Original article

Low dose mepolizumab is effective as an add-on therapy for treating long-lasting peripheral neuropathy in patients with eosinophilic granulomatosis with polyangiitis

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Abstract

Objective: To assess the effectiveness of low-dose mepolizumab as an add-on therapy for treating peripheral neurological symptoms in eosinophilic granulomatosis with polyangiitis (EGPA).

Methods: We prospectively studied 13 EGPA patients with conventional treatment-resistant peripheral neuropathy. Their symptoms (pain, numbness and muscle weakness) were assessed on a visual analog scale (VAS) before and after 12 months of mepolizumab therapy (100 mg every 4 weeks). Peripheral eosinophil levels and several biomarkers including urinary levels of eosinophil-derived neurotoxin (EDN) were measured before and after therapy.

Results: VAS scores for pain and numbness significantly improved after 12 months mepolizumab therapy (from 67.0 to 48.0; p=0.012, and from 67.0 to 51.0; p=0.017, respectively). However, the VAS score for muscle weakness did not improve (p=0.36). There were significant correlations between treatment-related changes in urinary EDN levels from baseline to 6 months later, and percent changes in the VAS scores of pain and numbness ($r = 0.75$, $p = 0.020$ and $r = 0.88$, $p = 0.002$).

Conclusions: Treatment-resistant peripheral neuropathy in EGPA was significantly improved by low dose mepolizumab, and effectiveness was correlated with decreased urinary EDN. Because the possibility of a placebo effect cannot be formally excluded, placebo-controlled studies will be required in the future.

1. Introduction

Eosinophilic granulomatosis with polyangiitis (EGPA, formerly Churg-Strauss syndrome) is a rare vasculitis. EGPA typically develops in three phases: first, asthma and upper airway lesions occur; second, eosinophilia and lung lesions appear; and third, systemic vasculitis with eosinophilic inflammation develops (1). Peripheral neuropathy is one of the most frequent EGPA manifestations; indeed, earlier reports indicated that it occurs in 51-98% of patients (2-7). Although peripheral neuropathy is not actually life-threatening, it greatly affects quality of life and impacts day-to-day functioning (8). Conventional treatments such as glucocorticoids and other immunosuppressants are almost always unable to accomplish complete patient recovery (9, 10).

The mechanisms responsible for systemic vasculitic neuropathy involve vascular occlusion which causes acute ischemia, and the activity of cytotoxic T cells that play a prominent role in vessel injury (11). This also occurs in the acute phase of EGPA (12). In addition, a higher incidence of peripheral neuropathy in patients positive for antineutrophil cytoplasmic antibody (ANCA) suggests that these antibodies may be involved (4, 13). Moreover, biopsies from patients with EGPA revealed eosinophilic infiltration not only of the extravascular epineurium but also the endoneurium, leading to the suggestion that toxic proteins released from eosinophils directly damage the nerves (14, 15). Although peripheral nerves that have undergone axonal degeneration due to ischemia are usually repaired (16), the cause of the remaining peripheral neuropathy in patients with EGPA has not been resolved to date.

Mepolizumab is a humanized monoclonal anti-IL 5 antibody that can reduce peripheral blood eosinophil levels, which has been shown to be effective not only for treating eosinophilic asthma but also for other eosinophilic diseases such as hypereosinophilic syndrome, eosinophilic esophagitis, and chronic rhinosinusitis with nasal polyposis (17-21). Thus far, to the best of our knowledge, there has been only one large-scale trial to investigate the efficacy of mepolizumab for treating EGPA (22). This trial, the MIRRA

study, concluded that relative to placebo, 300 mg of mepolizumab led to significant remission rates in participants with EGPA. However, treatment-related changes in peripheral neuropathic symptoms were not evaluated in that trial, so the efficacy of mepolizumab for relieving peripheral neuropathy in patients with EGPA remained unknown. Therefore, in the current study, we aimed to prospectively assess the effectiveness of a low dose of mepolizumab every 4 weeks for 12 months for treating conventional treatment-resistant peripheral neurological symptoms in EGPA over the long term.

2. Materials and Methods

2.1 Study design

This study was a prospective observational study selecting patients with EGPA who had suffered from long-lasting peripheral neuropathy resistant to conventional treatment and had then received add-on mepolizumab therapy. Patients with EGPA who had started mepolizumab treatment for the first time at National Hospital Organization Sagamihara National Hospital between June 2016 and December 2017 were enrolled in the study. At that time, there was no insurance coverage for mepolizumab use in EGPA patients in Japan. All patients had severe asthma. The dose of mepolizumab was 100 mg every 4 weeks, which was the standard dose for severe asthma. Participants were patients whose neurological symptoms had not completely disappeared even after receiving 20 mg or more of prednisolone equivalent per day in the past and due to their persistence had been continued on 4 mg or more prednisolone per day. Any changes in neurological and other symptoms in individual patients with EGPA were monitored over a 4-month screening period before enrollment. None of the patients were in either an acute or relapsing phase of EGPA when mepolizumab therapy was initiated **(**defined as **baseline)**. Patients received conventional treatments (glucocorticoids and other immunosuppressants) which was either increased or decreased at the discretion of the attending physician after initiation of mepolizumab as an add-on to the standard treatment. The study protocol (No. 2016-051) was approved by the Ethics Committee of National Hospital Organization

Sagamihara National Hospital. All subjects provided written informed consent.

2.2 Inclusion/exclusion criteria

EGPA was diagnosed according to the Lanham criteria and the American College of Rheumatology classification criteria for Churg-Struss Syndrome (1, 23). Patients were included if they met all of the following criteria: 18 years and older; a diagnosis of EGPA at least 2 years before enrollment; progress from onset to date of enrollment could be confirmed; treatment medication could be confirmed; patients had suffered from peripheral nerve symptoms due to EGPA despite receiving glucocorticoids equivalent to ≥20 mg per day of prednisolone previously for 4 months or more; patients required ≥4 mg of prednisolone equivalents per day to stabilize their EGPA; patients who started mepolizumab during the recruitment period (June 2016 to December 2017).

We excluded patients who had any of the following: they had developed EGPA less than 2 years previously; had no neurological symptoms due to EGPA; had neurological symptoms due to other diseases; had other diseases that were not associated with EGPA which had not been controlled with standard treatment; had developed peripheral neuropathy less than 2 years previously; had fluctuating peripheral neurological symptoms that could not be evaluated; had received anti-IL-5 antibody treatment at any time; or were pregnant, breastfeeding, or planned to become pregnant during the research period. Seventeen patients were recruited but two were excluded according to the above criteria. Of the latter, one had no neurological symptoms due to EGPA and the other suffered from neurological symptoms associated with rheumatoid arthritis. Hence, 15 patients met the inclusion criteria and were enrolled in the study.

2.3 Endpoints

The primary endpoint was the change in peripheral neurological symptoms between baseline and

after mepolizumab treatment, as evaluated by the VAS. Patients were asked to mark the level of their symptoms on a 100 mm, unhatched VAS scale marked at one end as “no symptoms” and at the other as “worst symptoms imaginable”(24). The items in the VAS questionnaire were as follows: “How much neuropathic pain do you feel?”, “How much numbness do you feel?” and “How much muscle weakness do you feel?” The VAS was assessed at baseline and 12 months after the initiation of mepolizumab treatment. The questionnaire was explained to patients by a dedicated nurse and was collected each time by the nurse and kept until the end of the research period. As secondary endpoints, we measured the change in Birmingham Vasculitis Activity Score (BVAS) version 3, change in glucocorticoid dose, change in biomarkers in blood and urine, and correlations between changes in neurological symptoms, changes in biomarkers and changes in asthma status. BVAS and glucocorticoid doses were measured at baseline and 12 months after the initiation of mepolizumab treatment. Details of the biomarkers are described in the next section. Patients′ asthma status was evaluated by the number of emergency outpatient visits or hospitalizations for asthma attacks, number of times temporary systemic glucocorticoid was administered, and the inhaled glucocorticoid dose before and after mepolizumab treatment.

2.4 Analysis of biomarkers in blood and urine

Peripheral eosinophils, urinary eosinophil-derived neurotoxin (EDN), serum Eotaxin-3, immunoglobulin G (IgG) 4, and plasma major basic protein (MBP) values were measured at baseline and 1, 3, 6 and 12 months after the start of mepolizumab therapy. Serum soluble IL-2 receptor (sIL-2R), IL-6, IL-15, tumor necrosis factor (TNF) -α, and granulocyte-colony stimulating factor (G-CSF), plasma P-selectin and urinary neutrophil gelatinase-associated lipocalin (NGAL) were also measured at baseline and 6 months after starting mepolizumab therapy (25-28). Peripheral blood eosinophils were analyzed in the clinical laboratory of our hospital using standard methods. Serum, plasma and urinary samples from patients

at baseline and at each time point were stored at –20°C until assayed. The lower limit of detection (LLD) for urinary EDN (MBL, Nagoya, Aichi, Japan) was 26.7 μg/mmol creatinine. This and LLD for other markers are shown in Table S1.

2.5 Statistical analysis

All data were analyzed using SPSS ver. 24.0 (IBM, Tokyo, Japan). VAS, BVAS, glucocorticoid dose (after conversion to prednisolone dose equivalent), peripheral eosinophil count and each blood and urinary marker level were compared between baseline and each predetermined time point after starting administration of mepolizumab. Wilcoxon signed-rank tests were used to analyze the statistical significance of differences before and after mepolizumab therapy. For repeated measures of urinary EDN, peripheral blood eosinophil levels, serum Eotaxin-3, IgG4 and plasma MBP, a linear mixed-effect model for analyzing sequential changes after adjustment for glucocorticoid doses at baseline was constructed with the patient as the random effect and visits as fixed effects, adjusted by Satterthwaite's approximation. Bonferroni correction was performed for comparisons between time points.

Correlations of changes in urinary EDN levels and peripheral blood eosinophil levels with percent changes in VAS from baseline to 12 months after the initiation of mepolizumab therapy were analyzed using Spearman’s correlation. P value <0.05 was considered statistically significant.

3. Results

3.1 Patients′ characteristics

Of the fifteen enrolled patients, one discontinued mepolizumab in less than 12 months and one lacked evaluation of VAS 12 months later. Thus, thirteen patients were finally the subject of the study. The median age of the patients and the duration since diagnosis of EGPA was 59.0 years and 11.0 years,

respectively (Table 1). All patients were being treated with systemic glucocorticoids at baseline (median prednisolone dose 5.0 mg per day). Immunosuppressants and omalizumab were administered to 3 and 4 patients, respectively. At the onset of EGPA, 10, 5, 3 and 2 patients suffered from sinonasal abnormalities, cardiomyopathy, gastrointestinal involvement and glomerulonephritis, respectively. Five patients were ANCA-positive at onset.

3.2 Peripheral neurological symptoms before and after mepolizumab treatment

Figure 1 shows changes in the VAS score for peripheral neurological symptoms of each patient at baseline and 12 months after the initiation of mepolizumab treatment. Pain and numbness significantly improved after mepolizumab treatment (from 67.0 to 48.0; p=0.012 and from 67.0 to 51.0; p=0.017, respectively), but muscle weakness did not show any improvement (from 51.0 to 50.0; p=0.36) (Table 2), and BVAS also failed to improve significantly (from 0 to 0; p=0.55) (Table 2). The prednisolone dose could be decreased (from 5.0 to 2.0 mg/day, p=0.001) and peripheral blood eosinophil levels declined significantly (from 370 to 50, p=0.003) (Table 2). By 12 months after the initiation of mepolizumab treatment, the number of patients receiving immunosuppressive and omalizumab therapy had decreased to one and zero, respectively.

3.3 Changes of other symptoms after treatment

The number of emergency outpatient visits or hospitalizations for asthma attacks and the number of temporary systemic glucocorticoid treatments during the 12 months of mepolizumab administration were significantly decreased relative to the 12 months before starting mepolizumab (data not shown). However, the inhaled glucocorticoid dose did not change significantly after starting mepolizumab treatment.

Exacerbation of nasal blockage was reported by two patients and chronic rhinosinusitis by one. There were no symptoms involving other organs such as the heart, gastrointestinal tract, kidney and skin in

any of the patients during the treatment period.

3.4 Changes in urinary EDN and numbers of peripheral eosinophils

As shown in Figure 2, peripheral blood eosinophil levels declined rapidly within one month of the initiation of mepolizumab therapy and low levels were maintained 12 months later. Urinary EDN levels also decreased significantly by 3 months and were maintained over the 12 months of mepolizumab therapy relative to baseline. On the other hand, there were no statistically significant changes in serum Eotaxin-3, IgG4, and plasma MBP. There were two ANCA-positive patients (both MPO-ANCA-positive) at baseline. In one of them, the ANCA titer decreased and in the other it increased after 12 months of treatment. Levels of serum sIL-2R, IL-6, IL-15, TNF-α, and G-CSF, plasma P-selectin and urinary NGAL did not show any statistically significant changes relative to baseline 6 months later (Figure S1).

3.5 Correlations between biomarkers and peripheral neurological symptoms

To explore biomarkers associated with peripheral neurological symptoms, we evaluated the correlation between changes in the levels of various markers and changes in peripheral neurological symptoms. First, changes in urinary EDN levels were evaluated. Figure 3 shows changes in peripheral neurological symptoms and EDN levels for each patient. Although no significant correlations were found between urinary EDN levels at baseline and each VAS score at baseline (data not shown), strong correlations were seen between changes in urinary EDN levels from baseline to 6 and 12 months after the initiation of mepolizumab treatment with percent change in VAS scores for pain from baseline to 12 months thereafter ($r = 0.75$, $p = 0.020$ and $r = 0.82$, $p = 0.023$, respectively) (Table 3). Similarly, there was a very strong correlation between changes in urinary EDN levels from baseline to 3 and 6 months later with percent change in VAS scores for numbness from baseline to 12 months later ($r = 0.96$, $p < 0.001$ and $r = 0.88$, $p =$

0.002, respectively). However, no significant correlation was found between changes in urinary EDN levels from baseline to each time point (1, 3, 6 and 12 months after) and percent change in VAS scores for muscle weakness. Also, neither peripheral blood eosinophil levels at baseline (data not shown) nor treatment-related changes in peripheral blood eosinophil levels (Table 3) were correlated with changes in VAS scores. Furthermore, there were no correlations between changes in the levels of any of the other markers studied, namely serum Eotaxin-3, IgG4, sIL-2R, IL-6, IL-15, TNF-α, and G-CSF, plasma P-selectin, MBP and urinary NGAL, and changes in the VAS scores for peripheral neurological symptoms.

Comparison of the patients with or without decreased VAS scores showed that peripheral blood eosinophil and urinary EDN levels at baseline were not significantly different (data not shown). Similarly, there were no significant differences between the two groups for sex, age, BMI, duration since diagnosis of EGPA, and other markers at baseline.

3.6 Safety and Adverse Events

Mild injection-site erythema was reported as an adverse event by one patient. Exacerbation of nasal blockage and chronic rhinosinusitis occurred in two patients and one patient, respectively. However, no moderate or severe adverse events related to mepolizumab use, including anaphylaxis, were reported during the study period.

4. Discussion

This is the first prospective study to document the effectiveness of mepolizumab for relieving EGPA-associated long-lasting peripheral neurological symptoms resistant to conventional treatment. We found that improvement in the VAS scores for peripheral neurological symptoms correlated significantly with decreased urinary EDN levels but not with blood eosinophil counts. Importantly, improvements in neurological symptoms which had been thought to be refractory were observed even for patients who had

239 previously undergone long-term conventional treatment. In addition, although a relationship between
240 neuropathy in EGPA and biomarkers had not been previously reported, we found strong correlations
241 between declining urinary EDN levels and improving neurological symptoms.

242 Although peripheral neuropathy is one of the most frequent and persistent disorders in EGPA,
243 effective treatment for this disorder has remained elusive. Some degree of recovery from peripheral
244 neuropathy in EGPA patients on conventional treatment with glucocorticoids or other immunosuppressive
245 drugs has been reported, but not always to the extent that there is no longer any interference with normal
246 daily life (29-31). Thus, reports have indicated that neurological symptoms persist in almost all patients
247 despite such treatment (9, 10). Although the MIRRA study indicated that treatment with 300 mg of
248 mepolizumab was effective for EGPA, treatment-related changes in peripheral neuropathy were not
249 evaluated, and the efficacy of mepolizumab for peripheral neuropathy remained undescribed.

250 Nerve ischemia in EGPA is known to result from vascular thrombosis and vessel wall necrosis
251 due to the action of T cells, eosinophils, and other cytotoxic cells. Nerve ischemia causes axonal
252 degeneration of the peripheral nerves, resulting in peripheral neuropathy (15, 32, 33). Furthermore, toxic
253 proteins released from eosinophils are also thought to damage nerves directly. Peripheral nerves with axonal
254 degeneration have been documented to undergo regeneration of 1 mm per day at least (16). This may result
255 in gradual improvement of peripheral neuropathy as the nerves are repaired. However, peripheral
256 neurological symptoms in EGPA persist in almost all patients despite treatment with glucocorticoids and
257 immunosuppressants, but the mechanism whereby this pathophysiology is resistant to conventional
258 treatment is unknown. Therefore, it is important to note that despite the fact that the long-term residual
259 peripheral neurological symptoms of the patients studied here at baseline appeared to be permanent, they
260 were nonetheless significantly improved by mepolizumab therapy (Figure 1). This finding encourages the
261 administration of mepolizumab as a new treatment for long-lasting conventional treatment-resistant

peripheral neuropathy and also contributes to elucidating the mechanism responsible. Although the mechanism of action for mepolizumab-mediated improvement of pain and numbness persisting after standard glucocorticoids and immunosuppressant treatment cannot be definitively stated, a likely reason is the reduction of eosinophil migration to peripheral nerves and surrounding tissues. This suggestion is based on findings that mepolizumab reduces not only peripheral blood eosinophils but also eosinophils that have migrated to tissues such as airway tissues and bone marrow (34, 35).

The current study also documented a correlation between improvement in neuropathy and reduction in EDN, which is a protein released from eosinophils on degranulation. We focused on changes in the levels of urinary EDN because it is a well-established biomarker for eosinophilic inflammation (36-41). EDN not only has antiparasitic effects, but also promotes allergic reactions through activation of dendritic cells and is closely related to Type 2 inflammation. Thus, it has been suggested that EDN has an important role in allergic diseases. When injected intradermally it can persist in the skin for 2.5 weeks and increase cutaneous vascular permeability (42). In the acute phase of EGPA, EDN released from eosinophils infiltrating the nerves is likely to directly cause nerve damage (14, 15). On the other hand, there have been no published studies showing a contribution of EDN to long-lasting peripheral neuropathy in patients with EGPA or other ANCA-associated vasculitides. In that respect, the current study presents novel data. If eosinophils infiltrate the nerves also in the non-acute phase of EGPA, EDN is a likely possible cause of long-lasting peripheral neuropathy. However, high baseline urinary EDN levels were not a good predictor of improvement in peripheral neurological symptoms following mepolizumab treatment in the current study (data not shown). This may be partially explained by the large variation in the baseline urinary EDN levels among patients and the small sample size. Moreover, it is difficult to understand why, unlike EDN, MBP did not decline. One possible reason might be that MBP is a protein derived not only from eosinophils but also from mast cells and basophils; localization of MBP in lesion tissue is different from localization of

EDN (43-45). Thus, it is possible that EDN reflects eosinophilic inflammation in patients with EGPA more specifically than MBP.

There are several limitations to the present study. First, this was a single-arm study. Thus, we cannot exclude the formal possibility of a placebo effect caused by intradermal injections especially when the outcome is measured solely based on the patient's own reporting. However, we enrolled only patients with treatment-resistant long-lasting peripheral neurological symptoms. In fact, before their enrolment into this study, most of the patients had received various different treatments including intravenous immunoglobulin therapy in order to improve their neurological symptoms but the response had been universally poor. Thus, we believe that the placebo effect was most likely minimal. Nonetheless, placebo-controlled trials need to be conducted in the future.

A second limitation is that there was no more appropriate method than VAS to assess peripheral neuropathy. Although some previous reports have evaluated peripheral neuropathy in EGPA using methods such as modified Rankin scores and manual muscle test (MMT), which are mainly methods to evaluate motor neuropathy (12, 46), there is no generally accepted questionnaire or scale for assessing sensory neuropathy. Third, the number of cases studied here was small and all were Japanese patients from a single center. Finally, only low dose mepolizumab was used here, but a follow-up study using the higher dose of 300 mg is necessary because the effect may be different.

In conclusion, to the best of our knowledge, this study documented the effectiveness of low dose mepolizumab for conventional treatment-resistant peripheral neurological symptoms due to EGPA for the first time in a prospective study. Moreover, a significant correlation between decreasing EDN and improving VAS-defined peripheral neurological symptoms suggests that occult steroid-resistant eosinophilic inflammation may be involved in persistent peripheral neuropathy with EGPA.

Some conclusions from the current study have been cited in unpublished presentations at

meetings (Nakamura et al., Japan Society of Allergology 2019, Nakamura et al. Japan Society of Allergology/World Allergy Organization 2020).

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Conflict of interest

Masami Taniguchi has received speakers' fees from GlaxoSmithKline, AstraZeneca, and Sanofi. The other authors declare no conflicts of interest.

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451

TABLE 1. Diagnostic and Baseline Characteristics of the Patients

Characteristics at the start of clinical trials	**(n = 13)**
Age—yr, median [IQR]	59.0 [52.0-66.5]
Female sex, n (%)	9 (69.2)
ANCA-positive status, n (%)	2 (15.4)
Peripheral blood eosinophil levels—/μL, median [IQR]	370 [200-880]
BVAS>0, n (%)	5 (38.5)
Peripheral neuropathy at baseline, n (%)	13 (100)
Type of peripheral neuropathy	
Mononeuritis multiplex, n (%)	7 (53.8)
Asymmetrical polyneuropathy, n (%)	2 (15.4)
Symmetrical polyneuropathy, n (%)	4 (30.8)
Prednisolone dose—mg/day, median [IQR]	5.0 [4.5-8.3]
Immunosuppressive therapy at baseline, n (%)	3 (23.1)
Background characteristics at onset	
Age at onset—yr, median [IQR]	49.0 [43.0-56.0]
Asthma with eosinophilia, n (%)	13 (100)
Biopsy evidence, n (%)	6 (46.2)
Peripheral neuropathy, n (%)	13 (100)
Nonfixed pulmonary infiltrates, n (%)	5 (38.5)
Sinonasal abnormality, n (%)	10 (76.9)
Cardiomyopathy, n (%)	5 (38.5)
Gastrointestinal involvement, n (%)	3 (23.1)
Glomerulonephritis, n (%)	2 (15.4)
Alveolar hemorrhage, n (%)	0 (0)
Palpable purpura, n (%)	3 (23.1)
ANCA-positive status, n (%)	5 (38.5)
Relapse and treatment history	
relapsing or refractory, n (%)	11 (84.6)
Immunosuppressive therapy, n (%)	8 (61.5)
Omalizumab therapy, n (%)	4 (30.8)
Immunoglobulin therapy, n (%)	11 (84.6)

IQR, interquartile range; BMI, body mass index; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; ANCA, antineutrophil cytoplasmic antibody

TABLE 2. Change in VAS, BVAS, prednisolone dose and peripheral eosinophils before and after 12 months of mepolizumab therapy

	Before	After	P value
Visual analog scale			
Pain, median (IQR)	67.0 (49.0-78.5)	48.0 (17.0-63.5)	0.012
Numbness, median (IQR)	67.0 (48.0-78.0)	51.0 (26.0-66.5)	0.017
Muscle weakness, median (IQR)	51.0 (27.5-82.5)	50.0 (27.0-75.0)	0.36
BVAS, median (IQR)	0 (0-2.0)	0 (0-1.5)	0.59
Prednisolone dose (mg/day), median (IQR)	5.0 (4.5-8.3)	2.0 (0.5-3.5)	0.001
Peripheral eosinophils (/μL), median (IQR)	370 (200-880)	50 (3-88)	0.003

Significance of the difference between before and after mepolizumab was assessed using the Wilcoxon signed rank test.

IQR, interquartile range; BVAS, Birmingham Vasculitis Activity Score

Table 3. Correlation of changes in urinary EDN and peripheral eosinophil levels with percent changes in VAS from baseline to 12 months after the initiation of mepolizumab therapy

	Percent changes in VAS from baseline to 12 months after the initiation of therapy					
	Pain		Numbness		Muscle weakness	
	r	P	r	P	r	P
Urinary EDN levels						
Change from baseline						
to 1 month after the initiation	0.29	0.49	0.41	0.32	0.11	0.82
to 3 months after the initiation	0.76	0.071	0.96	<0.001	-0.086	0.87
to 6 months after the initiation	0.75	0.020	0.88	0.002	0.048	0.91
to 12 months after the initiation	0.82	0.023	0.75	0.052	-0.086	0.87
Peripheral eosinophil levels						
Change from baseline						
to 1 month after the initiation	-0.007	0.98	0.35	0.27	-0.10	0.78
to 3 months after the initiation	-0.004	0.99	0.33	0.29	-0.030	0.93
to 6 months after the initiation	-0.081	0.80	0.25	0.44	-0.15	0.68
to 12 months after the initiation	-0.021	0.95	0.31	0.33	-0.097	0.79

Spearman's correlation coefficient (r) and its level of significance (P)

EDN, eosinophil-derived neurotoxin; VAS, visual analog scale

Figure 1

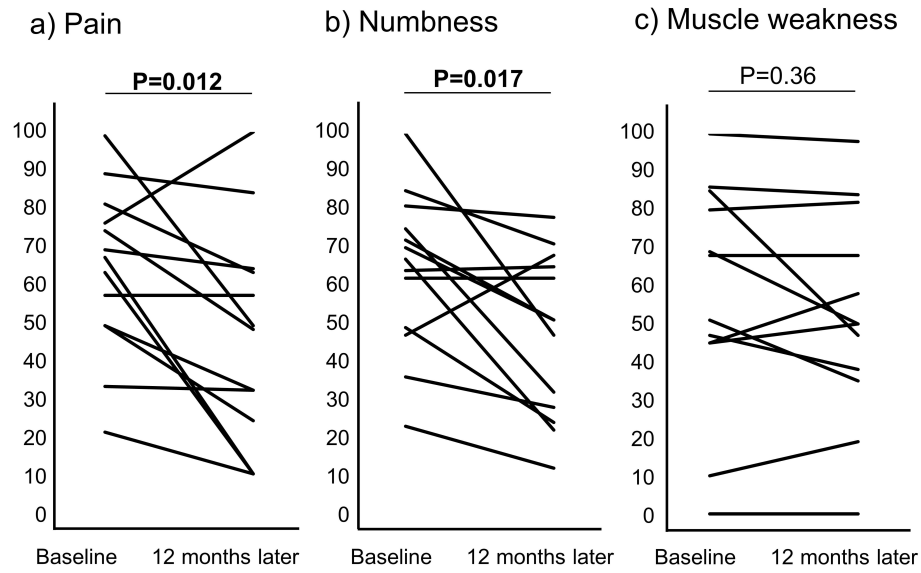

a) Pain
P=0.012
b) Numbness
P=0.017
c) Muscle weakness
P=0.36
100
90
80
70
60
50
40
30
20
10
0
Baseline
12 months later

Figure 2

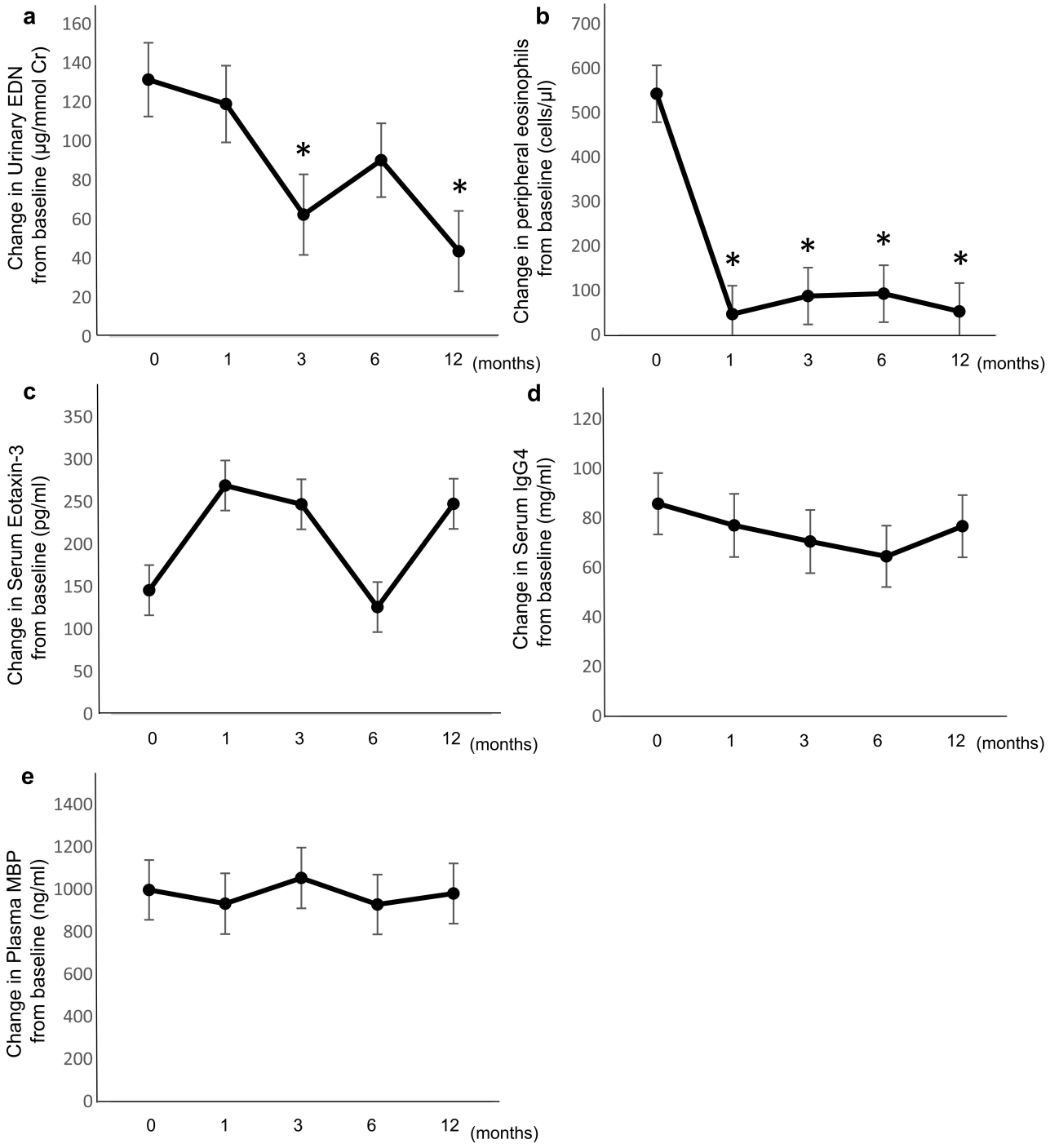

a
Change in Urinary EDN from baseline (μg/mmol Cr)
160
140
120
100
80
60
40
20
0
*
*
0
1
3
6
12
(months)
b
Change in peripheral eosinophils from baseline (cells/μl)
700
600
500
400
300
200
100
0
*
*
*
*
0
1
3
6
12
(months)
c
Change in Serum Eotaxin-3 from baseline (pg/ml)
350
300
250
200
150
100
50
0
0
1
3
6
12
(months)
d
Change in Serum IgG4 from baseline (mg/ml)
120
100
80
60
40
20
0
0
1
3
6
12
(months)
e
Change in Plasma MBP from baseline (ng/ml)
1400
1200
1000
800
600
400
200
0
0
1
3
6
12
(months)

Figure 3

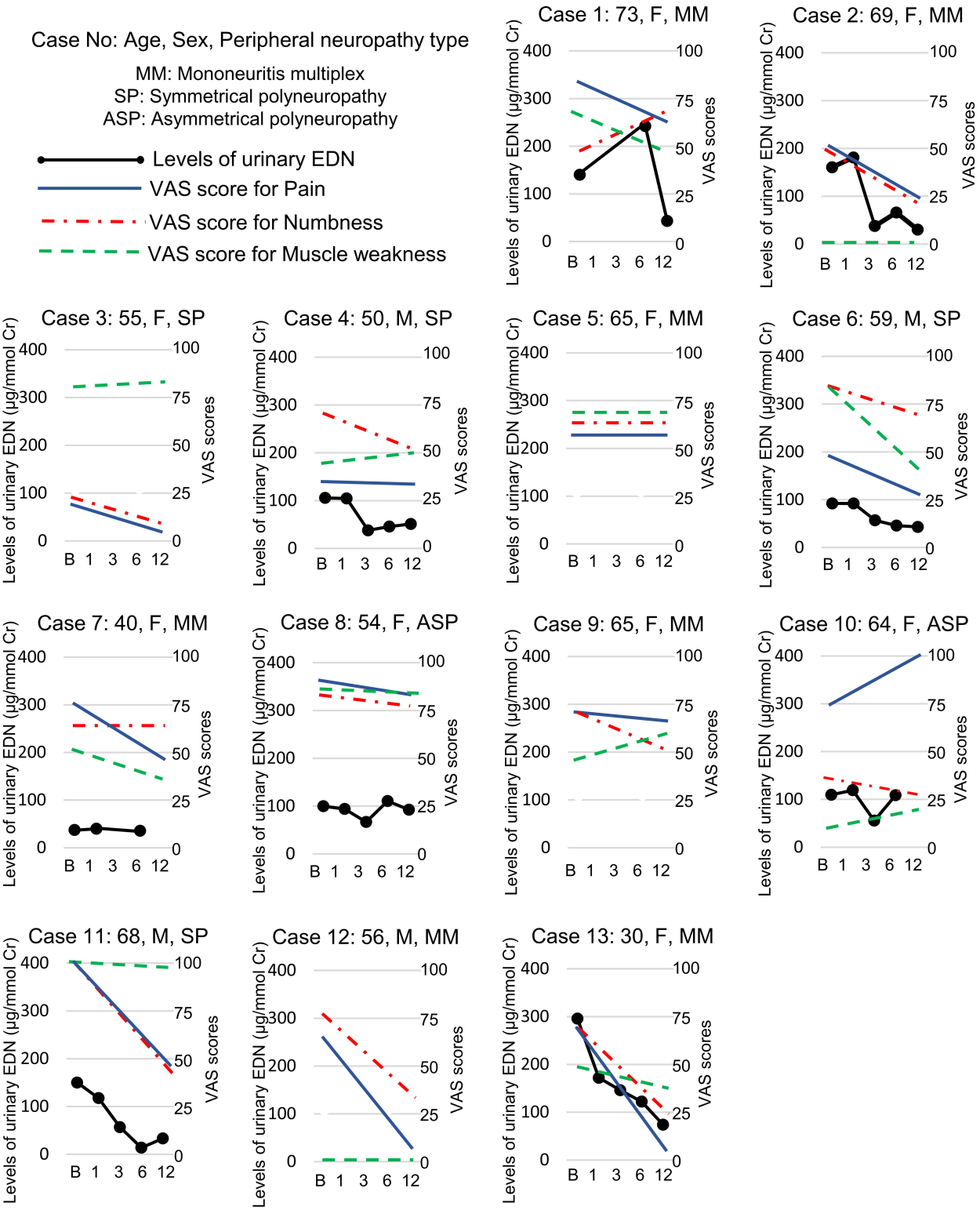
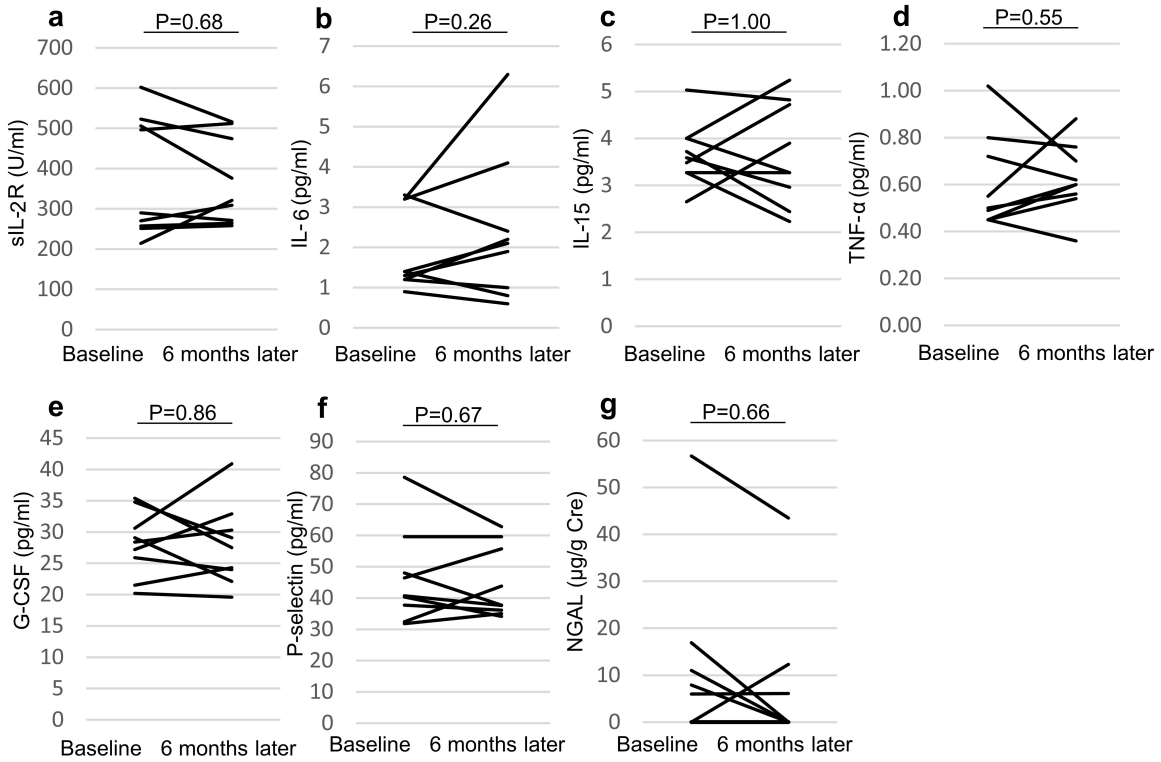

Case No: Age, Sex, Peripheral neuropathy type
MM: Mononeuritis multiplex
SP: Symmetrical polyneuropathy
ASP: Asymmetrical polyneuropathy
Levels of urinary EDN
VAS score for Pain
VAS score for Numbness
VAS score for Muscle weakness
Case 1: 73, F, MM
Case 2: 69, F, MM
Case 3: 55, F, SP
Case 4: 50, M, SP
Case 5: 65, F, MM
Case 6: 59, M, SP
Case 7: 40, F, MM
Case 8: 54, F, ASP
Case 9: 65, F, MM
Case 10: 64, F, ASP
Case 11: 68, M, SP
Case 12: 56, M, MM
Case 13: 30, F, MM
Levels of urinary EDN (μg/mmol Cr)
VAS scores
0 100 200 300 400
0 25 50 75 100
B 1 3 6 12

Figure S1


a
P=0.68
sIL-2R (U/ml)
700
600
500
400
300
200
100
0
Baseline
6 months later
b
P=0.26
IL-6 (pg/ml)
7
6
5
4
3
2
1
0
Baseline
6 months later
c
P=1.00
IL-15 (pg/ml)
6
5
4
3
2
1
0
Baseline
6 months later
d
P=0.55
TNF-α (pg/ml)
1.20
1.00
0.80
0.60
0.40
0.20
0.00
Baseline
6 months later
e
P=0.86
G-CSF (pg/ml)
45
40
35
30
25
20
15
10
5
0
Baseline
6 months later
f
P=0.67
P-selectin (pg/ml)
90
80
70
60
50
40
30
20
10
0
Baseline
6 months later
g
P=0.66
NGAL (μg/g Cre)
60
50
40
30
20
10
0
Baseline
6 months later