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Concise report

A multi-biomarker score measures rheumatoid arthritis disease activity in the BeSt study

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Abstract

Objective. To evaluate a multi-biomarker disease activity (MBDA) score, a novel index based on 12 serum proteins, as a tool to guide management of RA patients.

Methods. A total of 125 patients with RA from the Behandel Strategieën study were studied. Clinical data and serum samples were available from 179 visits, 91 at baseline and 88 at year 1. In each serum sample, 12 biomarkers were measured by quantitative multiplex immunoassays and the concentrations were used as input to a pre-specified algorithm to calculate MBDA scores.

Results. MBDA scores had significant correlation with DAS28-ESR (Spearman's $\rho = 0.66$, $P < 0.0001$) and also correlated with simplified disease activity index, clinical disease activity index and HAQ Disability Index (all $P < 0.0001$). Changes in MBDA between baseline and year 1 were also correlated with changes in DAS28-ESR ($\rho = 0.55$, $P < 0.0001$). Groups stratified by European League Against Rheumatism disease activity (DAS28-ESR $\leqslant 3.2$, 3.2–5.1 and > 5.1) had significantly different MBDA scores ($P < 0.0001$) and MBDA score could discriminate ACR/EULAR Boolean remission with an area under the receiver operating characteristic curve of 0.83 ($P < 0.0001$).

Conclusion: The MBDA score reflects current clinical disease activity and can track changes in disease activity over time.

Key words: rheumatoid arthritis, biomarkers, disability evaluation, outcome measures, bioinformatics, molecular biology, cytokines and inflammatory mediators.

CLINICAL SCIENCE

Introduction

The management of patients with RA has improved considerably in recent decades [1]. This is partly due to therapeutic strategies such as the combination of MTX and TNF inhibitors [2] and partly due to the introduction of composite measures to assess patients' disease activity and guide treatment decisions. In recent guidelines for RA it was recommended to evaluate disease activity by composite measures at regular intervals and adapt treatment decisions based on the results [3].

For example, the DAS, along with variants DAS28-ESR and DAS-CRP, has been shown to provide better clinical control [4], improved long-term physical ability [5], reduced radiographic progression [6] and lower costs [7]. Other composite measures have also been found to be valuable in the management of RA [8, 9].

Optimal management of RA requires the care of a specialist rheumatologist. However, it is difficult for some patients with RA to have frequent assessments because of the insufficient number of rheumatologists, long journeys to access or lack of time to assess disease activity. Thus alternative procedures for regular assessment could aid in optimal management.

Biomarkers are promising for assessing disease status in various chronic conditions. To date, no single biomarker has been proposed to adequately assess RA disease

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activity [10]. Therefore a combination of multiple biomarkers may provide richer information and be more robust than clinical composite indices.

Vectra DA is a novel blood test using measurements of 12 serum proteins to calculate a multi-biomarker disease activity (MBDA) score between 1 and 100 [11]. Using independent samples to confirm the relationship between MBDA scores and conventional disease activity indices such as DAS28 is crucial to validate the MBDA score. In this study we examined the validity of the MBDA score system as a novel index for evaluating disease activity and physical ability in patients with RA who participated in the Behandel Strategieën (BeSt) study [2].

Patients and methods

Patients and sample collection

A total of 125 patients with symptoms <2 years who fulfilled the 1987 ACR revised criteria for RA [12] and participated in the BeSt study [2] were analysed. For this study, ethics approval was obtained from the Leiden University Medical Center Ethics Committee and the ethics committees of all centres participating in the BeSt study. Patients provided informed consent before peripheral blood was collected. Serum was separated with centrifugation, dispensed and stored at −70°C. Clinical data and serum samples were available at 179 visits [91 at baseline (BL), 88 at year 1]. Clinical data and samples at both BL and year 1 were available in 54 patients (108 visits). Ethics approval was obtained from the Leiden University Medical Center Ethics Committee according to the Declaration of Helsinki, and all patients gave their written informed consent.

Conventional disease activity and physical disability assessment

In the single-blind BeSt study, specially trained nurses evaluated the tender joint count (TJC) and the swollen joint count (SJC). The participant and the evaluator registered general assessment [patient global (PG); evaluator global] on a visual analogue scale (VAS). ESR, CRP and the HAQ Disability Index (HAQ-DI) were also assessed.

Multiple biomarker-based disease activity assessment

The biomarker platform, assays and algorithm were the same as those used in Vectra DA (Crescendo Bioscience, South San Francisco, CA, USA). Assay development included studies to test for and block interference from RF and human anti-mouse antibodies. The finished assays were found to have no detectable interference [13]. The test algorithm was developed using DAS28-CRP as a reference standard. The algorithm was derived from statistical analysis of clinical data from three cohorts, the OKC (Oklahoma City Community), BRASS (Brigham & Women's RA Sequential Study) and InFoRM (Index For Rheumatoid Arthritis Measurement) cohorts [14]. Briefly, 396 candidate biomarkers were considered on the basis of literature review, screening experiments and bioinformatics databases. A series of studies was used to evaluate 130 of these as biomarkers of disease activity and to select the subset with the greatest utility. The final algorithm training process used penalized linear regression to select the combinations and weights of 12 biomarkers that give the best assessment of current disease activity. The concentrations of 12 serum proteins—serum amyloid A (SAA), IL-6, TNF receptor superfamily member 1A (TNF-RI), VEGFA, MMP1, human cartilage glycoprotein 39 (YKL40), MMP3, epithelial growth factor (EGF), vascular cell adhesion molecule 1 (VCAM1), leptin, resistin and CRP—were measured by customized immunoassays, quantified on a Sector Imager 6000 (Meso Scale Discovery, Gaithersburg, MD, USA) and transformed to the power 0.1 to achieve approximately normal distributions. The MBDA algorithm uses different subsets of biomarkers to estimate TJC, SJC and global health (GH), and then combines the estimates of these components into an overall score as follows:

$$\begin{aligned}\text{Prediction of TJC (PTJC)} = {} & -26.72 + 3.243 * [\text{YKL40}]^{1/10} \\ & - 11.97 * [\text{EGF}]^{1/10} + 15.72 * [\text{IL-6}]^{1/10} \\ & + 0.4594 * [\text{Leptin}]^{1/10} + 3.881 * [\text{SAA}]^{1/10} \\ & + 0.7388 * [\text{TNF-RI}]^{1/10} - 0.2557 * [\text{VCAM1}]^{1/10} \\ & + 0.7003 * [\text{VEGFA}]^{1/10}\end{aligned}$$

$$\begin{aligned}\text{Prediction of SJC (PSJC)} = {} & -26.63 + 3.232 * [\text{YKL40}]^{1/10} \\ & - 11.93 * [\text{EGF}]^{1/10} + 15.67 * [\text{IL-6}]^{1/10} \\ & + 0.4578 * [\text{Leptin}]^{1/10} + 3.868 * [\text{SAA}]^{1/10} \\ & + 0.7363 * [\text{TNF-RI}]^{1/10} - 0.2548 * [\text{VCAM1}]^{1/10} \\ & + 0.6979 * [\text{VEGFA}]^{1/10}\end{aligned}$$

$$\begin{aligned}\text{Prediction of PG Health (PGH)} = {} & -13.489 + 5.474 * [\text{IL-6}]^{1/10} + 0.486 * [\text{SAA}]^{1/10} \\ & + 2.246 * [\text{MMP1}]^{1/10} + 1.684 * [\text{Leptin}]^{1/10} \\ & + 4.14 * [\text{TNF-RI}]^{1/10} + 2.292 * [\text{VEGFA}]^{1/10} \\ & - 1.898 * [\text{EGF}]^{1/10} + 0.028 * [\text{MMP3}]^{1/10} \\ & - 2.892 * [\text{VCAM1}]^{1/10} - 0.506 * [\text{Resistin}]^{1/10}\end{aligned}$$

$$\begin{aligned}\text{MBDA score} = \text{round} < \max\{\min[(0.56 * \sqrt{\max(\text{PTJC}, 0)} \\ + 0.28 * \sqrt{\max(\text{PSJC}, 0)} + 0.14 * \text{PGH} \\ + 0.36 * \ln(\text{CRP}/10^6 + 1) + 0.96) * 10.53 \\ + 1, 100], 1\} >\end{aligned}$$

The predicted TJC, SJC and GH are combined with CRP in a formula analogous to that of the DAS28-CRP. The results are scaled and rounded to be integers on a scale of 1–100 such that an MBDA score of 1 would be equivalent to a DAS28-CRP value of 0 and an MBDA score of 100 would be equivalent to a DAS28-CRP value of 9.4. This mathematical relationship between MBDA and DAS28-CRP indicates that MBDA scores ⩽25 indicate remission, according to the DAS28-CRP thresholds of Inoue *et al*. [15].

Statistics

Spearman's rank correlation coefficients (Spearman's ρ) were calculated to evaluate the association between MBDA and clinical indices [DAS28, clinical disease activity index (CDAI), simplified disease activity index (SDAI) and HAQ-DI]. The values of MBDA stratified by EULAR disease activity (low, moderate or high disease activity) were compared by one-way analysis of variance with Tukey's multiple comparison. Statistical analyses were performed using JMP 9.0.3 (SAS Institute, Cary, NC, USA) and GraphPad Prism 5.0d (GraphPad Software, San Diego, CA, USA). All reported *P*-values are two sided; those <0.05 were considered significant.

Results

BL clinical characteristics

Representative clinical BL characteristics are summarized in Table 1. Since the BeSt study included patients with early active RA, median symptom duration of the disease was <6 months, disease activity was high and there was little joint destruction evident on radiographs.

The MBDA score reflects clinical disease activity and functional disability

The relationship between MBDA score and DAS28-ESR is shown in Fig. 1. The MBDA score was significantly correlated to DAS28-ESR, with a Spearman's rank correlation coefficient (ρ) of 0.66 ($P < 0.0001$, Fig. 1A). Similar results were obtained for correlation of MBDA score with SDAI ($\rho = 0.67$, $P < 0.0001$, Fig. 1B) and CDAI ($\rho = 0.56$, $P < 0.0001$, Fig. 1C). MBDA scores were also found to differ between EULAR disease activity strata (supplementary Table S1 and supplementary Fig. S1, available as supplementary data at *Rheumatology* Online) and were associated with HAQ-DI (supplementary Fig. S2, available as supplementary data at *Rheumatology* Online) in this early RA population. For further details see supplementary data available at *Rheumatology* Online.

A key requirement for a disease activity index is the ability to evaluate patients' changes in disease activity over time. The correlation of the change in DAS28-ESR (ΔDAS28) with the change in MBDA (ΔMBDA) between BL and year 1 was assessed in 54 patients (108 visits). A significant correlation of ΔMBDA was confirmed with ΔDAS28 ($\rho = 0.55$, $P < 0.0001$, Fig. 1D) and with ΔSDAI ($\rho = 0.35$, $P = 0.0158$, Fig. 1E), but the correlation with ΔCDAI was not significant ($\rho = 0.18$, $P = 0.2270$, Fig. 1F).

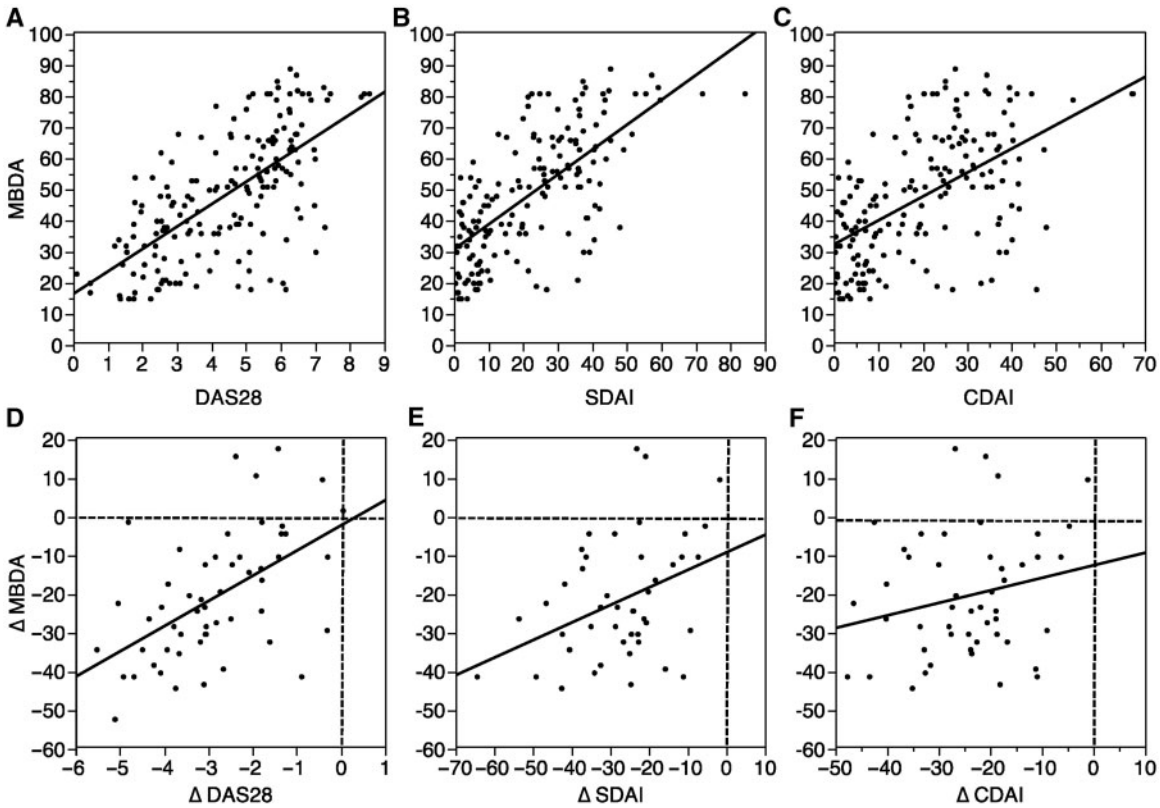
We also tested whether the MBDA score would distinguish patients in remission based on the ACR/EULAR criteria [16]. MBDA scores were associated with ACR/EULAR Boolean remission (TJC28 $\leqslant 1$, SJC28 $\leqslant 1$, VAS-GH $\leqslant 1$, CRP $\leqslant 1$ mg/dl) and with area under the receiver operating characteristic curve of 0.83 ($P < 0.0001$). Remission by MBDA score ($\leqslant 25$) was also associated with remission by DAS28-ESR, SDAI and CDAI

Table 1 Characteristics of the 125 patients with RA

Quantitative variable	Mean (s.d.)	Min, max	Median (IQR)
Age, years	52.9 (13.6)	21, 82	54 (45–63)
Symptom duration, weeks	43.8 (61.7)	3, 584	24.5 (13–57)
TJC28	11.1 (6.4)	0, 28	10 (6–15)
SJC28	10.15 (4.87)	0, 24	9 (7–13)
PG (VAS), mm	53.3 (22.0)	0, 100	53 (37–71)
CRP, mg/l	39.3 (46.2)	0, 228	21 (9–57)
ESR, mm/h	39.4 (25.3)	4, 126	37 (19–51)
DAS	4.29 (0.87)	1.90, 7.10	4.19 (3.67–4.91)
DAS28-CRP	5.45 (0.94)	2.35, 8.31	5.49 (4.90–6.02)
DAS28-ESR	5.80 (1.04)	1.43, 8.57	5.85 (5.20–6.45)
HAQ-DI	1.42 (0.71)	0, 3	1.38 (1.0–1.88)
SHS	5.5 (8.2)	0, 49	3 (1–7)
MBDA score	58.8 (17.1)	17, 88	60 (50–73)
Categorical variable	**Percentage**		
Treatment arms[a]			
Sequential monotherapy	17.6		
Step-up combination	24.0		
Initial combination with prednisone	30.4		
Initial combination with infliximab	28.0		
Gender (female)	74.4		
Presence of erosion at BL	67.2		
ACPA positivity	56.5		
RF positivity	62.4		

[a]Treatment arms in the BeSt study are described in detail in the original article [2]. Min: minimum; Max: maximum; IQR: interquartile range; SHS: Sharp–van der Heijde score.

FIG. 1 Relationship between the MBDA score and clinical disease activity indices.



(**A**–**C**) Correlation and linear regression of MBDA score with DAS28-ESR (**A**), SDAI (**B**) and CDAI (**C**). Spearman's rank correlation coefficient was 0.66 ($P < 0.0001$), 0.67 ($P < 0.0001$), 0.56 ($P < 0.0001$), respectively. (**D**–**F**) Correlation and linear regression of ΔMBDA score with ΔDAS28-ESR (**D**), ΔSDAI (**E**) and ΔCDAI (**F**). Spearman's rank correlation coefficient was 0.55 ($P < 0.0001$), 0.35 ($P = 0.0158$), 0.18 ($P = 0.2270$), respectively.

(supplementary Table S2, available as supplementary data at *Rheumatology* Online).

Discussion

In some circumstances, measurement of composite indices is not performed due to limited resources or lack of access to a rheumatologist. An objective biomarker-based DAS could complement clinical assessment and provide information to guide patient care when a composite clinical score is unfeasible.

We found that the MBDA score is associated with conventional clinical disease activity indices. It can track changes in disease activity over time, although small sample size was a limitation specifically for this analysis. Larger studies are required to clarify the relationship between ΔMBDA score and ΔCDAI, but we observed a consistent trend of stronger association with DAS28 (against which MBDA was trained) and a weaker association with CDAI (which does not include an acute-phase marker).

Our results demonstrate that the MBDA score reflects RA disease activity, and in the majority of patients it gives a similar result to the clinical indices. However, there are some cases in which the biomarkers and clinical assessment disagree. One potential explanation worthy of further study is that MBDA biomarkers could be affected by conditions such as vaccination or acute infection. On the other hand, since the conventional indices include subjective components such as TJC or PG, they may overestimate disease activity, especially in patients with pain due to accrued joint damage or other causes (e.g. FM, pain hypersensitivity) [17, 18]. In cases where the MBDA score and clinical assessment are discordant, it is unclear which is the more correct reflection of true RA disease activity. Independent outcomes such as imaging of synovitis or progressive joint damage may be used to evaluate which disease activity measures provide the best information to support clinical decision-making. Ultimately, since biomarkers and clinical examination are indicative of different aspects of disease activity, they may complement one another and provide the best information when used together.

This study was intended to determine whether the MBDA score is a valid measure of RA disease activity, and was not designed or powered to be a comparison of the performance of the MBDA score with that of other single biomarkers including ESR or CRP. It is challenging to conduct such a comparison by examining the agreement of the MBDA score and single acute-phase markers with clinical disease activity indices such as DAS28 or SDAI, because the acute-phase markers are used in the

calculation of these clinical indices and of the MBDA score. Effective comparison of the MBDA score with other biomarkers will require additional studies using independent disease outcomes such as joint damage progression or functional disability.

In recent years, patient-reported outcomes (PROs) such as RAPID3 have received attention as practical and low-cost tools for assessing the activity of RA [8, 19]. These PROs are important in providing consistent and quantitative information about the patient's experience of RA. In contrast, the MBDA score measures the biological pathways underlying the disease activity. These different types of information may be complementary and contribute to a more complete disease activity assessment. The comparison between PROs and the MBDA requires further investigation.

No biomarker assay can substitute for careful clinical judgement. However, our analysis suggests that a biomarker-based DAS could provide complementary information. The Tight Control for Rheumatoid Arthritis (TICORA) study [4] and the Computer Assisted Management in Early Rheumatoid Arthritis (CAMERA) study [20] showed that monthly assessment of disease activity and subsequent adaptation of therapy yielded improved outcomes. The MBDA score could be used to enable monthly monitoring of disease activity while allowing clinical assessment to take place less frequently. This would provide additional information about patient status and might allow more efficient use of precious health care resources.

Conclusion

The 12-biomarker based MBDA score can measure disease activity and track changes in disease activity in early RA.

Rheumatology key messages

- The MBDA algorithm reflects actual disease activity in RA.
- Biomarkers provide information about underlying RA pathophysiology that may complement clinical assessment.

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Supplementary data

Supplementary data are available at *Rheumatology* Online.

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