Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology) *2021 22(6):512-520*
www.jzus.zju.edu.cn; www.springer.com/journal/11585
E-mail: jzus_b@zju.edu.cn

Research Article

https://doi.org/10.1631/jzus.B2000555

Serum macrophage migration inhibitory factor as a potential biomarker to evaluate therapeutic response in patients with allergic asthma: an exploratory study

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Abstract: Background: Previous studies have shown that macrophage migration inhibitory factor (MIF) is involved in the pathogenesis of asthma. This study aimed to investigate whether serum MIF reflects a therapeutic response in allergic asthma. Methods: We enrolled 30 asthmatic patients with mild-to-moderate exacerbations and 20 healthy controls, analyzing the parameter levels of serum MIF, serum total immunoglobulin E (tIgE), peripheral blood eosinophil percentage (EOS%), and fractional exhaled nitric oxide (FeNO). Lung function indices were used to identify disease severity and therapeutic response. Results: Our study showed that all measured parameters in patients were at higher levels than those of controls. After one week of treatment, most parameter levels decreased significantly except for serum tIgE. Furthermore, we found that serum MIF positively correlated with EOS% as well as FeNO, but negatively correlated with lung function indices. Receiver operator characteristic (ROC) curve analysis indicated that among the parameters, serum MIF exhibited a higher capacity to evaluate therapeutic response. The area under the curve (AUC) of MIF was 0.931, with a sensitivity of 0.967 and a specificity of 0.800. Conclusions: Our results suggested that serum MIF may serve as a potential biomarker for evaluating therapeutic response in allergic asthma with mild-to-moderate exacerbations.

Key words: Macrophage migration inhibitory factor (MIF); Allergic asthma; Eosinophilic inflammation; Fractional exhaled nitric oxide; Immunoglobulin E (IgE)

1 Introduction

Asthma is a common respiratory disease worldwide, with an estimated 300 million affected individuals (To et al., 2012). Its prevalence has been increasing rapidly, resulting in a heavy financial burden on healthcare systems. Allergic asthma is the most prevalent phenotype of asthma and is characterized by airway hyperresponsiveness (AHR), eosinophilic inflammation, mucus hypersecretion, and high expression of T helper type 2 (Th2) cytokines and immunoglobulin E (IgE). A broad and complicated network of cells and cytokines is involved in the pathogenesis of allergic asthma (Agrawal and Shao, 2010; Zhao and Wang, 2018).

Macrophage migration inhibitory factor (MIF) is a type of upstream mediator which was initially found to inhibit macrophage movement (Bloom and Bennett, 1966; David, 1966) but later was re-evaluated as a pro-inflammatory cytokine and pituitary-derived hormone, potentiating endotoxemia (Bernhagen et al., 1993; Calandra and Roger, 2003). Nowadays, MIF is

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Received Sept. 12, 2020; Revision accepted Dec. 21, 2020;
Crosschecked Mar. 30, 2021

recognized as a cytokine that exhibits a broad range of immune and inflammatory activity, such as the induction of inflammatory cytokines and regulation of cell proliferation (Das et al., 2011). It has been found to be involved in the pathogenesis of various disorders, such as septic shock, acute respiratory distress syndrome, autoimmune diseases, cardiovascular diseases, and cancers (Calandra et al., 2000; Lai et al., 2003; Nishihira et al., 2003; Zernecke et al., 2008; Lang et al., 2015). Moreover, MIF can antagonize the anti-inflammatory effects of glucocorticoids (Al-Abed and VanPatten, 2011) and has the potential to exacerbate human allergic and inflammatory diseases, including allergic asthma (Rossi et al., 1998).

Results from animal models of allergic asthma indicated that both the levels of MIF protein in bronchoalveolar lavage fluid (BALF) and MIF messenger RNA (mRNA) in pulmonary tissues were significantly elevated in ovalbumin-challenged mice as compared with controls. Blockade with an anti-MIF antibody can dramatically decrease the number of inflammatory cells within BALF and attenuate AHR. Similarly, treatment with the MIF antagonist of (S, R)-3-(4-hydroxyphenyl)-4, 5-dihydro-5-isoxazole acetic acid methyl ester (ISO-1) can reduce transforming growth factor-β1 (TGF-β1) mRNA and protein expression levels in pulmonary tissues, thereby inhibiting asthmatic airway remodeling (Kobayashi et al., 2006; Amano et al., 2007; Chen et al., 2010). MIF-knockout mice exhibit less pulmonary inflammation and lower AHR as compared with wild-type controls. Furthermore, MIF deficiency also results in lower serum IgE and decreased levels of Th2 cytokine in BALF and pulmonary tissues (Mizue et al., 2005; Wang et al., 2006).

Clinical studies have shown that MIF levels in induced sputum, BALF, and serum are significantly higher in adult asthmatic patients compared with those in healthy controls. In addition, symptomatic asthmatic patients have higher MIF levels compared with asymptomatic patients (Rossi et al., 1998; Yamaguchi et al., 2000). Another research group has found that serum MIF levels are significantly higher in children with allergic asthma versus healthy controls (Tan et al., 2012). Genetic polymorphism studies have also indicated that MIF-173G/C promoter polymorphism is associated with the risk of developing childhood asthma (Wu et al., 2009; El-Adly et al., 2016).

The aforementioned studies have revealed that MIF plays an essential role in the pathogenesis of allergic asthma. However, the utility of MIF in clinical practice remains unclear. This study aimed to explore whether serum MIF could be a potential biomarker to reflect a therapeutic response in patients with allergic asthma.

2 Subjects and methods

2.1 Participants

We recruited a total of 30 hospitalized patients with exacerbations of allergic asthma and 20 matched healthy controls from Zhengzhou Second People's Hospital, China, between April 2018 and March 2019. All patients experienced at least one symptom of acute asthma such as breathlessness, wheezing, cough, or chest tightness, and were diagnosed via spirometry with bronchial reversibility or provocation tests according to the Global Initiative for Asthma (GINA) guidelines (GINA, 2019). In addition, all patients were categorized as mild-to-moderate exacerbations and they were able to pass initial lung function tests. Atopic status was identified by positive skin prick tests, and the allergens selected were house dust mites, animal dander, grass pollens, tree pollens, and molds. Exclusion criteria were as follows: (1) the presence of respiratory infections or other respiratory diseases, (2) existence of autoimmune diseases or other chronic severe systemic diseases, (3) use of systemic corticosteroids within one week before admission, (4) use of long-acting or short-acting bronchodilators within 24 h before admission, (5) pregnancy or lactation, (6) patients with such severe exacerbations that they could not tolerate lung function tests, and (7) smokers. Control individuals were non-allergic, non-smoking subjects, and without a history of chronic respiratory conditions or other systemic diseases.

2.2 Treatment protocol

According to the GINA guidelines for the therapy of asthma exacerbations (GINA, 2019), and keeping in mind the continuing deterioration risk to these patients, all enrolled subjects were intravenously treated with methylprednisolone (40 mg/d), accompanied with inhaled budesonide and terbutaline suspension. The total dose of corticosteroids was no more than 1 mg/(kg·d). Furthermore, the duration of treatment until the

symptoms of exacerbation were significantly controlled was one week.

2.3 Lung function tests

According to the operating standards for lung function examination, as recommended by American Thoracic Society and European Respiratory Society (ATS/ERS) guidelines (Miller et al., 2005), lung function tests were performed in asthmatic patients using a spirometer (JAEGER®, Germany) before and after treatment. The collected data included forced expiratory volume in 1 s (FEV_1), forced vital capacity (FVC), FEV_1/FVC, peak expiratory flow (PEF), mean forced expiratory flow between 25% and 75% of FVC ($MFEF_{25–75}$), forced expiratory flow when 25%, 50%, and 75% of FVC had been exhaled (FEF_{25}, FEF_{50}, and FEF_{75}, respectively), and the percentage of predicted values (%pred) for the above indices.

2.4 Detection of serum MIF, total IgE, and peripheral blood eosinophil percentage

Fasting peripheral venous blood samples were collected from both healthy controls and asthmatic patients before and after treatment. In addition to measuring eosinophil percentage (EOS%) using samples in ethylene diamine tetraacetic acid (EDTA) anticoagulant tubes, other blood samples in non-anticoagulant tubes were centrifuged at 500*g* for 10 min to isolate sera, which were then cryopreserved at −80 °C until analysis. All serum samples were subjected to enzyme-linked immunosorbent assay (ELISA; Boster Bio, China) and electrochemiluminescence immunoassay (ECLI; KingMed Diagnostics, China) to detect the levels of serum MIF and total IgE (tIgE), respectively.

2.5 Measurement of fractional exhaled nitric oxide

Fractional exhaled nitric oxide (FeNO) levels were measured in healthy controls and asthmatic patients before and after treatment via an exhaled nitric oxide analyzer (Sunvou®, China), according to the ATS/ERS guidelines (ATS/ERS, 2005) and the manufacturer's instructions. FeNO measurements were performed before lung function tests for asthmatic patients, using ppb (part per billion) as the measurement unit.

2.6 Statistical analysis

Data analysis was performed using SPSS 19.0 (SPSS Inc., Chicago, IL, USA), and graphics were generated by GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, CA, USA). Categorical data were described as number (percentage). In addition, the normally and non-normally distributed continuous data were described as mean±standard deviation (SD) and median (interquartile range (IQR)), respectively. The variables in asthmatic patients as determined during pre- and post-treatment were compared using a paired-sample *t*-test and Wilcoxon signed rank test for normally and non-normally distributed data, respectively. The data of asthmatic patients and healthy controls were compared using the Mann-Whitney *U* test. Differences in categorical data between groups were analyzed using a chi-squared (χ^2) test. Correlations between the indices were analyzed using Spearman's correlation analysis. Receiver operator characteristic (ROC) curve analysis was used to assess the capacity of different parameters to profile the response to therapy in asthmatic patients, based on the improvement of lung function tests before and after treatment. A *P*-value of <0.05 was considered to be statistically significant.

3 Results

3.1 Participants' characteristics

General characteristics such as age, height, weight, body mass index (BMI), and gender exhibited no statistically significant differences between asthmatic patients and matched healthy controls ($P>0.05$). Twenty-three patients (76.7%) were newly diagnosed with asthma, with onset less than three weeks prior to the study. Eleven patients (36.7%) also suffered from allergic rhinitis, while five (16.7%) had a family history of allergy and six (20.0%) had a history of atopy. The proportions of mild and moderate exacerbations in asthmatic patients were 46.7% and 53.3%, respectively (Table 1).

3.2 Comparisons of lung function indices before and after treatment in asthmatic patients

As expected, the post-treatment indices, including PEF %pred, FEV_1 %pred, FEV_1/FVC, FEV_1/FVC %pred, FEF_{25} %pred, FEF_{50} %pred, FEF_{75} %pred, and $MFEF_{25–75}$ %pred, were significantly improved compared with the pre-treatment indices in asthmatic patients ($P<0.01$; Table 2).

Table 1 Characteristics of participants

Characteristics	Asthmatic patients (*n*=30)	Healthy controls (*n*=20)	*P*-value
Age (year)	45.47±16.09	40.33±15.31	0.211*
Height (cm)	165.43±7.34	163.50±8.07	0.336*
Weight (kg)	68.60±13.55	64.00±10.14	0.142*
BMI (kg/m^2)	24.91±3.60	23.82±2.18	0.161*
Gender (M/F)	14/16	9/11	0.908**
Smoker	0	0	
Atopy	6 (20.0%)	0	
Allergic rhinitis	11 (36.7%)	0	
Allergic family history	5 (16.7%)	0	
Newly diagnosed asthma	23 (76.7%)		
Severity of exacerbations			
Mild	14 (46.7%)		
Moderate	16 (53.3%)		
Severe	0		

Data are expressed as number (percentage), number/number, or mean±standard deviation. * *t*-test; ** Chi-squared test. BMI: body mass index; M: male; F: female.

Table 2 Comparisons of lung function indices before and after treatment in asthmatic patients

Index	Pre-treatment (*n*=30)	Post-treatment (*n*=30)	*P*-value
PEF %pred	64.16±22.80	85.62±19.78	<0.001
FEV_1 %pred	66.38±20.94	85.91±16.33	<0.001
FEV_1/FVC	65.54±10.27	72.71±8.00	<0.001
FEV_1/FVC %pred	78.94±12.27	87.19±9.47	<0.001
FEF_{25} %pred	49.64±27.77	70.05±24.49	<0.001
FEF_{50} %pred	38.44±21.21	56.19±22.50	<0.001
FEF_{75} %pred	31.93±14.79	45.00±22.56	0.001
$MFEF_{25–75}$ %pred	37.50±17.82	53.18±20.87	<0.001

PEF: peak expiratory flow; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; FEF_{25}, FEF_{50}, FEF_{75}: forced expiratory flow when 25%, 50%, and 75% of FVC have been exhaled, respectively; $MFEF_{25–75}$: mean forced expiratory flow between 25% and 75% of FVC; %pred: percentage of predicted value.

3.3 Comparisons of serum MIF, tIgE, EOS%, and FeNO levels before and after treatment in asthmatic patients and healthy controls

The post-treatment levels of serum MIF decreased dramatically compared with the pre-treatment levels ((9.71±7.41) ng/mL vs. (29.20±14.42) ng/mL, $P<0.0001$; Fig. 1a). Nevertheless, they remained higher than those of healthy controls ((9.71±7.41) ng/mL vs. (3.44±1.03) ng/mL, $P<0.0001$; Fig. 2a).

Similarly, the post-treatment levels of both EOS% and FeNO decreased significantly compared with the pre-treatment levels ((3.44±1.78)% vs. (8.17±5.04)%, $P<0.0001$ and (51.47±22.77) ppb vs. (81.37±30.67) ppb, $P<0.0001$, respectively; Figs. 1b and 1c). Nevertheless, the post-treatment levels of both markers in asthmatic patients were still higher than those of healthy controls by about 50% ((3.44±1.78)% vs. (1.71±0.81)%, $P<0.0001$ and (51.47±22.77) ppb vs. (21.70±5.57) ppb, $P<0.0001$, respectively; Figs. 2b and 2c).

However, the post-treatment serum tIgE levels showed no significant difference compared with the pre-treatment levels (302.95 (275.45) IU/mL vs. 289.35 (307.98) IU/mL, $P=0.061$; Fig. 1d). Also, the post-treatment serum tIgE levels in asthmatic patients were significantly higher than those in healthy controls (302.95 (275.45) IU/mL vs. 46.45 (54.47) IU/mL, $P<0.0001$; Fig. 2d).

3.4 Correlations among the levels of serum MIF, EOS%, and FeNO in asthmatic patients

Spearman's correlation analyses showed that serum MIF, EOS%, and FeNO levels in asthmatic patients positively correlated with each other before and after treatment (Fig. 3). However, serum tIgE level did not correlate with serum levels of MIF, EOS%, or FeNO (data not shown).

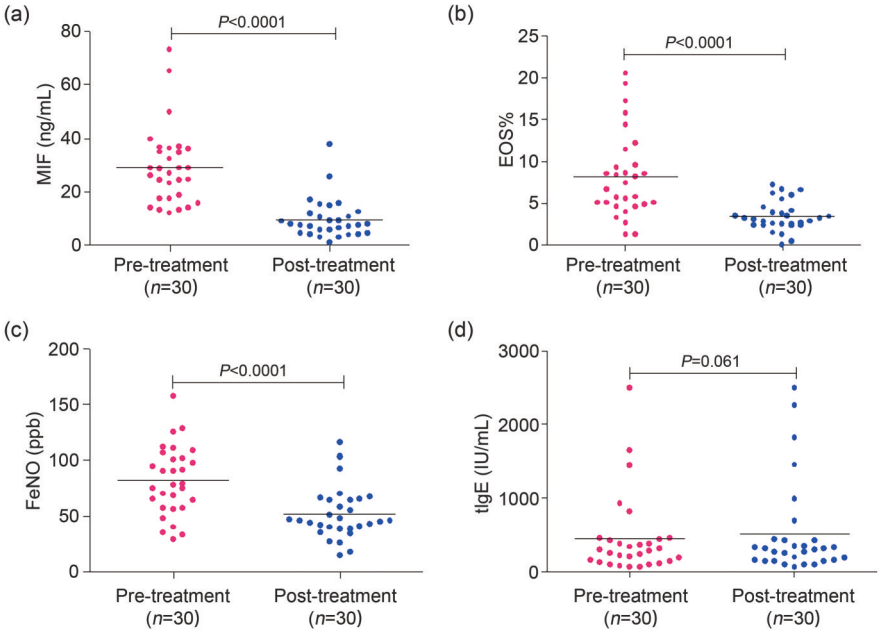

Fig. 1 Comparisons of serum MIF (a), EOS% (b), FeNO (c), and tIgE (d) levels before and after treatment in asthmatic patients (*n*=30). MIF, macrophage migration inhibitory factor; EOS%, eosinophil percentage; FeNO, fractional exhaled nitric oxide; ppb, part per billion; tIgE, total immunoglobulin E.

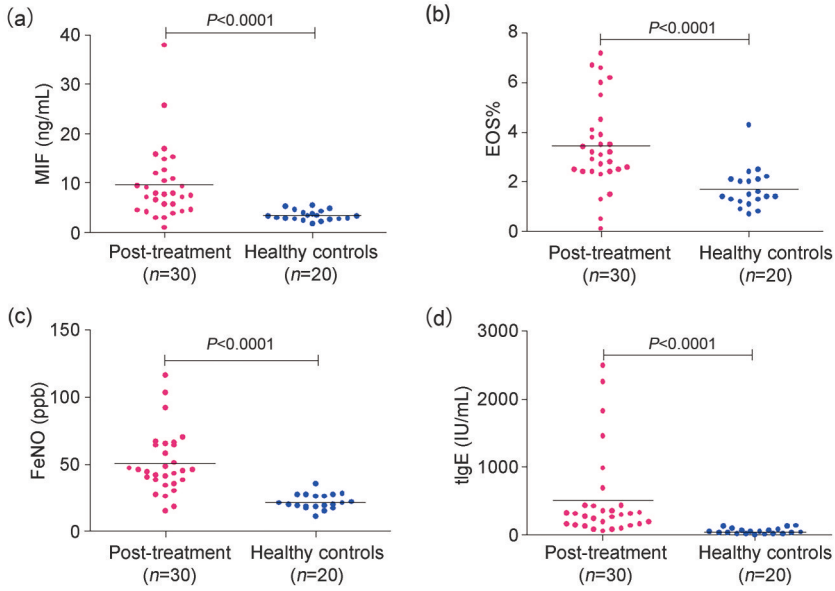

Fig. 2 Comparisons of serum MIF (a), EOS% (b), FeNO (c), and tIgE (d) levels between post-treatment asthmatic patients (*n*=30) and healthy controls (*n*=20). MIF, macrophage migration inhibitory factor; EOS%, eosinophil percentage; FeNO, fractional exhaled nitric oxide; ppb, part per billion; tIgE, total immunoglobulin E.

3.5 Correlations among serum levels of MIF and FeNO and lung function indices in asthmatic patients

Spearman's correlation analyses showed that serum MIF levels in asthmatic patients negatively correlated with lung function indices before and after treatment, including FEV_1 %pred (r=−0.379, P=0.003), PEF %pred (r=−0.396, P=0.002), and FEF_{50} %pred (r=−0.292, P=0.023) (Fig. 4a). Similarly, FeNO levels in asthmatic patients negatively correlated with lung function indices before and after treatment, including FEV_1/FVC (r=−0.358, P=0.005), FEV_1/FVC %pred (r=−0.350, P=

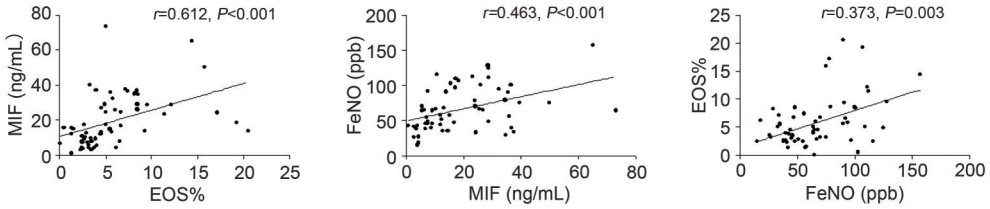

Fig. 3 Correlations among the levels of serum MIF, EOS%, and FeNO in asthmatic patients before and after treatment (n=60). Spearman's correlation analyses showed that serum MIF, EOS%, and FeNO levels positively correlated with each other. MIF, macrophage migration inhibitory factor; EOS%, eosinophil percentage; FeNO, fractional exhaled nitric oxide; ppb, part per billion.

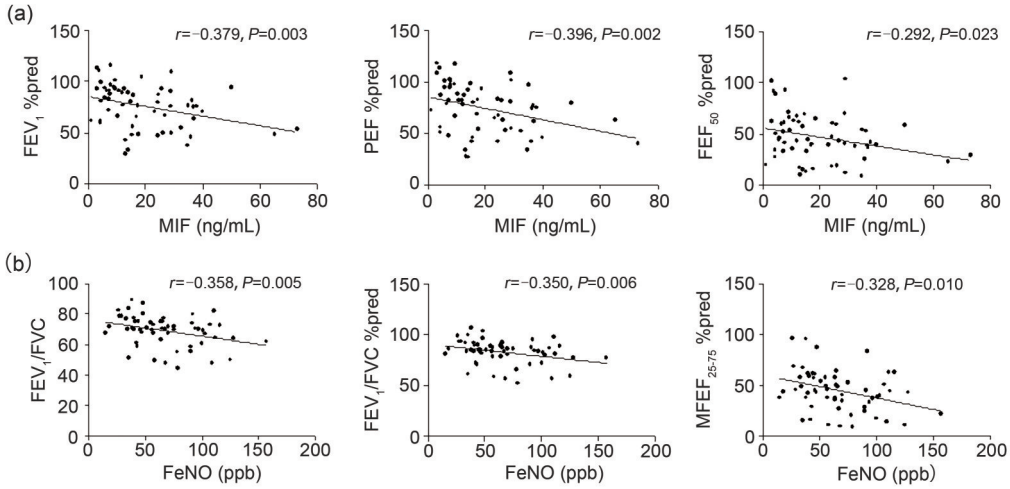

Fig. 4 Correlations among serum levels of MIF and FeNO and lung function indices in asthmatic patients before and after treatment (n=60). Spearman's correlation analyses showed that serum MIF levels in asthmatic patients negatively correlated with lung function indices, including FEV_1 %pred, PEF %pred, and FEF_{50} %pred (a), and that FeNO levels in asthmatic patients negatively correlated with lung function indices, including FEV_1/FVC, FEV_1/FVC %pred, and $MFEF_{25–75}$ %pred (b). FEV_1, forced expiratory volume in 1 s; MIF, macrophage migration inhibitory factor; PEF, peak expiratory flow; FVC, forced vital capacity; FeNO, fractional exhaled nitric oxide; ppb, part per billion; FEF_{50}, forced expiratory flow when 50% of FVC has been exhaled; $MFEF_{25–75}$, mean forced expiratory flow between 25% and 75% of FVC; %pred, percentage of predicted value.

0.006), and $MFEF_{25–75}$ %pred (r=−0.328, P=0.010) (Fig. 4b). However, neither the levels of serum tIgE nor EOS% correlated with any lung function indices in asthmatic patients (data not shown).

3.6 ROC curve analysis for evaluating the capacity of different parameters to profile therapeutic response

ROC curve analysis showed that for MIF, the area under the curve (AUC) was 0.931 (95% confidence interval (CI): 0.863–1.000, P<0.001), as determined by Youden index; the cut-off value was 12.8 ng/mL, with a sensitivity of 0.967 and a specificity of 0.800. Similarly, for EOS%, the AUC was 0.837 (95% CI: 0.731–0.942, P<0.001), with a sensitivity of 0.800 and a specificity of 0.567 at the cut-off value of 4.55%. For FeNO, the AUC was 0.783 (95% CI: 0.662–0.904, P<0.001), with a sensitivity of 0.833 and a specificity of 0.500 at the cut-off value of 55.5 ppb. For tIgE, the AUC was 0.479 (95% CI: 0.332–0.627, P=0.784), which indicated that it cannot be used to evaluate therapeutic response (Fig. 5).

4 Discussion

According to current clinical practice guidelines, blood eosinophil count and FeNO are the most useful biomarkers for evaluating airway eosinophil inflammation in allergic asthma (Dweik et al., 2011; GINA, 2019). A previous study has shown no difference between EOS% and blood eosinophil count in detecting eosinophilic inflammation (Zhang et al., 2014). In this

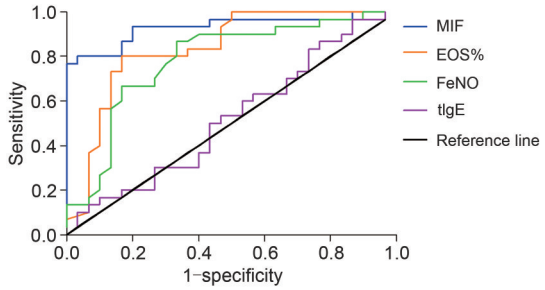

Fig. 5 ROC curve analysis for evaluating the capacity of different parameters to profile therapeutic response. ROC, receiver operator characteristic; MIF, macrophage migration inhibitory factor; EOS%, eosinophil percentage; FeNO, fractional exhaled nitric oxide; tIgE, total immunoglobulin E.

study, we found that asthmatic patients had higher serum MIF levels than the controls, and post-treatment levels of serum MIF decreased dramatically, accompanying reduced levels of EOS% and FeNO. Furthermore, serum MIF levels positively correlated with the levels of both EOS% and FeNO. These findings suggested that serum MIF correlates with eosinophilic inflammation and reflects inflammation severity, which is in agreement with the results of previous studies (Bozza et al., 2020).

We used corticosteroids and bronchodilators to treat asthmatic patients during exacerbations according to GINA clinical guidelines, and lung function indices were regarded as the objective indicators to identify disease severity and therapeutic response. We found that the significantly improved lung function indices following treatment paralleled the markedly decreased serum MIF levels. In addition, similar to the negative relationship between FeNO and lung function indices, there were also weak but significant inverse correlations between serum MIF levels and primary lung function indices, including FEV_1 % pred and FEV_1/FVC. These results showed that serum MIF is associated with disease severity and can serve as a biomarker to evaluate illness relief and therapeutic response in acute asthma. Also, serum MIF level negatively correlated with the lung function index of FEF_{50} % pred, which is often useful to assess the small airway (Bar-Yishay et al., 2003; Pellegrino et al., 2005), indicating that serum MIF might also reflect the functional improvement of the small airway.

To further evaluate the capacity of serum MIF to profile the response to therapy, we conducted ROC analysis and compared it with other measured biomarkers, such as EOS%, FeNO, and serum tIgE. Our results demonstrated that most parameters, except for serum tIgE, are valid for evaluating therapeutic response. In particular, we found that the AUC for MIF (0.931) was higher than those for EOS% (0.837) and FeNO (0.783), displaying a sensitivity of 0.967 and a specificity of 0.800 (using a cut-off value of 12.8 ng/mL). These results suggested that serum MIF exhibited a higher capacity among the parameters to evaluate therapeutic response in acute asthma.

It is known that serum IgE is a conventional biomarker in allergic asthma. However, it has certain limitations and might not be useful enough to assess disease severity and evaluate treatment response within acute asthma. Some studies have shown that serum IgE might differentiate severe asthma in children but not adults (Fitzpatrick et al., 2006; Chung et al., 2014). A two-week asthmatic treatment study reported that serum tIgE level could not predict response to routine treatment in acute asthma (Razi and Moosavi, 2010). Another four-week asthmatic treatment study indicated that clinical response was not associated with serum IgE level (Cai et al., 2007). In line with these results, our study showed that serum tIgE levels were significantly higher as compared with controls but did not markedly decrease after treatment and did not correlate with lung function indices. ROC curve analyses also revealed that serum tIgE was invalid for evaluating therapeutic response within asthma exacerbation. These results suggested that serum tIgE level might not accurately reflect the response to short-term treatment of allergic asthma. On the contrary, our one-week treatment study indicated that serum MIF exhibited higher sensitivity for evaluating therapeutic response in short-term treatment.

In conclusion, our study indicated that serum MIF correlates with eosinophilic inflammation and disease severity in allergic asthma. Serum MIF may serve as a potential biomarker for evaluating therapeutic response in allergic asthma with mild-to-moderate exacerbations. However, due to the limitations of our study, large-scale sample research and long-term studies still need to be pursued.

Acknowledgments

The study was supported by the Henan Province Medical Science and Technology Research Project (No. 2018020737), China.

Author contributions

Huiyuan ZHU and Shaochun YAN designed the study, wrote and edited the manuscript. Huiyuan ZHU performed the clinical and experimental research and data analysis. Zhong ZHANG, Xiaolin LI, Zheng LIU, Xing MA, Lina ZHOU, Lin ZHANG, Mingming FENG, Yiwei GENG, and Aixin ZHANG participated in the collection of clinical and experimental data. Aiguo XU, Jingshuo WU, and Sabina JANCIAUSKIENE coordinated the research project. All authors have read and approved the final manuscript, and therefore have full access to all the data in the study and take responsibility for the data's integrity and security.

Compliance with ethics guidelines

Huiyuan ZHU, Shaochun YAN, Jingshuo WU, Zhong ZHANG, Xiaolin LI, Zheng LIU, Xing MA, Lina ZHOU, Lin ZHANG, Mingming FENG, Yiwei GENG, Aixin ZHANG, Sabina JANCIAUSKIENE, and Aiguo XU declare that they have no conflict of interest.

All procedures were conducted in accordance with the Helsinki Declaration of 1975, as revised in 2008 (5). The medical ethics committee of Zhengzhou Second People's Hospital approved the study, and all participants signed the written informed consent.

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