Original Research Article

Cerebrospinal fluid biomarkers implicated in the pathogenesis of anti-neutrophil cytoplasmic antibody-related hypertrophic pachymeningitis

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Abstract

Objective: Hypertrophic pachymeningitis (HP) related to anti-neutrophil cytoplasmic antibody (ANCA) is the most frequently seen immune-mediated HP. We investigated cerebrospinal fluid (CSF) biomarkers related to the pathogenesis of ANCA-related HP (ANCA-HP).

Methods: The levels of B-cell activation factor of the tumor necrosis factor family (BAFF), a proliferation-inducing ligand (APRIL), and transforming growth factor beta 1 (TGF-β1) in the CSF were compared between patients with ANCA-HP (n = 12), other types of immune-mediated HP (other HP; n = 12), multiple sclerosis (MS; n = 14), and non-inflammatory neurological disorders (NIND; n = 10). In addition, we evaluated whether ANCA would be detected in CSF.

Results: CSF levels of BAFF, APRIL, and TGF-β1 were significantly increased in ANCA-HP and other HP. In particular, BAFF and APRIL levels were significantly correlated with the IgG index in ANCA-HP. In other HP, BAFF and APRIL levels were significantly correlated with cell counts and protein levels in CSF. Of 12 patients with ANCA-HP, the CSF of 7 patients (58%) tested positive for myeloperoxidase (MPO)- or proteinase 3 (PR3)-ANCA, while none of the CSF samples from other HP, MS, or NIND patients tested positive.

Conclusion: The levels of BAFF, APRIL, and TGF-β1 may serve as useful CSF biomarkers for assessing the disease activity of immune-mediated HP. Moreover, BAFF and APRIL in the CSF may be implicated in the pathogenesis of ANCA-HP via promoting autoreactive B cells, while detecting

MPO- or PR3-ANCA in the CSF may be found in some patients with ANCA-HP.

Key-points:

- CSF BAFF, APRIL, and TGF-β1 levels increase significantly in immune-mediated HP.
- CSF BAFF and APRIL levels are significantly correlated with IgG index in ANCA-HP.
- Detection of MPO- or PR3-ANCA in the CSF is found in some patients with ANCA-HP.
- BAFF, APRIL, and ANCA in the CSF may be implicated in the pathogenesis of ANCA-HP.

Keywords: hypertrophic pachymeningitis; anti-neutrophil cytoplasmic antibody; cerebrospinal fluid; B-cell activation factor of TNF family; a proliferation-inducing ligand; transforming growth factor beta 1

Introduction

Hypertrophic pachymeningitis (HP) is characterized by intracranial or spinal thickening of dura mater with pathological interstitial tissue fibrosis and inflammatory cells infiltration are pathologically involved [1]. This disorder usually causes headaches, cranial neuropathies, seizures, motor ataxia, consciousness disturbance, or spinal dysfunction. The development of HP is usually attributed to the immune-mediated disorders, intracranial infections, or neoplasms. The autoimmune diseases causing HP include anti-neutrophil cytoplasmic antibody (ANCA) associated vasculitis (AAV), IgG4-related disease (IgG4RD), sarcoidosis, and other rheumatic disorders. Notably, ANCA-related HP (ANCA-HP) occurs in 30–50% of patients with immune-mediated HP [1-3]. Additionally, some patients with immune-mediated HP are classified as idiopathic HP whose underlying disease is unknown. It has been demonstrated that approximately 15% of ANCA-HP cannot be classified as definite AAV [2,3]; moreover, HP often occurs as the initial clinical episode of AAV [4], indicating that it is difficult to fulfill the definite diagnostic criteria of AAV unless the pathological evidence or another surrogate marker exist. Furthermore, there is a concern that patients with central nervous system (CNS)-limited AAV may be classified as idiopathic HP [5], and IgG4RD also has similar histopathological and immunological aspects to idiopathic HP except for infiltration of IgG4 positive plasma cells [6,7]. Therefore, it is necessary to find useful biomarkers for diagnosing ANCA-HP even in early phase of the disease.

We previously reported increased levels of B-cell activation factor of the tumor necrosis factor family (BAFF) and a proliferation-inducing ligand (APRIL), which are members of the tumors necrosis factor superfamily, in the cerebrospinal fluid (CSF) of patients with ANCA-HP [2]. BAFF and/or APRIL is also expressed in the CSF of patients with active phase of neuro-Behçet disease (Neuro-BD), neuropsychiatric systemic lupus erythematosus (NPSLE), multiple sclerosis (MS), and neuromyelitis optica (NMO) [8-11]. However, there is no evidence for elevated levels of BAFF and APRIL in the CSF from patients with other types of immune-mediated HP (other HP). In addition, the type 1 helper T cells (Th1) seem to be predominant in ANCA-HP, while the Th2 cells are predominant in IgG4RD-related HP (IgG4-HP) and idiopathic HP [3,7], suggesting that the CNS immune environment is notably different in ANCA-HP and other HP. Conversely, fibrotic formation is common to the histology of thickened dura mater in ANCA-HP and other HP [1,3,12], whereas a definite biomarker for that is still uncertain. Therefore, specific CSF biomarkers associated with disease activity and pathogenesis of ANCA-HP must be found.

In the present study, we analyzed CSF biological markers which are implicated in the pathogenesis of ANCA-HP, and compared those in ANCA-HP with other HP, MS, or non-inflammatory neurological disorders (NIND).

Material and methods

Patients and samples

We recruited 24 Japanese patients with HP related to autoimmune disorders who were examined and treated at our hospital. HP was diagnosed by T1-weighted magnetic resonance imaging (MRI) with gadolinium-enhancement. Patients with intracranial hypotension, malignancy, or infection, which might cause thickening of dura mater based on MRI findings, were excluded. Twelve patients were categorized as ANCA-HP (mean age, 67 years; 7 men and 5 women), and the other 12 patients were categorized as other HP (mean age, 63 years; 9 men and 3 women) including IgG4-HP (n = 4), sarcoidosis-related HP (n = 2), relapsing polychondritis (RP)-related HP (n = 1), and idiopathic HP (n = 5). Nine of the patients with ANCA-HP and one case of RP-related HP were described in the previous study [2], review [13] and a case report [14] published by our department. Patients were diagnosed with ANCA-HP when they were either diagnosed with AAV according to the criteria of the Chapel Hill Consensus Conference [15] and/or the consensus algorithm proposed by the European Medicines Agency [16], or tested positive for myeloperoxidase (MPO) or proteinase 3 (PR3) -ANCA without other autoimmune diseases. Of the patients with ANCA-HP, 9 were diagnosed with granulomatosis with polyangiitis (GPA) and one with microscopic polyangiitis; in contrast, 2 patients who were PR3- or MPO-ANCA positive were categorized as unclassifiable. Patients with IgG4-HP or sarcoidosis-related HP were categorized based on the prevalent diagnostic criteria for IgG4RD [17], or sarcoidosis [18], respectively. The disease controls consisted of 14 patients with MS (mean age, 38 years; 3 men

and 11 women) and 10 patients with NIND (mean age, 56 years; 5 men and 5 women). Of patients with MS, 10 were classified as relapsing-remitting and 4 as secondary progressive. Their mean expanded disability status score was 4.1 ± 1.6. Patients with NIND included parkinsonian syndrome (n = 4), Parkinson disease (n = 2), progressive supranuclear palsy (n = 1), dementia (n = 1), amyotrophic lateral sclerosis (n = 1), and autonomic neuropathy (n = 1). CSF and serum samples were taken from the patients with ANCA-HP, other HP, MS, and NIND. All patients with ANCA-HP, other HP, or MS required immunosuppressive therapy for treating disease-related neurological symptoms, which were administered after CSF and serum sampling. CSF cell counts, protein levels, and IgG index ([CSF/serum IgG ratio]/[CSF/serum albumin ratio]) were examined, even though IgG index was not measured in NIND samples. The CSF and serum samples were immediately frozen at -80°C until further use. The present study was approved by the Local Ethics Committee of Shinshu University. Written informed consent was obtained from all participants.

CSF and serum BAFF, APRIL, TGF-β1, and ANCA levels

The levels of BAFF, APRIL, and transforming growth factor beta 1 (TGF-β1) in the CSF and serum were measured using commercially available ELISA kits as follows: Soluble (human) ELISA Kit (hypersensitive) (AdipoGen Life Science, Liestal, Switzerland) for BAFF; Human APRIL Platinum ELISA (eBioscience, Vienna, Austria) for APRIL; Quantikine ELISA Human TGF-β1 Immunoassay

(R&D system, Minneapolis, USA) for TGF-β1. The minimal detectable dose of BAFF, APRIL, and TGF-β1 were 8 pg/mL, 400 pg/mL, and 1.7 pg/mL, respectively. MPO- and PR3-ANCA were measured using a chemiluminescent enzyme immunoassay (STACIA MEBLux test MPO-ANCA/PR3-ANCA; Medical & Biological Laboratories, Nagoya, Japan). The cut-off value of MPO- or PR3-ANCA was established at 0.1 U/mL.

Statistical analysis

The data are presented as the mean ± standard deviation. *p*-values less than 0.05 were considered statistically significant. The Mann-Whitney U test was used to compare data from independent groups. The Steel-Dwass test was used for multiple comparison tests. The Spearman's rank correlation coefficient test was used to evaluate the correlation between two independent variables.

Results

Baseline and demographic features in ANCA- and other HP

Of the 12 patients with ANCA-HP, 10 were first diagnosed with HP in our hospital, while 2 were admitted because of HP exacerbation. Seven patients with ANCA-HP indicated that HP was the initial clinical episode (**Table 1**). All of the patients with other HP were initially diagnosed with HP in our hospital and 10 of them exhibited HP as the first episode of their underlying disease. As for prior

treatments in the underlying diseases, prednisolone had been given in 5 patients with ANCA-HP and 2 with other HP, while 3 with ANCA-HP had concomitant administration of immunosuppressants including methotrexate, azathioprine, or mizoribine. However, there were no significant differences in all analyses performed in this study between patients with and without prior treatment (data not shown). Headache and cranial neuropathy are frequent involvements related to HP; nevertheless, no significant differences were seen between patients with ANCA- and other HP. In patients with ANCA-HP, the mean value of Birmingham Vasculitis Activity Score (BVAS) [19] was 13.8 ± 4.9. Serum levels of C-reactive protein (CRP) were significantly higher in both patients with ANCA- and other HP than in those with MS or NIND ($p < 0.05$) (**Table 2**). Seven (58%) and 4 (33%) of patients with ANCA-HP tested positive for MPO- or PR3-ANCA, respectively. No patients with other HP were MPO- or PR3-ANCA positive.

Characteristics of CSF in ANCA-HP

Cell counts were significantly higher in both patients with ANCA- and other HP than in those with NIND ($p < 0.05$) (**Table 3**). CSF protein levels were also significantly higher in both patients with ANCA- and other HP than in those with MS or NIND ($p < 0.05$), while there was no significant differences in IgG index between patients with ANCA-, other HP, or MS. MPO- or PR3-ANCA was detected in the CSF of seven (58%) patients with ANCA-HP; 4 (33%) for MPO-ANCA and 3 (25%)

for PR3-ANCA. Moreover, one patient with PR3-ANCA positive CSF, who developed HP as the initial episode of GPA, had ANCA negative serum. The remaining 6 patients had the same phenotype of ANCA in their CSF and serum. Neither MPO- nor PR3-ANCA was detected in the CSF of patients with other HP, MS, or NIND.

CSF levels of BAFF, APRIL, and TGF-β1 are increased in immune-mediated HP

CSF levels of BAFF and APRIL were significantly higher in both patients with ANCA- and other HP than in patients with MS or NIND (mean values: 179 ± 80, 229 ± 173, 73 ± 125, and 41 ± 19 pg/mL, respectively) (ANCA-HP: vs. MS, $p = 0.008$; vs. NIND, $p = 0.0007$) (other HP: vs. MS, $p = 0.011$; vs. NIND, $p = 0.014$) (**Fig. 1a**). APRIL levels were also significantly higher in both patients with ANCA- and other HP than in patients with MS or NIND (mean values: 2510 ± 1425, 2986 ± 2527, 908 ± 524, and 862 ± 471 pg/mL, respectively) (ANCA-HP: vs. MS, $p = 0.002$; vs. NIND, $p = 0.006$) (other HP: vs. MS, $p = 0.011$; vs. NIND, $p = 0.014$) (**Fig. 1b**). However, neither BAFF nor APRIL levels indicated significant differences between patients with ANCA- and other HP ($p = 0.920$, $p = 0.999$, respectively). TGF-β1, which is generally recognized as a cytokine that promotes tissue fibrosis [20], was also measured in the four groups. CSF TGF-β1 levels were also significantly higher in patients with ANCA- and other HP than those with MS or NIND (mean values: 28.5 ± 17.5, 20.0 ± 8.3, 12.9 ± 7.1, and 12.7 ± 6.4 pg/mL, respectively) (ANCA-HP: vs. MS, $p = 0.007$; vs. NIND, $p =$

0.013) (other HP: vs. MS, $p = 0.040$; vs. NIND, $p = 0.034$) (**Fig. 1c**), while no significant differences were seen between patients with ANCA- and other HP ($p = 0.489$). Serum levels of BAFF were significantly higher in both patients with ANCA- and other HP than in those with NIND ($p < 0.05$) (**Table 2**). Serum levels of APRIL were significantly higher in patients with ANCA-HP than those with MS or NIND ($p < 0.05$). Serum levels of TGF-β1 were significantly higher in patients with ANCA-HP than those with MS or NIND ($p < 0.005$).

Correlation between BAFF or APRIL and CSF parameters in ANCA- and other HP

Regression analysis in BAFF, APRIL, or TGF-β1 levels identified no significant correlations between the CSF and serum in patients with ANCA-HP or other HP (ANCA-HP: $p = 0.403$, $p = 0.219$, $p = 0.296$, respectively) (other HP: $p = 0.931$, $p = 0.070$, $p = 0.535$, respectively). We then analyzed their correlations with some clinical CSF parameters. In patients with ANCA-HP, levels of BAFF and APRIL in the CSF were significantly correlated with IgG index (r = 0.545, $p = 0.035$; r = 0.622, $p = 0.019$, respectively) (**Table 4**). There were no correlations between CSF BAFF or APRIL levels and IgG index in patients with other HP ($p = 0.223$, $p = 0.082$, respectively). On the other hand, CSF levels of BAFF and APRIL were significantly correlated with CSF cell counts and protein levels in patients with other HP (BAFF: r = 0.748, $p = 0.007$; r = 0.867, $p = 0.002$, respectively) (APRIL: r = 0.888, $p = 0.001$; r = 0.783, $p = 0.005$, respectively). In patients with ANCA-HP, CSF levels of neither BAFF

nor APRIL were correlated with CSF cell counts or protein levels (BAFF: $p = 0.888$, $p = 0.935$, respectively) (APRIL: $p = 0.256$, $p = 0.231$, respectively). CSF levels of TGF-β1were not correlated with any of the tested CSF parameters (data not shown).

Comparison of ANCA-HP patients positive for MPO-ANCA and PR3-ANCA

To investigate the differences in ANCA-HP between patients who tested positive for MPO- and PR3-ANCA in their serum and/or CSF, their clinical parameters and cytokine levels were statistically analyzed. Neither BVAS nor CRP serum levels differed significantly between patients with MPO-ANCA and PR3-ANCA (13.0 ± 3.5 vs. 15.0 ± 6.6, $p = 0.871$; 7.9 ± 7.8 vs. 5.2 ± 5.4 mg/dL, $p = 0.465$, respectively). There were also no significant differences observed in the analyses of neurological involvement (Table 1) (data not shown). Mean CSF cell counts were higher in patients with MPO-ANCA than those with PR3-ANCA but the difference was not statistically significant (10.6 ± 9.0 vs. 3.4 ± 4.3 cells/μL, $p = 0.071$) (**Fig. 2a**). CSF levels of protein and IgG index were significantly higher in patients with MPO-ANCA than those with PR3-ANCA (90.9 ± 40.9 vs. 50.8 ± 11.1 mg/dL, $p = 0.041$; 1.30 ± 0.48 vs. 0.64 ± 0.17, $p = 0.012$, respectively) (**Fig. 2b, 2c**). There were no significant differences in levels of BAFF, APRIL, or TGF-β1 in the CSF and serum between patients positive for MPO- and PR3-ANCA.

Discussion

Given the findings obtained in the current study as well as in previous ones [2-4,21], increased CSF protein levels and cell counts are generally found in immune-mediated HP, suggesting that they have no specificity for distinguishing the underlying etiologies of HP. Furthermore, CNS immune responses ascribable to several autoimmune diseases, such as MS, NMO, NPSLE, or neuro-BD, nonspecifically produce mild to moderate pleocytosis as well as increased CSF protein levels, even though the CNS is essentially maintained as a protected environment by preventing the passage of immune cells or proteins through the blood-brain barrier [22,23]. Levels of CSF BAFF, APRIL, and TGF-β1 were significantly higher in both ANCA-HP and other HP than in MS or NIND. In addition, regression analyses found no significant correlation between CSF and serum levels of BAFF, APRIL, or TGF-β1, suggesting their local production in the CNS. TGF-β1 plays a crucial role in the development of several fibrotic diseases [20]. Notably, it contributes to the activation of fibroblasts for promoting tissue fibrosis [24,25]. Therefore, TGF-β1 may be a potential biomarker for immune-mediated HP because fibrosis is the main pathological feature seen in biopsied dura mater [3,6,12,21]. In addition, members of the TGF-β family are known to be crucial immune mediators which can both regulate and induce immune signaling [26,27]. TGF-β1 has been shown to promote macrophages to express BAFF and APRIL in *in vivo* experiments [28,29]. Combined with our results this suggests that TGF-β1 may be involved in inducing the intracranial production of BAFF and APRIL in immune-mediated HP.

However, further investigation is necessary to determine exactly how TGF-β1 relates to the development of HP because there were no significant correlations between TGF-β1 expression and other analyzed items in this study.

Expectedly, based on our previous investigation [2], CSF levels of BAFF were significantly correlated with IgG index in patients with ANCA-HP. The present study demonstrated that APRIL was also significantly correlated with IgG index. This suggests that not only BAFF but also APRIL expression in the CSF may impact the B cell-mediated immune response in the pathogenesis of ANCA-HP, because IgG index measures the synthesis of IgG from proliferated B cell lineage within the CNS as well as intracranial disease activation [30,31]. Both BAFF and APRIL play a critical role in the survival and maturation of B cells [32-34]. Conversely, neither BAFF nor APRIL levels in the CSF were significantly correlated with IgG index in other HP. Meanwhile, CSF levels of both BAFF and APRIL were significantly correlated with CSF cell count or protein levels in other HP, despite not being correlated in ANCA-HP. BAFF and APRIL are mainly produced from innate immune cells including macrophages, dendritic cells, monocytes, neutrophils, and NK cells, as well as activated lymphocytes [32,33], suggesting that the immune cells responsible for producing BAFF and APRIL in the CNS may be intimately involved in the intrathecal nonspecific immune response which leads to increased CSF cell counts and protein levels in other HP. Furthermore, the implication of BAFF and APRIL expression in the CSF is different in ANCA-HP and other HP; namely, intracranial production

of BAFF and APRIL may contribute to pathogenesis via autoreactive B cells in ANCA-HP. Activated autoreactive B cells are implicated in the pathogenesis of AAV by producing the specific antibodies including MPO- or PR3-ANCA [35]. Significant levels of BAFF and APRIL, which are capable of promoting autoreactive B cells, were previously found in serum and pathological tissues of patients with AAV, so their significant expression is associated with the disease activity of AAV and therefore is a useful biomarker [36-40]. The present study demonstrated detection of MPO- or PR3-ANCA in CSF, suggesting that BAFF and APRIL may contribute to promoting autoreactive B cells which impact the intracranial production of ANCA. To the best of our knowledge, there are only two case reports of patients with GPA indicating CNS involvement, with PR3-/c-ANCA expression in their CSF. One patient had thickened meninges around the right cavernous sinus and temporal lobe, and the other had Wallenberg syndrome due to vasculitis [41,42]. Consequently, detecting MPO- or PR3-ANCA in CSF may be useful for verifying the pathogenesis that underlies ANCA-related CNS manifestations regardless of visceral complication and/or as surrogate markers for diagnosing AAV. In fact, the present study identified PR3-ANCA in the CSF of one patient who indicated an initial episode of GPA despite an absence of ANCA in the serum. Meanwhile, a small number of patients with ANCA-HP exhibited the finding that the CSF tested positive for MPO- or PR3-ANCA in our study. Therefore, accumulation of this finding is required for providing evidence that testing CSF for MPO- and PR3-ANCA may be useful for validating the disease specificity of ANCA-HP.

Intracranial production of BAFF and APRIL may be implicated in another immunological feature of ANCA-HP, Th1 dominancy, which was demonstrated previous studies [3,7]. Although the innate immune system, including activated neutrophils and autoreactive B cells related to the production of ANCA, may affect the activation of T cell lineages [35,39], BAFF and APRIL were also found to induce the Th1 response *in vivo* study [33,39,43,44], suggesting that BAFF and APRIL in the CNS may have roles in the development of ANCA-HP.

In the clinical analysis of ANCA-HP, protein levels and IgG index were significantly higher in patients with MPO-ANCA than in those with PR3-ANCA. BVAS and serum CRP levels, which are known to be general activation markers in AAV [45,46], were not significantly different between patients with MPO-ANCA and those with PR3-ANCA, allowing for the evaluation to focus on neurological impairments due to ANCA-HP. In addition, neither clinical symptoms nor levels of CSF cytokines, including BAFF, APRIL, or TGF-β1, were significantly different. We considered two hypotheses to explain the ANCA-HP results. First, the immune response in the CNS may be more intense in patients with MPO-ANCA than those with PR3-ANCA, even though no differences were seen in BAFF, APRIL, or TGF-β1 levels. Second, significant differences might not be detected in CSF levels of BAFF, APRIL, or TGF-β1 because of the limited number of patients with ANCA-HP. The other HP group consisted of heterogeneous diseases, although it is difficult to recruit enough patients in a single institute because immune-mediated HP is a rare disease. Moreover, Neuro-BD, NPSLE,

and NMO, in which BAFF and APRIL were also found to be useful CSF biomarkers [8-11], were not included in the present study. Considering such limitations, future studies should involve more patients to study pathogenesis in immune-mediated HP including ANCA-HP.

In conclusion, CSF levels of BAFF, APRIL, and TGF-β1 are significantly increased in immune-mediated HP, and may be useful biomarkers in the active phase of disease. Notably, those of BAFF and APRIL were significantly correlated with IgG index in ANCA-HP, suggesting that they may be implicated in the pathogenesis of ANCA-HP through activating autoreactive B cells. In addition, MPO- or PR3-ANCA in the CSF was detected in some patients with ANCA-HP. Therefore, the levels of BAFF and APRIL, and the presence of MPO- or PR3-ANCA in the CSF may serve as effective biomarkers for assessing ANCA-HP development.

Conflicts of Interest

The authors declare that they have no financial or personal conflicts of interest.

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Figure legends

Fig. 1: CSF levels of BAFF (**a**), APRIL (**b**), and TGF-β1 (**c**) in patients with ANCA-HP, other HP, MS, and NIND. CSF, cerebrospinal fluid; BAFF, B-cell activation factor of the TNF family; APRIL, a proliferation-inducing ligand; TGF-β1, transforming growth factor beta 1; HP, hypertrophic pachymeningitis; ANCA, anti-neutrophil cytoplasmic antibody; ANCA-HP, ANCA-related HP (n = 12); other HP, other types of immune-mediated HP except for ANCA-HP (n = 12); MS, multiple sclerosis (n = 14); NIND, non-inflammatory neurological disorders (n = 10). *$p < 0.05$; **$p < 0.01$; ***$p < 0.005$; ****$p < 0.001$.

Fig. 2: Comparison of CSF cell counts (**a**), levels of protein in CSF (**b**), and IgG index (**c**) between ANCA-HP patients positive for MPO-ANCA (n = 7) and PR3-ANCA (n = 5). MPO, myeloperoxidase-ANCA; PR3, proteinase 3-ANCA.

Table 1 Demographic characteristics and neurological symptoms related to HP in ANCA- and other HP

	ANCA-HP n = 12	Other HP n = 12	*p* value
Male : Female	7 : 5	9 : 3	n.s.
Age [years, mean ± SD]	67 ± 13	63 ± 19	n.s.
HP was the initial episode‡ (%)	7 (58)	10 (83)	n.s.
Duration* [years, mean ± SD] (range)	3.0 ± 5.6 (0–17)	0.3 ± 1.0 (0–3.5)	n.s.
Neurological involvements related to HP			
Headache (%)	10 (83.3)	6 (50.0)	n.s.
Seizure (%)	3 (25.0)	2 (16.6)	n.s.
Consciousness disturbance (%)	2 (16.7)	1 (8.3)	n.s.
Cranial neuropathy, total (%)	10 (83.3)	10 (83.3)	n.s.
II (%)	2 (16.7)	2 (16.7)	n.s.
III (%)	3 (25.0)	1 (8.3)	n.s.
IV (%)	2 (16.7)	2 (16.7)	n.s.
V (%)	3 (25.0)	2 (16.7)	n.s.
VI (%)	2 (16.7)	3 (25.0)	n.s.
VII (%)	2 (16.7)	0	n.s.
VIII (%)	7 (58.3)	3 (25.0)	n.s.
IX,X (%)	1 (8.3)	2 (16.7)	n.s.
XII (%)	1 (8.3)	0	n.s.

HP, hypertrophic pachymeningitis; ANCA, anti-neutrophil cytoplasmic antibody; ANCA-HP, ANCA-related HP; other HP, other types of immune-mediated HP; n.s., not significant.

‡in the clinical course of the underlying disease.

*between initial diagnosis of the underlying disease and HP onset.

<0.05 is statistically significant.

Table 2 Laboratory findings and cytokine levels in the serum from patients with ANCA-HP, other HP, MS, and NIND

	ANCA-HP	Other HP	MS	NIND	*p* value		
	n = 12	n = 12	n = 14	n = 10	ANCA vs. Other	ANCA vs. *	Other vs. *
Parameters							
CRP (mg/dL)	6.81 ± 6.77	5.67 ± 6.63	0.09 ± 0.18	0.08 ± 0.12	n.s.	0.0001 / 0.002	0.012 / 0.023
Positive for							
MPO-ANCA (%)	7 (58)	0	—	—	0.045	—	—
PR3-ANCA (%)	4 (33)	0	—	—	0.006	—	—
Cytokine levels (pg/mL)							
BAFF	86.3 ± 55.1	129.7 ± 112.8	66.7 ± 70.7	17.3 ± 20.5	n.s.	n.s. / 0.008	n.s. / 0.042
APRIL	3855 ± 2568	2265 ± 1278	1525 ± 743	1146 ± 413	n.s.	0.026 / 0.017	n.s. / n.s.
TGF-β1	851 ± 200	716 ± 252	550 ± 189	496 ± 956	n.s.	0.008 / 0.038	n.s. / n.s.

MS, multiple sclerosis; NIND, non-inflammatory neurological disorders; CRP, C-reactive protein; MPO, myeloperoxidase; PR3, proteinase 3;

BAFF, B-cell activation factor of the TNF family; APRIL, a proliferation-inducing ligand; TGF-β1, transforming growth factor beta 1; n.s., not significant.

*controls (MS / NIND); <0.05 is statistically significant.

Table 3 Laboratory findings in CSF from patients with ANCA-HP, other HP, MS, and NIND

	ANCA-HP	Other HP	MS	NIND	*p* value		
	n = 12	n = 12	n = 14	n = 10	ANCA vs. Other	ANCA vs. *	Other vs. *
Cells (/μL)	7.6 ± 8.0	11.0 ± 17.0	6.6 ± 8.9	0.6 ± 1.0	n.s.	n.s. / 0.006	n.s. / 0.011
Protein (mg/dL)	78 ± 41	73 ± 51	34 ± 16	37 ± 20	n.s.	0.005 / 0.016	0.006 / 0.022
IgG index	1.03 ± 0.51	1.17 ± 1.23	1.20 ± 1.03	—	n.s.	n.s. / —	n.s. / —
Positive for							
MPO-ANCA (%)	4 (33)	0	0	0	0.046	—	—
PR3-ANCA (%)	3 (25)	0	0	0	n.s.	—	—

CSF, cerebrospinal fluid; n.s., not significant.

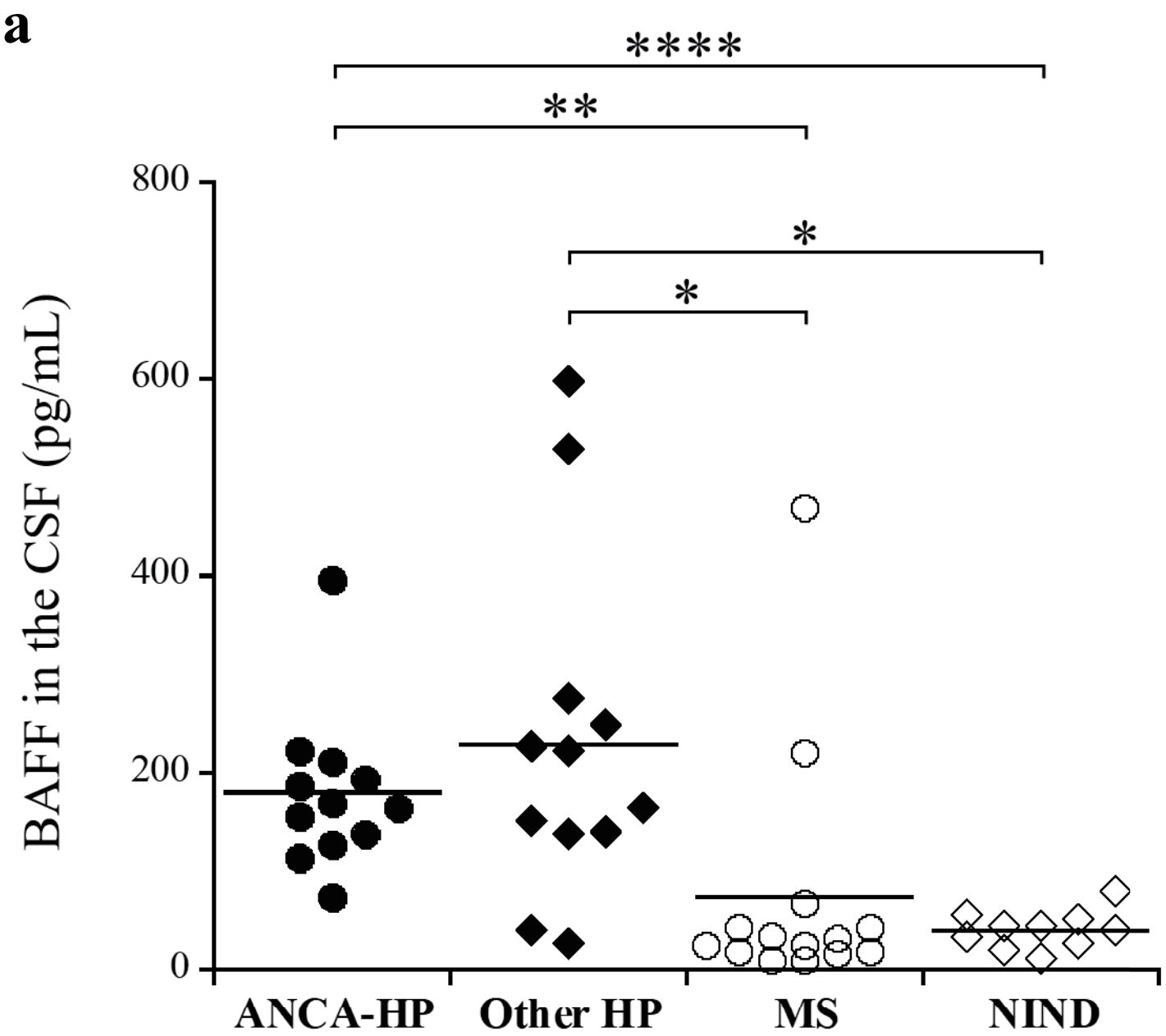
*controls (MS / NIND); <0.05 is statistically significant.

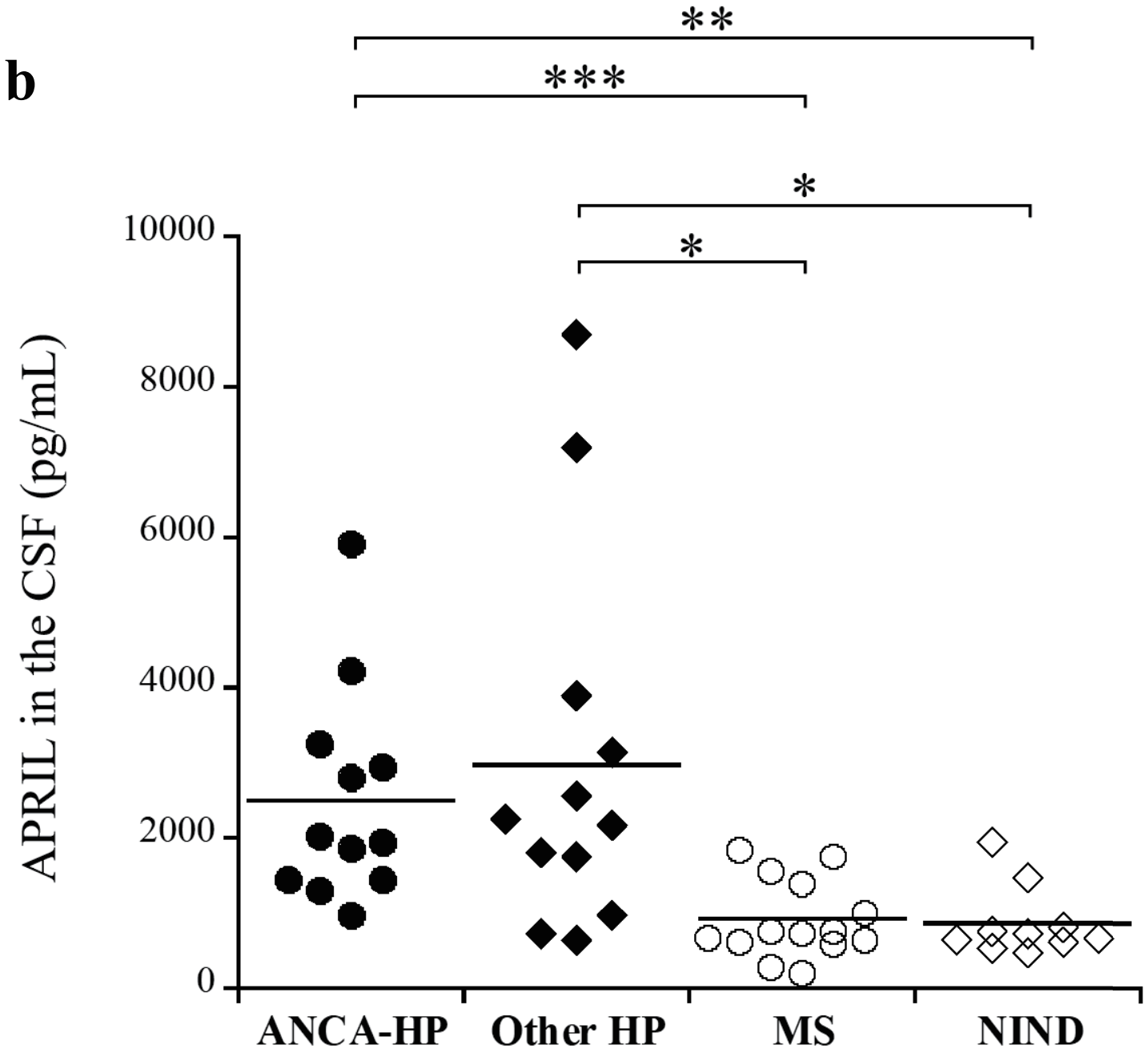
Table 4 Correlations between CSF levels of BAFF or APRIL and CSF parameters in ANCA-HP or other HP

	ANCA-HP (n = 12)		Other HP (n = 12)	
	Coefficient	*p* value	Coefficient	*p* value
CSF BAFF vs.				
IgG index	0.545	0.035	0.379	n.s.
CSF Cell counts	0.052	n.s.	0.748	0.007
CSF protein levels	0.026	n.s.	0.867	0.002
CSF APRIL vs.				
IgG index	0.622	0.019	0.524	n.s.
CSF Cell counts	0.349	n.s.	0.888	0.001
CSF protein levels	0.362	n.s.	0.783	0.005

CSF, cerebrospinal fluid; Coefficient, correlation coefficient; n.s., not significant. <0.05 is statistically significant.

Fig. 1

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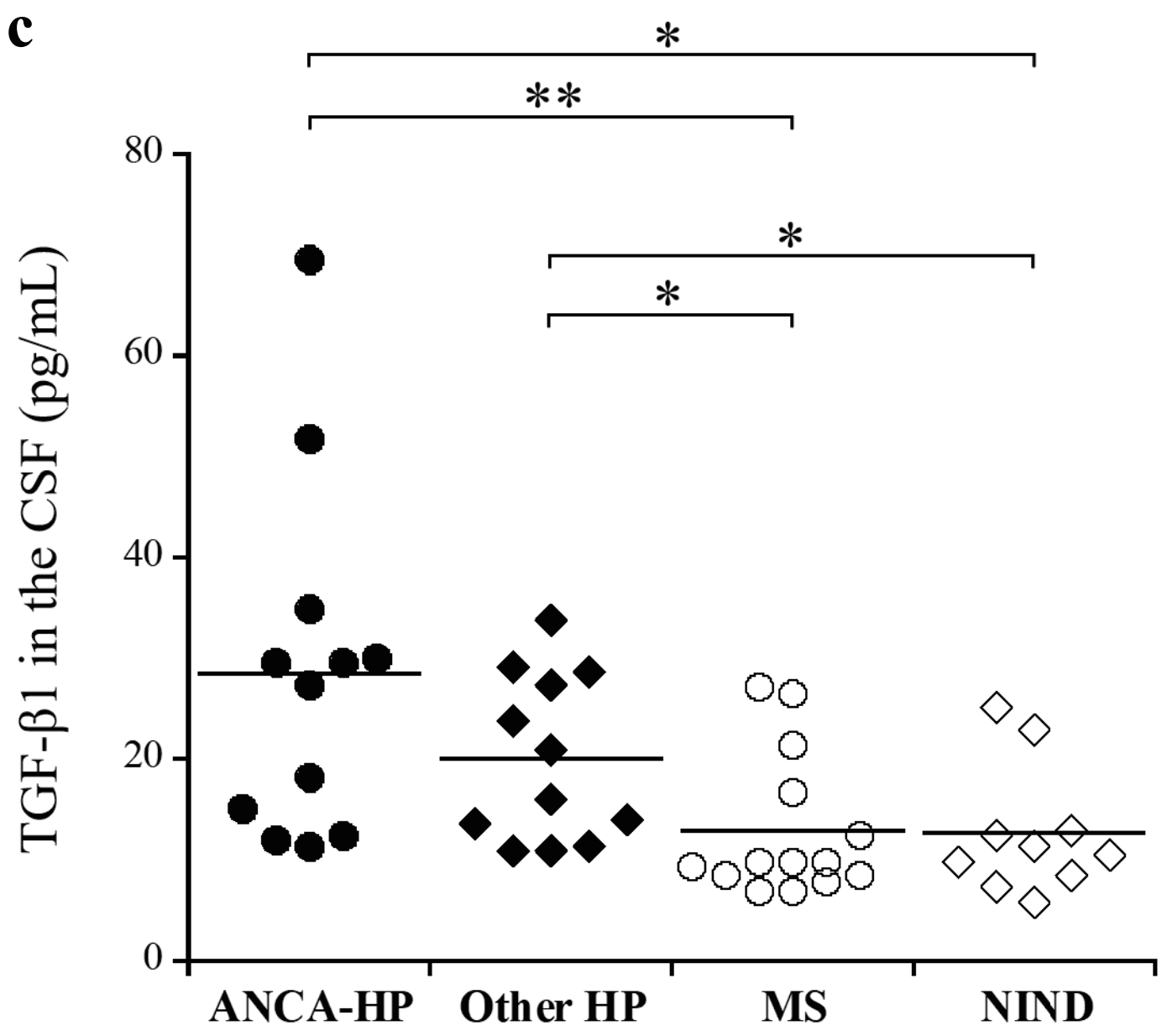
*
10000
8000
6000
4000
2000
0
APRIL in the CSF (pg/mL)
ANCA-HP
Other HP
MS
NIND


c
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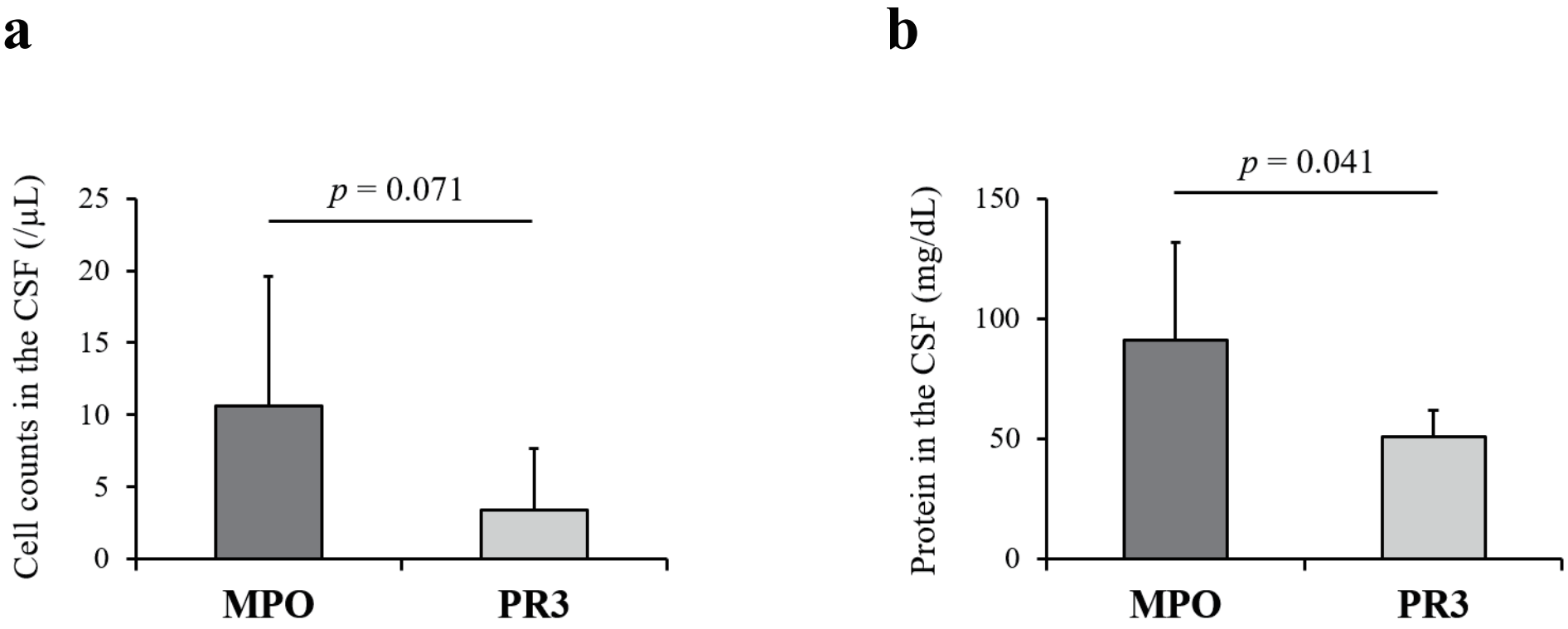
80
60
40
20
0
TGF-β1 in the CSF (pg/mL)
ANCA-HP
Other HP
MS
NIND

Fig. 2


a
b
p = 0.071
p = 0.041
Cell counts in the CSF (/μL)
Protein in the CSF (mg/dL)
25
20
15
10
5
0
150
100
50
0
MPO
PR3
MPO
PR3

c