinearity with sex, and age and HAQ were significant predictors, we further stratified the analyses for the biomarker score using those 2 fundamental variables, as presented in the risk matrices (Supplementary Figure 2, available from the authors on request). We found strong and clinically meaningful differences in the likelihood of LDA and EULAR response, especially in patients with high HAQ score, although numbers were limited in certain subgroups.

The size of the study group as well as the posthoc design are the main limitations of our study. Another limitation is that no standard cutoff values for the 4 biomarkers are available, so we used ROC curve analysis for dichotomization. Therefore, these threshold values need to be validated in another RA cohort.

The strength of our current study is the SWEFOT trial design, because the patients were recruited with very limited selection bias, as close as possible to real-life situations. Also, inclusion of DMARD-naive patients with eRA allowed us to evaluate the treatment outcome caused purely by MTX monotherapy, with only a minority of patients (12%) receiving low-dose cortisone.

Lower baseline levels of CRP and leptin as well as higher baseline levels of TNF-RI and VCAM-1 were associated, independently from each other, with higher likelihood for response to MTX monotherapy in patients with eRA. Combination of these 4 biomarkers increased the predictive value of response to MTX for patients in addition to clinical baseline factors. If validated, these biomarkers could become a useful complement when choosing the treatment strategy for patients with eRA.

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