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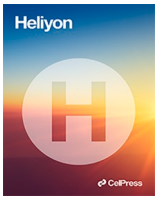

Research article

Serum cytokines profile changes in amyotrophic lateral sclerosis

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ABSTRACT

Background: Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disorder, characterized by progressive limb weakness, dysphagia, dysphonia, and respiratory failure due to degeneration of upper and lower motor neurons. The pathogenesis of ALS is still unclear. Neuroinflammation has been found to be involved in its development and progression. Cytokines play a significant role in the inflammatory process. This study aims to identify novel biomarkers that may assist in the diagnosis of ALS.

Methods: In Fujian Medical University Union Hospital and Huashan Hospital Fudan University, two independent centers, we prospectively recruited 50 ALS patients, and 41 healthy controls (25 ALS and 26 controls in the first stage and 25 ALS and 15 controls in the validation stage). An 18-plex Luminex kit was used to screen the serum cytokines levels in the first stage. Commercial ELISA kits were used to measure the levels of target cytokines in the validation stage. A single-molecule array HD-X platform was applied to assess the levels of serum neurofilament light chain (NFL).

Results: The levels of serum IL-18 were markedly increased in patients with ALS in the first stage ($p = 0.016$). The ROC curve showed an area under the curve at 0.695 (95% CI 0.50–0.84) in distinguishing ALS patients from healthy controls. The IL-21 was decreased in elderly patients when grouped by 55 years old (the medium age). Furthermore, the IL-5, IL-13, IL-18, and NFL had a positive relationship with the disease progression of ALS. We also found that serum IL-18 was markedly increased in ALS patients in the validation stage (167.67 [148.25–175.59] vs 116.44 [102.43–122.19]pg/ml, $p < 0.0015$).

Conclusion: In this study, we identified systemic cytokine profile changes in the serum of ALS patients, especially the elevated IL-18, as well as the decreased IL-21 in elder patients. These changes in serum cytokine profiles may shed new light on an in-depth understanding of the immunopathogenic characteristics of ALS.

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1. Background

Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disorder characterized by progressive limb weakness, dysphagia, dysphonia, and respiratory failure due to the degeneration of upper and lower motor neurons [1]. ALS is diagnostically difficult and rapidly progressive, with most patients dying within 3–5 years of onset [2]. As an incurable disorder, there are only three drugs that have been approved for the clinical use of sporadic ALS, including riluzole, edaravone, and AMX0035 [3], which only alleviate the progression of ALS. Nevertheless, its etiology and mechanism haven't been thoroughly elucidated. The genetic [4] and environmental [5] factors were proven to play crucial parts in the pathogenesis of ALS. There is high heterogeneity among affected individuals as well. The unclear pathogenesis and high heterogeneity make it difficult to recognize, particularly in the early stages. Therefore, new biomarkers are needed for early diagnosis and disease course monitoring for ALS [6].

Increasing evidence has demonstrated the immune system contributes to the pathogenesis and progression of ALS [7,8]. Neuroinflammation, characterized by the activation of astrocytes and microglia, as well as the infiltration of macrophages and lymphocytes into the spinal cord and brain of ALS mouse models and patients, has been documented in multiple studies [9,10]. Furthermore, it has been reported that leukocytes, neutrophils, and monocytes increased in patients with ALS [11], and amplifying the regulatory T lymphocytes (Tregs) population can slow down the progression rates of ALS during both early and later stages [12,13]. In addition, in ALS patients, some cytokines, which participate in the inflammatory process and immune response, are altered in the cerebrospinal fluid (CSF) or serum [14,15]. For example, the serum and/or CSF levels of tumor necrosis factor α (TNF-α) [16], interleukin-18 (IL-18) [17], and monocyte chemoattractant protein-1 [18] have been noticed to be increased in ALS patients.

The neurofilaments, in which neurofilament light chain (NFL) was included, have been proposed to be potential biomarkers for ALS in diagnosis [19], prognosis prediction [20–22], and pharmacodynamic assessment [23]. The NFL in serum and CSF [15] has been found to increase in ALS patients compared to healthy controls (HC) and patients with other neurodegenerative diseases [24]. Otherwise, levels of NFL were rapidly elevated in the early stage of ALS and kept stable over the course of the disease, with a significant association between baseline NFL and rate of disease progression [24,25].

Limited studies have evaluated the correlation between serum cytokine profile and serum NFL levels. This study aims to investigate the serum cytokine profile in the Chinese ALS population. Furthermore, we expect to find a potential correlation between these cytokines and NFL, a sensitive marker of neurodegeneration in ALS. In this study, we collected samples from two independent neuromuscular referral centers and analyzed the levels of a series of serum cytokines from ALS patients in the first stage, which was used for the preliminary investigation of cytokine profiles in serum. Then in the validation stage, we further validated the target cytokines that may have potential significance in the first stage.

2. Methods

2.1. Participants and sampling

We recruited a total of ninety-one participants (25 ALS patients and 26 healthy controls in the first stage, 25 ALS patients and 15 healthy controls in the validation stage) from Fujian Medical University Union Hospital and Huashan Hospital Fudan University, two independent neuromuscular referral centers. Accompanying demographics (such as sex and age) and clinical characteristics (age of disease onset, disease duration, site of onset, and the ALSFRS-R score), blood samples in the first stage were obtained from February 2019 to November 2019, and the blood samples in the validation stage were collected from March 2020 to April 2021 for a biomarker study. According to the revisited EI Escorial criteria, patients diagnosed with definite, probable, and clinically probable laboratory-supported ALS were recruited [26]. We used the Revised Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R) to assess the clinical severity of the disease. The decreasing rate of ALSFRS-R was calculated at the visit as 48 minus the baseline ALSFRS-R score, divided by the disease duration from the onset of the symptoms (months). The exclusion criteria for ALS patients were the following. First, patients were verified as having familial ALS. Second, patients with confounding conditions affect the immune system, including a recent infection or a history of autoimmune disease. Third, patients received noninvasive or invasive ventilation for more than 20 h per day. Last, patients withdraw informed consent. The healthy controls were selected to be individuals aged 40 to 70 (mostly 50–60 years), with no recent infection, no history of autoimmune diseases, no history of neurodegenerative diseases, no family history of ALS, and voluntarily enrolled in this study. The sample size was determined according to the formula "Sample size for adequate sensitivity/specificity" [27] and the sensitivity and specificity of serum NFL were measured by SIMOA in ALS patients in a previous study [28]. The study was approved by the ethics committee of both hospitals (No.2019GZR032 and No.2019–603). Informed consents were obtained from all participants.

2.2. Sample analysis

Serum was aliquoted within 2 h from the collection and frozen at −80 °C. An 18-plex *Luminex* kit from eBioscience (Hunam ProcartaPlex Panel #EPX180-12165-901) was used to measure serum cytokines levels on the Bio plex 200 platform (BioRad, the United States) according to the manufacturer protocols in the first stage, including Th1/Th2 cytokines (GM-CSF, IFN γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-12p70, IL-13, IL-18, TNF α) and Th9/Th17/Th22/Treg cytokines (IL-9, IL-10, IL-17A (CTLA-8), IL-21, IL-22, IL-23, IL-27). In the validation stage, we focused on the target cytokines to further explore their clinical value. Commercial enzyme-linked immunosorbent assay (ELISA) kits (RayBio® Human IL-18 ELISA Kit ELH-IL-18 and RayBio® Human IL-21 ELISA Kit ELH-IL21)

were used to measure the target cytokines levels according to the manufacturer's instructions in the validation stage (Table 1). Measurement of serum NFL was performed on an ultra-sensitive Single-molecule array (SIMOA) HD-X platform with a commercial NFL assay kit (Cat No: 103186, Quanterix the United States) to assess the levels of serum NFL. Technical replicates were run for all samples, and all values were within the linear range of the determination.

2.3. Statistical analysis

R 4.2.2 and GraphPad Prism9 were used to perform statistical analysis. The data was tested by the Normality Test in R. If the data was normally distributed, it was displayed as mean [standard deviation (SD)] and compared using the T-test. If the data was not normally distributed, it was displayed as median [lower quartile, upper quartile] and compared using the Wilcoxon test. The correlation was calculated using Spearman correlation analysis. The statistically significant difference was indicated by $p < 0.05$. And used the Receiver operating characteristic curve (ROC) analysis to determine the cut-off values and assess the sensitivity and specificity of parameters, and the performance of IL-18 was assessed by the area under the curve (AUC).

3. Result

3.1. Demographics

The demographic characteristics of the study population were summarized in Tables 1 and in which the ALS patients and healthy controls were similar. Most of the patients are in the early stage of the disease (median of 41 for ALSFRS-R score) and the site of onset was the limb.

3.2. Comparison of cytokine levels between ALS and HC

The cytokines levels in both stages were summarized in Table 2. We used an 18-plex *Luminex* kit to detect 18 cytokines in serum in the first stage. We only found circulating levels of IL-18 were significantly increased in patients with ALS (13.31 [10.10–16.36] vs 9.43 [6.63–13.32] pg/ml, $p = 0.016$, Fig. 1A). The ROC curve analysis showed an AUC at 0.695, the cut-off value was 9.435. The serum IL-18 levels ≥9.435 were considered positive. At this value, the Luminex gave 84% (21/25) sensitivity and 50% specificity (13/26) respectively. (Fig. 2A). What's more, in the first stages, IL-18 was positively correlated with the decreasing rate of ALSFRS-R ($p = 0.012$, Fig. 3), indicating its potential role in progression prediction. We also explored the effect of age and gender on the levels of cytokines as well. When grouped by the cut-off level (55 years old of stage 1), the levels of IL-21 were significantly decreased in elder ALS patients compared to both younger ALS patients ($p = 0.033$, Fig. 4) and elder HC ($p = 0.034$, Fig. 4). Furthermore, the levels of GM-CSF, IL-1b, IL-2, IL-4, IL-12, IL-13, and TNF-α were higher in female ALS patients ($p < 0.05$, Fig. 5A–G).

Correlation analyses were performed between cytokines, NFL, and the decreasing rate of ALSFRS-R. The NFL was correlated with the ALSFRS-R decrease score per month (Fig. 6A). We also noticed that IL-5 (Fig. 6B), IL-13 (Fig. 6C), and IL-18 (Fig. 3) were correlated with the ALSFRS-R decrease score per month. Unfortunately, none of the correlation between NFL and cytokines was found in these two stages.

Based on the serum cytokine data, in the validation stage, we used commercial ELISA kits to assess the IL-18 and IL-21 levels. The part of IL-21 failed to be detected. We found the IL-18 levels were still remarkably increased in patients with ALS (167.67 [148.25–175.59] vs 116.44 [102.43–122.19]pg/ml, $p < 0.001$[5], Fig. 1B), and the ROC curve analysis showed an AUC at 0.859, the cut-off value was 124.120. The serum IL-18 levels ≥124.120 were considered positive. At this value, the ELISA gave 100% (25/25) sensitivity and 80% specificity (12/15) respectively. (Fig. 2B). Because of the low precision of ELISA, no efficient values were concluded for other cytokines in the validation stage.

Table 1
Demographics.

	Stage 1			Stage 2		
	ALS(n = 25)	HC(n = 26)	p value	ALS(n = 25)	HC(n = 15)	p value
Sex, male, no. (%)	14 (56.00)	12 (46.15)	0.493	19 (76.00)	10 (66.67)	0.539
Age, years	55.00 [39.00–54.75]	55.00 [50.25–57.00]	0.924	61.00 [51.00–66.00]	56.00 [51.50–61.00]	0.567
Age of onset, years	54.00 [38.00–60.00]	–	–	60.00 [50.00–65.00]	–	
Disease duration, months	16.00 [10.00–20.00]			12.00 [9.00–6.00]		
Site onset, no. (%)						
Bulbar	4 (16.0)	–		5 (20.0)	–	
Limb	21 (84.0)	–		20 (80.0)	–	
ALSFRS-R	41.00 [35.00–40.00]	–		42.00 [39.00–44.00]	–	
ALSFRS-R decrease rate, %	0.58 [0.38–1.00]	–		0.53 [0.44–0.70]	–	

ALS, Amyotrophic Lateral Sclerosis; ALSFRS-R, Amyotrophic Lateral Sclerosis.

Table 2
The results of cytokines and NFL (median [lower quartile, upper quartile]).

	Stage 1			Stage 2		
	ALS	HC	p value	ALS	HC	p value
NFL	81.12 [49.97–114.16]	–	–	74.18 [41.20–115.28]	–	–
IL-1beta	4.36 [2.53–6.61]	3.45 [2.53–4.36]	0.195	–	–	–
IL-2	23.99 [23.99–46.07]	23.99 [18.69–35.83]	0.184	–	–	–
IL-4	27.35 [23.62–38.03]	27.68 [27.35–34.54]	0.617	–	–	–
IL-5	1.42 [0.00–9.36]	0 [0.00–4.25]	0.065	–	–	–
IL-6	1.60 [0.00–4.80]	1.60 [0.00–4.40]	0.454	–	–	–
IL-9	4.59 [3.58–9.45]	3.58 [2.24–7.53]	0.090	–	–	–
IL-10	0.92 [0.75–1.10]	0.38 [0.00–0.97]	0.161	–	–	–
IL-12p70	0.17 [0.06–0.50]	0.13 [0.02–0.28]	0.261	–	–	–
IL-13	0.80 [0.00–4.26]	1.43 [0.00–2.05]	0.244	–	–	–
IL-17A	4.50 [0.00–14.40]	2.25 [0.00–8.55]	0.311	–	–	–
IL-18	13.31 [10.10–16.36]	9.44 [6.63–13.31]	0.017*	167.67 [148.25–175.59]	116.44 [102.43–122.19]	<0.001*
IL-21	2.38 [1.66–3.71]	3.41 [1.84–3.77]	0.432	0.00 [0.00–3263.87]	0.00 [0.00–0.00]	0.231
IL-22	15.48 [13.44–18.51]	15.48 [13.44–19.13]	0.872	–	–	–
IL-23	4.91 [0.00–5.90]	4.29 [3.66–4.91]	0.848	–	–	–
IL-27	6.78 [0.00–11.29]	2.26 [0.00–6.78]	0.174	–	–	–
IFN	11.75 [10.22–13.29]	12.05 [10.22–13.44]	0.985	–	–	–
TNF-α	1.97 [1.97–5.99]	1.97 [0.33–3.39]	0.159	–	–	–
GM-CSF	42.16 [42.16–60.56]	42.16 [34.79–54.88]	0.053	–	–	–

Median [lower quartile, upper quartile] concentrations of cytokines and NFL were reported (pg/ml). ALS, Amyotrophic Lateral Sclerosis; ALSFRS-R, Amyotrophic Lateral Sclerosis.

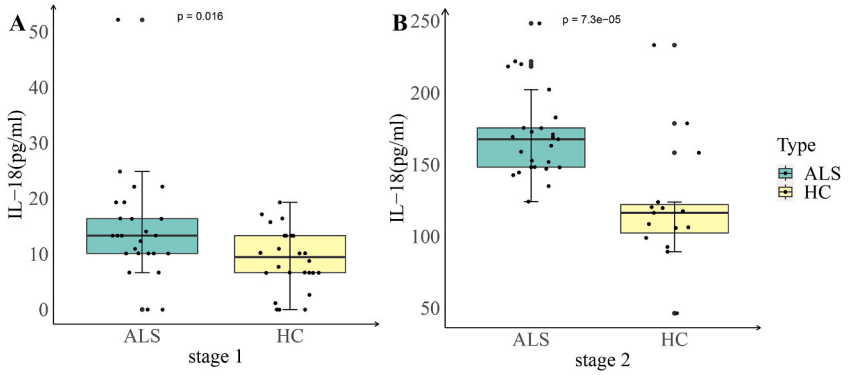

Fig. 1. IL-18 between ALS and HC. (A) The level of IL-18 in serum of ALS patients and healthy controls in first stage measured by an 18-plex Luminex kit; (B) The level of IL-18 in serum of ALS patients and healthy controls in the validation measured by commercial ELISA kit.

4. Discussion

Given the unclear pathogenesis of ALS and the lack of effective biomarkers, it is difficult to identify in the early stages until symptoms like limb weakness and dysarthria. Therefore, the search for new biomarkers to assist in ALS diagnosis is extremely important. The inflammation mediated by cytokines has been suggested to play an important role in the onset and progression of neurodegeneration diseases, such as Amyotrophic lateral sclerosis, Alzheimer's disease (AD), and Parkinson's disease (PD) [18]. Some cytokines were reported to have intimate correlations with neurodegeneration disease and had the potential to become biomarkers of these diseases [18]. The TNF-α and IFNγ were elevated in the plasma of patients with PD, which may contribute to its inflammatory process [29]. A meta-analysis indicated the IL-6, TNF, IL-1β, IL-2, IL-10, C-reactive protein, and RNATES were significantly elevated in patients with PD, these results enhance our knowledge of the participation of inflammatory response, especially the T cell-related cytokines, in PD [30]. In addition, it was suggested that IL-1beta, IL-6, IL-12, IL-18, TNFα, and TGF-β were significantly higher in peripheral blood of AD, while the IL-4, IL-8, and IL-10 have no significant differences between AD and controls [31]. The serum IL-1α and IL-10 were significantly lower in patients with AD, while the TNFα was increased, these altered in cytokines indicated the involvement of neuroinflammatory in AD [32]. In view of the contribution of peripheral inflammatory response in ALS [33,34], there may be important cytokine biomarkers correlated with ALS. Previous studies found that the increase in Th1 cells and Th17 cells [35], which evert proinflammation and upregulate inflammation, and the decrease in Th2 cells, Tregs [36] that play a suppressive role in inflammation are associated with the disease progression of ALS. They may affect ALS through the secreting of cytokines. In this study, we investigated the serum Th1/Th2/Th9/Th17/Th22/Treg cytokines in the first stage with a Luminex multiplex kit, and the cytokines that may have potential significance in the first stage were picked up for further investigation in the validation stage. Our study was

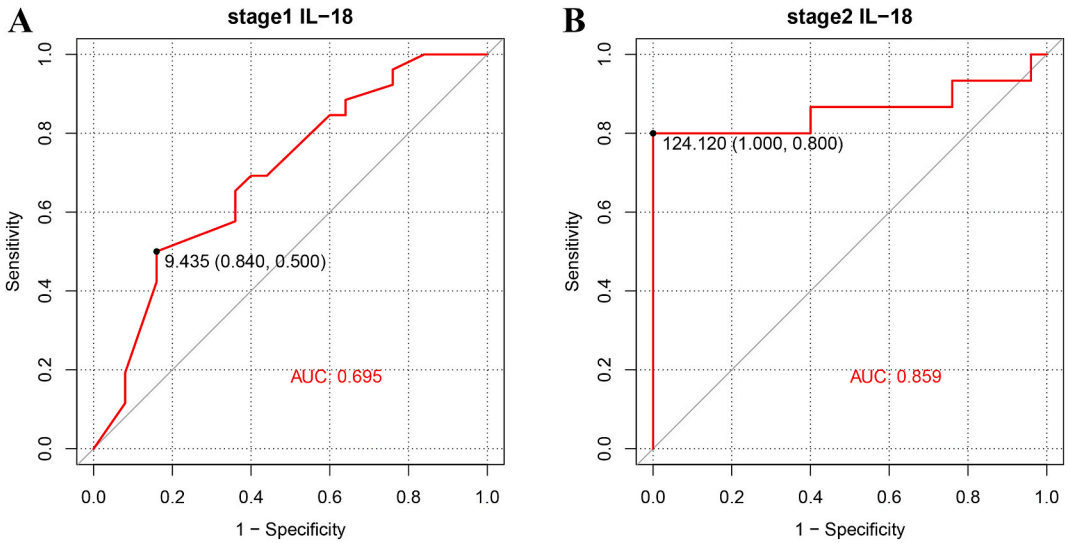

Fig. 2. Receiver operating characteristic (ROC) curves of serum IL-18 between ALS and HC in both first stage(A) and validation stage(B).

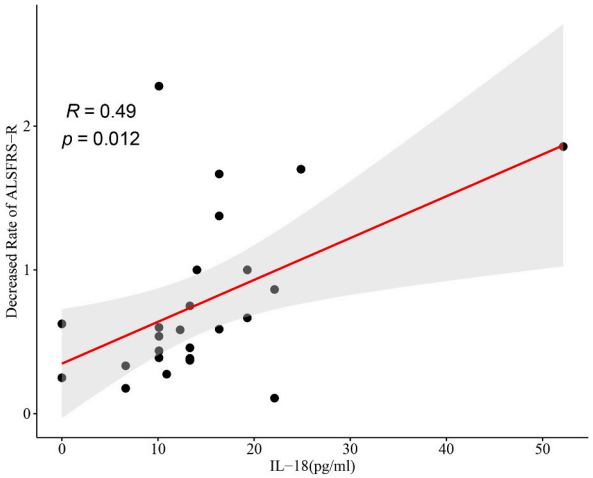

Fig. 3. Correlation between IL-18 and the decrease rate of ALSFRS-R. The correlation between IL-18 and decreased rate of ALSFRS-R in the first stage.

intended to extend the evidence for the role of these biomarkers in assisting in ALS diagnosis and evaluation of progression by quantifying cytokine profiles in serum samples from ALS patients and analyzing the correlation between biomarkers and ALS progression.

Previous studies have found the activation of central and peripheral immune cells in ALS [37,38], as well as the elevation of cytokines like TNF-α, IL-1β, IL-2, IL-6, and IL-18 [15,39]. For example, the Th17 cells may induce degeneration of motor neurons through the secreting of IL-17A in ALS [33]. Therefore, cytokines have the potential to be biomarkers for ALS differential diagnosis, progression, and prognosis prediction. A meta-analysis suggested that TNF-alpha, TNF receptor 1, IL-1beta, IL-6, IL-8, and vascular endothelial growth factor were significantly increased in the blood of ALS patients compared to controls, these results extended the role of inflammatory response in ALS and the potential of circulating cytokines as diagnosis biomarkers for ALS [40]. In this study, compared to HC, we detected a remarkable elevation of proinflammatory cytokine IL-18 in the serum of ALS. As a member of the IL-1 family, IL-18 recognizes IL-18 receptor α and β and then forms a high-affinity heterodimeric complex, that induces multiple inflammatory cytokines [41,42]. In the brain of $SOD1_{G93A}$ rats, the NLRP-3 inflammasome, which is of great importance in inducing active caspase-1, was found in turn cleaving the precursor molecules of IL-18 and resulting in an increase in IL-18 levels [43]. Moreover, IL-18 was found significantly elevated in the CSF of ALS patients [15], correlated with this disease [17]. What's more, whole-genome sequencing indicates that IL-18R accessory protein is associated with a reduced risk of ALS [44]. Together with our results, the increased IL-18 in serum supported the activation of inflammasome-mediated mechanisms in ALS [17,45]. Besides, the association between IL-18 and the decreasing rate of ALSFRS-R suggested its potential role in recognizing patients and predicting their prognosis.

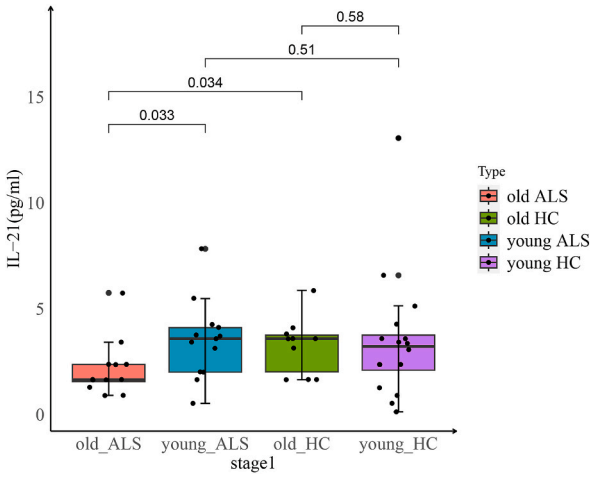

Fig. 4. IL-21 in serum of ALS and HC group by 55-age-old (the medium age). The level of IL-21 in serum of ALS patients and healthy controls in the first stage group by gender.

There was no correlation between age and cytokine levels, but when divided into two groups according to medium age, the levels of IL-21 were remarkably decreased in elder ALS patients compared to both younger ALS patients and healthy controls. Meanwhile, no difference was noticed in IL-21 levels between older HCs and younger HCs. IL-21, a cytokine with both proinflammatory and anti-inflammatory activities, is mainly produced by $CD4^+$ T cell subsets [46], and it was found can inhibit the Th1 differentiation and the pathogenic Th1/Th17 cell initiation [47]. Interestingly, studies had found the peripheral immune profile in ALS was transferred to the proinflammatory immune response that was mediated by T helper (Th) 1/Th17 cells, correlated to disease progression and severity [33,35]. Otherwise, a previous study had reported the dual-specific phosphatase 4 increased in CD4 memory T cells from older individuals, shortened the expression of CD40-ligand and inducible T-cell costimulatory, and decreased the production of IL-4, IL17A, and IL-21, which may be associated with adaptive immunodeficiency in elderly individuals and partly explain why they are less resistant to infections [48]. In patients with AD, elevated sTREM2 was found to correlate with tau pathology and negatively correlate with cerebrospinal fluid IL-21 levels, suggesting that reduced IL-21 may be associated with aging [49]. Thus, we speculated that the decline of IL-21 in elder ALS patients was potentially correlated with a different immune status during the aging process. Moreover, previous studies found there were differences in cytokines between genders [50], which may be the result of the influence of estrogen [51]. In consistency with previous research, we observed multiple cytokines were higher in female ALS patients, such as GM-CSF, IL-1b, IL-2, IL-4, IL-10, IL-12, IL-13, and TNF-α. The positive correlations of the decreasing rate of ALSFRS-R and IL-5, IL-13, and IL-18 suggested they may be useful in predicting the disease progression rate.

The NFL is a cytoskeletal protein in large-diameter axons of neurons [52]. Studies have found that NFL increased in serum and CSF of patients with ALS [22], which could reflect axonal degeneration and neuron damage [25]. Given the role in diagnosis [25], progression prediction [53], and pharmacodynamic evaluation [23], NFL in both serum and CSF were proposed as potential ALS biomarkers. Baseline serum NFL concentration was capable of predicting ALSFRS-R slope and survival [23]. In our study, we found the NFL was positively associated with the decreasing rate of ALSFRS-R, this is consistent with previous results [25]. Unfortunately, none of the correlation between NFL and cytokines was found in these two stages. The possible reasons may be as follows. First, serum inflammatory factors may create an inflammatory microenvironment but are not the direct cause of motor neuron injury. Second, the levels of inflammation from our cross-sectional study might be not sufficient to reflect long-term outcomes and require longitudinal follow-up. Last, studies have shown that the level of NFL rises after the onset of ALS, then remains stable after 12 months [54]. In the present study, most patients were in the early stage with a median ALSFRS-R score of 41, which may cause the levels of cytokines not parallel to the NFL.

Other limitations in our study might lie in the different methods used in these two stages, and the cut-off value of these two methods quite different, which made the results barely compatible. In addition, the sensitivity and specificity of these two methods were different as well, which made the results of these two methods cannot be combined in the present study. Moreover, some of the results of cytokines, especially those measured by IL-21 Human ELISA kits, were under the detection limit. This may be because the background of the standard curve was relatively high, and the low target signal was covered by the high background, resulting in the sample value not being detected, which suggested that some commercial ELISA kits were not sensitive enough for the detection of serum cytokines. The ELISA method, which is commonly used, has high specificity but low sensitivity. More sensitive detection methods, like single molecule array (SIMOA), may contribute to reducing the cut-off value of cytokine in ALS diagnosis, and contribute to the early diagnosis and intervention of ALS.

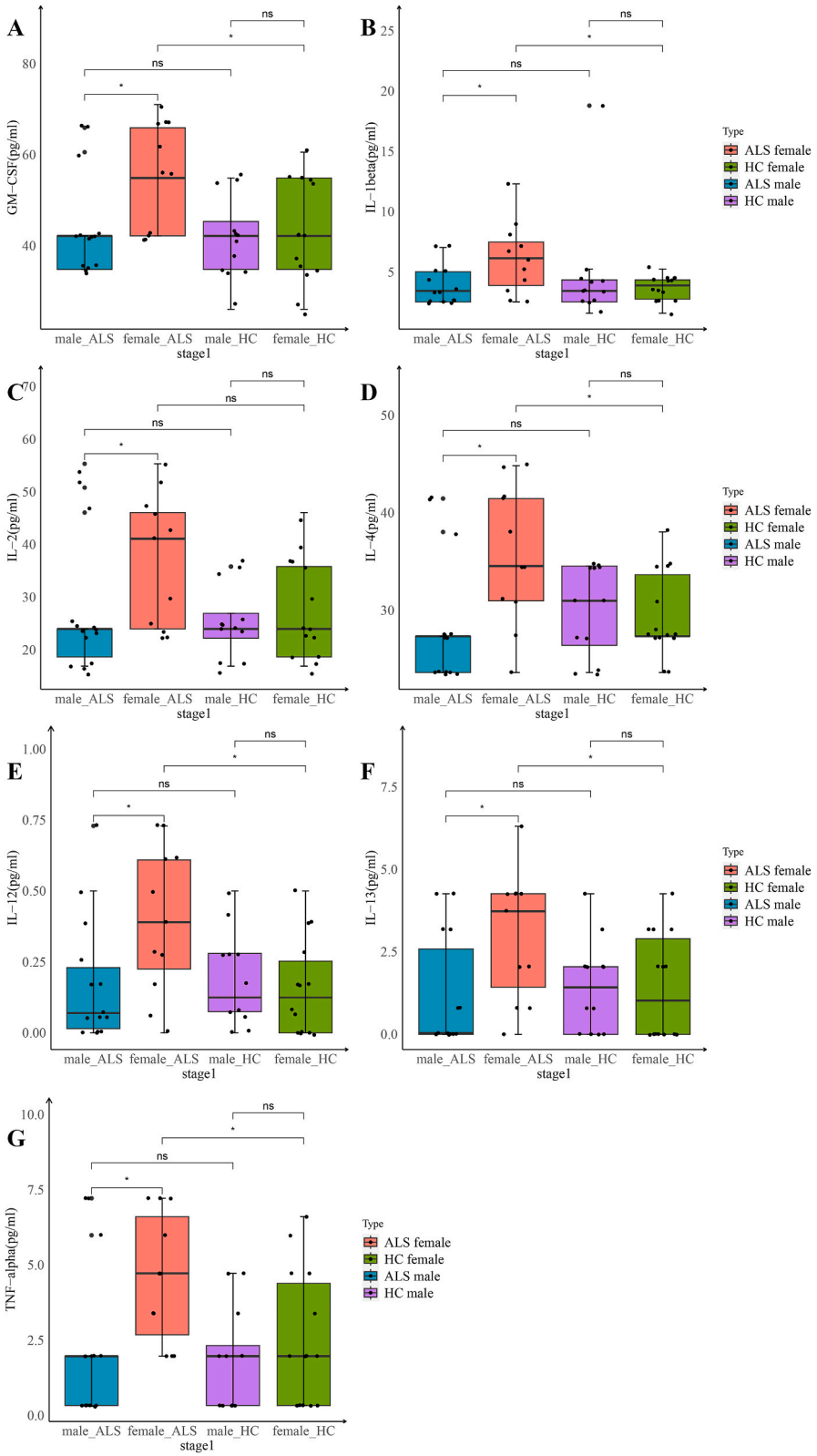

Fig. 5. GM-CSF, IL-1b, IL-2, IL-4, IL-12, IL-13 and TNF-α of ALS and HC group by gender. The level of GM-CSF(A), IL-1b(B), IL-2(C), IL-4(D), IL-12(E), IL-13(F), TNF-α(G) in serum of ALS patients and healthy controls in the first stage group by gender.

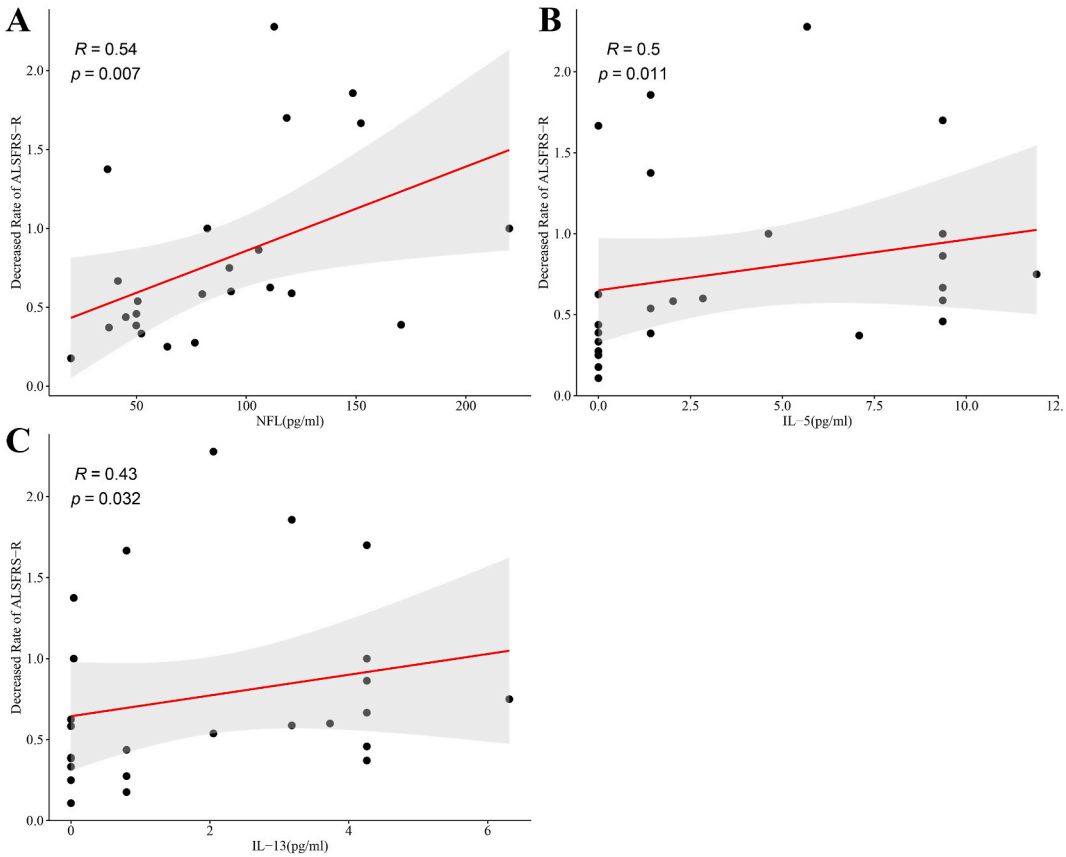

Fig. 6. Correlation between NFL, IL-5, IL-13 and decrease rate of ALSFRS-R. The correlation between NFL(A), IL-5(B), IL-13(C) and decreased rate of ALSFRS-R in the first stage.

5. Conclusions

In summary, for the purpose of exploring a new biomarker to assist diagnosis, prognosis, and response assessment, we measured 18 cytokines in serum from patients of ALS and healthy controls. Our data supported the participation of inflammatory mechanisms in ALS pathogenesis, particularly in disease progression. Specifically, the IL-18 in serum, which was significantly elevated in patients of ALS in both stages, appears to be a marker of assisting in diagnosis. Furthermore, the positive correlation between IL-18 and the decreasing rate of ALSFRS-R in the first stage suggested it is possible to be utilized to predict the rate of progression and survival. Meanwhile, we observed a decline of IL-21 in elderly ALS patients which might be correlated with a different immune status. Our results might shed new light on discovering inflammation biomarkers in ALS.

Ethics declarations

The study was reviewed and approved by the ethics committees of Fudan University Huashan Hospital and Fujian Medical University Union Hospital, with the approval number: 2019–603 and 2019GZR032. All participants/patients (or their proxies/legal guardians) provided informed consent to participate in the study. The Declaration of Helsinki and Good Practice Guidelines were followed in all study procedures.

Consent for publication

Not applicable.

Data availability statement

The authors do not have permission to share data due to Chinese laws. However, anonymized data can be made available to qualified investigators on request from the corresponding author.

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CRediT authorship contribution statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Abbreviations

ALS	Amyotrophic Lateral Sclerosis
CSF	Cerebrospinal fluid
TNF-α	Tumor necrosis factor α
IL	Interleukin
NFL	Neuro filament light chain
ALSFRS-R	Revised Amyotrophic Lateral Sclerosis Functional Rating Scale
ELISA	Enzyme-linked immunosorbent assay
SIMOA	Single-molecule Array
HC	Healthy controls
SD	Standard deviation
ROC	Receiver operating characteristic curve
AUC	Area under the curve
AD	Alzheimer's disease
PD	Parkinson's disease

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