EXTENDED REPORT

Macrophage migration inhibitory factor polymorphisms do not predict therapeutic response to glucocorticoids or to tumour necrosis factor α -neutralising treatments in rheumatoid arthritis

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Background: Macrophage migration inhibitory factor (MIF) is an inflammatory mediator associated with RA severity. In various diseases, MIF polymorphisms are associated with clinical response glucocorticoid (GC) treatment. It is unclear whether MIF polymorphisms determine GC response in rheumatoid arthritis (RA) and to other RA treatments. Therefore, the question of whether two functional variants in MIF are associated with the response to tumour necrosis factor (TNF) α -neutralising and GC treatments in RA was investigated.

Methods: Data from two cohorts of an RA registry were used. For patients who started with TNF α -neutralising (infliximab) or GC treatment, courses with a duration of at least 3 months were included and response to TNF α blockers or GC was calculated according to the European League Against Rheumatism response criteria. MIF –173G $\rightarrow$ C genotyping was achieved using an assay-on-demand allelic discrimination assay, and alleles of the CATT repeat element were identified using a fluorescently labelled PCR primer and capillary electrophoresis. Logistic-regression modelling was used for the statistical analysis.

Results: In total, 192 courses of oral prednisone or methylprednisolone injections in 98 patients with RA and 90 patients with RA who were on TNF α -neutralising treatments were documented. In all, 27% of the patients with RA were found to be heterozygous for seven CATT repeats (CATT₇) and 31% were heterozygous for –173C. Respectively, 4% and 6% of the patients with RA were homozygous for the MIF CATT₇ repeat or the MIF –173C allele. Carrier status and homozygosity for CATT₇ repeat and the MIF –173C allele were not associated with response to GC (odds ratios (ORs) close to 1) or to TNF α -neutralising treatment (ORs close to 2).

Conclusion: The MIF-CATT₇ repeat and the MIF –173G $\rightarrow$ C functional variant are not strongly associated with a decreased clinical response to TNF α -neutralising or GC treatment in RA.

Macrophage migration inhibitory factor (MIF) is a pleiotropic inflammatory mediator that is thought to play a role in the innate and adaptive immune responses, and has been implicated in various inflammatory conditions, such as psoriasis,¹ multiple sclerosis,² inflammatory bowel diseases³ and arthritis.^{4–9} Rheumatoid arthritis (RA) is a chronic autoimmune condition with a complex pathogenesis that has not been fully elucidated. However, substantial evidence currently suggests the involvement of many immunological effector cells, including macrophages, T cells, B cells and dendritic cells, which infiltrate the synovial tissue underlying the vicious cycle of inflammation. Although treatment strategies have evolved tremendously during recent years, a substantial proportion of patients still does not respond to disease-modifying antirheumatic drugs (DMARDs) and/or biological treatments including anti-interleukin (IL)-1 β or TNF α -neutralising agents.

In these patients, the need for glucocorticoid (GC) treatment is still high, coinciding with a wide spectrum of side-effects and comorbidity. In fact, despite the use of these novel therapeutic regimens, 56–68% of patients with RA continue to require GC. To avoid unnecessary complications, good clinical markers that enable rheumatologists to distinguish between aggressive and mild disease are essential. Recently, two genetic variants of MIF (the –173G $\rightarrow$ C allele and the CATT_{5–8} tetranucleotide repeat) and the circulating levels of MIF were found to be clearly associated with the rate of radiological joint damage, suggesting

that MIF plays a role in the RA disease process and could be a valuable marker in the assessment of RA prognosis.¹⁰

There are many characteristics of MIF that advocate its role both in the onset and the perpetuation of inflammation during RA. MIF induces activation of several inflammatory mediators such as phospholipase A2, which are involved in the secretion of prostaglandins and leucotrienes.¹¹ Secondly, MIF induces the secretion of a plethora of proinflammatory cytokines including TNF α , IL-6, and IL-1 β , which are key inflammatory mediators in RA¹² (also reviewed by Feldmann and Maini¹³). In addition, MIF is known to be involved in the regulation of Toll-like receptors (TLR), which are increased in synovial tissue in patients with RA, and a substantial amount of evidence supports a role for TLR in arthritis.^{14–17} Finally, MIF was found to inhibit nitric oxide-induced intracellular accumulation of p53, a pivotal molecule that commences the orchestration of apoptosis, resulting in diminished p53-mediated apoptosis.¹⁸ Altogether, a large body of evidence supports a role for MIF in arthritis, a finding that was further substantiated by observations

Abbreviations: DAS, Disease Activity Score; DMARD, disease-modifying antirheumatic drug; ESR, erythrocyte sedimentation rate; EULAR, European League Against Rheumatism; GC, glucocorticoid; IL, interleukin; JIA, juvenile idiopathic arthritis; MIF, migration inhibitory factor; RA, rheumatoid arthritis; RUNMC, Radboud University Nijmegen Medical Centre; SF, synovial fluid; SLE, systemic lupus erythematosus; TLR, Toll-like receptor; TNF, tumour necrosis factor

using experimental models of arthritis in which neutralisation of MIF delayed the onset, lowered the incidence and led to a significant reduction in severity of arthritis.⁸

MIF is produced by various effector cells of the immune system including macrophages, T cells and dendritic cells. Intriguingly, resident cells of the anterior pituitary gland were found to secrete MIF after challenge with endotoxins, providing preliminary evidence for MIF as a key player bridging the gap between the endocrine and immune system (reviewed by Calandra and Roger¹⁹). In this regard, it is interesting to note that MIF expression can be induced by GC, whereas other proinflammatory cytokines are uniformly suppressed by GC. In turn, MIF can override the immune dampening effects of GC both in vitro and in vivo, as shown by studies involving cytokine production by T cells, macrophages and synovial fibroblasts, and in experimental models of sepsis.^{12, 20–22} In keeping with this, the increased joint inflammation and lethality in rat adjuvant arthritis in the absence of GC could be reversed by the inhibition of MIF.⁸ Recently, these observations were extended to in vivo studies in humans. Berdeli *et al* reported an association between the MIF –173G→C polymorphism and GC resistance in children with nephrotic syndrome,²³ an observation that followed another study by De Benedetti *et al*, who showed that the MIF –173 G→C SNP and the protein level of MIF in the synovial fluid of patients with juvenile arthritis was closely linked with the level of GC response.²⁴ Therefore, we investigated the role of MIF variants in determining the response to response to TNF α -neutralising and GC treatment.

We investigated whether the functional MIF-CATT_{5–8} repeat and –173G→C variants of MIF are associated with the 3-month response to TNF α -neutralising and GC treatments in RA. We show, for the first time, that carriage of either the MIF –173 C allele or the CATT_{5–8} repeats in MIF is not associated with a decreased response to GC or TNF α neutralisation. Therefore, our data do not support the notion that MIF variants interfere with the clinical effects of TNF α -neutralising or GC treatments in RA.

METHODS

Patient cohorts

The study comprised two cohorts: one cohort (A) of patients with RA who were treated with ≥ 1 courses of GC treatment, and another cohort (B) of patients with RA starting their first course of a TNF-blocking agent. The study was approved for both cohorts by the local ethics committee in The Netherlands.

For cohort A, data were retrieved from a long-term observational study of early RA that was started at the Department of Rheumatology, Radboud University Nijmegen Medical Centre (RUNMC), in 1985.^{25, 26} Patients were included in this cohort if they fulfilled the 1987 American College of Rheumatology criteria for diagnosis of RA (disease duration <1 year) and had no prior use of DMARDs. MIF genotyping had been performed for the first 300 patients with RA in the cohort.

Since April 1997, all patients with RA who start treatment with a TNF-blocking agent according to the indication in the Netherlands (a Disease Activity Score (DAS)-28 >3.2 and failure on at least two DMARDs, of which one is methotrexate) in the RUNMC and the St. Maartenskliniek in Nijmegen are included in a biologicals registry, and these patients formed cohort B.

In both cohorts, clinical assessments were performed by specially trained research nurses. Disease activity was measured using 28 joint counts (DAS28) for tenderness and for swelling, erythrocyte sedimentation rate (ESR) (the Westergren method), and a visual analogue scale for general health. Data

on type of medication, start date, dose, stop date, change of dose or change of medication were collected, and the reason for changing dose or medication was registered.

Patients on glucocorticoid treatment

Within cohort A, 621 courses of prednisone in 463 patients and 1774 intramuscular methylprednisolone injections in 521 patients were documented. For prednisone, only patients with a follow-up duration of ≥ 3 months were included. Use of DMARDs and TNF inhibitors was registered in a standardised fashion. A new variable (DM) was created. If a DMARD or TNF inhibitor was started or the dose of a DMARD was increased, this variable was 1; otherwise, this variable was 0. RA disease activity was measured as described above.²⁷ The DAS28 scores at the start date of the prednisone course or methylprednisolone injection (120 mg intramuscular, maximum 10 days before injection) and the DAS28 score 3 months later were used to calculate clinical response according to the European League Against Rheumatism (EULAR) response criteria.²⁸ Complete data concerning the course of oral prednisone, response at 3 months, and MIF measurement were present for 48 courses in 44 patients. Complete data concerning methylprednisolone injection, response at 3 months, and MIF measurement were present for 144 injections in 78 patients. In total, there were 192 (48+144) assessable courses of either oral prednisone or intramuscular methylprednisolone injections in 98 patients available. The number of courses per patient varied from one to five. MIF had previously been measured in frozen blood samples in a subsample of patients from the entire cohort, without knowledge of clinical status, treatment use or treatment outcome.

Patients on TNF α -neutralising treatment (infliximab)

Within cohort B, 98 patients who started their first TNF-blocking agent were documented. Only courses of at least 3 months' duration and data on response to treatment were included. The DAS28 scores at the start date of the TNF-blocking agent and the DAS28 score at 3 months were used to calculate clinical response as described above. MIF was measured in frozen blood samples in all patients from this cohort.

Genotyping of MIF –173G→C and CATT_{5–8} repeat polymorphisms

The genotyping of the MIF –173G→C and MIF-CATT_{5–8} polymorphism was performed using essentially the same protocols as described previously.¹

MIF –173G→C.

Genotyping was achieved using an assay-on-demand allelic discrimination assay and detection system (Taqman 7700) according to the manufacturer's instructions (Applied Biosystems, Warrington, Cheshire, UK). The PCR reaction contained 10 ng of genomic DNA, 10 μ l TaqMan master mix and 0.125 μ l of 40 $\times$ assay mix. PCR was performed using 96-well plates on a thermal cycler (ABI 9700; Applied Biosystems). Reaction conditions were 50°C for 2 min and 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. The Taqman 7700 system was used to perform end-plate reading using the allelic discrimination option.

MIF-CATT_{5–8} repeat genotyping

Alleles of the CATT repeat element were identified using a fluorescently labelled PCR primer and capillary electrophoresis. Genomic DNA (50 ng) was amplified by PCR in a total reaction volume of 10 μ l containing 5 pmol of both forward and reverse primers: (forward 5'-TTG CAC CTA TCA GAG ACC-3'; reverse

5'-TCC ACT AAT GGT AAA CTC G-3'). The forward primer was prelabelled with FAM fluorescent dye. The PCR mix included 4 nmol of each of the four dNTPs, 0.2 U Taq polymerase, 1.5 mM MgCl₂ 1× KCl buffer and 1 mmol/l betaine. The PCR was performed in 96-well microtitre plates on a thermal cycler (Tetrad; MJ Research, Waltham, Massachusetts, USA). In total, 40 PCR cycles were carried out, each with denaturation at 95°C for 1 min, primer annealing at 54°C for 1 min and extension for 45 s at 72°C, followed by a final extension for 5 min. The amplified product was pooled with the TAMRA 350 size standard. Gel electrophoresis was performed on a 0.4 mM 6% polyacrylamide gel on a DNA sequencer (Model 377; PE Applied Biosystems, Foster City, California, USA). Gels were run at 1200 V for 2 h. Semi-automated genotyping was carried out using Genescan analysis and Genotyper V.3.6 software (Applied Biosystems), with all genotyping manually checked.

Statistical analysis

In the analysis of Cohort A, a longitudinal logistic regression model was used (generalised linear mixed models) to account for multiple observations within the same patients. The primary outcome variable was predefined as clinical response (EULAR non-response versus EULAR moderate or good response)²⁸ at 3 months. The two independent variables were the MIF polymorphisms MIF -173G→C and the CATT₅₋₈ repeat. The relationship between a MIF polymorphism and response was corrected for DAS28 at treatment start, change in DMARD use and mode of administration (oral prednisone or methylprednisolone injection). In addition, the relationship between a MIF polymorphism and EULAR response was corrected for age at diagnosis, gender, disease duration and rheumatoid factor positivity, if addition of these variables to the model changed the β value in the model for MIF polymorphism by at least 10%. In addition, differences between MIF functional variants using the DAS28 as continuous measure were analysed using a generalised linear model correcting for repeated observations per subject and other confounders (generalised estimating equations).

Using an uncorrected logistic regression model including MIF polymorphism and response on 192 courses of prednisone or methylprednisolone injections and adopting the usual α level of 0.05 and power (1-β) of 0.80 and a prevalence of the MIF polymorphism of 30%, an odds ratio (OR) of at least 0.33 (or 2.5 in reverse) would be statistically significant, which corresponds to a moderate effect. The correction for additional variables such as baseline DAS28 would increase the power, and correction for multiple observations would decrease the power.

In the analysis of cohort B, a logistic regression model was used with predefined clinical response (EULAR non-response versus EULAR moderate or good response) at 3 months as outcome variable and the MIF polymorphisms 173G→C and CATT₅₋₈ repeat as independent variables. Correction for

confounding was applied as described above. In addition, differences between MIF functional variants in the continuous DAS28 were analysed using a linear-regression model.

Using an uncorrected logistic-regression model including MIF polymorphism and response on 90 cases, and adopting an α level of 0.05, a power (1-β) of 0.80 and a prevalence of the MIF polymorphism of 23% (lowest prevalence), an OR of at least 0.23 (or 4.3 in reverse) would be statistically significant.

RESULTS

Patients

In total, 192 courses of prednisone or methylprednisolone injections in 98 patients with RA were documented. The frequency of the MIF polymorphisms CATT₇ and -173G→C among these patients is shown in table 1. In all, 27% of the patients with RA were found to be heterozygous for the MIF CATT₇ allele, and 31% carried one MIF -173C allele. Respectively, 4% and 6% of the patients with RA were homozygous for the MIF CATT₇ or MIF -173G→C alleles, corroborating previous findings from our group and others.^{10 29} Carriage of the MIF-CATT₇ allele was strongly associated with carriage of the MIF-173C allele (p<0.001; Fisher exact test). In the further analyses of CATT₇ and -173G→C, patients who were homozygous or heterozygous for the CATT₇ repeat and -173C allele (GC + CC) were pooled together as carriers and compared with individuals not carrying any risk allele.

MIF variants and response to GC

Carriers of the MIF -173C risk allele or CATT₇ repeat did not differ from non-carriers in age at diagnosis or in distribution of gender and rheumatoid factor positivity (table 2). In addition, corticosteroid treatment was similar for carriers and non-carriers, according to the numbers of oral prednisone and methylprednisolone injections and dosages. The median prednisone dose was 7.5 mg/day in all groups, and most of the patients received 120 mg injections of methylprednisolone. The median disease duration at the start of a course of corticosteroid treatment was slightly longer in patients carrying one of each the risk alleles. The mean DAS28 at start of corticosteroid treatment was similar for carriers and non-carriers. In all groups, the DAS28 decreased to a similar level after 3 months of GC treatment. Accordingly, no differences in response to GC treatment were observed between patients carrying and those not carrying the MIF CATT₇ repeat or MIF -173C allele. The mean difference in DAS28 reduction (table 2) was 0.10 (p = 0.59) for MIF CATT₇ and -0.04 (p = 0.72) for -173G→C, as calculated using generalised estimating equations. The proportion of EULAR good or moderate responders was about 50% for both carriers and non-carriers of the CATT₇ repeat and -173C allele, respectively. This resulted in an (uncorrected) OR of 0.8 (p = 0.53) for the CATT₇ repeat and an OR of 1.1 (p = 0.79) for the -173G→C polymorphism (table 3). Correction for baseline DAS28, concurrent changes in DMARD treatment and mode of corticosteroid administration (oral prednisone or methylprednisolone injection did not change the ORs to a relevant degree, in contrast to the correction for repeated observations within single patients ("subject") was applied (table 3).

Several studies have shown that stratification for the presence of CATT₅ or presence over all CATT variants except CATT₅ is necessary. In our study, stratification for carriage of the CATT₅ variant did not result in any difference in response to GC treatment between patients carrying or not carrying CATT₅ (data not shown). As there were no differences in response to GC between patients with or without carriage of one of the MIF variants (heterozygous patients), we explored whether patients who were homozygous for either MIF CATT₇ or MIF -173G→C

Table 1 Frequencies of genotypes for the MIF CATT₇ repeat and MIF -173C allele and distribution among patients with RA

Polymorphism	Alleles	n (%)
MIF-CATT ₇	0	67 (69)
	1	26 (27)
	2	4 (4)
	Total	97
MIF-173C	0	62 (63)
	1	30 (31)
	2	6 (6)
	Total	98

MIF, migration inhibitory factor; RA, rheumatoid arthritis.

Table 2 Phenotypic disease characteristics, GC use and response to GC treatment of patients with RA with or without the CATT₇ repeat* or MIF -173C allele†

	Total	MIF CATT ₇		MIF -173G→C	
		Non-carrier	Carrier	Non-carrier	Carrier
Patients, n (%)	98	67 (69)	30 (31)	62 (63)	36 (37)
Age (years), mean (SD)	51 (14)	52 (15)	48 (12)	52 (15)	48 (12)
Female gender, n (%)	68 (70)	44 (66)	24 (80)	40 (65)	28 (78)
RF-positive, n (%)	79 (82)	55 (85)	23 (77)	51 (85)	28 (78)
Courses, n	192	130	61	121	71
Dosing route, n (%)					
Oral	48 (25)	34 (26)	14 (23)	33 (27)	15 (21)
Intramuscular	144 (75)	96 (74)	47 (77)	88 (73)	56 (79)
Oral dose (mg/day), mean (range)	7.5 (2.5 to 15)	7.5 (2.5 to 15)	7.5 (5 to 12.5)	7.5 (2.5 to 15)	7.5 (5 to 12.5)
Intramuscular dose (120 mg), n (%)	128 (90)	89 (95)	38 (81)	81 (94)	47 (84)
DMARD change, n (%)	28 (15)	18 (14)	10 (16)	17 (14)	11 (15)
Disease duration (years), mean (range)	6 (0 to 30)	4 (0 to 29)	6 (0 to 31)	4 (0 to 29)	6 (0 to 31)
DAS28					
At baseline, mean (SD)	5.1 (1.3)	5.1 (1.3)	5.1 (1.2)	5.2 (1.3)	5.1 (1.3)
After 3 months, mean (SD)	4.4 (1.2)	4.4 (1.2)	4.5 (1.3)	4.5 (1.2)	4.4 (1.2)
EULAR response, n (%)					
Moderate	76 (40)	55 (42)	20 (33)	51 (42)	25 (35)
Good	19 (10)	11 (8)	8 (13)	8 (7)	11 (16)

DAS, Disease Activity Score; DMARD, disease-modifying anti-rheumatic drug; EULAR, European League Against Rheumatism; GC, glucocorticoid; MIF, migration inhibitory factor; RA, rheumatoid arthritis; RF, rheumatoid factor.

*CATT₇ heterozygous and homozygous patients added together.

†Carriers were defined as patients having either GC or CC genotype.

or carrying both the MIF CATT₇ and MIF -173G→C risk alleles (inferred haplotype, defined as having at least one of either the MIF CATT₇ or MIF -173G→C alleles) showed a different response to GC from that of patients not carrying either of the MIF risk alleles. Neither homozygosity nor carriage of the inferred haplotype led to a decreased response to GC treatment (data not shown).

MIF variants and response to TNF α -neutralising treatments

Table 4 shows the results for the patients starting a TNF α inhibitor (infliximab). Carriers and non-carriers of the MIF CATT₇ or MIF -173C allele were similarly distributed with regard to age, gender, rheumatoid factor positivity and disease duration at start of the TNF-inhibitor treatment. There were also no relevant or large between-group differences in baseline DAS28 or DAS28 at 3 months. The mean difference in DAS28 reduction was -0.21 ($p = 0.38$) for the MIF -173G→C allele and -0.11 ($p = 0.72$) for MIF CATT₇ (table 4) as calculated using linear regression. Response seemed to be higher in the carrier groups, due to a relatively low rate of non-responders in

the patients treated with a TNF inhibitor, giving rise to positive (ie the variant protects for non-response, contrary to the a priori expectation) but non-significant ORs. For the CATT₇ repeat, the OR for predicting clinical response was 2.0 (95% CI 0.6 to 6.6, $p = 0.26$) and for the -173C allele the corresponding OR was 2.3 (95% CI 0.81 to 6.5, $p = 0.12$). Corrected models did not lead to relevant changes in OR values. Thus, carriage of the MIF CATT₇ or MIF -173C allele did not lead to a diminished response to TNF α -neutralising treatment. Stratification of patients for carriage of the CATT5 or CATT6-8 variants or for the inferred MIF -173C/MIF CATT₇ haplotype did result in significant differences in response to TNF α blockade.

DISCUSSION

In the current study, we show that the CATT₇ repeat and the -173G→C polymorphism in MIF are not associated with clinical response to either TNF α -neutralising or GC treatment in RA. We expected to find moderate to large effects on response, and the study was powered accordingly. However, the effects we found in this study were small and statistically non-significant. The ORs were close to 1 (no effect) for response to GC, and close to 2 (small effect) for response to TNF α -neutralising treatment. With a larger sample size, these effects might have become significant.

To date, the association between the functional variants of MIF and GC response has been suggested in non-RA diseases such as systemic lupus erythematosus (SLE), asthma and juvenile idiopathic arthritis (JIA).^{24 30 31} Finding no differences between MIF variants and clinical response was contrary to the a priori expectation. There are several possible explanations for the discrepancies between the current study and the literature. RA is likely to have a underlying pathophysiology that is distinct from that seen in SLE and JIA. In RA, MIF -173 was recently shown to be a clear predictor of RA severity; this was not as clear in SLE and JIA. This observation supports the notion that MIF has a distinct role in the inflammatory cascade in RA compared with other diseases. Perhaps other proinflammatory mediators override certain MIF-induced effects including the regulation of GC response. It has been shown that patients with MIF variants have more severe RA with greater progression of joint damage.¹⁰ It could be argued that patients

Table 3 Results from logistic regression models of MIF variants to predict EULAR response.

Model	OR (95% CI)	p Value
1 MIF-CATT ₇ carrier	0.82 (0.45 to 1.51)	0.53
2 + DAS28, DM, mode	0.82 (0.43 to 1.54)	0.54
3 + Subject	0.83 (0.42 to 1.62)	0.58
1 MIF -173C allele carrier	1.08 (0.60 to 1.94)	0.79
2 + DAS28, DM, mode	1.15 (0.62 to 2.12)	0.66
3 + Subject	1.17 (0.60 to 2.28)	0.65

DAS, Disease Activity Score; DM, change in disease-modifying anti-rheumatic drug; EULAR, European League Against Rheumatism; MIF, migration inhibitory factor.

p Value shows whether the OR deviates from 1.

The models started with MIF variant as the only predictor, then disease activity score (DAS28 at baseline), disease-modifying anti-rheumatic drug changes (DM), and oral prednisone or methylprednisolone injections (mode) were added to correct for confounding.

The third models were corrected for multiple courses within individual patients (subject).

Table 4 Phenotypic disease characteristics and response upon TNF α -neutralising treatment of patients with RA with or without the MIF CATT $_7$ or MIF -173G $\rightarrow$ C variants

	Total	MIF CATT $_7$		MIF -173G $\rightarrow$ C	
		Non-carrier	Carrier	Non-carrier	Carrier
Number, n (%)	90	69 (77)	21 (23)	58 (65)	32 (35)
Age (years), n (%)	56 (12)	55 (12)	58 (11)	54 (13)	58 (10)
Female gender, n (%)	65 (72)	49 (71)	16 (76)	41 (71)	24 (75)
RF-positive, n (%)	73 (82)	57 (83)	16 (80)	46 (79)	27 (87)
Disease duration (years), range	9 (0 to 36)	9 (0 to 36)	10 (2 to 36)	9 (0 to 36)	8 (1 to 26)
DAS28					
At baseline	5.7 (1.2)	5.6 (1.2)	5.8 (1.1)	5.6 (1.1)	5.7 (1.4)
After 3 months	4.0 (1.5)	4.0 (1.6)	4.1 (1.2)	4.1 (1.6)	4.0 (1.3)
EULAR response					
Moderate	44 (49)	29 (42)	15 (71)	22 (38)	22 (69)
Good	20 (22)	18 (26)	2 (10)	16 (28)	4 (13)

DAS, Disease Activity Score; EULAR, European League Against Rheumatism; MIF, migration inhibitory factor; RA, rheumatoid arthritis; RF, rheumatoid factor; TNF, tumour necrosis factor.

carrying MIF variants are likely to be more intensively treated with other DMARDs and thus this would confound the relationship between MIF variants and response. However, in our study, the treating physicians were not aware of the MIF genotype of their patients, there were no differences in disease activity at baseline and we applied correction for DMARD changes in the analysis. In addition, the frequency of the MIF -173C allele and MIF CATT $_7$ repeat in the cohort of patients who were given TNF α -neutralising or GC treatment was comparable with that seen in our prospective cohort, as published previously,¹⁰ and none of the patients included in the analysis for GC response was treated with YNF α -neutralising treatments or vice versa. Therefore, we believe that our study and the subsequent conclusions are unbiased.

In the Netherlands, GCs are only very rarely given as monotherapy to patients with RA. Therefore, delineating the exact role of MIF in the GC response is a hazardous and daunting task taking into account the required number of patients treated with GCs alone. In this light, the current study is likely to reflect a "real-life" population and thus clearly shows the applicability and value of MIF as a predictor for GC response in daily clinical practice.

Previous studies dealing with the relation between MIF and GC response were largely based on small study populations comprising patients with several, sometimes not well-characterised conditions. These small numbers might lead to spurious observations based on chance rather than on a real genetic effect. In addition, the studies that addressed a role for MIF in GC response in JIA were based on MIF levels measured in the synovial fluid (SF) of actively inflamed joints, and were correlated with GC response after intra-articular injection of GC. The measurement of MIF in SF versus that in peripheral blood, and the measurement of disease activity in one joint versus multiple joints (DAS28) might not be comparable. It is therefore tempting to speculate that MIF effects in the peripheral circulation are "simply" over-ruled by other inflammatory mediators (eg acute-phase proteins) that are likely to be more prominent in the circulation compared with the joint, which might be the primary location where MIF is secreted. Therefore, this study cannot exclude a role for MIF in the determination of GC response intra-articularly, as suggested by other groups. However, the clinical response to GC was not influenced by MIF variants, and MIF is unlikely to be suitable as a clinical predictor for response to GC treatment in RA.

In contrast to that shown for MIF and GC metabolism, the role for MIF in the signalling cascade of TNF α has not been delineated in detail to date. Hence, the absence of a clear correlation between the functional variants of MIF and clinical

response to TNF-neutralising treatment is therefore less surprising and might not need further explanation.

In conclusion, we provide evidence that the functional variants of MIF are not strongly associated with response to TNF α -neutralising or GC treatment using a large and well-documented cohort of patients with RA.

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