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A Multi-Biomarker Disease Activity Score and the Choice of Second-Line Therapy in Early Rheumatoid Arthritis After Methotrexate Failure

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Objective. **To investigate whether the Multi-Biomarker Disease Activity (MBDA) score predicts optimal add-on treatment in patients with early rheumatoid arthritis (RA) who were inadequate responders to MTX (MTX-IRs).**

Methods. **We analyzed data from 157 MTX-IRs (with a Disease Activity Score using the erythrocyte sedimentation rate [DAS28-ESR] >3.2) from the Swedish Pharmacotherapy (SWEFOT) trial who were randomized to receive triple therapy (MTX plus sulfasalazine plus hydroxychloroquine) versus MTX plus infliximab. The MBDA score as a predictor of the subsequent DAS28-based response to each second-line treatment was analyzed at randomization with the Breslow-Day test for 2 × 2 groups, using both validated categories (low [<30], moderate [30–44], and high [>44]) and dichotomized categories (lower [≤38] versus higher [>38]).**

Results. **Among the 157 patients, 12% had a low MBDA score, 32% moderate, and 56% high. Of those with a low MBDA score, 88% responded to subsequent triple therapy, and 18% responded to MTX plus infliximab ($P = 0.006$); for those with a high MBDA score, the response rates were 35% and 58%, respectively ($P = 0.040$). When using 38 as a cutoff for the MBDA score (29% patients with lower scores versus 71% with higher scores), the differential associations with response to triple therapy versus MTX plus infliximab were 79% versus 44% and 36% versus 58%, respectively ($P = 0.001$). Clinical and inflammatory markers had poorer predictive capacity for response to triple therapy or MTX plus infliximab.**

Conclusion. **In patients with RA who had an inadequate response to MTX, the MBDA score categories were differentially associated with response to subsequent therapies. Thus, patients with post-MTX biochemical improvements (lower MBDA scores) were more likely to respond to triple therapy than to MTX plus infliximab. If confirmed, these results may help to improve treatment in RA.**

In the standard care of patients with early rheumatoid arthritis (RA) (1,2), inadequate response to methotrexate (MTX) monotherapy is followed by a further intensification

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Table 1. Baseline characteristics and demographic data of the study patients, by clinical response at 1 year in the SWEFOT trial*

Baseline characteristic	All SWEFOT patients (n = 487)†	MTX-IR subset in the present study		
		All MTX-IR patients (n = 157)	Responders (DAS28 ≤3.2) (n = 79)	Nonresponders (DAS28 >3.2) (n = 78)
No. (%) female	344 (70)	125 (79.6)	54 (68.4)	71 (91)‡
Symptom duration, mean ± SD months	6.2 ± 4.6	6.1 ± 3.5	6.2 ± 3. 8	6.0 ± 3.2
Anti-CCP status, no. (%)				
Positive	275 (57)	87 (55)	45 (57)	42 (54)
Negative	157 (32)	62 (40)	30 (38)	32 (41)
Not available	55 (11)	8 (5)	4 (5)	4 (5)
RF status, no. (%)				
Positive	330 (68)	97 (62)	47 (60)	50 (64)
Negative	152 (31)	59 (38)	32 (40)	27 (35)
Not available	5 (1)	1 (1)	0 (0)	1 (1)
Joint counts, mean ± SD of 28 joints				
Swollen joints	10.8 ± 5.3	11.6 ± 5.4	11.3 ± 5.3	11.9 ± 5.4
Tender joints	9.6 ± 6.1	10.6 ± 6.1	9.6 ± 6.4	11.7 ± 5.6§
ESR, mean ± SD mm/hour	39.9 ± 25.9	44.3 ± 27.0	39.7 ± 22.2	48.9 ± 30.6
CRP, mean ± SD mg/liter	33.8 ± 36.8	37.0 ± 38.2	34.5 ± 35.4	39.6 ± 4.1
PGA, mean ± SD mm (0–100-mm VAS)	56.0 ± 23.9	57.6 ± 25.1	55.0 ± 25.5	60.1 ± 24.6
DAS28, mean ± SD	5.7 ± 1.0	6.0 ± 1.0	5.8 ± 0.9	6.2 ± 0.9§
MBDA score, mean ± SD	58.6 ± 15.1	59.2 ± 15.7	58.9 ± 13.8	59.5 ± 17.5

* In the present study, a subset of 157 patients with early rheumatoid arthritis who participated in the Swedish Pharmacotherapy (SWEFOT) trial and were inadequate responders to methotrexate monotherapy (MTX-IRs) at 3 months were evaluated according to the Multi-Biomarker Disease Activity (MBDA) score. Anti-CCP = anti–cyclic citrullinated peptide; RF = rheumatoid factor; VAS = visual analog scale.
† Patients in the main study group were missing data for the following assessments: swollen and tender joint counts (n = 2), erythrocyte sedimentation rate (ESR; n = 5), C-reactive protein (CRP; n = 3), patient's global assessment (PGA; n = 3), Disease Activity Score in 28 joints (DAS28; n = 8), and MBDA score (n = 185).
‡ $P < 0.001$ versus responders, by chi-square test.
§ $P = 0.006$ versus responders, by Mann-Whitney U test (tender joint count) or Student's *t*-test (DAS28).

of treatment by adding conventional, nonbiologic disease-modifying antirheumatic drugs (cDMARDs) such as sulfasalazine and hydroxychloroquine, also known as triple therapy (3–5), or biologic medications, such as anti–tumor necrosis factor (anti-TNF), including infliximab (1,2,6). The relative strengths of these 2 options were compared in several trials. In the open-label randomized Swedish Pharmacotherapy (SWEFOT) trial, the addition of infliximab was significantly more effective after 1 year, but the difference was no longer significant after 2 years (7). Addition of infliximab to triple therapy for 6 months in patients from the New Finnish RA Combination Therapy (NEO-RACo) trial showed a clinically beneficial trend at 2 years as compared with the group of patients who received triple therapy plus placebo (8). Following further yearly examinations, however, the slope between the 2 arms merged closer, resulting in a disappearance of the trend at 5 years. In the randomized double-blind trials Treatment of Early Aggressive RA (TEAR) and RA: Comparison of Active Therapies in Patients With Active Disease Despite Methotrexate Therapy (RACAT), the addition of etanercept to MTX was not more effective than the addition of sulfasalazine plus hydroxychloroquine with regard to the primary end point, establishing formal noninferiority in the latter trial but identifying some 2-year differences in radiographic progression (in only the TEAR trial) that differed between the treatments (9,10). Moreover, even if a true difference might be present between these options, the cost difference between cDMARDs and biologic drugs is so large that use of the latter has not been shown to be cost-effective (11).

Results from all these trials, however, apply on a group level, and it stands to reason that for each patient, the 2 treatment options may have different likelihoods of response (10). Although some clinical and serologic factors have been shown to be associated with response to certain treatment options (12,13), there have yet been no consistent predictors that could identify an individual patient with a higher chance of responding to a particular therapy compared with another (14,15).

The Multi-Biomarker Disease Activity (MBDA) score is a disease activity measure based on the measurement of 12 serum biomarkers that was designed to correlate with the Disease Activity Score in 28 joints using the C-reactive protein level (DAS28-CRP) (16,17). The MBDA score includes measurement of the levels of acute-phase reactants, inflammatory cytokines, cell-adhesion molecules,

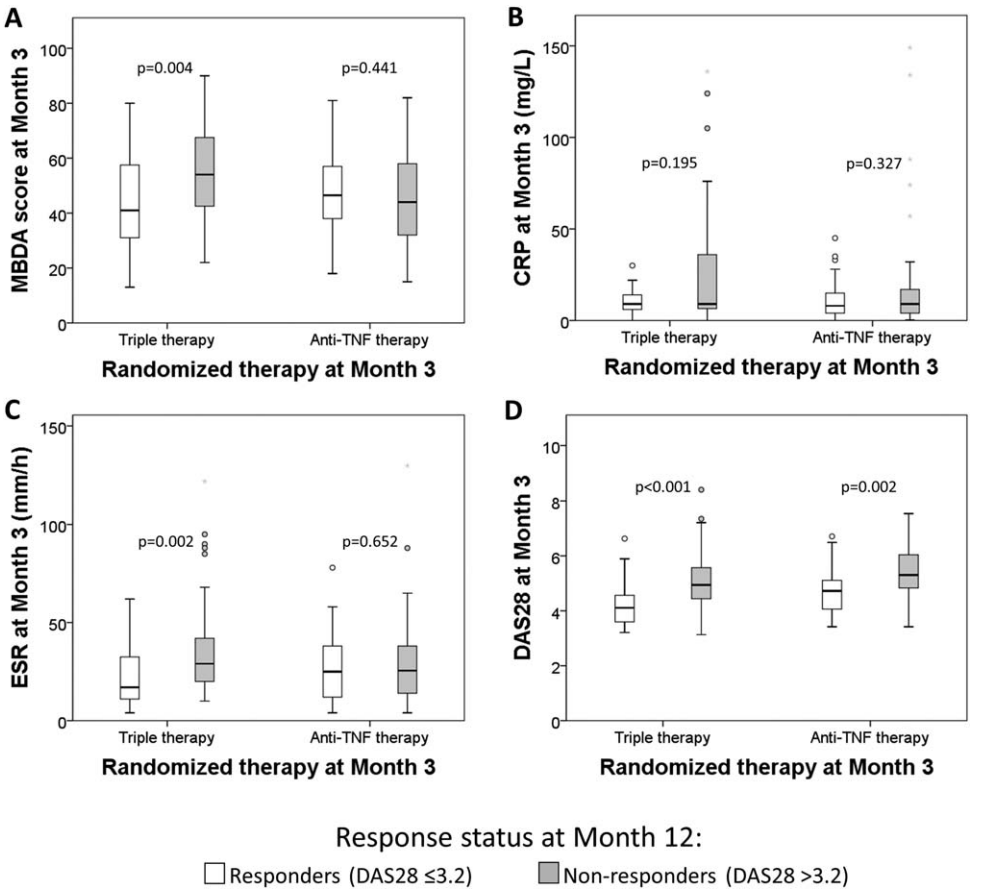

Figure 1. Distribution of disease activity measures at month 3 in responders and nonresponders to second-line therapy at year 1. The Multi-Biomarker Disease Activity (MBDA) score (**A**), C-reactive protein (CRP) level (**B**), erythrocyte sedimentation rate (ESR) (**C**), and Disease Activity Score in 28 joints (DAS28) (**D**) at month 3 (at the time of randomization) among responders and nonresponders to triple therapy or anti–tumor necrosis factor (anti-TNF) therapy are shown. Data are shown as box plots. Each box represents the upper and lower interquartile range (IQR). Lines inside the boxes represent the median. Whiskers represent 1.5 times the upper and lower IQRs. Circles indicate outliers.

adipose tissue products, and matrix metalloproteinases. We previously showed that in the SWEFOT clinical trial (7,18), low and moderate MBDA scores (<30 and 30–44, respectively) were associated with a very low risk of subsequent radiographic joint damage (19). However, baseline MBDA scores did not predict the clinical response to MTX monotherapy or to second-line therapies.

We reasoned that rather than focusing on the baseline score, the MBDA score at month 3 of MTX monotherapy might provide useful clues as to the efficacy of subsequent treatments in inadequate responders to MTX (MTX-IRs). Thus, in the present study, we investigated whether an MBDA score at the time of randomization to second-line therapy might be predictive of subsequent clinical responses to triple therapy versus MTX plus anti-TNF therapies and whether it might guide the optimal choice of treatment strategy.

PATIENTS AND METHODS

Study design. This was a post hoc study done on samples and clinical data from the SWEFOT trial. Patients with early RA (n = 487) diagnosed according to American College of Rheumatology (ACR) criteria were recruited to the SWEFOT trial. Inclusion criteria were active disease (DAS28 using the erythrocyte sedimentation rate [DAS28-ESR] >3.2), age ≥18 years, and symptom duration <1 year (7). Patients started MTX monotherapy for 3 months, and those with a DAS28 of ≤3.2 at month 3 (responders) continued the MTX monotherapy, while those with a DAS28 of >3.2 at month 3 (n = 258) were randomized to receive intensified treatment: either MTX plus sulfasalazine plus hydroxychloroquine (triple therapy) or MTX plus infliximab (anti-TNF). Samples from 157 of the 258 randomized MTX-IR patients were analyzed using the MBDA, based on availability of serum samples and completeness of the available clinical data (data available upon request from RFvV, the senior author and coordinating investigator of the SWEFOT trial).

The SWEFOT trial was registered at the World Health Organization database at Karolinska Institute (CT20080004)

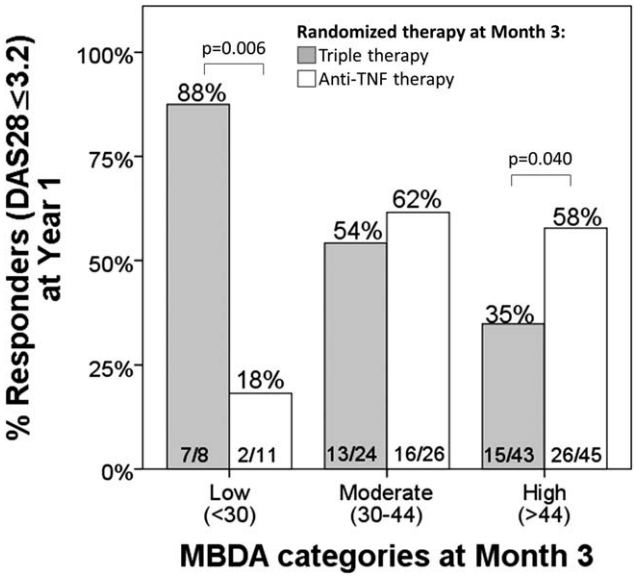

Figure 2. Proportion of patients with a clinical response to second-line therapy at year 1 according to a DAS28 score of ≤3.2, stratified by conventional cutoffs of the MBDA score at the start of treatment intensification. Responders at year 1 were evaluated according to low (<30), moderate (30–44), or high (>44) scores on the MBDA at month 3. See Figure 1 for definitions.

and at the ClinicalTrials.gov database (NCT00764725). All patients gave their written informed consent before the start of the SWEFOT trial. The trial was approved by the regional ethics committees of all participating units. See Appendix A for the names of the principal investigators.

Outcomes measures. The MBDA score at month 3 was measured and related to the likelihood of low disease activity (DAS28 ≤3.2) or good response according to the European League Against Rheumatism (EULAR) criteria (20,21) at year 1 in the 2 separate groups of MTX-IR patients: those receiving triple therapy (n = 75) and those receiving anti-TNF (n = 82). The same analysis was done after stratification of patients according to rheumatoid factor (RF) and anti–cyclic citrullinated peptide (anti-CCP) status. For comparison, we also studied values for the CRP, ESR, and DAS28 at month 3. Two of the 157 MTX-IR patients had missing CRP data (n = 155) and 3 had missing ESR data (n = 154). Categorization of the DAS28 values at month 3 was based on standard cutoffs recommended by EULAR (20,21): >3.2–5.1 for moderate disease activity and >5.1 for high disease activity. For the MBDA score, CRP level, and ESR, receiver operating characteristic (ROC) curve analysis yielded the following cutoffs (based on the largest sum of the sensitivity plus the specificity) for lower versus higher disease activity categories: for the MBDA score, ≤38 versus >38; for the CRP level, ≤32 mg/liter versus >32 mg/liter; and for the ESR, ≤25.5 mm/hour versus >25.5 mm/hour.

MBDA scores. Serum samples from the SWEFOT trial were analyzed for components of the MBDA score by Crescendo Bioscience using electrochemiluminescence-based multiplexed immunoassay on a Meso Scale Discovery Multi-Array platform (22). The MBDA score (Vectra DA disease activity test) is based on serum levels of the following 12 biomarkers: vascular cell adhesion molecule 1, epidermal growth factor, vascular endothelial growth factor, interleukin-6, TNF receptor 1, matrix metalloproteinases 1 and 3, cartilage glycoprotein 39 (YKL-40), leptin, resistin, serum amyloid A, and CRP. The scale of the MBDA score has a range of 1–100, and validated cutoffs for different categories of disease activity are as follows: low = <30, moderate = 30–44, and high = >44 (16,17). In addition to the cutoff based on ROC curve analysis mentioned above, these validated cutoffs were used for further analyses.

Statistical analysis. Baseline characteristics and demographic data were analyzed by *t*-test for normally distributed variables, Mann-Whitney U test for non-normally distributed variables, and chi-square test for categorical variables. For the comparison of continuous values of the MBDA score, the CRP level, and the ESR at month 3 between the responders and the nonresponders to triple therapy or anti-TNF treatment at year 1, the Mann-Whitney U test was used, and for the DAS28, Student's *t*-test was used. Categories of the MBDA score, the CRP level, and the ESR were obtained from ROC curve analysis. Based on this analysis, we selected the cutoff values that corresponded to the highest sum of the sensitivity plus the specificity. For the MBDA score and the DAS28, validated cutoffs were also used. The proportion of clinical responders (DAS28 ≤3.2) to triple therapy or anti-TNF therapy within patient groups with different disease activity categories was compared using chi-square test or Fisher's exact test. The homogeneity of odds ratios for clinical response or a EULAR good response at year 1 to triple therapy or anti-TNF therapy among patients with lower or higher levels of the MBDA score, CRP level, ESR, or DAS28 was determined by Breslow-Day test. All statistical analyses were done using IBM SPSS Statistics 22 software.

RESULTS

Baseline characteristics. The baseline characteristics and demographic data for the entire SWEFOT cohort (n = 487) and for the 157 MTX-IR patients included in the present study were similar (Table 1). Characteristics at month 3 between randomized patients who were included in this study (n = 157) and those who were not (n = 101) were also similar, with the patient's global assessment by visual analog scale being the only significantly different variable (lower among the patients in the present study; data available upon request from the corresponding author).

Relationship between the MBDA score, CRP level, ESR, and DAS28 and a subsequent clinical response to triple therapy or anti-TNF therapy. Overall, there was no significant difference in the proportion of responders at year 1 between the triple therapy (n = 75) and the anti-TNF therapy (n = 82) groups (47% versus 54%; $P = 0.381$). At month 3, the MBDA score, ESR, and DAS28 values were significantly lower in subsequent responders versus nonresponders to triple therapy at year 1 (Figures 1A, C, and D), whereas this was only observed for the DAS28 value in those receiving anti-TNF therapy (Figure 1D). When stratified according to established cutoffs for the MBDA score, 12%

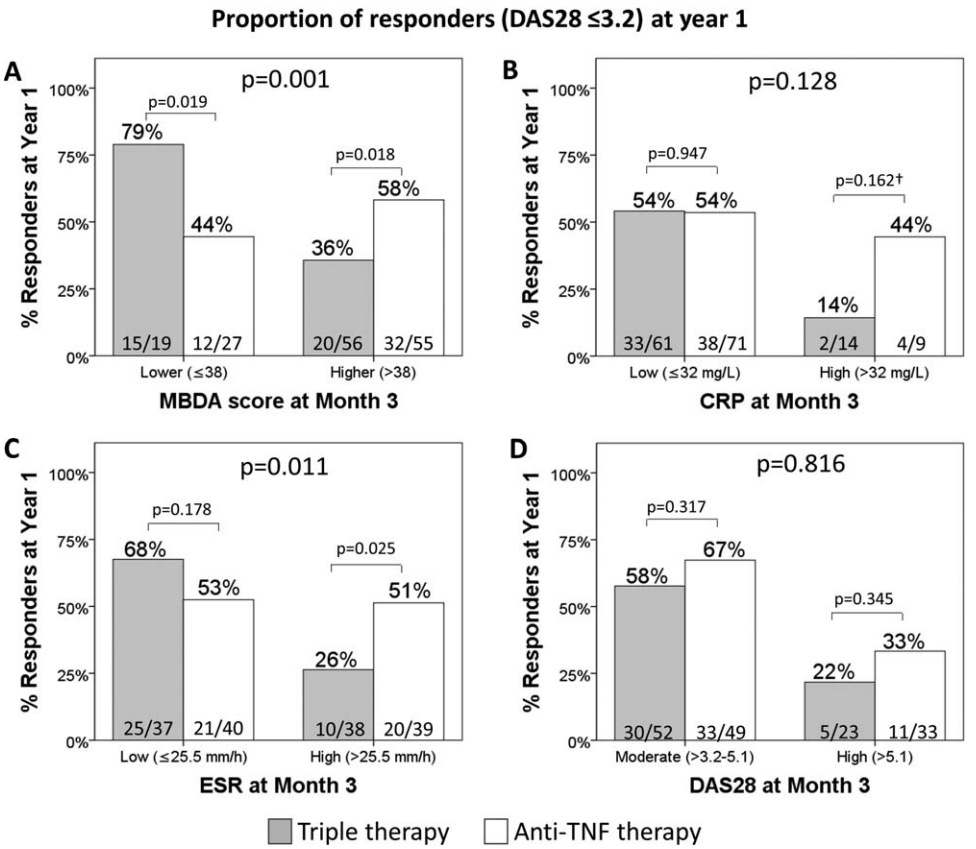

Figure 3. Proportion of patients with a clinical response to second-line therapy at year 1 according to a DAS28 score of ≤3.2, stratified by receiver operating characteristic curve–based cutoffs of disease activity measures at month 3. Responders at year 1 were evaluated according to the MBDA score (**A**), CRP level (**B**), ESR (**C**), and DAS28 (**D**) at month 3. Overall *P* values for the 4 groups were calculated using the Breslow-Day test; *P* values for triple therapy versus anti-TNF therapy were calculated using the chi-square test, except where indicated otherwise. † = *P* value was calculated using Fisher's exact test. See Figure 1 for definitions.

of patients had low (<30), 32% had moderate (30–44), and 56% had high (>44) MBDA scores at treatment escalation.

Patients with low MBDA scores included a significantly greater proportion of subsequent responders at year 1 to triple therapy as compared with anti-TNF therapy (88% versus 18%; $P = 0.006$). Patients with high MBDA scores responded better to anti-TNF (35% versus 58%; $P = 0.040$) (Figure 2).

Similar results were obtained using ROC-based cutoffs; patients with lower MBDA scores (≤38) at month 3 (29% of 157 patients) had a higher likelihood of response at year 1 to triple therapy (79%) as compared with anti-TNF (44%) (Figure 3A). For patients with higher (>38) MBDA scores (71% of 157 patients), the response rates were 36% and 58%, respectively ($P = 0.001$ for comparison across all 4 groups). Using the same approach, we analyzed the CRP, ESR, and DAS28 values (Figures 3B–D). Only the ESR resulted in a similar, although weaker, association, with 68% responding to triple therapy and 53% responding to anti-TNF therapy for patients with lower ESRs and 26% versus 51% responding to the respective therapies among those with higher ESRs ($P = 0.011$ for comparison across all 4 groups) (Figure 3C).

Impact of autoantibodies on the association of the MBDA score with subsequent clinical response to triple therapy or anti-TNF therapy. Of the 157 MTX-IR patients, RF status was missing in 1 and anti-CCP in 8 (Table 1). When grouped according to RF or anti-CCP status, the pattern of associations of the MBDA score at month 3 with the subsequent achievement of a low disease activity score (DAS28 ≤3.2) for each therapeutic group was similar between seropositive and seronegative patients (Figure 4). Thus, among the RF-negative patients with lower MBDA scores (n = 19), the proportions achieving a DAS28 of ≤3.2 at year 1 were 78% of those receiving triple therapy and 50% of those receiving anti-TNF, while for patients with higher MBDA scores (n = 40), the proportions were 37% and 62%, respectively ($P = 0.055$) (Figure 4A). Among RF-positive patients, these proportions were 80% versus 41% and 35% versus 58%, respectively

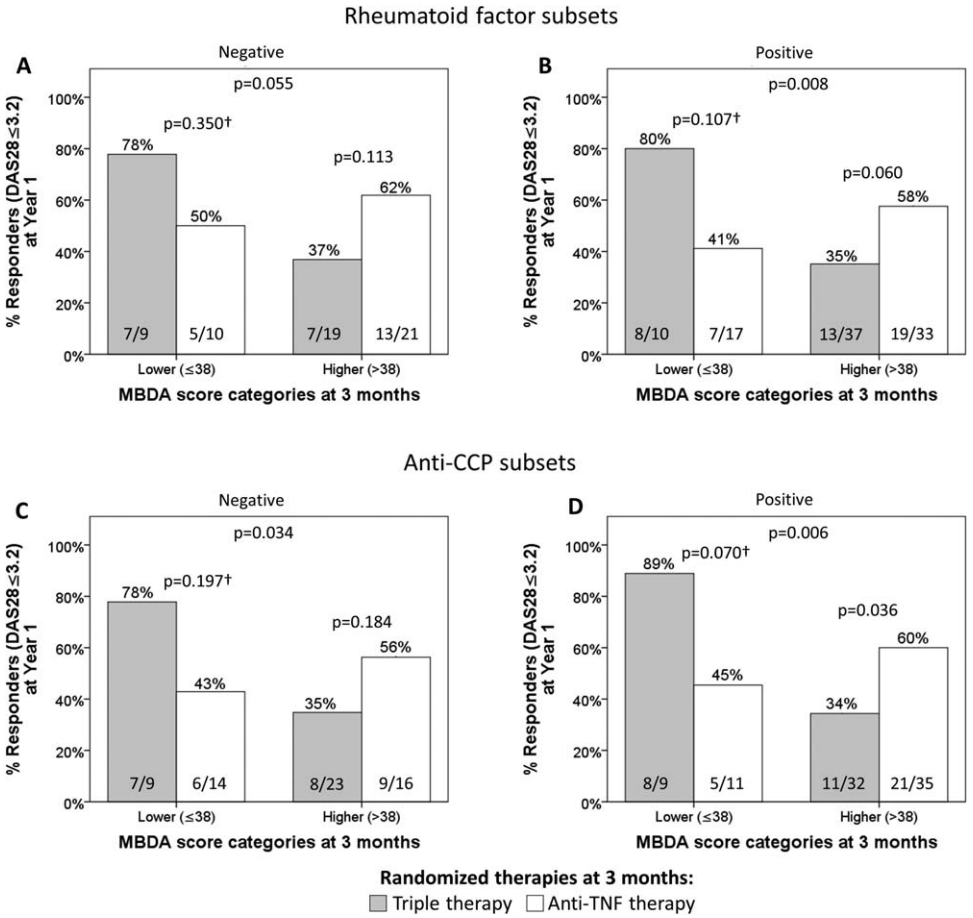

Figure 4. Proportion of patients achieving low levels of disease activity at year 1 according to a DAS28 score of ≤3.2, stratified by the MBDA scores at month 3 in seropositive and seronegative subsets. Responders at year 1 were evaluated according to rheumatoid factor (RF)–negative (**A**), RF-positive (**B**), anti–cyclic citrullinated peptide (anti-CCP)–negative (**C**), and anti-CCP–positive (**D**) status. Overall *P* values for the 4 groups were calculated using the Breslow-Day test, and *P* values for triple therapy versus anti-TNF therapy were calculated using the chi-square test, except where indicated otherwise. † = *P* value was calculated using Fisher's exact test. See Figure 1 for other definitions.

($P = 0.008$) (Figure 4B). When stratified according to anti-CCP status, among the anti-CCP–negative patients with lower MBDA scores (n = 23), the proportion who achieved a DAS28 of ≤3.2 at year 1 was 78% of those receiving triple therapy and 43% of those receiving anti-TNF, while for those with higher MBDA scores (n = 39), the proportions were 35% and 56%, respectively ($P = 0.034$) (Figure 4C). Among anti-CCP–positive patients, these proportions were 89% versus 45% and 34% versus 60%, respectively ($P = 0.006$) (Figure 4D).

Relationship between the MBDA score, CRP level, ESR, and DAS28 and a good clinical response at year 1 according to the EULAR criteria. Among patients with lower (≤38) MBDA scores, the proportions of EULAR good responders at year 1 in the triple therapy and anti-TNF therapy arms were 58% and 30%, respectively, while among those with higher (>38) MBDA scores, the proportions were 34% and 53%, respectively ($P = 0.007$) (Figure 5A). Similar but weaker patterns were obtained from analyses according to the CRP level ($P = 0.034$) (Figure 5B) or the ESR ($P = 0.012$) (Figure 5C). Analyses according to the DAS28 did not reveal a similar pattern (Figure 5D).

DISCUSSION

The aim of this study, which was based on the early RA SWEFOT trial, was to investigate whether the MBDA score is a valuable tool for predicting which of the second-line treatments (anti-TNF or triple therapy) is preferable for the individual patient in whom MTX monotherapy has failed. We have previously shown that smoking, functional impairment, and female sex strongly predict a nonresponse to MTX at 3 months of follow-up (13), whereas those who

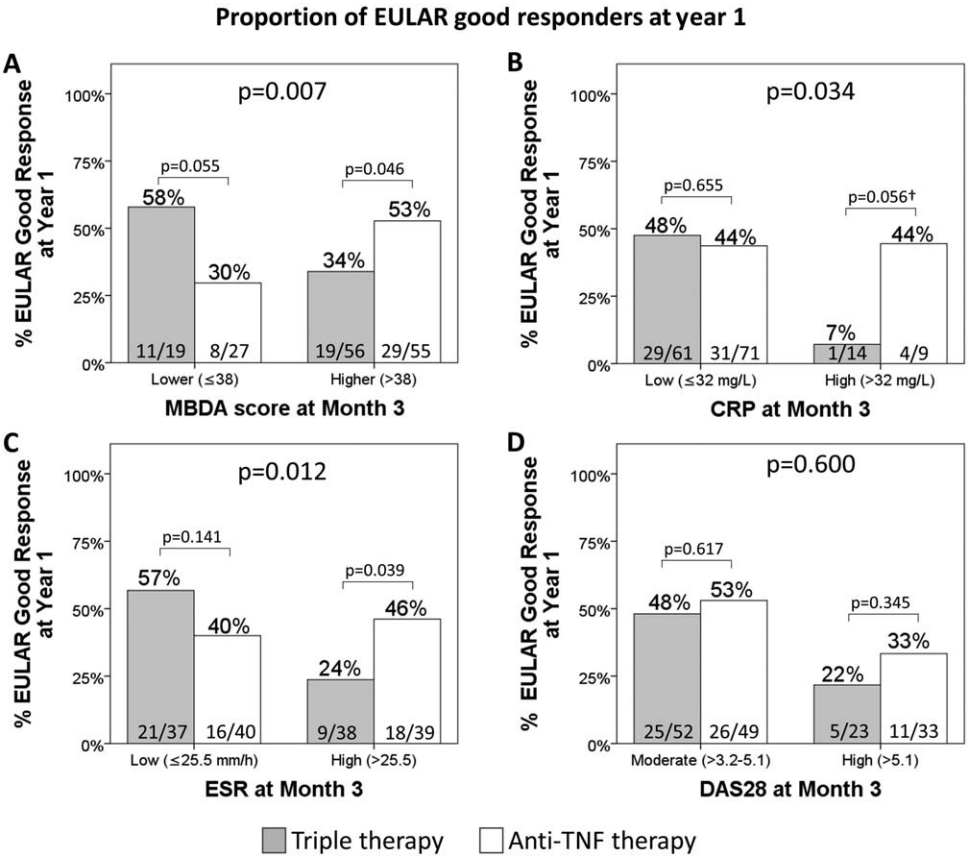

Figure 5. Proportions of patients with a good clinical response to second-line therapy at year 1 according to the European League Against Rheumatism (EULAR) criteria, among those with lower versus higher disease activity at month 3. Responders at year 1 were evaluated according to the MBDA score (**A**), CRP level (**B**), ESR (**C**), and DAS28 (**D**) at month 3. Overall *P* values for the 4 groups were calculated using the Breslow-Day test, and *P* values for triple therapy versus anti-TNF therapy were calculated using the chi-square test, except where indicated otherwise. † = *P* value was calculated using Fisher's exact test. See Figure 1 for other definitions.

responded well to MTX did well during 2 years of follow-up under standard care (23). All clinical guidelines recommend that treatment start with MTX, while there are several options to choose from in cases of nonresponse: cDMARD combinations and different biologic drugs. Predictors of the optimal choice are scarce.

In the present study, we found that a validated score based on a panel of biomarkers could help predict in a differential manner which subsequent therapy would be most effective in early RA patients with an insufficient response to MTX monotherapy. Thus, while overall, second-line therapy with anti-TNF was better in terms of the DAS28 (although perhaps only marginally so) at the group level, we found that for patients with lower MBDA scores (≤38) at treatment escalation, triple therapy was not only equal but was in fact a better therapeutic option than anti-TNF in terms of clinical response. In contrast, in patients with higher MBDA scores (>38), anti-TNF was more efficient in achieving low DAS28 values at year 1.

The conventional markers of inflammation did not show any preferential outcome for triple therapy. Among them, only higher ESRs (>25.5 mm/hour) showed similar, though weaker, associations with response (either a DAS28 of ≤3.2 or a EULAR good response) to therapy with anti-TNF. The observed significance regarding the homogeneity of odds ratios for a EULAR good response to triple therapy or anti-TNF therapy in patients with higher (>32 mg/liter) versus lower (≤32 mg/liter) CRP levels could be explained by the fact that among the 14 patients with higher CRP levels, only 1 responded to triple therapy. However, comparison of the proportion of responders between the triple therapy and anti-TNF therapy groups within each CRP category did not reveal any significant differences (Figure 5B).

Several trials have shown that early and aggressive treatment of RA increases the chances of achieving remission or low levels of disease activity. However, the superiority of biologic drugs versus combination cDMARDs was

observed only for the first few months of treatment, gradually losing their advantage after further follow-up (7–10). Moreover, O'Dell and colleagues (10) showed in the RACAT trial that MTX nonresponders who received triple therapy and failed to respond improved significantly after switching to MTX plus etanercept. Similarly, those who received MTX plus etanercept and did not respond had better clinical outcomes after they were switched to triple therapy.

The results presented herein may, if confirmed, have a major bearing on clinical practice. According to widely accepted guidelines, initial (first-line) therapy for RA is usually MTX, but if this yields an insufficient response, several second-line options are available, including the addition of anti-TNF or escalation to triple therapy (1). While both of these options are superior to placebo, their head-to-head efficacy has been a matter of some debate. The data currently available indicate either that the 2 options are equivalent or that anti-TNF is only marginally better than triple therapy but at a very large cost, and it has therefore been argued that triple therapy should be attempted first. However, as demonstrated in the RACAT trial, where patients failing anti-TNF therapy could respond to triple therapy and vice versa, the equivalence of the 2 options is only true at the group level; individual patients may show different responses to triple therapy versus anti-TNF therapy (10).

Because escalation of therapy in clinical practice is usually from conventional drugs to biologic drugs rather than vice versa, clinicians are often keenly aware of patients who responded much better to anti-TNF than to triple therapy and much less aware of the reverse scenario. This may be a reason that clinicians have been reluctant to adopt triple therapy. The findings in our study allow the identification of a patient subgroup that is much more likely to respond to triple therapy than to anti-TNF, and this may help clinicians choose the better option. The MDBA score cutoff of ≤ 38 and >38, which was defined here based on ROC curve analyses, needs to be confirmed in other patient populations; however, the findings were also striking for the already validated cutoffs and for continuous levels of the MBDA score.

Clinical outcomes do not always reflect radiologic data (23–27). We previously showed in the SWEFOT trial that MTX-IR patients receiving anti-TNF therapy had a significantly lower proportion of radiographic progression at year 2 compared with those receiving triple therapy (18). However, in a later study of the same patients, it was shown that patients with low MBDA scores at the time of randomization did not progress radiographically during 2 years from baseline, regardless of the choice of therapy (28). Neither differed in the proportion of patients with 2-year radiographic progression among those with moderate MBDA scores between the anti-TNF and triple therapy groups (24% and 25%, respectively). The superiority of anti-TNF versus triple therapy was obvious only in patients with high MBDA scores (32% and 57%, respectively; $P = 0.038$). Thus, it may be speculated that giving preference to triple therapy over anti-TNF for patients with lower MBDA scores is not likely to have a negative effect on radiographic outcome.

There have indeed been many studies performed on biomarkers as potential predictors of response to biologic therapies. Trocme and colleagues (29) demonstrated that increased levels of plasma apolipoprotein A-I were an indicator of response to infliximab therapy (according to the ACR criteria for 70% improvement), while platelet factor 4 was associated with nonresponse. Another study on cytokines showed that a simultaneous increase in the levels of monocyte chemoattractant protein 1 and epidermal growth factor (EGF) or in the levels of CRP and EGF was associated with a response to etanercept therapy (30). In a randomized trial, different biomarkers measured at baseline or during the first 4 weeks of treatment correlated with a subsequent clinical response to the anti-TNF agent golimumab (31). Likewise, Hueber et al (32) demonstrated that a panel of 24 biomarkers (13 autoantibodies and 11 cytokines) predicted response to etanercept. However, none of these studies analyzed whether the biomarkers differentially predicted response to triple therapy versus anti-TNF.

Thus, in this study, the MBDA score was differentially associated with the likelihood of response to one or the other second-line treatment. A possible explanation for these findings might lie in the fact that an inadequate response to MTX monotherapy was based on the DAS28, which is mostly based on symptomatic parameters. Some of these patients might have experienced improvements during the 3 months of MTX monotherapy, but of a minor magnitude, making them symptomatically undetectable at the time of the month 3 evaluation (lagging response). The MBDA blood test, on the other hand, shows changes on a molecular level, which can show early improvements that are not yet detectable on physical examination. Therefore, patients who achieved lower MBDA scores by the end of MTX monotherapy but still had moderate/high disease activity based on the DAS28 value were able to accelerate their improvements after addition of other nonbiologic DMARDs. One of the components of the triple therapy, sulfasalazine, has a pharmacokinetic/pharmacodynamic interaction with MTX (33,34), which could further support this theory. If this was indeed the case, for those who responded to triple therapy, taking MTX longer or escalating the dosage might be another option for achieving clinical response.

It is therefore conceivable that patients who show biochemical improvements with MTX treatment, even when they have insufficient clinical responses, are more likely to respond to intensification of treatment with a drug that acts by the same mechanism rather than switching to a drug with a different mechanism of action. In contrast, patients with a lack of biochemical improvements during MTX monotherapy may need a drug with a completely different treatment mechanism (e.g., TNF inhibition) to achieve low levels of disease activity. This hypothesis could also apply to the prediction of responders to anti-TNF according to the ESR, which also detects changes on a molecular/cellular level.

There were some limitations in this study which could affect the results. This was a post hoc analysis, prompted by novel biomarker findings that did not exist when the SWEFOT trial was designed. There is no validated threshold value for defining patients with low or high CRP or ESR values. Therefore, we used ROC curve analysis to define the best cutoffs. Even though the MBDA score had validated categories, those were developed to monitor RA. We therefore also defined new cutoffs using ROC curve analysis for the MBDA scores. This allowed us to create 2 groups (lower and higher) instead of 3 (low, moderate, and high), which led to a more comparable sample size in each group. Another limitation was that we did not have an opportunity to study these relationships for other anti-TNF medications or for biologic drugs other than anti-TNF. Similar studies using other anti-TNF, anti–interleukin-6, T cell–modulating, or anti–B cell therapies would give us further opportunities to explore predictive patterns of the MBDA score. And finally, because of missing data, we were unable to analyze 40% of patients who were randomized to second-line therapy. This fact may generate uncertainty concerning the reliability of the results. We tried to address this challenge by comparing characteristics at the time of randomization between these 40% of patients and the remaining 60% who were included in this study (data available upon request from the corresponding author). The results did not show striking differences, which allowed us to assume that the inclusion of these patients would be less likely to affect the results presented here.

The main strength of this study was that the patient population included in the SWEFOT trial is from standard care with little selection; almost all patients in the areas of the participating centers were referred directly there, and the only inclusion criteria were an age $\geq$18 years, a symptom duration of $<$12 months, a DAS28 of $>$3.2, and a stable low dosage of prednisolone in those who were taking it. Another strength of this study was a reasonable sample size and comparable numbers of patients in each treatment arm (n = 75 for triple therapy versus n = 82 for anti-TNF), which allowed us to obtain reliable statistical results. However, of the entire group of MTX-IR patients randomized to triple therapy or anti-TNF (n = 258), 40% were not included in the analyses because complete data at 3 and 12 months were lacking. The characteristics at 3 months did not differ between the 2 groups, except for global health assessment in the patients who were not analyzed (n = 101) versus those who were (n = 157) (data available upon request from the corresponding author).

In conclusion, in patients with early RA who had an insufficient clinical response to first-line therapy with MTX, the MBDA score was significantly and differentially associated with subsequent response to triple therapy or to anti-TNF therapy. A subset of patients (29% of the study population) had lower MBDA scores and a higher proportion of responders to triple therapy than to anti-TNF. In contrast, patients with higher MBDA scores (71% of the study population) had greater benefit from anti-TNF than triple therapy. We believe this is the first identification of a biomarker test that identifies a group of patients in whom conventional therapy is more optimal than biologic therapy.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. van Vollenhoven had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Van Vollenhoven.

Acquisition of data. Hambardzumyan, Saevarsdottir, Forslind, Petersson, Wallman, Ernestam, Bolce, van Vollenhoven.

Analysis and interpretation of data. Hambardzumyan, Saevarsdottir, Forslind, Petersson, Wallman, Ernestam, Bolce, van Vollenhoven.

ADDITIONAL DISCLOSURES

Crescendo Bioscience, Inc. performed the serum analyses for the MBDA scores at no cost to the investigators. Author Bolce, an employee of Crescendo Bioscience, Inc., had a role in the analysis and interpretation of the data and the writing of the manuscript, but had no role in collection of the data. Publication of this article was not contingent upon approval by Crescendo Bioscience, Inc. Schering-Plough Sweden provided an unrestricted grant for the original SWEFOT trial (2003–2010).

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APPENDIX A: THE SWEFOT TRIAL INVESTIGATORS

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