Original Article

Macrophage colony-stimulating factor could evaluate both disease activity and renal involvement in systemic lupus erythematosus

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Background: Aberrations of monocyte/macrophage-associated cytokines are increasingly recognized in systemic lupus erythematosus (SLE). However, the combined expression of these cytokines has not been sufficiently studied in relation to either disease activity or renal involvement in SLE. Here, we explored clinical values of monocyte/macrophage-associated cytokines for monitoring of disease activity and renal involvement in SLE patients.

Methods: A total of 44 healthy people and 100 SLE patients were enrolled in this study. The serum levels of 8 monocyte/macrophage-associated cytokines [interferon gamma (IFN-γ), granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α), macrophage colony stimulating factor (M-CSF), interleukin-34 (IL-34), interleukin-10 (IL-10), and urokinase-type plasminogen activator receptor (uPAR)] were measured, and correlations between their levels and both SLE activity and renal involvement were analyzed using receiver operating characteristic (ROC) curves. Additionally, we analyzed the correlation between M-CSF level and laboratory or clinical data and used real time-polymerase chain reaction (RT-PCR) to assess M-CSF messenger RNA (mRNA) expression in sorted candidate cells.

Results: The levels of IL-6, IFN-γ, and TNF-α were significant for predicting SLE activity, while the M-CSF level was significant for predicting both SLE activity and renal involvement. Furthermore, the mRNA expression of M-CSF in monocytes from SLE patients was higher than that of healthy people, and the M-CSF level was positively correlated with monocyte numbers.

Conclusions: The cytokine M-CSF is a promising marker to evaluate both disease activity and renal involvement in SLE, and the high level of M-CSF in SLE patients was mainly derived from monocytes.

Keywords: Macrophage colony stimulating factor (M-CSF); systemic lupus erythematosus (SLE); disease activity; renal involvement; monocyte/macrophage

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Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disorder characterized by autoantibody production and chronic inflammation targeting multiple organs (1,2). The accurate assessment of disease activity in patients with systemic lupus erythematosus (SLE) is a critical tool for clinical practice, observational studies and clinical trials. The SLE disease activity index (SLEDAI), which includes the SLEDAI 2000 (SLEDAI-2K) and Safety of Estrogens in Lupus Erythematosus National Assessment (SELENA)-SLEDAI versions, is the most widely used SLE disease activity measure (3,4). The development of comorbidities and tissue damage, especially renal involvement, can be the direct consequence of SLE or a result of SLE medication (5). Renal involvement is a serious manifestation of systemic lupus erythematosus (SLE), it may portend a poor prognosis as it may lead to end-stage renal disease (6). At present, renal biopsy remains the "gold standard" for the diagnosis of lupus nephritis, but not all patients accept this method because of its invasive nature. However, few reliable biomarkers have been validated for the evaluation of SLE progression (7,8); therefore, there is a need to develop new biomarkers for both disease activity and renal involvement.

In patients with SLE, the immune system is subverted to target numerous self-antigens, and the ensuing inflammatory response elicits a vicious cycle of immune cell activation. Aberrations of monocyte/macrophage phenotype and function are increasingly recognized in patients with SLE and animal models of the disease (9,10). Under physiological conditions, monocytes and macrophages represent an essential arm of the innate immune system with a multitude of immunological functions, including antigen presentation, phagocytosis, and cytokine production. They are heterogeneous cells whose phenotype and functions are regulated by the surrounding micro-environment (11-13). Classically activated or M1 macrophages, which are polarized by interferon-gamma (IFN-γ) or granulocyte-macrophage colony-stimulating factor (GM-CSF), possess high pro-inflammatory, tumoricidal and anti-microbial activities and produce cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α). Alternatively activated or M2 macrophages, which are immune-regulatory and polarized by interleukin-4 (IL-4) and macrophage colony-stimulating factor (M-CSF), are related to the wound healing and anti-inflammatory role and produce cytokines such as interleukin-10 (IL-10) (14,15). Additionally, interleukin-34 (IL-34), sharing the same receptor with M-CSF, is reported to polarize macrophages to an M2-like regulatory phenotype (16,17). Furthermore, the soluble urokinase plasminogen activator receptor (uPAR), expressed on macrophages, is positively related to M2 phenotype macrophages (18,19).

The implications of monocyte/macrophage abnormalities in the pathogenesis of SLE have been discussed in previous studies (20-23). These aberrations include macrophage polarization that is augmented by dysregulated cytokine production (24), and several macrophage-associated cytokines are reported to reach high levels in SLE patients (22,23), e.g., the increased expression of M-CSF, and the correlation between the M-CSF level and SLE activity, are reported in SLE patients (25). However, neither the combined expression of monocyte/macrophage-associated cytokines, nor the direct relationship between monocyte/macrophage and the cytokines, have been sufficiently studied in SLE patients.

In this study, we measured the levels of a panel of 8 cytokines (IFN-γ, GM-CSF, IL-6, TNF-α, M-CSF, IL-34, IL-10, and uPAR) that are characteristic of different macrophage polarization phenotypes. We found that M-CSF was a promising marker to evaluate both disease activity and renal involvement in SLE, with the high level of M-CSF in SLE patients being predominantly derived from monocytes. Our findings emphasize the value of M-CSF in the clinical management of SLE and provide clues regarding key roles of the monocyte/macrophage lineage in SLE progression.

We present the following article in accordance with the MDAR reporting checklist (available at http://dx.doi.org/10.21037/apm-20-2607).

Methods

Data collection

A total of 100 patients diagnosed with SLE and 44 healthy people were analyzed in Henan Provincial People's Hospital (Zhengzhou, China). All participants met the 1997 American College of Rheumatology (ACR) revised criteria for the classification of SLE (26); SLE patients with cancer, infectious diseases, and other rheumatic diseases, were excluded. For some analyses, the 100 SLE patients were divided into an inactive group (n=50) and an active group (n=50) according to their SLEDAI score [if ≥10, they were considered clinically active (27)]. For other analyses, these participants were grouped into a non-lupus nephritis group

(non-LN, n=52) and lupus nephritis group (LN, n=48) according to the results of renal biopsy in department of pathology in our hospital [including1 class II, 9 class III, 25 class IV, 6 class V, 5 class III + V, and 2 class VI, using the International Society of Nephrology/Renal Pathology Society (ISN/RPS) 2003 lupus nephritis classification (28)]. The pathological activity of lupus nephritis was also evaluated by active index (AI) and chronic index (CI) (29). Demographic information, clinical data, and laboratory findings were extracted from electronic medical records. All participants provided written informed consent, and the research was approved by the Ethics Committee of Henan Provincial People's Hospital (No: 2019-50). The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Cytokine detection

Luminex liquid suspension chip technology (Luminex 200, Millipore, Burlington, MA, USA) was used to detect the expression of 8 macrophage-associated cytokines (M1 macrophage: IFN-γ, GM-CSF, IL-6, TNF-α; M2 macrophage: M-CSF, IL-34, IL-10, uPAR) in serum from both SLE and healthy groups. The samples were performed in duplicate according to the manufacturer's instructions (LXSAHM-08, Research and Diagnostic Systems, Inc., Minneapolis, MN, USA). Data were analyzed using cytokine standard protocols.

Cell sorting

Peripheral blood cells were treated by red blood cell lysis buffer according to standard protocols. The cells were labeled with antibodies to determine their phenotypes ($CD15^+CD16^+$ for neutrophils, $CD14^{hi}$ for monocytes, $CD3^+CD4^+$ for CD4 T cells, $CD3^+CD8^+$ for CD8 T cells, and $CD20^+$ B cells), then sorted using Aria III (Becton, Dickinson and Co., Franklin Lakes, NJ, USA) according to standard protocols.

The antibodies used in fluorescence-activated cell sorting (FACS) (Biolegend, USA) were: CD14 (FITC, clone: M5E2), CD16 (PE, clone: 3G8), CD15 (APC, clone: W6D3), CD3 (PE-cy7, clone: HIT3a), CD4 (PE, clone: OKT4), CD8 (FITC, clone: SK1), CD20 (APC, clone: 2H7).

Real-time polymerase chain reaction

Total RNA was extracted from the respective sorted cells using the RNeasy Mini Kit (Qiagen, Valencia, CA, USA), and reverse transcription was performed using the Super Script First-Strand Synthesis System for real-time polymerase chain reaction (RT-PCR) (Invitrogen, Carlsbad, CA, USA) according to suggested protocols. The RT-PCR was performed using an ABI Prism 7500 Sequence Detector (Applied Biosystems, Foster City, CA, USA). The messenger RNA (mRNA) expression of M-CSF was analyzed by the relative quantification (RQ) value, calculated by normalization to the internal control glyceraldehyde-3-phosphate dehydrogenase (GAPDH) by the $2^{-\Delta\Delta Ct}$ method [$^{\Delta\Delta}Ct = (Ct_{TARGET} - Ct_{GAPDH})_{sample} - (Ct_{TARGET} - Ct_{GAPDH})_{calibrator}$]. The relative expression of M-CSF mRNA in SLE patients was $2^{-\Delta\Delta Ct}$.

The human primers used in the study were:

GAPDH-Forward (5'-3') GGAGTCCACTGGCGTCTTCA;

GAPDH-Reverse (5'-3') TTGCTGATGATCTTGAGGCTGTTG;

M-CSF-Forward (5'-3') CCAGAAGGAGGACCAGCAAGT;

M-CSF-Reverse (5'-3') CCAGCAAGACCAGGATGACACT.

Statistical analysis

The software SPSS 17.0 (SPSS, IBM, Chicago, IL, USA) was used for statistical analysis. Categorical variables were presented as n (%), and continuous variables were presented as mean ± SEM, respectively. We used the chi-squared (χ^2) test, Fisher's exact test, unpaired Student's *t*-test, and one-way analysis of variance (ANOVA) to evaluate the significance of differences. Detector performance analysis of the cytokine levels for predicting disease activity or renal involvement in SLE was performed using receiver operating characteristic (ROC) curves. The value at which the sum of sensitivity and specificity reached the maximum was considered to be the optimal cutoff according to Youden's index ($\mathcal{J}$ = sensitivity + specificity – 1). Correlation analysis of continuous variables was performed by Pearson correlation analysis. A P value <0.05 was considered statistically significant.

Results

Demographic characteristics and laboratory findings in SLE patients

A total of 100 SLE patients and 44 healthy people were

enrolled in this study. To reduce bias, we matched the factors of age and gender upon collection of information and serum from healthy people. Thus, there was no significant difference between healthy controls and SLE patients for age or gender (Table S1).

The 100 SLE patients were divided into an inactive group (n=50) and an active group (n=50) according to the SLEDAI score. There was no significant difference between the two SLE groups regarding age or gender. Additionally, the disease duration of active SLE patients was not different from that of the inactive group. Notably, there were more LN participants in the active group (n=36) than the inactive group (n=12), indicating the close relationship between renal involvement and SLE activity. The features of laboratory findings were also analyzed. The levels of 24-hour urinary protein, serum creatinine (Scr), and C-reactive protein (CRP) and the erythrocyte sedimentation rate (ESR), double-stranded DNA (dsDNA), and complement 3 (C3) were all significantly higher in the active SLE group than in the inactive SLE group (Table S2), suggesting the clinical importance of these laboratory indicators for SLE activity.

The high level of monocyte/macrophage-associated cytokines in SLE patients

In this study, we analyzed the levels of the 8 cytokines related to SLE activity (*Figure 1A*). Of the 8 cytokines, 6 (all but GM-CSF and IL-34) had higher levels in the active SLE group than in both the inactive SLE group and the healthy control group. The cytokines IFN-γ, IL-6, and TNF-α, are involved in M1 macrophage polarization, while M-CSF, IL-10, and uPAR are involved in M2 macrophage polarization. The results indicated the predictive role of these 6 cytokines for SLE activity.

We also analyzed the levels of these 8 cytokines in the non-LN and LN groups to explore their association with renal involvement (*Figure 1B*). Among the 8 cytokines, only M-CSF had a higher level in the LN group than in the non-LN group. Accordingly, M-CSF can be used to distinguish renal involvement in SLE patients.

The detection efficiency analysis of the cytokine levels for predicting disease activity and renal involvement in SLE patients

To verify the detection efficiency of these cytokine levels for predicting disease activity in SLE patients, the area under the ROC (AUROC) curve was used to evaluate specificity and sensitivity. Additionally, the optimal cut-off value was calculated according to Youden's index. The AUROC curves for M-CSF (0.755), IL-6 (0.742), IFN-γ (0.704), and TNF-α (0.702) were significant for predicting disease activity in SLE patients. The optimal cut-off values were 68.750, 3.575, 10.275, and 3.165, respectively (*Figure 2A* and *Table 1*), suggesting their utility in predicting SLE activity.

The detection efficiency of M-CSF level for predicting renal involvement in SLE patients was also evaluated by its ROC curve. The AUROC curve was 0.667 and the optimum cut-off value was 68.750 (*Figure 2B*), indicating the utility of M-CSF in predicting renal involvement in SLE. These results indicated that M-CSF can predict both disease activity and renal involvement in SLE patients.

The correlation between serum M-CSF level and laboratory or clinical data of SLE patients

Based on the predictive effect of M-CSF level for both disease activity and renal involvement in SLE patients, the correlation between the serum M-CSF level and laboratory or clinical data was explored to confirm the clinical value of the M-CSF level in SLE (*Table 2*). For laboratory data, the M-CSF level was positively correlated with the levels of 24 hour urinary protein, Scr, and CRP, dsDNA, and was negatively correlated with the C3 level. For the clinical data, SLE activity was evaluated by SLEDAI scores, and the pathological activity of lupus nephritis was evaluated by renal activity index (AI) and chronicity index (CI) scores (*Figure 2C*). The results showed that the M-CSF level was positively correlated with the SLEDAI and renal AI scores and negatively correlated with the renal CI scores. This confirmed the potential of M-CSF level to predict both disease activity and renal involvement in SLE patients.

The high level of M-CSF in SLE patients was closely associated with monocytes

The cytokine M-CSF is reported to be synthesized by mesenchymal cells such as fibroblasts, osteoblasts, and endothelial cells (30). It can also be produced by activated macrophages, B cells, and T cells (31,32). Based on the results above, we identified a close relationship between the serum M-CSF level and cell subpopulations in the peripheral blood of SLE patients. Neutrophils, monocytes, and lymphocytes were quantified in peripheral blood to

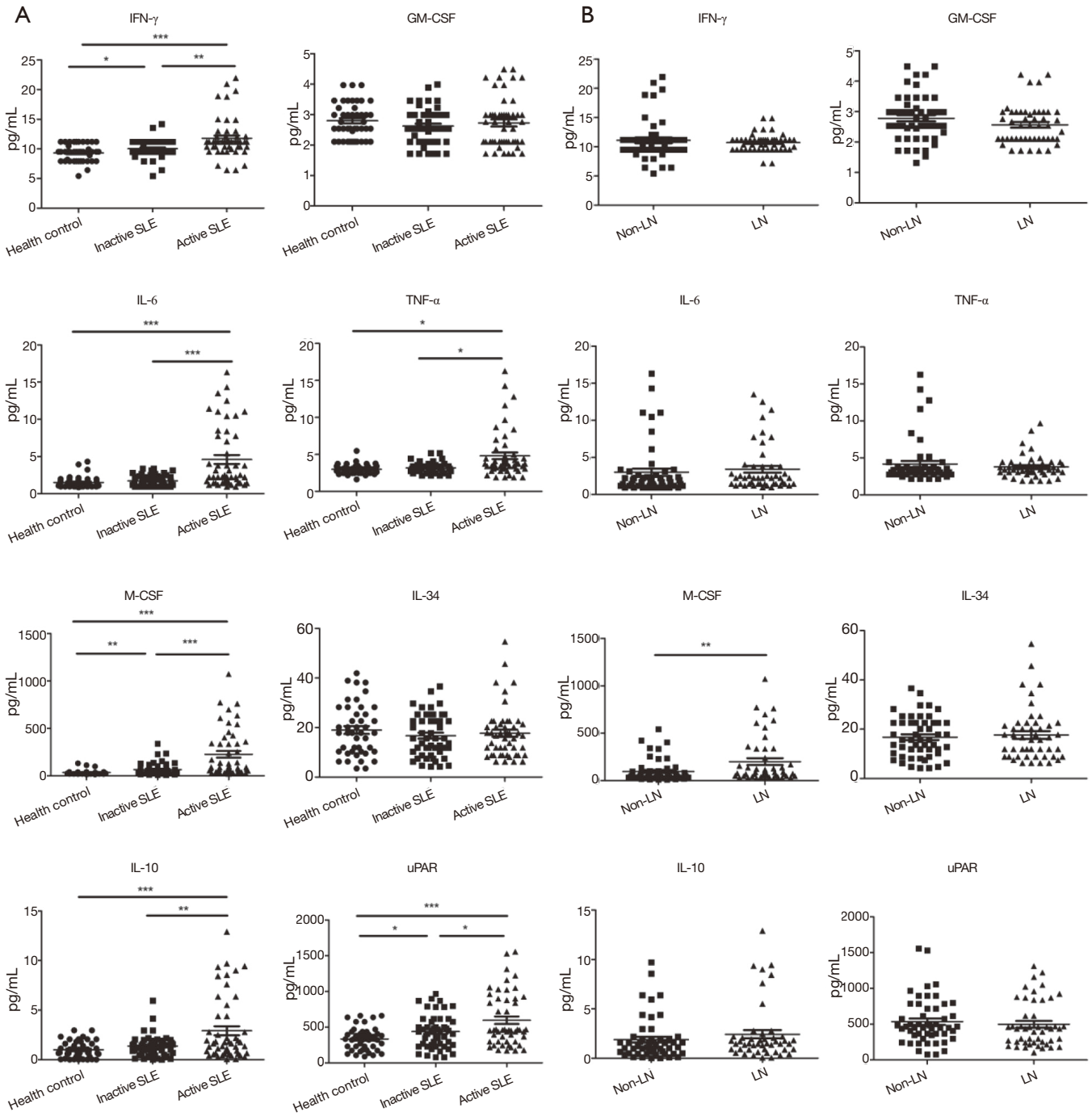

Figure 1 The levels of monocyte/macrophage-associated cytokines in SLE patients. (A) SLE patients were classified into inactive and active groups according to their SLEDAI scores. Levels of the 8 macrophage-associated cytokines were detected by Luminex liquid suspension chip technology in three groups (healthy controls, n=44; inactive group, n=50; active group, n=50). (B) SLE patients were classified into non-LN and LN groups according to their renal involvement. The levels of the 8 monocyte/macrophage-associated cytokines were analyzed in two groups (non-LN group, n=52; LN group, n=48). Data in columns are shown as mean ± SEM. *, P<0.05; **, P<0.01; ***, P<0.001. M1 macrophage polarization: IFN-γ, GM-CSF, TNF-α, and IL-6. M2 macrophage polarization: M-CSF, IL-34, IL-10, and uPAR. SLE, systemic lupus erythematosus; SLEDAI, SLE disease activity index; non-LN, non-lupus nephritis; LN, lupus nephritis; SEM, standard error of measurement; IFN-γ, interferon gamma; GM-CSF, granulocyte-macrophage colony-stimulating factor; TNF-α, tumor necrosis factor alpha; IL-6, interleukin-6; M-CSF, macrophage colony-stimulating factor; IL-34, interleukin-34; IL-10, interleukin-10; uPAR, urokinase plasminogen activator receptor.

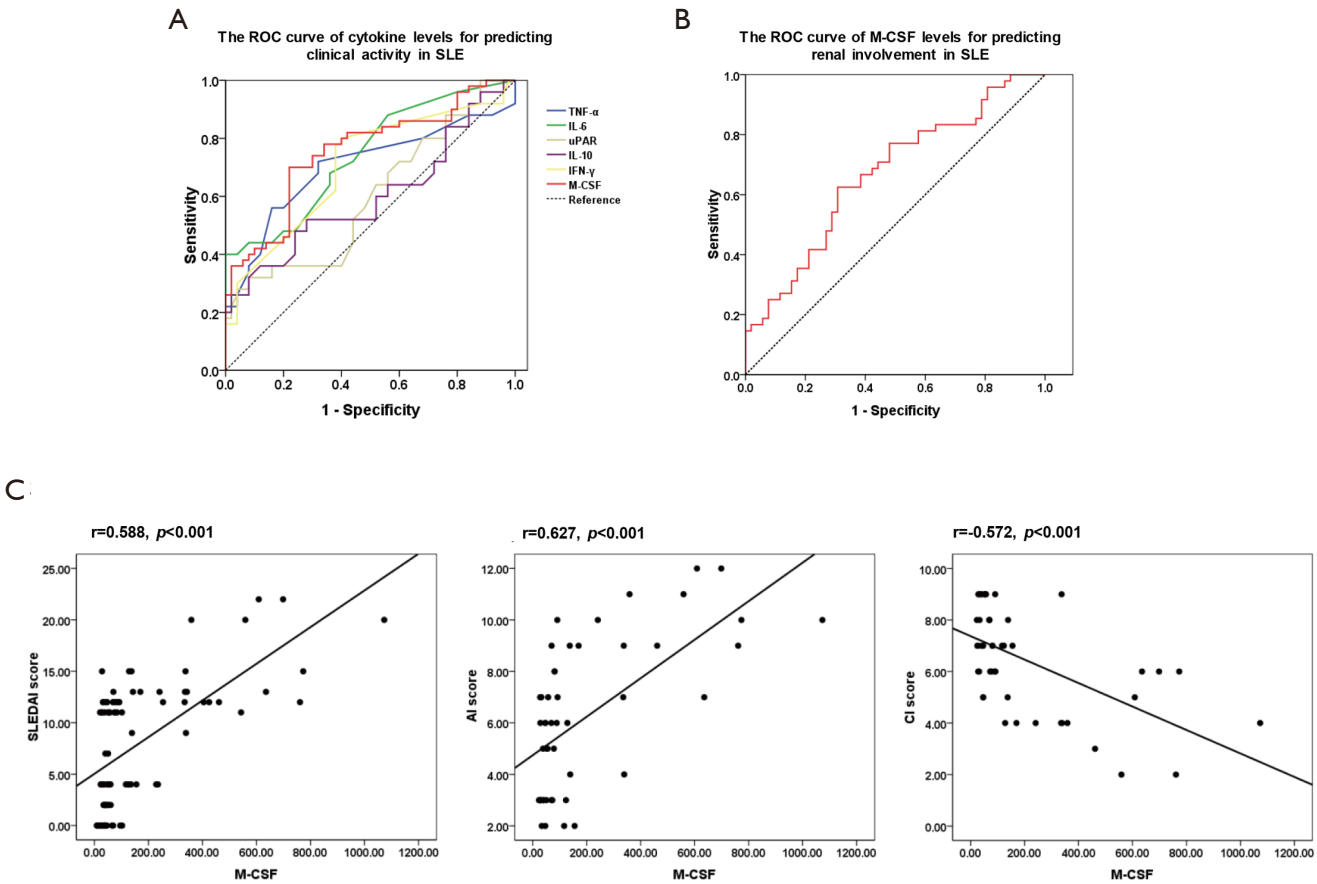

Figure 2 The detection efficiency analysis and the correlation analysis of M-CSF with laboratory or clinical data of SLE patients. (A) The ROC curve of cytokine levels for predicting clinical activity in SLE patients; (B) the ROC curve of M-CSF levels for predicting renal involvement in SLE patients; (C) the correlation between serum M-CSF level and clinical data (SLEDAI scores, renal AI and CI scores) was performed by Pearson correlation analysis. A P value <0.05 was considered statistically significant. SLE, systemic lupus erythematosus; ROC, receiver operating characteristic; M-CSF, macrophage colony-stimulating factor; SLEDAI, SLE disease activity index, AI, activity index; CI, chronicity index.

explore the source of the high level of M-CSF in SLE. The results showed that the numbers of neutrophils and monocytes in SLE patients were significantly higher than in healthy people, indicating that neutrophils and monocytes may contribute to the high level of M-CSF in SLE patients (*Figure 3A*).

Next, the candidate cells from peripheral blood in both SLE patients and healthy people were sorted (gating strategies in Figure S1) to detect the mRNA expression of M-CSF by RT-PCR. The results showed that the mRNA expression of M-CSF in monocytes from the SLE group was significantly higher than that from the healthy group (*Figure 3B*). Furthermore, M-CSF level was positively correlated with monocyte numbers (*Figure 3C*), indicating that the high level of M-CSF in SLE patients was closely associated with monocytes, specifically, the high level of M-CSF in SLE patients was derived mainly from monocytes.

Discussion

The disease SLE is a multisystem autoimmune disorder associated with widespread inflammation and is often progressive. Aberrations of monocyte and macrophage phenotype and function have been increasingly recognized in SLE (33). These aberrations include macrophage

Table 1 The detection efficiency analysis of cytokine levels for predicting clinical activity in SLE patients

Variables	ROC area	Std. error	Asymptotic sig.	Asymptotic 95% confidence interval	Optimal cut off
M-CSF	0.755	0.049	0.000***	0.659–0.850	68.750
IL-6	0.742	0.049	0.000***	0.646–0.837	3.575
IFN-γ	0.704	0.053	0.000***	0.601–0.807	10.275
TNF-α	0.702	0.054	0.000***	0.596–0.809	3.165
IL-10	0.598	0.058	0.091	0.485–0.711	NA
uPAR	0.597	0.057	0.094	0.485–0.709	NA

The detection efficiency of cytokine levels for predicting clinical activity in SLE patients was analyzed by ROC curve (***, P<0.001). The optimal cut-off value is calculated according to Youden's index. SLE, systemic lupus erythematosus; ROC, receiver operating characteristic; IFN-γ, interferon gamma; GM-CSF, granulocyte-macrophage colony-stimulating factor; TNF-α, tumor necrosis factor alpha; IL-6, interleukin-6; M-CSF, macrophage colony-stimulating factor; IL-34, interleukin-34; IL-10, interleukin-10; uPAR, urokinase plasminogen activator receptor; NA, not applicable (the AUROC curves for IL-10 and uPAR weren't significant for predicting disease activity in SLE patients, so there was no optimal cut-off values for the two cytokines).

Table 2 The correlation between serum M-CSF level and clinical or laboratory data of SLE patients

Variables	M-CSF	
	r	P value
24-hour urinary protein	0.405	<0.001***
Scr	0.320	0.001***
CRP	0.266	0.008**
ESR	0.146	0.148
dsDNA	0.672	0.001***
C3	−0.407	<0.001***
C4	−0.191	0.114

Correlation analysis of continuous variables was performed by Pearson correlation analysis (**, P<0.01; ***, P<0.001). SLE, systemic lupus erythematosus; M-CSF, macrophage colony-stimulating factor; Scr, serum creatinine; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; dsDNA, double-stranded DNA; C3, complement 3; C4, complement 4.

polarization augmented by the dysregulated production of cytokines (24,32). Herein, we explored the levels of 8 cytokines associated with macrophage polarization in SLE patients and analyzed the data according to SLE activity or renal involvement. Interestingly, the levels of 4 cytokines (IL-6, IFN-γ, M-CSF, and TNF-α) were related to SLE activity, while only the level of M-CSF was related to renal involvement. In line with other reports (34-36), the predictive role of M-CSF in particular for both SLE activity and renal involvement, cannot be overemphasized.

As an important cytokine for the differentiation and function of the monocyte/macrophage lineage, M-CSF regulates the production and activation phenotype of macrophages (32) and is considered a marker of M2 macrophages (24). Furthermore, M-CSF could be produced by activated macrophages and lymphocytes in addition to mesenchymal cells (37,38). However, unlike M-CSF, GM-CSF plays a minor role in the physiological support of myeloid cells. It is becoming increasingly clear that GM-CSF is a major mediator of tissue inflammation though neutrophils from SLE patients display resistance possibly to the apoptosis-inhibiting effects of GM-CSF (39,40). In this study, the high level of M-CSF, but not GM-CSF, in serum of SLE patients was confirmed to have come mainly from monocytes, which emphasized the involvement of the monocyte/macrophage lineage in SLE progression.

Previous studies have demonstrated that monocyte/macrophage associated cytokines are important participants in SLE. For example, IL-34 has been reported to be correlated with SLE activity (41), the baseline serum levels of IL-6, uPAR and IFN-γ can be used as sensitive biomarkers for disease activity, as well as predictors of remission of lupus nephritis (22,42), the involvement of IL-10 and TNF- genetic variants on SLE appearance, clinical phenotype, and outcome, showed a relevant role of the cytokines in SLE (43). However, neither was correlated with SLE activity or renal involvement in our current study. The differences from previous studies might have been due to the sample size and tissue differences in this research.

There were several limitations to our study. Certain cytokines involved in the study are not specific to

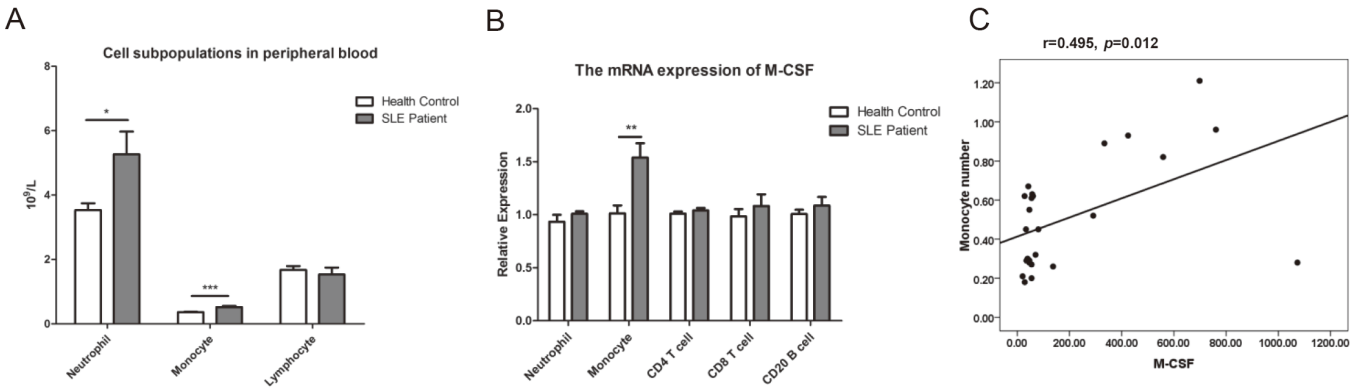

Figure 3 Exploring the source of the high level of M-CSF in SLE patients. (A) The numbers of neutrophils, monocytes, and lymphocytes in peripheral blood were detected by an automated blood cell analyzer in healthy controls and SLE patients. Data in columns are shown as mean ± SEM. *, P<0.05; ***, P<0.001. (B) The cell subpopulations in peripheral blood were labeled with different antibodies. The mRNA expression of M-CSF in sorted candidate cells from both healthy controls and SLE patients was detected by RT-PCR (n=5). The data are representative of three independent experiments. Data in columns are shown as mean ± SEM. **, P<0.01. (C) The correlation between serum M-CSF level and the monocyte numbers was performed by Pearson correlation analysis. A P value <0.05 was considered statistically significant. SLE, systemic lupus erythematosus; SEM, standard error of measurement; mRNA, messenger RNA; M-CSF, macrophage colony-stimulating factor; RT-PCR, real-time polymerase chain reaction.

macrophages; e.g., IL-6 and TNF-α, are produced by multiple cell types and have a variety of cell targets (24,44-46). Our results showed that 6 of 8 cytokines in a panel had high levels in active SLE patients, indicating that cells of the monocyte/macrophage lineage contribute at least partly to SLE progression. Additionally, drug effects were ignored in this study. The use of anti-inflammatory drugs and immunosuppressants, e.g., methotrexate, may have influenced the levels of these cytokines. Further investigations are needed to evaluate the effects of different drugs.

Conclusions

In summary, our study demonstrated that M-CSF is a promising effective marker to evaluate both disease activity and renal involvement in SLE, providing clues for clinical diagnosis and treatment. The high level of M-CSF in SLE patients was predominantly derived from monocytes, revealing the involvement of the monocyte/macrophage lineage in SLE.

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Footnote

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Data Sharing Statement: Available at http://dx.doi.org/10.21037/apm-20-2607

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/apm-20-2607). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All participants provided written informed consent, and the research was approved by the Ethics Committee of Henan Provincial People's Hospital (No: 2019-50). The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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Supplementary

Table S1 The age and gender distribution of healthy controls and SLE patients

Characteristic	Healthy control (n=44)	SLE patients (n=100)	P value
Age, years (mean ± SEM)	33.636±7.409	33.680±12.900	0.983
Gender (male/female)	4/40	10/90	0.865

Continuous variables are presented as mean ± SEM, P values were calculated by unpaired Student's test, as appropriate. The P value for the categorical variable was calculated by χ^2 test, or Fisher's exact test, as appropriate. SLE, systemic lupus erythematosus.

Table S2 Demographics and clinical characteristics in SLE patients

Characteristic	Inactive SLE (n=50)	Active SLE (n=50)	P value
Age, years	33.280±12.265	34.080±13.618	0.758
Gender (male/female)	4/46	6/44	0.505
Disease duration, years	2.2±3.4	2.4±3.2	0.832
Lupus nephritis (n=48)	12/50 (24%)	36/50 (72%)	<0.001***
Laboratory findings			
24-hour urinary protein, g	0.252±0.138	1.771±2.152	<0.001***
Scr, μmol/L	50.240±17.354	76.080±49.168	0.001***
CRP, mg/L	1.112±1.021	18.406±26.868	<0.001***
ESR, mm/h	9.320±7.322	16.760±12.227	<0.001***
dsDNA, IU/mL	92.942±94.086	163.934±102.240	0.030*
C3, g/L	0.831±0.131	0.727±0.254	0.046*
C4, g/L	0.158±0.079	0.146±0.061	0.486

Categorical variables are presented as n (%), P values were calculated by χ^2 test, or Fisher's exact test, as appropriate. Continuous variables are presented as mean ± SEM, P values were calculated by unpaired Student's test, as appropriate (*, P<0.05; ***, P<0.001). SLE, systemic lupus erythematosus; Scr, serum creatinine; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; dsDNA, double-stranded DNA; C3, complement 3; C4, complement 4.

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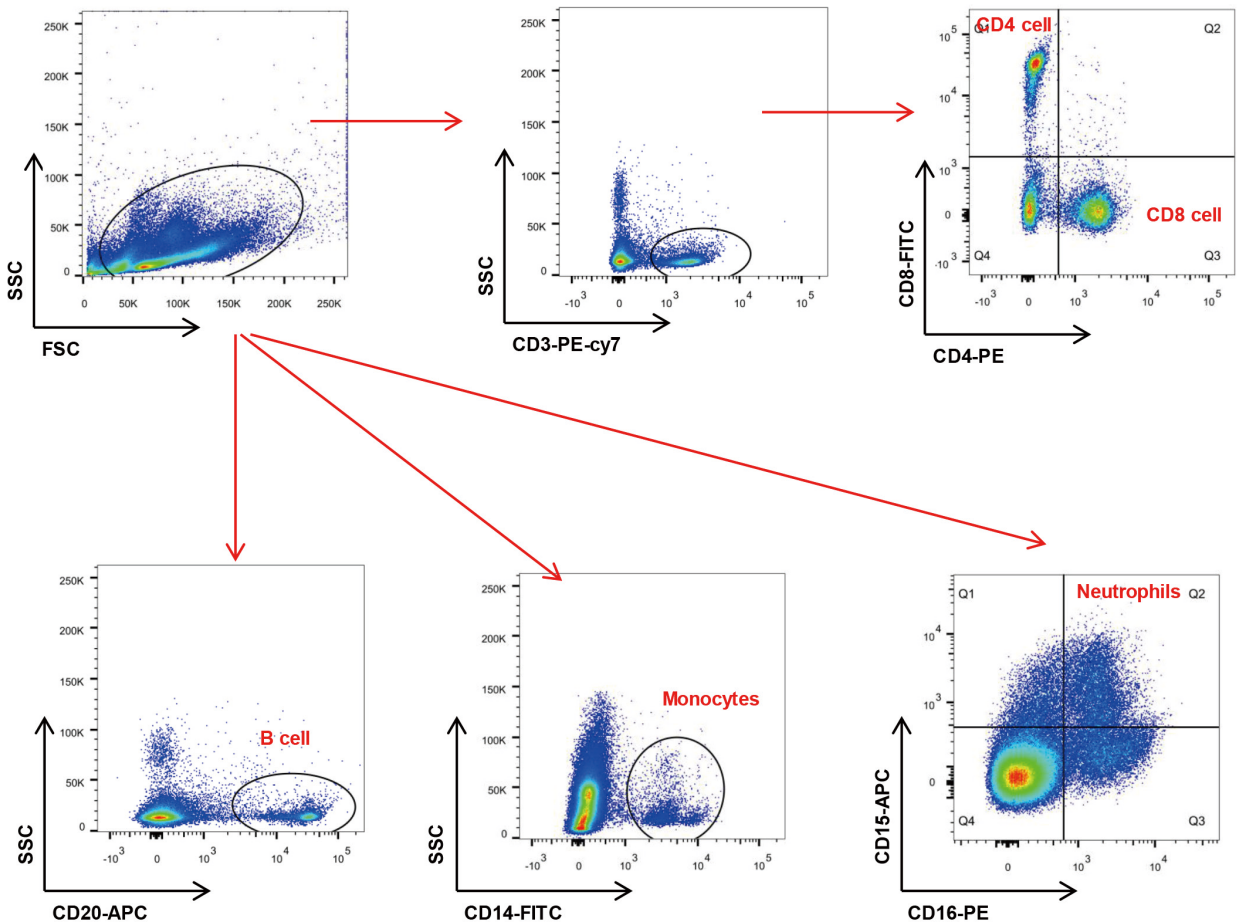

Figure S1 Gating strategies for sorted cells. Gating strategies for CD4 T cells ($CD3^+$ $CD4^+$), CD8 T cells ($CD3^+$ $CD8^+$), B cells ($CD20^+$), monocytes ($CD14^{hi}$) and neutrophils ($CD15^+CD16^+$) are shown.

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