Neurotoxicity Research (2019) 36:1–11
https://doi.org/10.1007/s12640-018-9984-1

CLINICAL RESEARCH REPORT

Macrophage Migration Inhibitory Factor Levels Correlate with Stroke Recurrence in Patients with Ischemic Stroke

Guangjie Wang[1] · Chuanbin Li[1] · Yashou Liu[1] · Lei Xia[1]

Received: 8 October 2018 / Revised: 13 November 2018 / Accepted: 22 November 2018 / Published online: 1 December 2018

Introduction

Stroke is the leading cause of death and long-term disability in China and has conferred a huge economic and societal burden (Bai et al. 2017). China has 2.5 million new stroke cases and 1.6 million deaths caused by stroke each year and 7.5 million stroke survivors (Tu et al. 2017). Survivors often require long-term care, and they are at high risk of recurrent stroke (Levine et al. 2007). Importantly, ischemic stroke is a devastating disease with few effective treatment strategies. Intravenous thrombolysis with alteplase is the standard medical treatment for acute ischemic stroke within 4.5 h after the onset of symptoms (Thomalla et al. 2018). This time window is hard to reach. Thus, it is very urgent to find a new target for drug development.

Macrophage migration inhibitory factor (MIF), a central cytokine of the innate immunity, includes 114 amino acid (12.5-kDa) and is expressed in a diversity of cell types, such as T cells, monocytes, macrophages, endothelial cells, and activated platelets (Calandra and Roger 2003; Chatterjee et al. 2014). Furthermore, it is recognized as a multifunctional cytokine participating in both immune and inflammatory responses (Liu et al. 2018).

Macrophage migration inhibitory factor (MIF) is an integral component of the host antimicrobial alarm system and plays a pivotal role in the development of inflammatory processes (Donnelly and Bucala 1997), sepsis (Chuang et al. 2014), rheumatoid arthritis (Baugh et al. 2002), autoimmune diseases (Assis et al. 2014), autosomal dominant polycystic kidney disease (Chen et al. 2015a, b), obesity (Morrison and Kleemann 2015), diabetes (Sánchez-Zamora and Rodriguez-Sosa 2014), and cancer (Lv et al. 2016). Another study suggested that MIF may protect the kidney from ischemia-reperfusion injury after cardiac surgery (Stoppe et al. 2018). Previous studies supported a regulatory role for MIF in the process of atherosclerosis (Boekholdt et al. 2004), acute coronary syndromes (Müller et al. 2012), art failure (Luedike et al. 2018a). Burger-Kentischer et al. (2002) found that MIF may play an important role in early plaque development and advanced complicated lesions. Moreover, MIF has emerged as a key player in cardiovascular disease and might be an indicator of disease severity (Zernecke et al. 2008). Herder et al. (2008) proposed that MIF played a role in local vascular inflammation and atherogenesis but is not a novel biomarker for coronary heart disease (CHD) risk. Another study suggested that that the relation between MIF and the risk of myocardial infarction or death due to coronary artery disease in adults was not very strong (Boekholdt et al. 2004).

Interestingly, Lin et al. (2017) reported that MIF in serum might be a potential biomarker for reflecting inflammation, severity, and prognosis of acute intracerebral hemorrhage patients. Furthermore, it has been reported that MIF is dysregulated in rodent ischemic stroke model (Wang et al. 2009). One study demonstrated that serum MIF levels at admission were positively correlated with infarct volume and long-term outcome in patients with acute ischemic stroke (AIS) (Li et al. 2017), while another study confirmed that elevated plasma levels of MIF at admission were associated with increased risk of post-stroke depression (PSD) in the next 3 months (Xu et al. 2018). Therefore, we hypothesized that MIF might be play role in the pathophysiology of ischemic stroke. The present study thus aimed to investigate the possibility of MIF, a central cytokine of the innate immunity, proposed to play role in the development of stroke recurrence events in a 12-month follow-up study in Chinese patients with first-ever ischemic stroke.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s12640-018-9984-1) contains supplementary material, which is available to authorized users.

✉ Lei Xia
tomjikzhu876@163.com

[1] Department of Neurology II, Zhoukou Central Hospital, No. 26 Renmin Road East, Zhoukou 466000, Henan Province, People's Republic of China

Methods

Study Design and Included Patients

This prospective cohort study was conducted at a single hospital. From September 2015 to December 2016, all consecutive first-ever acute ischemic stroke patients without any premorbid handicap who were admitted to the Emergency Department of the Zhoukou Central Hospital (Henan, China) were identified. Ischemic stroke was diagnosed according to World Health Organization (WHO) recommendations (neurological deficit of cerebrovascular cause that persists beyond 24 h or is interrupted by death within 24 h) (Hatano 1976). The clinical diagnoses were validated on the basis of computed tomography (CT) and/or magnetic resonance imaging (MRI; assessed by the Zhang and Liu). The patients with the following criteria were excluded: (1) malignant tumor (2) and a history of recent surgery or trauma during the preceding 3 months; (3) liver and kidney function insufficiency; (4) acute and chronic inflammation; metabolic abnormalities (not included diabetes); (5) other neurological diseases (cerebral hemorrhage, Parkinson's disease, and Alzheimer's disease); and (6) lost blood samples, lost flow-up, and unexplained death during follow-up. The study was approved by the ethics committee of Zhoukou Central Hospital. The patients or their relatives gave written informed consent prior to entering the study.

Clinical Variables and Neuroimaging

Demographical and clinical data including age, sex, body mass index (BMI), race, conventional vascular risk factors (hypertension, diabetes mellitus, atrial fibrillation, coronary heart disease, smoking habit, and a history of transient ischemic attack (TIA)) were recorded. Pre-stroke therapy, including oral anticoagulants, antiplatelet agents, antihypertensive treatment, and statins, as well as acute treatment (IV thrombolysis and/or mechanical thrombectomy), were obtained. The stroke severity was evaluated with the National Institute of Health Stroke Scale (NIHSS, range from 0 to 42) (Brott et al. 1989) score at their admission by a stroke neurologist (Liu). Stroke subtype and syndrome were defined according to TOAST (trial of org 10,172 in acute stroke treatment) criteria (Adams et al. 1993) and the Oxfordshire Community Stroke Project [total anterior circulation syndrome (TACS), partial anterior circulation syndrome (PACS), lacunar syndrome (LACS), and posterior circulation syndrome (POCS)] (Bamford et al. 1991), respectively. The OCSP and TOAST classifications were verified by brain imaging (either CT or MRI) within 24 h after admission. MRI with diffusion-weighted imaging (DWI) was available for some patients (n = 233). The infarct volume was calculated by using the formula $0.5 \times a \times b \times c$ (a = the maximal longitudinal diameter; b = the maximal transverse diameter perpendicular to a; c = the number of 10-mm slices containing infarct) (Tu et al. 2014).

End Points and Follow-Up

We followed the participants for a median of 12 months (range, 11–13 months) using a standard questionnaire and telephone or household contact by physician investigators. The follow-up protocol after hospital discharge included phone interviews at 1 month, 3 months, 6 months, 9 months, and 12 months thereafter to ascertain vital status and occurrence of stroke events. The primary endpoint was stroke recurrence. The secondary endpoint was all-cause death. Stroke recurrence was defined as a sudden functional deterioration in neurological status with a decrease of the NIHSS score of 4 or more, or a new focal neurological deficit of vascular origin lasting > 24 h (Zhang et al. 2011). Death was assessed by vascular death (including fatal stroke, other cardiovascular death, or death by any causes). In patients who had a recurrent stroke, medical records from the stroke admission were reviewed by the investigators. If the patients had unexplained death during follow-up, they would be excluded from our study.

Laboratory Analyses

For the purpose of this study, serum samples were drawn from the antecubital vein at the first morning after admission. After centrifugation, the serum samples were immediately stored at − 80 °C before assay. Blood samples from some patients (N = 33) were collected on 12 h, 24 h, 48 h, 72 h, and 96 h after admission for MIF tested. Serum levels of MIF were tested by Quantikine Human MIF Immunoassay using a commercially available ELISA kit (Catalog Number DMF00B; R&D Systems, Inc. Minneapolis, USA). The measuring range of the MIF was between 0.2 ng/ml and 10 ng/ml. Due to the high levels of MIF (ng/ml) in the human blood sample, serum and platelet-poor plasma samples require a tenfold dilution. Thus, the test range of the MIF was between 2 and 100 g/ml. The coefficients of variation (CVs) for the intra- and inter-assay reproducibility were 4.5–6.0% and 6.0–9.0%, respectively. In addition, fasting blood glucose (FBG) and C-reactive protein (CRP) were also tested using standard laboratory methods. Serum interleukin 6 (IL-6) was tested by ELISA method. All those tested were done in duplicates and samples with a CV exceeding 10% were reanalyzed.

Statistical Analysis

The results were expressed as percentages for categorical variables and as medians (interquartile ranges [IQRs]) for continuous variables. The Mann-Whitney U test and chi-square test were used to compare the two groups. Spearman's rank

correlation was used for bivariate correlations. In addition, association between MIF and NIHSS score was also assessed using ordered logistic regression models in multivariate adjustment with possible confounder (including age, sex, BMI, vascular risk factors, stroke etiology, stroke syndrome, acute treatment, pre-stroke treatment, and serum levels of Hs-CRP, IL-6, and FBG).

To investigate whether MIF allows predicting of stroke recurrence after 1 year in patients with stroke, different statistical methods were used. First, the relation of MIF with the stroke recurrence was investigated with the use of logistic regression models. Common logarithmic transformation (i.e., Log) was performed to obtain normal distribution for skewed variables (i.e., MIF concentrations). We used crude models and multivariate models adjusted for all significant predictors and report odds ratios (ORs) with the corresponding 95% confidence interval (CI). For multivariate analysis, we included confounders, known risk factors, and other predictors as assessed in univariate analysis. Note that the OR corresponds to a one-unit increase in the explanatory variable; for the log-transformed MIF values, this corresponds to a tenfold increase. For a more detailed exploration of the MIF and stroke recurrence, we also used multivariate analysis models to estimate adjusted OR and 95% CIs of stroke recurrence for MIF quartiles (with lowest quartile as reference).

Second, we compared different prognostic risk scores from different predictive models by calculating receiver operating characteristic curve (ROC) analysis. ROC was used to test the overall prognostic accuracy of MIF and other markers and results were reported as area under the curve (AUC). To test whether the MIF levels improves score performance, we considered the nested models with MIF, CRP, and IL-6 as compared with MIF only. Furthermore, care was taken to adjust for the optimistic bias of in-sample prediction error estimates using a five-fold cross-validation scheme. In addition, the net reclassification improvement (NRI) and the integrated discrimination improvement (IDI) were used to measure the quantity of improvement for the correct classification and sensitivity according to the addition of serum MIF levels to the prediction model I, including risk factors which confirmed in the univariate analysis (Pencina et al. 2008).

Lastly, the influence of elevated levels of MIF(≥cut-off) on stroke recurrence was also performed. The positive predictive value (PPV), negative predictive value (NPV), and the diagnostic accordance rate were calculated. All statistical analyses were performed with SPSS for Windows, version 22.0 (SPSS Inc., Chicago, IL, USA) and the ROCR package (version 1.0–2), which is available from CRAN repository (http:// cran.r-project.org/) for evaluating and visualizing the performance of scoring classifiers (Katan et al. 2009). Statistical significance was defined as $P < 0.05$.

Results

Patient Characteristics

In this study, we recorded 469 patients with ischemic stroke and 423 completed follow-ups (26 were lost to follow-up and 20 withdrew from the study). Furthermore, 37 patients had died and were without detailed reasons for death. Finally, 386 stroke patients were included in our study. The median age was 64 (IQR, 56–73) years, and 50.8% were male. The median NIHSS score on admission was 7 points (IQR, 4 to 13). The serum level of MIF in those patients was with a median value of 21.5 ng/ml (IQR, 17.5–27.8 ng/ml). The baseline characteristics of the 386 patients were described in Table 1.

Main Results

Serum levels of MIF increased with increasing severity of stroke as defined by the NIHSS score, and there was a modest correlation between serum levels of MIF and NIHSS score (r[spearman] = 0.441, $P < 0.001$). The positive correction still exists even after adjusted for possible confounders (including age, sex, BMI, vascular risk factors, stroke etiology, stroke syndrome, acute treatment, pre-stroke treatment, and serum levels of Hs-CRP, IL-6, and FBG) by ordered logistic regression ($P = 0.013$). In addition, serum levels of MIF were also correlated with CRP ($P < 0.001$), IL-6($P < 0.001$), FBG ($P = 0.003$), stroke etiology ($P = 0.001$), and stroke syndrome ($P < 0.002$); Sup Table I. As shown in the Sup Table I, no correlations between serum levels of MIF and others factors, such as, sex, age, BMI, time from admission to blood collected, pre-stroke treatment, and vascular risk factors ($P > 0.05$, respectively). In patients for whom MRI data were available ($n = 233$), there was a positive correlation between levels of MIF and the infarct volume ($r = 0.285$, $P < 0.001$).

Daily blood samples were obtained for 96 h after admission in a subgroup of 33 patients, 6 of whom subsequently experienced stroke recurrence events. The result illustrates the time course of serum MIF, showing significant changes with day of sampling ($P < 0.01$), with peak concentrations on day 1 ($P < 0.01$, compared to days 0, 0.5, and 2–4, respectively), falling to a plateau by days 3 to 4 (Fig. 1).

MIF and 1-Year Stroke Recurrence

In our study, 66 patients had a stroke recurrence. Thus, the incidence was 17.1% (95%CI 13.3–20.9%). Interestingly, we found that the nearly half of the stroke recurrence occurred in 1 month after admission (7.3%), and the rates of new occurred stroke recurrence among 3 months, 6 months, 9 months, and 12 months were 3.1%, 2.6%, 2.3%, and 1.8%, respectively; Fig. 2. Serum levels of MIF in patients

Table 1 Baseline characteristics of the included ischemic stroke patients

	N = 386
Age, medians (IQRs), years	64 (56–73)
Sex-male, *n* (%)	196 (50.8)
Race-Han, *n* (%)	355 (92.0)
BMI, medians (IQRs) (kg/m^2)	26.8 (24.9–29.2)
Vascular risk factors	
Hypertension	248 (64.2)
Diabetes	161 (41.7)
Coronary heart disease	89 (23.1)
Atrial fibrillation	71 (18.4)
Myocardial infarction	44 (11.4)
Previous TIA	47 (12.2)
Smoking habit	55 (14.3)
Time from admission to blood collected, medians (IQRs), (h)	5.5 (3.5–12.0)
Pre-stroke treatment	
Antihypertensive	198 (51.3)
Oral hypoglycemic drugs and/or insulin	139 (36.0)
Antiplatelet agents	101 (26.2)
Anticoagulants	33 (8.5)
Statins	125 (32.4)
Acute treatment, TPA-T no. (%)	85 (22.0)
NIHSS at admission, medians (IQR)	7 (4–13)
Lesion volumes (*N* = 233), median (IQR) (ml)	24.9 (9.6–38.8)
Stroke syndrome, *N* (%)	
TACS	77 (19.9)
PACS	115 (29.8)
LACS	87 (22.5)
POCS	107 (27.7)
Stroke etiology, *N* (%)	
Small-vessel occlusive	75 (19.4)
Large-vessel occlusive	91 (23.6)
Cardioembolic	137 (35.5)
Other	43 (11.1)
Unknown	40 (10.4)
Laboratory findings, medians (IQR)	
CRP, mg/l	6.4 (4.2–10.3)
IL-6, pg/ml	7.1 (5.2–8.9)
FBG, ng/ml	5.4 (4.9–6.1)
MIF, ng/ml	21.5 (17.5–27.8)
Incidence of stroke recurrent at 1-year follow-up, *n* (%; 95%CI)	66 (17.1%; 13.3%–20.9%)

IQR interquartile range, *NIHSS* National Institutes of Health Stroke Scale, *LACS* lacunar syndrome, *PACS* partial anterior circulation syndrome, *POCS* posterior circulation syndrome, *TACS* total anterior circulation syndrome, *TPA-T* tissue plasminogen activator-treated, *CRP* C-reactive protein, *MIF* macrophage migration inhibitory factor, *FBG* fasting blood glucose, *TIA* transient ischemic attack

with recurrent stroke were significantly higher as compared with those in patients without recurrent stroke [26.9 ng/ml (IQR, 21.6–37.7) vs. 21.0 ng/ml (IQR, 17.1–25.8); Z = 5.705, $P < 0.001$; Sup Fig. I).

In univariate logistic regression analysis, we calculated the odds ratio (OR) of log-transformed MIF levels as compared with the NIHSS score and other risk factors as presented in Table 2. With an unadjusted OR of 16.25 (95% CI, 6.88–40.15; $P < 0.001$), MIF had a strong association with stroke recurrences. After adjusting for all other significant outcome predictors in univariate analysis, MIF remained an independent stroke recurrence predictor with an adjusted OR of 5.15

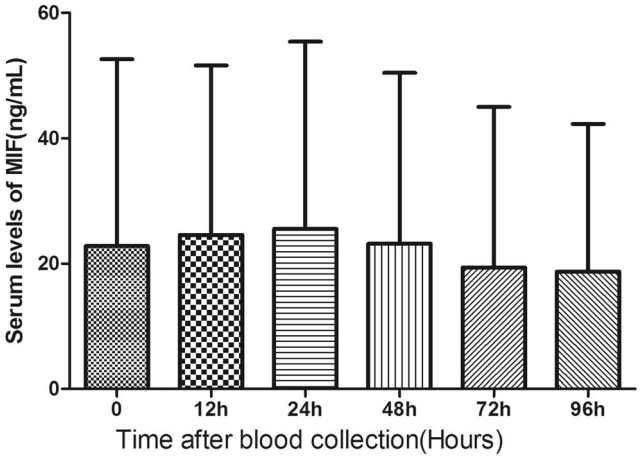

Fig. 1 Boxplots (ranges) of serum levels of MIF in the first 4 days after stroke ($N = 33$). MIF = macrophage migration inhibitory factor

(95% CI, 2.09–12.33; $P < 0.001$). In the subgroup of patients ($n = 233$) in whom MRI evaluations were performed, MIF was an independent stroke recurrence predictor with an OR of 8.18 (95% CI, 3.76–18.27; $P < 0.001$) after adjustment for both lesion size and the NIHSS score. In addition, age, atrial fibrillation, the NIHSS score, TACS, and laboratory findings, such as CRP and IL-6 remained significant predictors (Table 2).

For a more detailed exploration of the MIF and stroke recurrence relationship, we also used multivariate analysis models to estimate adjusted OR and 95% CIs of stroke recurrence for MIF quartiles (with first quartile as reference). In multivariate models comparing the second (Q2), third, and fourth quartiles against the first quartile of the MIF (Table 3), MIF in Q3 and Q4 were associated with stroke recurrence and increased risk of stroke recurrence by 211%(OR 3.11; 95%CI 1.62–5.76; $P = 0.016$) and 325% (4.25; 2.42–9.18; $P < 0.001$), respectively. MIF in Q2 was not associated with stroke recurrence (OR 1.40; 95%CI 0.85–2.99; $P = 0.25$). The independent association was confirmed using the likelihood ratio test ($P < 0.001$).

Based on ROC curves, the optimal cutoff value of serum level of MIF to diagnose the stroke recurrence was 21.3 ng/ml, which yielded the highest sensitivity and specificity [81.3 and 53.1%, respectively; area under the curve (AUC) = 0.73, 95%CI 0.66–0.79; $P < 0.001$; Fig. 3]. With an AUC of 0.73, MIF showed a significantly greater discriminatory ability as compared with CRP (AUC, 0.63; 95% CI, 0.56–0.71; $P < 0.001$), FBG (AUC, 0.59; 95% CI, 0.51–0.67; $P < 0.001$), IL-6 (AUC, 0.65; 95% CI, 0.57–0.72; $P = 0.002$), and NIHSS score (AUC, 0.61; 95% CI, 0.56–0.68; $P < 0.01$). Interestingly, the combined model (MIF/CRP/IL-6) improved the MIF (AUC of the combined model, 0.77; 95% CI, 0.71–0.85; $P = 0.012$). This improvement was stable in an internal fivefold cross validation that resulted in an average AUC (standard error) of 0.73 (0.033) for the MIF and 0.77 (0.030) for the combined model, corresponding to a difference of 0.04 (0.003). In addition, a significant difference in the AUC between the established risk factors alone and the addition of MIF concentrations was observed (difference, 0.03 [95% CI, 0.02–0.04]; $P = 0.009$). In addition, the NRI and the IDI were used to test the quantity of improvement for the correct reclassification and sensitivity according to the addition of MIF serum levels to the prediction model I. As shown in Table 4, the inclusion of MIF in the prediction model I (including age, hypertension, atrial fibrillation, previous TIA, anticoagulant treatment, TPA-T, NIHSS at admission, TACS, cardioembolic etiology, CRP, IL-6, and FBG) for the prediction of stroke recurrence enhanced the NRI ($P = 0.005$) and IDI ($P = 0.001$) values, confirming the effective reclassification and discrimination.

Furthermore, in our study, we found that an increased risk of stroke recurrence was associated with MIF levels ≥ 21.3 ng/ml, and increased risk of stroke recurrence by 331% (OR unadjusted 4.31; 95%CI 2.29–8.10) and 201% (OR adjusted 3.01; 1.64–5.04), respectively (Table 3). Interestingly, patients with MIF levels ≥ 21.3 ng/ml were considered as an indicator to predict stroke recurrence; the positive predictive value (PPV) and negative predictive value (NPV) were 25.5 and 92.6%, respectively. The diagnostic accordance rate was 61.4%.

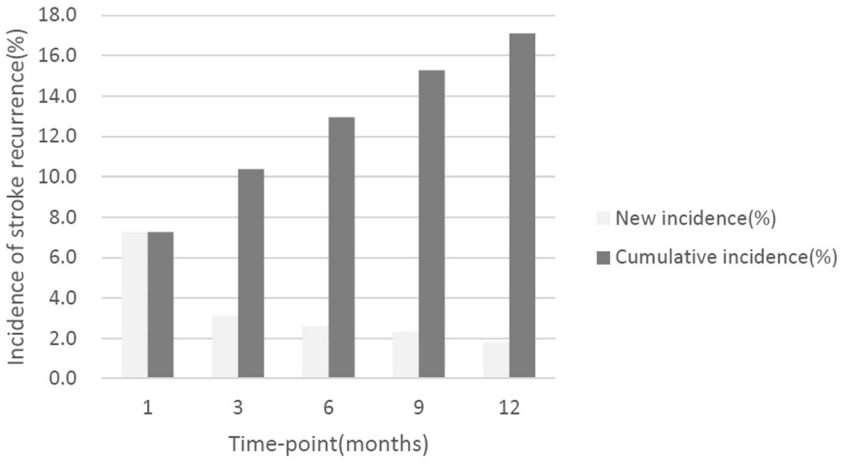

Fig. 2 The new and cumulative incidence of stroke recurrence at different follow-up time point

Table 2 Univariate and multivariate logistic regression analysis of predictors for stroke recurrence

Predictors	Univariate analysis		Multivariate analysis[a]	
	OR(95%CI)	*p* value	OR(95%CI)	*p* value
Age (per unit increase)	1.38 (1.18–1.59)	0.003	1.21 (1.03–1.44)	0.015
Sex (male vs. female)	1.06 (0.94–1.29)	0.27	–	
Race (Han vs. others)	1.16 (0.93–1.44)	0.16	–	
BMI (per unit increase)	0.88 (0.75–1.44)	0.39	–	
Vascular risk factors				
Hypertension	1.62 (1.15–2.43)	0.035	1.37(0.94–1.86)	0.073
Diabetes	1.35 (0.85–2.39)	0.47	–	
Coronary heart disease	1.17 (0.90–1.44)	0.15	–	
Atrial fibrillation	1.91 (1.21–3.04)	0.009	1.55 (1.19–2.11)	0.015
Myocardial infarction	1.34 (0.85–2.11)	0.26	–	
Previous TIA	1.77 (1.70–2.66)	0.011	1.22 (0.78–1.93)	0.25
Smoking habit	0.71 (0.50–1.34)	0.30	–	
Time from admission to blood collected (per unit increase)	0.77 (0.43–1.33)	0.22	–	
Pre-stroke treatment				
Antihypertensive	0.66 (0.31–1.74)	0.37	–	
Oral hypoglycemic drugs and/or insulin	0.93 (0.73–1.44)	0.55	–	
Antiplatelet agents	0.68 (0.45–1.15)	0.44	–	
Anticoagulants	0.48 (0.31–0.84)	0.012	0.69 (0.55–0.89)	0.042
Statins	1.05 (0.90–1.44)	0.75		
Acute treatment, TPA-T	0.83 (0.71–0.94)	< 0.001	0.92 (0.85–0.98)	0.003
NIHSS at admission (per unit increase)	1.55 (1.21–1.84)	0.005	1.21 (1.03–1.52)	0.033
Lesion volumes ($N = 233$, per unit increase)	1.48 (1.16–1.69)	0.003	1.16 (1.04–1.43)	0.019
Stroke syndrome and etiology				
TACS	3.38 (1.76–7.65)	< 0.001	2.26 (1.35–3.87)	0.004
PACS	1.09 (0.80–1.76)	0.59	–	
LACS	0.73 (0.45–1.54)	0.27	–	
POCS	0.55 (0.29–1.33)	0.092	–	
Small-vessel occlusive	0.60 (0.31–1.09)	0.075	–	
Large-vessel occlusive	1.04 (0.90–1.55)	0.17	–	
Cardioembolic	1.62 (1.19–2.04)	0.018	1.29 (1.03–1.53)	0.039
Laboratory findings (per unit increase)				
CRP	1.15 (1.05–1.31)	0.001	1.06 (1.01–1.15)	0.013
IL-6	1.33 (1.17–1.50)	< 0.001	1.19 (1.08–1.36)	0.008
FBG	1.07 (1.02–1.20)	0.025	1.04 (0.97–1.15)	0.072
MIF (increase per log unit)[b]	16.25 (6.88–40.15)	< 0.001	5.15 (2.09–12.33)	<0.001

OR odds ratio, *CI* confidence interval, *NIHSS* National Institutes of Health Stroke Scale, *LACS* lacunar syndrome, *PACS* partial anterior circulation syndrome, *POCS* posterior circulation syndrome, *TACS* total anterior circulation syndrome, *TPA-T* tissue plasminogen activator-treated, *CRP* C-reactive protein, *MIF* macrophage migration inhibitory factor, *FBG* fasting blood glucose, *IL-6* interleukin-6

[a] Multivariable model included significant risk factors which confirmed in the univariate analysis

[b] Note that the odds ratio corresponds to a unit increase in the explanatory variable; for MIF, this corresponds to an increase per unit of the log transformation of MIF (thus, a log-transformed increase of 1 corresponds to a MIF increase of 10 ng/ml)

Discussion

MIF has been proposed as a pro-inflammatory cytokine and can produce lots of pro-inflammatory molecules (Leyton-Jaimes et al. 2018). Thus, previous studies had suggested that MIF derived from glial cells might be an important player in pro-inflammatory diseases (Conroy et al. 2010; Leyton-Jaimes et al. 2018) and further can damage neurons (Koda et al. 2004). To the best of our knowledge, it is the first time to measure serum MIF levels after AIS and further assess its

Table 3 Multivariate logistic regression analysis for stroke recurrence according to MIF quartiles

MIF[a]	SR/N, (%)	Crude OR (95%CI), *P*#	Multivariable-adjusted[b], *P*#
Quartile 1	5/96, (5.2)	Reference	Reference
Quartile 2	11/97, (11.3)	2.33 (0.78–6.98), 0.073	1.40 (0.85–2.99), 0.25
Quartile 3	19/95, (20.0)	4.55 (1.62–12.76), 0.002	3.11 (1.62–5.76), 0.016
Quartile 4	31/98, (31.6)	8.42 (3.11–22.79), < 0.001	4.25 (2.42–9.18), < 0.001
Elevated vs. normal	50/196 vs. *16*/190	4.31 (2.29–8.10), < 0.001	3.01 (1.64–5.04), 0.006

SR stroke recurrence; *OR* odds ratio; *CI* confidence interval; *MIF*, Macrophage migration inhibitory factor; *TIA*, transient ischemic attack; *TPA-T*: Tissue plasminogen activator-treated; *NIHSS*, National Institutes of Health Stroke Scale; *TACS*, total anterior circulation syndrome; *CRP*, C-reactive protein; *FBG*, Fasting blood glucose; *IL-6*, Interleukin-6

#*P* value for the trend < 0.001

[a] MIF in quartile 1 (< 17.5 ng/ml), quartile 2 (17.5–21.5 ng/ml), quartile 3 (21.5–27.8 ng/ml), and quartile 4 (> 27.8 ng/ml). Elevated MIF level was defined as ≥ 21.5 ng/ml (median)

[b] Adjusted for those significant risk factors which confirmed in the univariate analysis (Table 2), including age, hypertension, atrial fibrillation, previous TIA, anticoagulants treatment, TPA-T, NIHSS at admission, TACS, cardioembolic etiology, CRP, IL-6, and FBG

association with further stroke recurrence in AIS. The main findings of the current study were that (1) increasing levels of MIF were correlated to the severity of stroke and peaked 24 h after stroke; (2) 17.1% of the stroke patients suffered from stroke recurrence, and nearly half of the stroke recurrence occurred in 1 month after admission (7.3%); (3) serum levels of MIF in patients with recurrent stroke were significantly higher as compared with those in patients without recurrent stroke ($P < 0.001$); (4) patients in the highest quartile of MIF had the greatest likelihood of stroke recurrence (adjusted OR = 4.25; 95% CI 2.42 to 9.18).

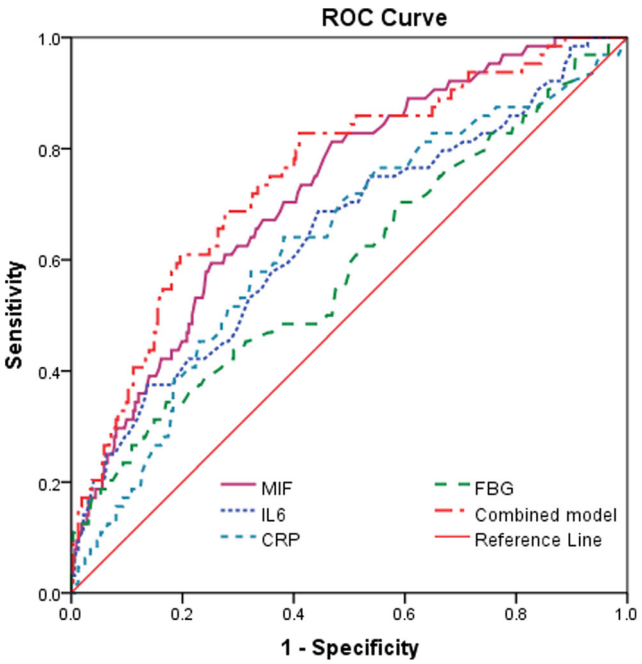

Fig. 3 Receiver operator characteristic curve demonstrating sensitivity as a function of 1-specificity for predicting the stroke recurrence based on the different biomarkers. CRP = C-reactive protein; IL-6 = interleukin 6; MIFM = macrophage migration inhibitory factor; FBG = fasting blood glucose

In a follow-up to a 1-year study involving patients who had a TIA or minor stroke, the rate of cardiovascular events including stroke in a selected cohort was 6.4% in the first year and 6.4% in the second through fifth years (Amarenco et al. 2018). In this study, we found that the rate of 1-year stroke recurrence events was 7.3% in the first month and 9.8% in the second through 12th months. Another study in Chinese population suggested that 10.6% of stoke patients had a stroke recurrence in the 3-month follow-up (Huang et al. 2016). In fact, the previous studies had reported that the rate of recurrence events after stroke was in the range of 6.2–17.7% (Cabral et al. 2018; Fernández-Cadenas et al. 2017; Sposato et al. 2018; Wang et al. 2013). Different populations, sample sizes, follow-up time, geographical regions, health status, ELISA testing kits, and ethnicity may justify the differences results observed in those studies.

In this study, we found that the optimal cutoff value of serum level of MIF to diagnose the stroke recurrence was 21.3 ng/ml, which yielded the highest sensitivity and specificity [81.3 and 53.1%, respectively; area under the curve (AUC) = 0.73, 95%CI: 0.66–0.79]. Similarly, a previous study showed that cut-off value of MIF to predict the development of depression after stroke was 21.5 ng/ml (Xu et al. 2018). In addition, MIF levels correlated with the severity and outcome of various disease states with different cut-off value. Kaplan-Meier analysis revealed an increased mortality rate in the high MIF group (> 51 ng/ml) in patients with heart failure (Luedike et al. 2018b). Another study identified a cut-off value of 1.22 ng/mL with a sensitivity of 82% and a specificity of 56% for the diagnosis of acute coronary syndrome compared to patients with stable coronary artery disease and healthy controls (Müller et al. 2012). Boekholdt et al. (2004) reported

Table 4 Statistics for model fit and improvement with addition of MIF predicted on the prediction of stroke recurrence

	Prediction Model I[a]	Prediction model II (Model I + MIF)	*p* value
NRI (95% CI)	–	0.396(0.172–0.605)	0.005
IDI (95% CI)	–	0.149(0.083–0.255)	0.001

IDI integrated discrimination improvement, *NRI* net reclassification improvement, *CI* confidence interval, *MIF* macrophage migration inhibitory factor, *TIA* transient ischemic attack, *TPA-T* tissue plasminogen activator-treated, *NIHSS* National Institutes of Health Stroke Scale, *TACS* total anterior circulation syndrome, *CRP* C-reactive protein, *FBG* fasting blood glucose, *IL-6* interleukin-6

[a] Prediction model I included age, hypertension, atrial fibrillation, previous TIA, anticoagulants treatment, TPA-T, NIHSS at admission, TACS, cardioembolic etiology, CRP, IL-6, and FBG

that among men and women, the unadjusted odds ratio for future myocardial infarction or death from coronary artery disease was elevated in the highest quartiles (> 166.6 μg/l) (P for linearity = 0.003 for men, 0.006 for women), while another study demonstrated that high plasma levels of MIF (> 1100 pg/ml) had a sensitivity of 100% and a specificity of 64% to identify the patients who eventually would evolve to a fatal outcome sepsis (Bozza et al. 2004). Furthermore, increased serum MIF concentrations have been suggested close relation to clinical outcomes (> 33.8 ng/ml) and mortality (> 28.0 ng/ml) after traumatic brain injury (Yang et al. 2017).

Interestingly, in this study, we found that the serum level of MIF in those stroke patients was with a median value of 21.5 ng/ml (IQR, 17.5–27.8 ng/ml). When studies in different diseases were compared, the levels of MIF were inconsistent. Lehmann et al. (2001) found that the median MIF plasma level was four to five times higher in patients with severe sepsis (2.70 ng/ml; range, 0.31–19.59) and in critically ill non-septic postsurgical patients (2.43 ng/ml, 0.49–4.31) than in healthy blood donors (0.56 ng/ml, 0.16–1.68), while another study reported that the plasma MIF levels were significantly higher in the acute myocardial infarction group than in the control group [5.08(SD: 23) vs. 2.21(23) pg/ml; $P < 0.001$](Yüksel et al. 2015). In patient with coronary artery disease, the data showed that baseline concentrations of MIF were higher in cases (median,107.4 ng/ml; IQR, 62.5–185.3 ng/ml) than in controls (90.7; 62.5–156.4 ng/ml) (Boekholdt et al. 2004). Patients with acute coronary syndromes showed higher plasma levels of MIF compared to patients with stable coronary artery disease and control subjects (median 2.85 vs. 1.22 ng/mL vs. 0.1 ng/ml) (Müller et al. 2012). There were variations in study diseases or population, sampling and testing methods, including criteria, racial or ethnic, culture, and economic status between those various studies (Sobierajski et al. 2013). Those variations make it difficult to compare different studies.

A previous study found that MIF protein and mRNA were significantly increased in stroke patients (Wang et al. 2009). Another study reported that high MIF levels were an independent risk factor for future coronary events in coronary artery disease patients with impaired glucose tolerance or type 2 diabetes mellitus (IGT/T2DM) (Makino et al. 2010). Similarly, in this study, we proposed that high MIF levels were an independent risk factor for future stroke recurrence events in patients with AIS. However, Turtzo et al. (2013) presented that loss of MIF exacerbated injury in the female mice brain after experimental stroke, which was independent of changes in pro-inflammatory cytokine levels. On the contrary, another study found that MIF exacerbates neuronal cell death and neurological deficits in the focal cerebral ischemia (MCAo) rodent model (Liu et al. 2018). Intriguingly, both protective and pathological roles of MIF have been implicated in stroke and cerebral ischemia (Koga et al. 2011; Leyton-Jaimes et al. 2018; Wang et al. 2009). Previous studies suggest that MIF plays an important role in neurogenesis and neural protection by supporting the proliferation and survival of neural stem cells via multiple signaling pathways (Okazaki et al. 2018). More work should be carried out to resolve the disputations.

The cross-sectional design prevented us from inferring any cause-effect relationship of MIF with stroke recurrence, However, our results and previous studies indicated that MIF may be cause rather than consequence of stroke recurrence events. A study suggested that MIF knockout mice reduces neuronal death and promotes recovery of stroke-induced neurologic deficits following tMCAo (Inácio et al. 2011), while another study found that administration of MIF antagonist ISO-1 has the profound neuroprotective effect (Liu et al. 2018). Furthermore, previous studies had suggested that MIF was involved in the pathogenesis of non-insulin-dependent diabetes mellitus (NIDDM)(Sanchez-Zamora et al. 2010) and idiopathic pulmonary arterial hypertension (iPAH) (Le Hiress et al. 2015). Hypertension and diabetes were strongly related to stroke risk (Sacco et al. 1999). Lastly, administration of MIF following MCAo showed the deleterious influence on stroke-induced injury by destroying the tight junction of blood-brain barrier and increasing the infarct size (Liu et al. 2018). In addition, another study found that MIF triggered autophagic degradation of endothelial cells, resulting in vascular leakage (Chen et al. 2015a, b).

This is the largest study to date to validate the prognostic efficacy of MIF in stroke patients. In addition, some limitations should be considered. First, this study measured MIF in serum, not in cerebral spinal fluid (CSF). It was still uncertain whether peripheral MIF levels reflect similar changes in the central nervous system (CNS). A previous study showed that MIF was

significantly upregulated in focal ischemic rat brains (Wang et al. 2009). In addition, in this study, the serum samples were collected. However, a previous validation study found that MIF serum concentrations (1) were higher than plasma concentrations and show broader ranges, (2) were higher in samples processed with latency than in those processed directly, (3) were strongly correlated with hemoglobin in plasma, suggesting use plasma and not serum samples when determining circulating MIF and avoiding hemolysis by processing samples immediately after blood drawing (Sobierajski et al. 2013). Second, the study was lack of functional assessment of HPA axis in parallel and no information about MIF gene expression. One study demonstrated that MIF gene expression was altered during stroke, and dysregulation of the hypoxia signaling-induced MIF expression plays an important role in neuronal death in stroke (Zis et al. 2015). Another study showed that MIF gene expression was upregulated after stroke, and hypoxia signaling plays an important role in upregulation of MIF expression under stroke (Wang et al. 2009). Therefore, further studies should be carried out to determine the association of those factors (such as HPA axis and MIF gene expression) with plasma levels of MIF and stroke recurrence. Lastly, observational study design did not allow drawing secure conclusions for a causal relationship.

Conclusions

The present study demonstrated that elevated serum levels of MIF were associated with increased risk of stroke recurrence in the next 12 months and might be useful in identifying stroke at risk for recurrence events for early prevention strategies. Further studies are proposed to confirm this association, which may open a good drug target for the therapy of stroke.

Acknowledgments We also express our gratitude to all the patients who participated in this study, and thereby made this work possible. All authors have contributed significantly, and that all authors are in agreement with the content of the manuscript. The content has not been published or submitted for publication elsewhere.

Role of the Sponsor The funding organizations had no role in the design and concept of the study; the collection, management, analysis, and interpretation of the data; or the preparation, review, or approval of the manuscript.

Funding This study was supported by grants from National Natural Science Foundation of China (No. 81671079).

Compliance with Ethical Standards

Conflict of Interest The authors have no relevant potential conflicts of interest to declare.

Ethics Approval The study was approved by the ethics committee of Zhoukou Central Hospital. The patients or their relatives gave written informed consent prior to entering the study.

References

Adams HP, Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL, Marsh EE (1993) Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of org 10172 in acute stroke treatment. Stroke 24(1):35–41

Amarenco P, Lavallée PC, Monteiro Tavares L, Labreuche J, Albers GW, Abboud H et al (2018) Five-year risk of stroke after TIA or minor ischemic stroke. N Engl J Med 378(23):2182–2190

Assis DN, Leng L, Du X et al (2014) The role of macrophage migration inhibitory factor in autoimmune liver disease. Hepatology 59(2): 580–591

Bai B, Yan Z, Hao Y, Zhang Z, Li G, Dekker J, Qiu C (2017) A randomised controlled multimodal intervention trial in patients with ischaemic stroke in Shandong, China: design and rationale. Lancet 390:S13

Bamford J, Sandercock P, Dennis M, Warlow C, Burn J (1991) Classification and natural history of clinically identifiable subtypes of cerebral infarction. Lancet 337(8756):1521–1526

Baugh JA, Chitnis S, Donnelly SC, Monteiro J, Lin X, Plant BJ, Wolfe F, Gregersen PK, Bucala R (2002) A functional promoter polymorphism in the macrophage migration inhibitory factor (MIF) gene associated with disease severity in rheumatoid arthritis. Genes Immun 3(3):170–176

Boekholdt SM, Peters RJ, Day NE, Luben R, Bingham SA, Wareham NJ, Khaw KT (2004) Macrophage migration inhibitory factor and the risk of myocardial infarction or death due to coronary artery disease in adults without prior myocardial infarction or stroke: the EPIC-Norfolk prospective population study. Am J Med 117(6):390–397

Bozza FA, Gomes RN, Japiassú AM, Soares M, Castro-Faria-Neto HC, Bozza PT, Bozza MT (2004) Macrophage migration inhibitory factor levels correlate with fatal outcome in sepsis. Shock 22(4):309–313

Brott T, Adams HP Jr, Olinger CP, Marler JR, Barsan WG, Biller J, Spilker J, Holleran R, Eberle R, Hertzberg V (1989) Measurements of acute cerebral infarction: a clinical examination scale. Stroke 20(7):864–870

Burger-Kentischer A, Goebel H, Seiler R, Fraedrich G, Schaefer HE, Dimmeler S, Kleemann R, Bernhagen J, Ihling C (2002) Expression of macrophage migration inhibitory factor in different stages of human atherosclerosis. Circulation 105(13):1561–1566

Cabral NL, Nagel V, Conforto AB, Amaral CH, Venancio VG, Safanelli J et al (2018) Five-year survival, disability, and recurrence after first-ever stroke in a middle-income country: a population-based study in Joinvile, Brazil. Int J Stroke 1747493018763906

Calandra T, Roger T (2003) Macrophage migration inhibitory factor: a regulator of innate immunity. Nat Rev Immunol 3(10):791–800

Chatterjee M, Borst O, Walker B, Fotinos A, Vogel S, Seizer P et al (2014) Macrophage migration inhibitory factor (MIF) limits activation-induced apoptosis of platelets via CXCR7-dependent Akt signaling. Circ Res 115:939–949

Chen HR, Chuang YC, Chao CH, Yeh TM (2015a) Macrophage migration inhibitory factor induces vascular leakage via autophagy. Biology open 4(2):244–252

Chen L, Zhou X, Fan LX, Yao Y, Swenson-Fields KI, Gadjeva M, Wallace DP, Peters DJM, Yu A, Grantham JJ, Li X (2015b) Macrophage migration inhibitory factor promotes cyst growth in polycystic kidney disease. J Clin Invest 125(6):2399–2412

Chuang TY, Chang HT, Chung KP, Cheng HS, Liu CY, Liu YC et al (2014) High levels of serum macrophage migration inhibitory factor

and interleukin 10 are associated with a rapidly fatal outcome in patients with severe sepsis. Int J Infect Dis 20:13–17

Conroy H, Mawhinney L, Donnelly SC (2010) Inflammation and cancer: macrophage migration inhibitory factor (MIF)—the potential missing link. QJM: An International Journal of Medicine 103(11):831–836

Donnelly SC, Bucala R (1997) Macrophage migration inhibitory factor: a regulator of glucocorticoid activity with a critical role in inflammatory disease. Molecular medicine today 3(11):502–507

Fernández-Cadenas, I., Mendióroz, M., Giralt, D., Nafria, C., Garcia, E., Carrera, C., ... & Castellanos, M. (2017). GRECOS project (genotyping recurrence risk of stroke) the use of genetics to predict the vascular recurrence after stroke. Stroke, 48(5), 1147–1153

Hatano S (1976) Experience from a multicentre stroke register: a preliminary report. Bull World Health Organ 54(5):541–553

Herder C, Illig T, Baumert J, Müller M, Klopp N, Khuseyinova N, Meisinger C, Martin S, Thorand B, Koenig W (2008) Macrophage migration inhibitory factor (MIF) and risk for coronary heart disease: results from the MONICA/KORA Augsburg case-cohort study, 1984–2002. Atherosclerosis 200(2):380–388

Huang H, Zheng T, Wang S, Wei L, Wang Q, Sun Z (2016) Serum 25-hydroxyvitamin D predicts early recurrent stroke in ischemic stroke patients. Nutr Metab Cardiovasc Dis 26(10):908–914

Inácio AR, Ruscher K, Leng L, Bucala R, Deierborg T (2011) Macrophage migration inhibitory factor promotes cell death and aggravates neurologic deficits after experimental stroke. J Cereb Blood Flow Metab 31(4):1093–1106

Katan M, Fluri F, Morgenthaler NG, Schuetz P, Zweifel C, Bingisser R et al (2009) Copeptin: a novel, independent prognostic marker in patients with ischemic stroke. Ann Neurol 66(6):799–808

Koda M, Nishio Y, Hashimoto M, Kamada T, Koshizuka S, Yoshinaga K, Onodera S, Nishihira J, Moriya H, Yamazaki M (2004) Up-regulation of macrophage migration-inhibitory factor expression after compression-induced spinal cord injury in rats. Acta Neuropathol 108(1):31–36

Koga K, Kenessey A, Powell SR, Sison CP, Miller EJ, Ojamaa K (2011) Macrophage migration inhibitory factor provides cardioprotection during ischemia/reperfusion by reducing oxidative stress. Antioxid Redox Signal 14(7):1191–1202

Le Hiress M, Tu L, Ricard N, Phan C, Thuillet R, Fadel E et al (2015) Proinflammatory signature of the dysfunctional endothelium in pulmonary hypertension. Role of the macrophage migration inhibitory factor/CD74 complex. Am J Respir Crit Care Med 192(8):983–997

Lehmann L, Novender U, Schroeder S, Pietsch T, von Spiegel T, Putensen C, Hoeft A, Stüber F (2001) Plasma levels of macrophage migration inhibitory factor are elevated in patients with severe sepsis. Intensive Care Med 27(8):1412–1415

Levine DA, Kiefe CI, Houston TK, Allison JJ, McCarthy EP, Ayanian JZ (2007) Younger stroke survivors have reduced access to physician care and medications: National Health Interview Survey from years 1998 to 2002. Arch Neurol 64(1):37–42

Leyton-Jaimes MF, Kahn J, Israelson A (2018) Macrophage migration inhibitory factor: a multifaceted cytokine implicated in multiple neurological diseases. Exp Neurol 301:83–91

Li YS, Chen W, Liu S, Zhang YY, Li XH (2017) Serum macrophage migration inhibitory factor levels are associated with infarct volumes and long-term outcomes in patients with acute ischemic stroke. Int J Neurosci 127(6):539–546

Lin Q, Cai JY, Lu C, Sun J, Ba HJ, Chen MH, Chen XD, Dai JX, Lin JH (2017) Macrophage migration inhibitory factor levels in serum from patients with acute intracerebral hemorrhage: potential contribution to prognosis. Clin Chim Acta 472:58–63

Liu YC, Tsai YH, Tang SC, Liou HC, Kang KH, Liou HH, Jeng JS, Fu WM (2018) Cytokine MIF enhances blood-brain barrier permeability: impact for therapy in ischemic stroke. Sci Rep 8(1):743

Luedike P, Alatzides G, Papathanasiou M, Heisler M, Pohl J, Lehmann N, Rassaf T (2018a) Predictive potential of macrophage migration inhibitory factor (MIF) in patients with heart failure with preserved ejection fraction (HFpEF). Eur J Med Res 23(1):22

Luedike P, Alatzides G, Papathanasiou M, Heisler M, Pohl J, Lehmann N, Rassaf T (2018b) Circulating macrophage migration inhibitory factor (MIF) in patients with heart failure. Cytokine 110:104–109

Lv W, Chen N, Lin Y, Ma H, Ruan Y, Li Z, Li X, Pan X, Tian X (2016) Macrophage migration inhibitory factor promotes breast cancer metastasis via activation of HMGB1/TLR4/NF kappa B axis. Cancer Lett 375(2):245–255

Makino A, Nakamura T, Hirano M, Kitta Y, Sano K, Kobayashi T et al (2010) High plasma levels of macrophage migration inhibitory factor are associated with adverse long-term outcome in patients with stable coronary artery disease and impaired glucose tolerance or type 2 diabetes mellitus. Atherosclerosis 213(2):573–578

Morrison MC, Kleemann R (2015) Role of macrophage migration inhibitory factor in obesity, insulin resistance, type 2 diabetes, and associated hepatic co-morbidities: a comprehensive review of human and rodent studies. Front Immunol 6:308

Müller II, Müller KA, Schönleber H, Karathanos A, Schneider M, Jorbenadze R et al (2012) Macrophage migration inhibitory factor is enhanced in acute coronary syndromes and is associated with the inflammatory response. PLoS One 7(6):e38376

Okazaki S, Hishimoto A, Otsuka I, Watanabe Y, Numata S, Boku S et al (2018) Increased serum levels and promoter polymorphisms of macrophage migration inhibitory factor in schizophrenia. Prog Neuro-Psychopharmacol Biol Psychiatry 83:33–41

Pencina MJ, D'Agostino RB Sr, D'Agostino RB Jr, Vasan RS (2008) Evaluating the added predictive ability of a new marker: from area under the ROC curve to reclassification and beyond. Stat Med 27(2): 157–172

Sacco RL, Wolf PA, Gorelick PB (1999) Risk factors and their management for stroke prevention: outlook for 1999 and beyond. Neurology 53(7 Suppl 4):S15–S24

Sanchez-Zamora Y, Terrazas LI, Vilches-Flores A, Leal E, Juárez I, Whitacre C, Kithcart A, Pruitt J, Sielecki T, Satoskar AR, Rodriguez-Sosa M (2010) Macrophage migration inhibitory factor is a therapeutic target in treatment of non-insulin-dependent diabetes mellitus. FASEB J 24(7):2583–2590

Sánchez-Zamora YI, Rodriguez-Sosa M (2014) The role of MIF in type 1 and type 2 diabetes mellitus. J. Diabetes Res. 2014:1–6

Sobierajski J, Hendgen-Cotta UB, Luedike P, Stock P, Rammos C, Meyer C, Kraemer S, Stoppe C, Bernhagen J, Kelm M, Rassaf T (2013) Assessment of macrophage migration inhibitory factor in humans: protocol for accurate and reproducible levels. Free Radic Biol Med 63:236–242

Sposato LA, Cerasuolo JO, Cipriano LE, Fang J, Fridman S, Paquet M et al (2018) Atrial fibrillation detected after stroke is related to a low risk of ischemic stroke recurrence. Neurology 90(11):e924–e931

Stoppe C, Averdunk L, Goetzenich A, Soppert J, Marlier A, Kraemer S et al (2018) The protective role of macrophage migration inhibitory factor in acute kidney injury after cardiac surgery. Sci Transl Med 10(441):eaan4886

Thomalla G, Simonsen CZ, Boutitie F, Andersen G, Berthezene Y, Cheng B et al (2018) MRI-guided thrombolysis for stroke with unknown time of onset. N Engl J Med 379:611–622. https://doi.org/10.1056/NEJMoa1804355

Tu WJ, Zhao SJ, Xu DJ, Chen H (2014) Serum 25-hydroxyvitamin D predicts the short-term outcomes of Chinese patients with acute ischaemic stroke. Clin Sci 126(5):339–346

Tu WJ, Zeng XW, Deng A, Zhao SJ, Luo DZ, Ma GZ, Wang H, Liu Q (2017) Circulating FABP4 (fatty acid–binding protein 4) is a novel prognostic biomarker in patients with acute ischemic stroke. Stroke 48(6):1531–1538

Turtzo LC, Li J, Persky R, Benashski S, Weston G, Bucala R, Venna VR, McCullough LD (2013) Deletion of macrophage migration inhibitory factor worsens stroke outcome in female mice. Neurobiol Dis 54:421–431

Xu T, Pu S, Ni Y, Gao M, Li X, Zeng X (2018) Elevated plasma macrophage migration inhibitor factor as a risk factor for the development of post-stroke depression in ischemic stroke. J Neuroimmunol 320: 58–63

Wang L, Zis O, Ma G, Shan Z, Zhang X, Wang S, Dai C, Zhao J, Lin Q, Lin S, Song W (2009) Upregulation of macrophage migration inhibitory factor gene expression in stroke. Stroke 40(3):973–976

Wang Y, Xu J, Zhao X, Wang D, Wang C, Liu L, Wang A, Meng X, Li H, Wang Y (2013) Association of hypertension with stroke recurrence depends on ischemic stroke subtype. Stroke 44(5):1232–1237

Yang DB, Yu WH, Dong XQ, Zhang ZY, Du Q, Zhu Q et al (2017) Serum macrophage migration inhibitory factor concentrations correlate with prognosis of traumatic brain injury. Clin Chim Acta 469:99–104

Yüksel A, Bilgir F, Bilgir O, Calan M, Bozkaya G (2015) Increased circulating macrophage migration inhibitory factor levels are associated with coronary artery disease. Clinics 70(3):169–172

Zernecke A, Bernhagen J, Weber C (2008) Macrophage migration inhibitory factor in cardiovascular disease. Circulation 117(12):1594–1602

Zhang Q, Ding H, Yan J, Wang W, Ma A, Zhu Z, Cianflone K, Hu FB, Hui R, Wang DW (2011) Plasma tissue kallikrein level is negatively associated with incident and recurrent stroke: a multicenter case–control study in China. Ann Neurol 70(2):265–273

Zis O, Zhang S, Dorovini-Zis K, Wang L, Song W (2015) Hypoxia signaling regulates macrophage migration inhibitory factor (MIF) expression in stroke. Mol Neurobiol 51(1):155–167