tomography (CT) scan revealed a massive pleural effusion and ascites. Additional treatment with melphalan (20 mg/m^2; day 7), and dexamethasone (20 mg/kg; days 7–10), was administered. Notably, the level of serum VEGF at the time of PBSC collection was reduced to 1210 pg/mL. Although the patient became oxygen-free after the treatment, a considerable volume of pleural effusion and ascites remained. In addition, the level of serum VEGF was increased to 4770 pg/mL on day 35 after the administration of G-CSF. Autologous stem cell transplantation was performed on day 39. The patient developed a sudden cardiopulmonary arrest with an unknown cause on day 85, and died on day 91 after the administration of G-CSF for PBSC collection.

3. Case 2

The second case (case 2) is a 64-year-old woman who was diagnosed with POEMS syndrome with monoclonal gammopathy (IgG-λ), polyneuropathy, a left pleural effusion, and pericardial effusion at a different hospital in October 2005. She was suffering from refractory pleural effusion after receiving 2 courses of steroid-pulse therapy and melphalan (4 mg/day × 4 days) + prednisolone (20 mg/day × 4 days) therapy, and was referred to our hospital in September 2009. Her clinical course is shown in Fig. 2. The day of administration of G-CSF for second attempt of PBSC collection is defined as day 0. The patient presented with systemic edema, left pleural effusion, pericardial effusion, and polyneuropathy. Her serum VEGF level on day minus 163 was 1360 pg/mL and her performance status was 3. Thalidomide monotherapy of 100 mg/day facilitated a slight improvement in the extravascular volume overload, and the patient's serum VEGF level was decreased to 883 pg/mL on day minus 122. She underwent PBSC collection by G-CSF alone using the COBE Spectra cell separator on day minus 94, although the number of CD34$^+$ cells was insufficient. Because serum VEGF level was increased to 2540 pg/mL on day minus 70, 3 courses of additional thalidomide (200 mg/day × 28 days) + DEX (12 mg/m^2/day × 4 days) therapy were performed. Serum VEGF level was mildly reduced to 1850 pg/mL on day minus 30, and the patient received HD-CY (CY: 2 g/m^2; days minus 11 to minus 10), followed by G-CSF (10 μg/kg; days 0–2) for PBSC collection. On day 3, she developed fever, hypoxia, acute renal failure, and hypotension, and CT scan revealed a deteriorated pleural and pericardial effusion. Mechanical ventilation and continuous hemodiafiltration in the intensive care unit (ICU) were required to support her life. Her serum VEGF level was reduced to 117 pg/mL, while her serum IL-6 level increased to 63.0 pg/mL on day 3. PBSC collection was cancelled because of her life-threatening condition, and additional treatment with thalidomide (100 mg/day) and DEX (40 mg/day; days 5–8) was initiated. Her respiratory distress and acute renal failure improved after the treatment,

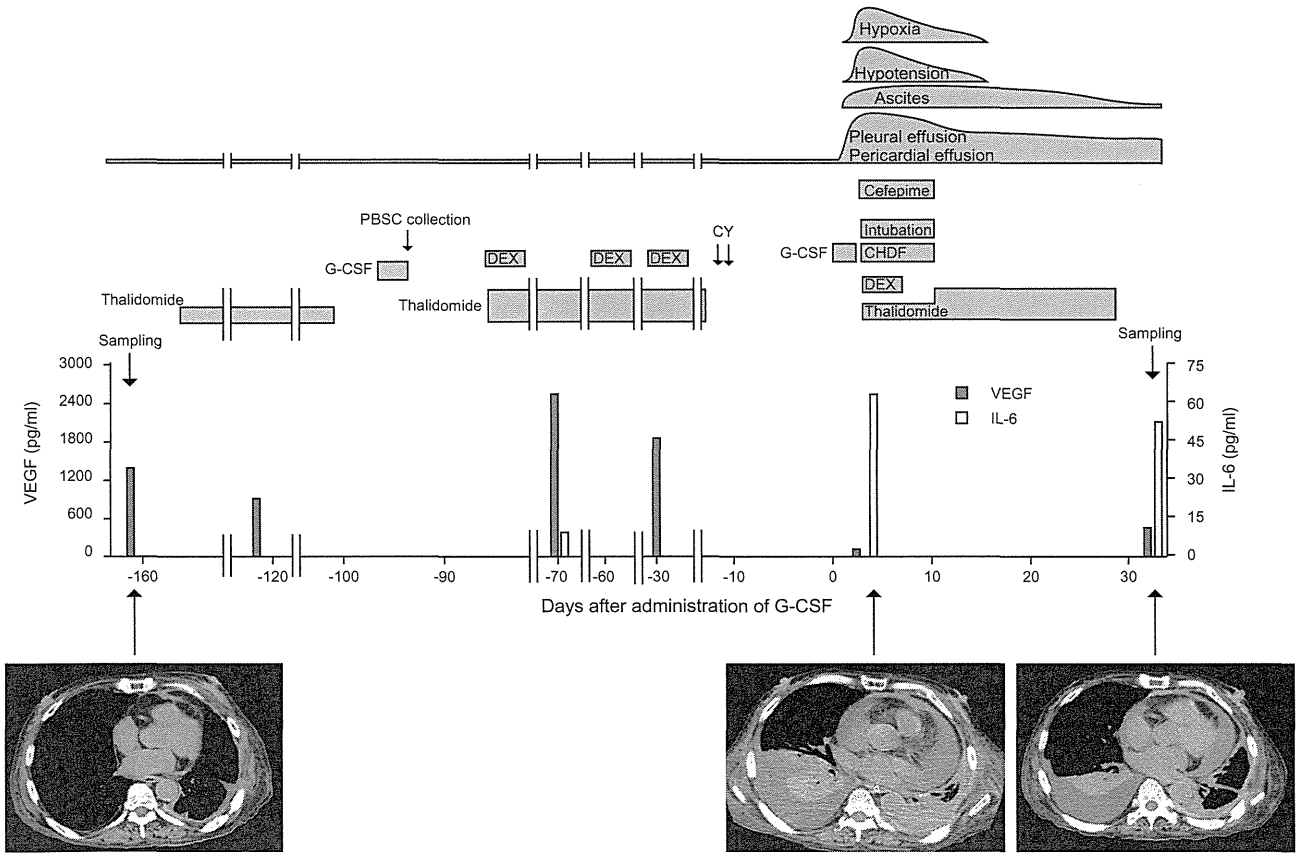

Fig. 2. Serial changes in CT scan images and cytokine levels, and the therapeutic course of case 2. IL-6, interleukin-6; CHDF, continuous hemodiafiltration.

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and she was discharged from the ICU on day 11 after the administration of G-CSF. Despite the continuation of the treatment by thalidomide monotherapy (200 mg/day), pleural and pericardial effusion still remained on day 33. Serum levels of IL-6 and VEGF also remained elevated and reduced, respectively (51.2 pg/mL and 453 pg/mL, respectively). She was transferred to a hospital closer to her hometown on day 137 after the administration of G-CSF.

4. Cytokine analysis

The serum concentrations of 27 cytokines and chemokines from these two cases were measured using a multiplex suspension array system (Table 1). The sera in case 1 were obtained 29 days before and 35 days after the administration of G-CSF for PBSC collection (Fig. 1). The sera in case 2 were obtained 163 days before and 33 days after the administration of G-CSF for second attempt of PBSC collection (Fig. 2). Sera from normal controls were obtained from people without malignancies at medical check-ups after informed consent was obtained. The average age of normal controls (n = 20) was 57.4 years. We defined the upper and lower limits of normal for each cytokine as the mean ± 3 standard deviations of normal control values in the same manner as our previous study [8]. The study was approved by the Ethics Committee of the Chiba University School of Medicine, and we received consent from each patient. In case 1, the levels of five cytokines, including IL-6, were elevated after CG (black colors in Table 1). The levels of four cytokines, including VEGF, were decreased, but remained abnormally high after CG (pale gray color in Table 1), while the abnormally high levels of two cytokines (IL-13 and monocyte chemotactic protein–1) were decreased to normal levels after CG. In case 2, the abnormally high levels of three cytokines (IL-10, IL-12, and VEGF) were decreased to normal levels after CG, while the level of IL-6 was increased to an abnormally high level after CG (black color in Table 1).

5. Discussion

To our knowledge, this is the first analysis of cytokine profiles in POEMS syndrome patients who developed acutely deteriorated extravascular volume overload. The development of capillary leak symptoms without increased levels of VEGF prompted us to speculate that the cytokine profiles in our cases may be altered after the administration of CG. Although the cytokine profiles of two patients in this study were different, it is noteworthy that levels of IL-6 after CG were elevated in both cases. IL-6 is a proinflammatory cytokine involved in pathogenesis of various diseases,

Table 1
Serum cytokine levels[a] in control samples and POEMS syndrome patients with severe capillary leak symptoms.

	Control (n = 20)			Case 1			Case 2		
	Mean	SD	Mean + 3SDs	Before	After	Fold change (before/after)	Before	After	Fold change (before/after)
VEGF	134	76.6	363	3593	1753	0.49	564	107	0.19
IL-12 (p70)	82	42.7	210	797	510	0.64	224	51.6	0.23
IL-10	19.2	10.6	51	132	109	0.83	55	9.3	0.17
IL-7	11.1	3.7	22.4	51.3	31.8	0.62	16.3	6.9	0.42
MCP-1	45.7	15.2	91.4	103	47.7	0.46	78.7	23.9	0.30
IL-13	15	4.8	29.4	32.9	24	0.73	16.1	7.1	0.44
IL-8	24.7	12.6	62.4	165	380	2.30	32.7	27.7	0.85
MIP-1β	438	225	1112	1209	2478	2.05	481	332	0.69
IL-6	8	3.6	18.9	16.6	45	2.71	8.6	30	3.49
MIP-1α	9.2	8.3	34.1	32.6	100	3.07	5.4	3.8	0.70
IL-1β	2	0.82	4.4	4.3	7.5	1.74	1.6	0.68	0.43
IL-1ra	114	95.3	400	214	102	0.48	94	16.9	0.18
IL-2	3.7	5.2	19.1	ND	ND	–	ND	ND	–
IL-4	5.1	1.3	8.9	6.9	3.9	0.57	4.4	3.8	0.86
IL-5	2.3	1.2	6	3.6	2.1	0.58	1.5	ND	–
IL-9	31.2	50.5	182	42.5	33.3	0.78	18.6	8.8	0.47
IL-15	ND			ND	ND	–	ND	ND	–
IL-17	25.8	23.4	96	47.3	10.6	0.22	20	ND	–
Eotaxin	77.2	35.7	184	62.2	54.2	0.87	138	60	0.43
FGF basic	42.9	24.5	116	72.2	27.9	0.39	63.9	10.2	0.16
G-CSF	60.3	28.2	145	114	68.9	0.60	53.5	22.8	0.43
GM-CSF	13	31.1	106	27.4	6.9	0.25	ND	ND	–
IFN-γ	66.7	23.4	136	120	64.4	0.54	48.8	25.8	0.53
IP-10	1643	1808	7067	1813	3388	1.87	6298	6730	1.07
PDGF-BB	759	240	1479	853	348	0.41	381	400	1.05
TNF-α	44.4	26.3	123	65.3	118	1.81	38.5	78.5	2.04
RANTES	12363	1183	8814[b]	8877	6127	0.69	8738	9268	1.06

Serum cytokine levels of control samples and POEMS syndrome patients before and after cyclophosphamide (CY) and G-CSF (CG) were administered. Gray colors represent the cytokines with abnormally high levels before CG. Pale gray colors represent the cytokines with reduced levels after CY, but still in the abnormal range. Black colors represent the cytokines with elevated levels after CY as compared to those before CY, and in the abnormal range. [a]pg/mL; [b]Mean-3SD. FGF, fibroblast growth factor; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; IL, interleukin; IL-1ra, interleukin-1 receptor antagonist; IP, induced protein; MCP, monocyte chemotactic protein; MIP, macrophage inflammatory protein; PDGF, platelet-derived growth factor; RANTES, regulated upon activation, normal T cell expressed and secreted; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor; ND, not detected.

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including multiple myeloma and Castleman's disease [10]. In addition, the level of IL-6 is also frequently elevated in POEMS syndrome patients [11]. Thus, IL-6 might play an important role of developing capillary leak symptoms after CG in both cases. Other cytokines with changed levels before and after CG in the present study are also informative. For instance, the level of inflammatory protein (MIP)-1α in case 1 was increased to abnormally high level after CG. MIP-1α has been reported to be a key proinflammatory cytokine in the pathogenesis of inflammatory diseases, such as multiple sclerosis [12]. Additionally, the ratio of IL-1β to IL-1 receptor antagonist (IL-1ra) was increased after CG. In fact, the results of previous studies have implicated an imbalance of IL-1β and IL-1ra as a contributing factor in several inflammatory diseases [13,14]. Thus, elevated level of MIP-1α and an increased ratio of IL-1β to IL-1ra may contribute to the acutely deteriorated extravascular volume overload after CG as well as elevated level of IL-6 in case 1.

The factor that induced the change of the cytokine profile, leading to acutely deteriorated extravascular volume overload observed in this study, is crucial. It has been speculated that the use of G-CSF in POEMS syndrome patients may increase the release of proinflammatory cytokines and lead to worsened clinical symptoms [15,16]. In addition, POEMS syndrome is known to have high rates of engraftment syndrome after autologous stem cell transplantation [17]. As exaggerated cytokine responses during marrow reconstitution after autologous stem cell transplantation are suspected to contribute to developing engraftment syndrome, an overlap in mechanism of exaggerated production of proinflammatory cytokines between the clinical symptoms in our cases and engraftment syndrome may exist. Indeed, the level of IL-6, which is considered an important factor of engraftment syndrome [18], was increased after CG in both cases in this study. Taken together, G-CSF and hematological recovery from bone marrow suppression induced by HD-CY might cooperate to increase the release of proinflammatory cytokines, resulting in developing capillary leak symptoms in our cases.

Other than exaggerated production of proinflammatory cytokines described above, the reduction of VEGF level may play some role of the deteriorated extravascular volume overload in our cases. VEGF is an important survival factor for newly formed blood vessels. Of note, removing VEGF leads to apoptosis of endothelial cells [19,20]. Therefore, it is speculated that sudden VEGF removal may induce sudden collapse of a newly formed fragile vessels and an increase of capillary leakiness [6,21]. Remarkable reduction of VEGF levels by HD-CY in the present cases might also contribute to microvascular hyperpermeability.

The current study has some limitations. The number of patients is small, and the treatments for capillary leak symptoms after the administration of CG probably affect the results of multiplex suspension array system. In addition, it is an important issue how our findings in this study should be applied to understand the pathogenesis of POEMS syndrome not via VEGF. Thus, a future study on a large scale using sera at appropriate timings from POEMS syndrome patients who develop capillary leak symptoms without increased VEGF levels, such as a relapsed patient after autologous stem cell transplantation, should be performed [22]. Despite these limitations, our findings indicated that exaggerated production of proinflammatory cytokines may have contributed to acutely deteriorated extravascular volume overload during stem cell mobilization, which may improve our understanding of the pathogenesis of POEMS syndrome not attributable to VEGF.

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Correlation between peripapillary retinal thickness and serum level of vascular endothelial growth factor in patients with POEMS syndrome.
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Correlation between peripapillary retinal thickness and serum level of vascular endothelial growth factor in patients with POEMS syndrome

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ABSTRACT

Aims To determine whether there is a significant correlation between the peripapillary retinal thickness (pRT) and the serum level of vascular endothelial growth factor (VEGF) in patients with polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy and skin changes (POEMS) syndrome.

Methods This was a cross-sectional, observational case series. We studied 34 eyes of 17 treatment-naïve patients with POEMS syndrome whose intracranial pressure was within the normal range. The spectral-domain optical coherence tomographic (SD-OCT) examinations consisted of circle scans of 3.45 mm diameter around the optic disc. The pRT was automatically measured in the SD-OCT images and was used for the statistical analysis. The serum level of VEGF was measured by ELISAs, and the correlation between the pRT and the serum level of VEGF was determined. Multivariable logistic regression analyses were used to identify independent factors that were correlated with the pRT.

Results There was a significant positive correlation between the serum levels of VEGF and the average pRT of the two eyes of each patient ($r=0.81$, $p<0.0001$). There was a significant correlation between the pRT of the right and left eyes with an intraclass correlation coefficient of 0.839. Multiple regression analysis showed that the serum levels of VEGF were independent contributors to the pRT (standard regression coefficient=0.59, $p=0.012$).

Conclusions The significant correlation between the pRT and the serum level of VEGF suggests that the higher serum level of VEGF might be associated with the development of the optic disc oedema in patients with POEMS syndrome.

INTRODUCTION

Patients with polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy and skin changes are diagnosed with POEMS syndrome, and they are associated with plasma cell dyscrasia.[1] The overproduction of vascular endothelial growth factor (VEGF) has been suggested to be the cause of POEMS syndrome, although the pathogenesis has not been determined definitively. VEGF promotes neovascularisation and enhances vascular permeability,[1–3] and these alterations have been suggested to be responsible for the signs of POEMS syndrome, such as oedema, angiomata, organomegaly and pleural effusion/ascites.[2]

The pathogenesis of the ocular abnormalities associated with POEMS syndrome has not been determined, and data on the incidence and spectrum of these associations are limited. The major ocular finding in POEMS syndrome is optic disc oedema (ODO) as reported in several single case reports and small case series.[1 4–8] It has been suggested that an elevated VEGF level or elevated intracranial pressure (ICP) might play a role in the development of ODO.[4 7–11]

Optical coherence tomography (OCT) is a non-invasive imaging technique producing high-resolution cross-sectional images of the retina. The retinal thickness measurements obtained by OCT have been used mainly to evaluate macular oedema, and the measurements are accurate for the distance between the internal limiting membrane (ILM) and retinal pigment epithelium (RPE). In addition, the measurements are useful for evaluating oedema in the peripapillary region. The peripapillary thickness measurements by OCT have been used to evaluate the optic disc in patients with idiopathic intracranial hypertension,[12] and the total peripapillary thickness has also been used to diagnose papilloedema.[13 14]

The purpose of this study was to determine whether the peripapillary retinal thickness (pRT) in patients with POEMS syndrome with normal ICP was significantly correlated with the serum level of VEGF.

MATERIALS AND METHODS

We reviewed the medical records of 34 eyes of 17 treatment-naïve patients with the POEMS syndrome at the Chiba University Hospital from September 2013 to March 2015. The diagnosis of POEMS syndrome was made according to published criteria in 2013.[15]

The design and protocol of this study was approved by the Institutional Review Board of Chiba University Graduate School of Medicine. All procedures conformed to the tenets of the Declaration of Helsinki, and patients were informed about the aim of the study, and written consents were obtained.

Patients were excluded if even one eye had: an axial length >26.5 mm, intraocular pressure (IOP) >21 mm Hg, history of intraocular surgery, history of retinal or choroidal vascular diseases and glaucoma. In addition, patients were excluded if the opening lumbar puncture (LP) pressure was >20 cm H_2O. Cranial CT and MRI did not find any intracranial pathology or optic nerve infiltration in any of the patients.

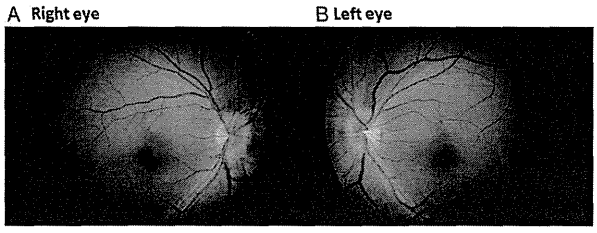

Figure 1 Ocular findings in a patient with polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy and skin changes (POEMS) syndrome (case 8). Fundus photographs showing bilateral optic disc oedema; (A) right eye, (B) left eye.

Dilated ophthalmoscopy was performed by one experienced neuro-ophthalmologist (TO) using a 90-diopter lens.[16] ODO was diagnosed when one of the following was present: hyperaemia of the disc, disc blurring, or absence of spontaneous venous pulsations[16] (figure 1A, B).

The data collected from all patients included the best-corrected visual acuity (BCVA) measured with a Snellen chart, IOP, axial length, slit lamp biomicroscopy and ophthalmoscopic findings. Blood samples were collected from all of the patients, and the opening LP pressure was measured. The major outcomes were the pRT thickness and the serum level of VEGF.

Measurements of serum level of VEGF

Blood samples were collected and allowed to clot at room temperature for about 1 h. The samples were then centrifuged at 3000×g for 10 min, and the sera collected. The serum samples were stored in aliquots at −80°C until analyses. ELISAs were used to determine the serum level of VEGF (Quantikine HS, R&D Systems, Minneapolis, Minnesota, USA).

Optical coherence tomography

The pRT was measured with the RS3000 advance spectral domain OCT (SD-OCT; NIDEK, Gamagori, Japan). A circular scan centred on the optic disc (3.45 mm diameter, 'Disc Circle' mode, software NAVIS-EX V.1.5.0) was recorded through a dilated pupil. The scan consisted of 1024-A scan with high-definition (50 HD) frame enhancement software that measured the pRT along a circle of 3.45 mm diameter around the optic disc. The pRT was measured automatically as the distance between the ILM and the outer border of the RPE with the software in the RS3000 SD-OCT (figure 2A, B). The average pRT was used for the statistical analyses. All images were acquired by a single well-trained operator (MK) who was masked to the diagnosis of POEMS syndrome and other clinical findings.

Intracranial pressure

After the ophthalmic examinations, LP was performed in all patients using a standard 18-guage or 20-gauge spinal needle and a manometer positioned at a 90-degree angle to the spine. The opening LP pressure was measured while the subject was placed in lateral decubitus position with legs extended, head and spine strictly horizontal, and as relaxed as possible. Sufficient time was allowed for the pressure to stabilise. The pRT and the opening LP pressure were measured within 2 week after the blood samples were collected. The opening LP pressure was defined as the ICP.

Statistical analyses

Statistical analysis was performed using SPSS software for Microsoft Windows (SPSS V.20, IBM Japan, Tokyo). The correlations between the pRT and the serum level of VEGF in POEMS patients were determined by the Spearman's rank-correlation coefficient. The correlation between the pRT of the right and left eyes in POEMS patients was determined by the

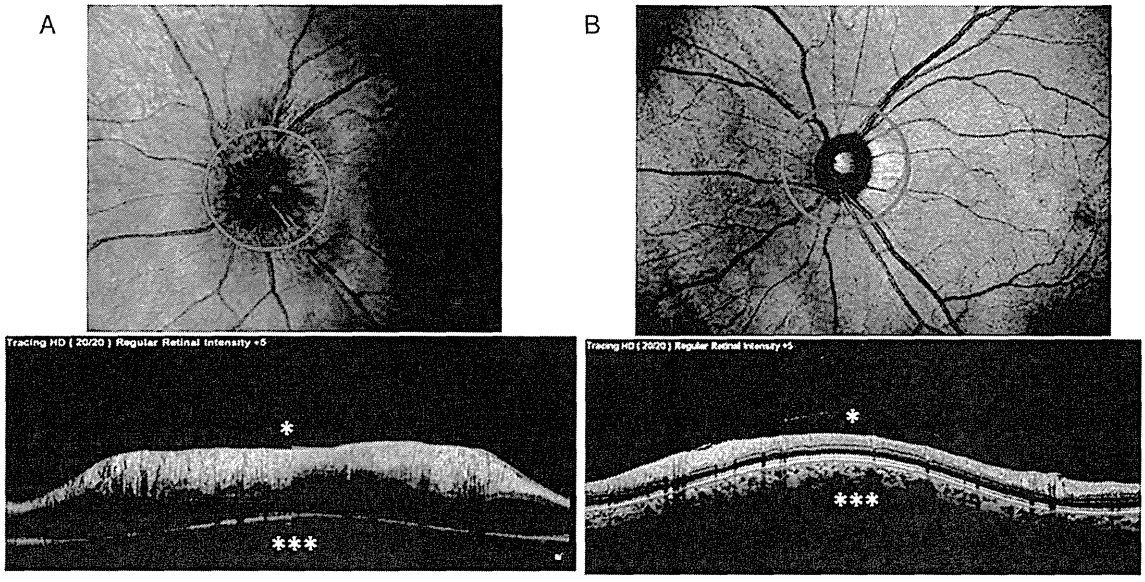

Figure 2 Peripapillary retinal thickness (pRT) analysis. Optical coherence tomographic (OCT) images of patients with polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy and skin changes (POEMS) syndrome with papillary oedema (A; left eye of case 16) and without papillary oedema (B; left eye of case 4). Disc Circle protocol was centred on the optic nerve head to measure the pRT around a 3.45 mm-diameter circle. Average pRT thickness measurement was used for the statistical analyses. Red line and purple line represent the OCT algorithm outlining the pRT: the anterior border (*) towards the internal limiting membrane and the posterior border (***) at the outer border of retinal pigment epithelium (RPE) line.

intraclass correlation coefficient (ICC). Mann–Whitney U tests were used to determine if the differences between groups were statistically significant. In addition, multivariable logistic regression analysis was performed to identify independent factors, for example, serum VEGF, ICP, age and IOP, which were correlated with the average pRT of the two eyes of each patient. Statistical significance was defined as $p<0.05$.

RESULTS

The demographics of the 17 Japanese patients with POEMS syndrome (9 men, 8 women) are shown in table 1.

Their mean age was 51.4±13.5 years with a range from 36 to 73 years. The mean IOP was 11.9±2.0 mm Hg with a range from 9 to 16 mm Hg. The mean pRT was 344.8±100.7 μm for the right eyes and 362.8±124.1 μm for the left eyes. The difference in the mean pRT between the right and the left eyes was not significant ($p=0.55$, Mann–Whitney U test), but there was a significant correlation between the pRT of the right and left eyes with an ICC of 0.839 (figure 3). Thirteen patients (76%) had bilateral ODO that was detected by indirect ophthalmoscopy.

The mean serum level of VEGF in all patients was 6085±3332 pg/mL with a range of 1380–12 000 pg/mL, which is almost 30-fold higher than that of normal subjects (219 pg/mL).[17] The mean opening LP pressure in all patients was 137.9±33.7 mm H_2O with a range of 100–195 mm H_2O, which is almost within normal range of 120–206 mm H_2O.[12]

There was a significant positive correlation between serum levels of VEGF and the average pRT of the two eyes of each patient ($r=0.81$, $p<0.0001$, Spearman's rank correlation; figure 4).

The difference in the serum VEGF concentrations between patients with ODO (7086±3115 pg/mL) and without ODO (2835±1408 pg/mL) was also significant ($p=0.029$, Mann–Whitney U test).

Multiple regression analysis showed that the serum level of VEGF was an independent contributor to the average pRT of the two eyes of each patient (VEGF, standard regression coefficient=0.59, $p=0.012$; ICP, standard regression coefficient=0.076, $p=0.73$; age, standard regression coefficient=0.052, $p=0.81$; IOP, standard regression coefficient=0.21, $p=0.34$).

DISCUSSION

The pathogenesis of POEMS syndrome is complex, and several systemic factors are thought to be involved. The results of

Table 1 Patient characteristics and optical coherence tomography data and serum levels of VEGF

Patient	Age	Sex	Eye	BCVA	Intraocular pressure (mm Hg)	Peripapillary retinal thickness (μm)	Intracranial pressure (mm H_2O)	Serum levels of VEGF (pg/mL)	Optic disc oedema
1	57	F	OD	1	11	301	125	6400	+
			OS	1.2	13	328			+
2	61	M	OD	1.2	11	388	110	11 600	+
			OS	1.2	11	298			+
3	69	F	OD	1.2	13	287	105	3150	+
			OS	1.2	13	294			+
4	56	M	OD	0.6	9	217	120	1960	–
			OS	0.6	10	254			–
5	73	M	OD	1.2	10	287	140	3910	+
			OS	1.2	10	311			+
6	39	M	OD	1.2	15	316	195	2230	+
			OS	1.2	14	334			+
7	63	M	OD	1.2	16	323	160	5220	+
			OS	1.2	15	291			+
8	38	F	OD	1.2	9	341	190	6420	+
			OS	1.2	9	347			+
9	36	F	OD	1.2	12	263	195	1380	–
			OS	1.2	12	250			–
10	38	M	OD	1.2	12	324	140	6110	+
			OS	1.2	11	299			+
11	57	M	OD	1.2	10	447	170	10 600	+
			OS	1.2	9	553			+
12	38	M	OD	1.2	12	266	140	4420	–
			OS	1.2	11	266			–
13	34	F	OD	1.2	10	328	105	12 000	+
			OS	1.2	11	327			+
14	49	F	OD	1.2	14	291	100	3580	–
			OS	1.2	15	287			–
15	65	F	OD	0.7	14	361	120	7250	+
			OS	1.2	14	503			+
16	64	F	OD	1.2	15	479	90	8170	+
			OS	1.2	10	635			+
17	36	M	OD	1.2	13	643	140	9060	+
			OS	1.2	12	592			+

BCVA, best-corrected visual acuity; OD, right eye; OS, left eye; VEGF, vascular endothelial growth factor.

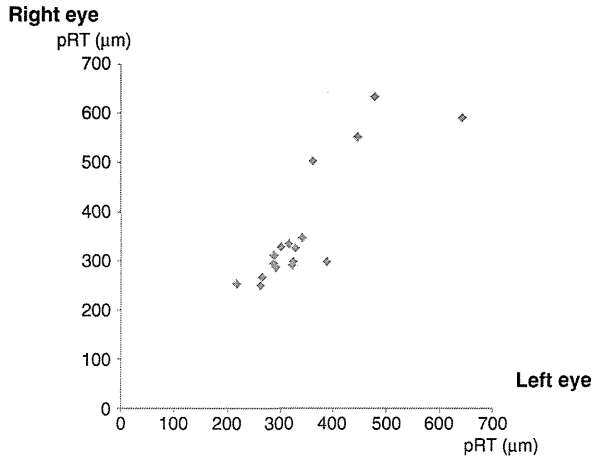

Figure 3 Correlation between the peripapillary retinal thickness (pRT) of right and left eyes in patients with polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy and skin changes (POEMS) syndrome. There was a significant correlation between the pRT of right and left eyes in patients with POEMS syndrome with an intraclass correlation coefficient of 0.839.

several studies suggested that a hyperproduction of VEGF by abnormal plasma cells was the major contributor to the development of POEMS syndrome.[9] The elevated levels of VEGF may also account for the ODO as it has been suggested that ODO is due to higher levels of VEGF.

ODO was detected bilaterally in 13 patients (76%), which is consistent with earlier reports that bilateral ODO was the most common (30%–70%) sign in POEMS syndrome.[1 4 6 8 11] The cause of the ODO in POEMS syndrome has not been determined, but it has been suggested that an elevated ICP,[7–9 11] vasculitis,[18] presence of cerebrospinal fluid proteins,[11] infiltrations into the optic nerve,[19] or increased VEGF levels[4 8 10 20] may be involved.

It has also been reported that the increased vascular permeability associated with elevated VEGF serum levels may contribute to the development of ODO.[4 8 10 20] This concept of

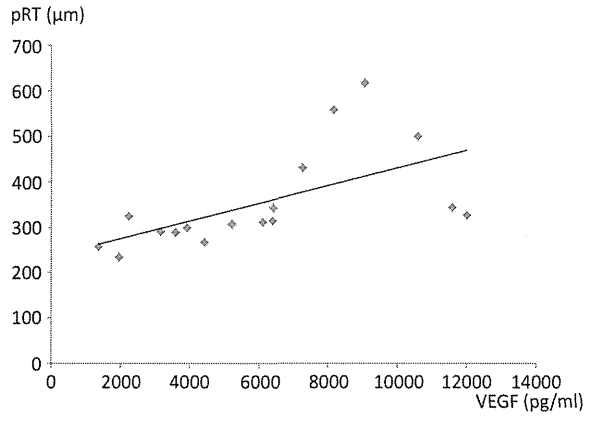

Figure 4 Correlation between serum levels of vascular endothelial growth factor (VEGF) and the average peripapillary retinal thickness (pRT) of two eyes of each patient with polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy and skin changes (POEMS) syndrome. There was a significant positive correlation between serum levels of VEGF and the average pRT of the two eyes of each patient ($r=0.81$, $p<0.0001$, Spearman's rank correlation).

microvascular hyperpermeability is supported by the presence of oedema elsewhere in the body of patients with POEMS syndrome.[4]

Okada reported that the ODO in POEMS syndrome was correlated with the serum level of VEGF as we found, and patients with ODO responded well to systemic autologous peripheral blood stem cell transplantation.[20] Kaushik also suggested a possible association between plasma VEGF and ODO because the median plasma VEGF level at the initial presentation was 300 pg/mL, which was significantly higher than the median VEGF level after the ODO resolved (51 pg/mL).[8]

However, these earlier reports were small case series or single case reports, and to the best of our knowledge, our study is the first to investigate the relationship between the serums levels of VEGF and the pRT by SD-OCT in a large number of patients with POEMS syndrome. The reason why the pRT was used in this study was that some patients had bilateral high grade of papilloedema with a Modified Frisen Scale of grade 4 or 5. Colin *et al* reported that the failure rates of retinal nerve fibre layer (RNFL) thickness measurements increased as the degree of disc oedema increased, and that the algorithm defining total retinal thickness had a lower failure rate than that defining the RNFL thickness.[14] Therefore, Colin *et al* reported that for lower grade papilloedemas, the RNFL thickness measurement was useful, but with higher-grade papilloedemas, the total retinal thickness was obtained more accurately,[14] and that the OCT total retinal thickness measurements might be more useful especially for higher grades of papilloedemas.[14]

The significantly higher levels of serum VEGF in patients with than without ODO and the significant positive correlation between the pRT and the serum levels of VEGF suggest that the elevated serum VEGF was the cause of the ODO in patients with POEMS syndrome as reported.

However, some earlier case reports, a small series study[7–9 21] and a clinical study[11] suggested that the presence of intracranial hypertension may be a contributing factor to the ODO in POEMS syndrome, although some small series have found no relationship between ODO and raised ICP.[5 6 18 22 23] These results suggest that an elevated ICP may be a mechanical explanation for the development of ODO in POEMS syndrome because the ICP can be transmitted to the optic disc causing ODO.[8]

Kaushik reported that patients with ODO had a higher mean opening LP (276 mm H_2O, three patients) than patients without ODO (176 mm H_2O, two patients), although the difference was not statistically significant ($p=0.08$).[8] Wiaux also reported that two patients had elevated ICP of 300 and 390 cm H_2O.[7]

In our patients, the mean opening LP pressure in all patients was almost within the normal range of ICP,[12] and was lower than these case reports.[7 8] In addition, the difference in the mean opening LP pressure between patients with ODO (132 mm H_2O, 13 patients) and patients without ODO (120 mm H_2O, four patients) was not significant ($p>0.05$, Mann–Whitney U test).

In addition, the correlation between the pRT and the opening LP pressure was not significant in our patients ($p>0.05$, Spearman's rank-correlation coefficient; data not shown). The multiple regression analysis showed that the ICP was not an independent contributor to the pRT in our patients (standard regression coefficient=0.076, $p=0.73$). Thus, we believe that the effect of ICP had a minimal effect on the ODO in our patients.

There was a significant correlation between the serum levels of VEGF and the average pRT of the two eyes of each patient

(r=0.81, p<0.0001, figure 4). However, the pRT peaked at about 9000 pg/mL and then decreased (figure 4). The reason why the pRT peaked at about 9000 pg/mL and then fell is that Case 2 (11 600 pg/mL) and Case 13 (12 000 pg/mL) had myopia with axial lengths of 26.04 and 26.1 mm, respectively and their retinas appeared to be thin. To exclude the effect of high myopia, all of our patients whose axial length was >26.5 mm were excluded, but we believe that it may not have been completely excluded in these eyes.

Our study has several limitations. First, the possible effects of factors other than the ICP and level of VEGF on the ODO must be taken into consideration. Other factors hypothesised to contribute to ODO, such as infiltration by abnormal proteinaceous material,[19] were not examined, although we could find no lesions suggesting infiltrations into the choroid, orbit, or optic nerve in our cases. In addition, we did not find any systemic abnormalities such as differences in the body mass index. Second, our results cannot answer the question of a cause–effect relationship between ODO and raised VEGF. Further studies are needed to determine whether treatment of POEMS syndrome, such as high-dose chemotherapy with autologous peripheral blood stem-cell transplantation, anti-VEGF monoclonal antibody (bevacizumab) therapy and thalidomide therapy, can lead to a decrease in the pRT thickness by reducing the serum VEGF level. Finally, we were not able to determine whether ODO is more likely related to VEGF concentration, and further studies with a larger sample size may help to explain this and also explain the mechanism of ODO in patients with POEMS syndrome.

In conclusion, we found a significant correlation between the pRT and serum level of VEGF in patients with POEMS syndrome and we also found the significantly higher serum VEGF concentrations in patients with POEMS syndrome and ODO. In addition, the multiple regression analysis showed that the serum level of VEGF was an independent contributor to the pRT. These results suggest that raised VEGF might explain the development and mechanism of ODO in patients with POEMS syndrome. In addition, our findings indicate that OCT can be helpful in the ophthalmic evaluations of patients with POEMS syndrome.

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Contributors Design and conduct of study: HY, TB and SS. Collection, management, analysis and interpretation of the data: HY, TB, MK, TO and SM. Preparation, review or approval of the manuscript: HY, TB, SY and SK.

Competing interests None declared.

Patient consent Obtained.

Ethics approval Institutional Review Board of Chiba University Graduate School of Medicine.

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RETINAL DISORDERS

Correlation between serum level of vascular endothelial growth factor and subfoveal choroidal thickness in patients with POEMS syndrome

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Abstract
Purpose The study was conducted to determine whether serum vascular endothelial growth factor (VEGF) levels are significantly correlated with subfoveal choroidal thickness (CT) and foveal thickness (FT) in patients with polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes (POEMS) syndrome.
Patients and methods In this cross-sectional observational case series, we studied 31 eyes of 16 treatment-naïve patients with POEMS syndrome with no evidence of fundus abnormalities. Subfoveal CT and FT were measured using enhanced depth imaging optical coherence tomography (EDI-OCT), and correlations between serum VEGF levels and subfoveal CT and FT were determined.
Results The mean subfoveal CT was 417.9±73.5 μm (right eye, 416.7±81.2 μm; left eye, 419.0±68.1 μm), and the mean FT was 243.8±35.2 μm (right eye, 248.8±22.0 μm; left eye, 239.1±44.6 μm). There was a significant positive correlation between the serum VEGF level and subfoveal CT (right eye, r=0.58, p=0.021; left eye, r=0.60, p=0.012), but the correlation between the level of serum VEGF and FT was not significant (right eye, r=0.007, p>0.05; left eye, r=0.25, p> 0.05).
Conclusions The significant correlation between the serum VEGF level and subfoveal CT in patients with POEMS syndrome suggests that choroidal thickness is influenced by the level of serum VEGF. These results not only aid in an understanding of the pathogenesis of ocular changes in patients with POEMS syndrome, but also offer clues regarding the pathogenesis of other choroidal diseases.

Keywords POEMS syndrome · Vascular endothelial growth factor (VEGF) · Subfoveal choroidal thickness · Foveal thickness

Introduction

The term "POEMS" syndrome refers to a multi-system disorder that is characterized by polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes, and which is associated with plasma cell dyscrasia [1, 2]. Although the pathogenesis of the disease has not been definitively determined, it has been suggested that overproduction of vascular endothelial growth factor (VEGF) plays an important role. VEGF strongly promotes neovascularization and enhances vascular permeability [3–5], and these changes are responsible for the characteristic signs of POEMS syndrome, including angiomata, pleural effusion/ascites, edema, and organomegaly [3]. It is thought that VEGF is inappropriately secreted by monoclonal plasma cells [4, 5]; if this can be proven, these cells could be targeted for the treatment of POEMS syndrome [5].

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Data on the incidence and the spectrum of ocular abnormalities associated with POEMS syndrome are limited, and the pathogenesis of the disease has not been determined. The major ocular finding in POEMS syndrome is optic disc edema [5–7]. Examination of the eyes of patients with POEMS syndrome using spectral-domain optical coherence tomography (SD-OCT) has shown serous retinal detachment (SRD) [8] and cystoid macular edema (CME) [6]. Conventional SD-OCT cannot provide clear imaging of the entire choroid, but such images can be obtained using enhanced depth imaging (EDI)-OCT, and these images can be used to measure the thickness of the choroid [9]. As such, EDI-OCT has been used to measure choroidal thickness in normal eyes [10, 11], in eyes with central serous chorioretinopathy (CSC) [12] and Vogt–Koyanagi–Harada (VKH) disease [13], in highly myopic eyes [14], and in eyes with retinitis pigmentosa (RP) [15]. However, EDI-OCT has not been used to study the choroid in eyes with POEMS syndrome.

The purpose of this study, therefore, was to determine the subfoveal choroidal and foveal thickness in the eyes of patients with POEMS syndrome, and also to determine whether there was a significant correlation between subfoveal choroidal thickness and serum VEGF levels.

Methods

We reviewed the medical records of 31 eyes of 16 treatment-naïve patients with POEMS syndrome at the Chiba University Hospital from November 2011 through September 2013. The diagnosis of POEMS syndrome was made using criteria established by Dispenzieri in 2007 [5].

The design and protocol of the study were approved by the Institutional Review Board of Chiba University Graduate School of Medicine. All procedures conformed to the tenets of the Declaration of Helsinki; patients were informed of the nature of the study, and written consent was obtained.

Patients were excluded if even one eye had any of the following: (1) axial length greater than 26.5 mm, (2) refractive error (spherical equivalent)>−6.0 diopters (D); (3) intraocular pressure >21 mmHg; (4) history of intraocular surgery, history of retinal or choroidal vascular disease, or glaucoma.

Serum samples were obtained from all of the patients, and data was collected, including best-corrected visual acuity (BCVA) using Snellen charts, intraocular pressure, refractive errors, axial length, and slit-lamp and fundus findings.

The major outcomes were subfoveal choroidal thickness (CT), foveal thickness (FT), and VEGF serum levels. The changes in the subfoveal CT and FT were determined using SD-OCT (Heidelberg Spectralis OCT; Heidelberg, Germany) images (Fig. 1). Each image was obtained using the eye tracking system, and 100 scans were averaged to increase the signal-to-noise ratio [9] using an EDI-OCT algorithm. The subfoveal CT was measured from the outer border of RPE to the inner border of sclera using software in the Heidelberg Spectralis OCT. The subfoveal CT and FT were measured vertically in a horizontally scanned image through the center of the fovea. Measurements of OCT images were made by two of the authors (MK, TO), who were masked to VEGF serum levels. The average of the two measurements was used. Differences between the readings of the two observers were found to be within 10 % of the mean.

Measurement of VEGF serum levels

After the blood samples were collected, they were allowed to clot at room temperature for about one hour, and were then centrifuged at 3,000×g for 10 minutes. Serum samples were stored in aliquots at −80 ° C until analyses were performed. Enzyme-linked immunosorbent assays (ELISAs) were used to determine serum VEGF levels (Quantikine HS®, R&D Systems, Minneapolis, MN, USA). The subfoveal CT and FT were measured within one week after collection of blood samples.

Statistical analyses

The correlations between the serum VEGF levels and subfoveal CT and FT in POEMS patients were determined

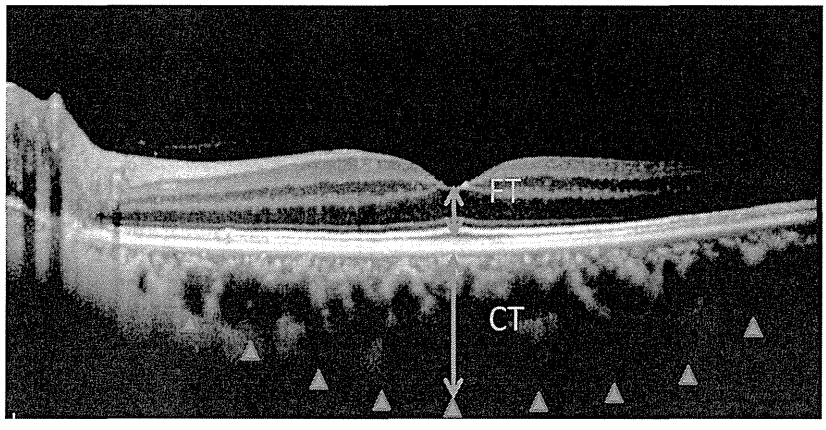

Fig. 1 Enhanced depth images of a patient with POEMS syndrome (Case 12, left eye) with choroidal thickening. CT choroidal thickness, FT foveal thickness

using Spearman's rank correlation coefficient. Statistical significance was defined as $p < 0.05$.

Results

Sixteen Japanese patients with POEMS syndrome (12 men, 4 women) were studied. The demographics of the patients are shown in Table 1.

The mean age of patients was 56.3±11.4 years, with a range from 36 to 75 years, and the mean intraocular pressure was 12.3±2.8 mmHg, with a range from 9 to 20 mmHg. Eleven patients (68.7 %) had optic disc edema detected by indirect ophthalmoscopy, and the edema was bilateral in seven patients (43.7 %) and unilateral in four patients (25 %). Cystoid macular edema (CME) and serous retinal detachment (SRD) were not detected in any of the eyes by ophthalmoscopy and SD-OCT. The mean refractive error (spherical equivalent) was −0.40±1.55 diopters (D), with a range from −4.75 to 2.75 D, and the mean axial length was 23.6±1.00 mm, with a range from 21.8 to 25.7 mm. The mean subfoveal CT was 417.9±73.5 μm for both eyes; it was 416.7±81.2 μm for the right eye and 419.0±68.1 μm for the left eye. The mean FT

Table 1 Patient characteristics, OCT data, and serum VEGF levels

Patient	Age	Sex	Eye	BCVA	AL(mm)	IOP(mmhg)	SE(D)	FT(μm)	SCT(μm)	VEGF(pg/ml)	ODE
1	54	F	OD	1	23.5	11	−0.5	280	361	3,380	+
			OS	1.2	23.2	13	−0.25	275	379		+
2	59	M	OD	1.2	25.1	12	−2	285	465	11,000	+
			OS	1.2	25.6	12	−2.25	278	432		+
3	66	F	OD	1.2	22.2	13	−0.25	206	396	5,590	−
			OS	1.2	22.2	13	−0.75	224	381		+
4	75	M	OD	1.2	23.7	11	2.75	257	399	9,690	−
			OS	1.2	23.5	12	2.75	250	408		−
5	50	M	OD	0.6	23.1	17	1.5	249	236	1,330	−
			OS	0.6	22.6	19	2.5	122	297		−
6	56	M	OD	1	21.8	9	−0.5	233	387	1,760	−
			OS	1.2	21.9	10	0.5	235	419		+
7	66	M	OS	0.6	21.9	10	−1	156	409	1,350	−
8	72	M	OD	1	23.2	11	−1	270	350	2,460	−
			OS	1	23.4	11	−1	285	367		+
9	47	F	OD	1.2	23.7	12	−0.25	218	465	4,590	−
			OS	1.2	23.7	13	0	238	389		−
10	62	M	OD	1.2	24.3	11	−1	252	463	8,430	+
			OS	1.2	23.9	12	−0.75	282	448		+
11	36	M	OD	1.2	23.6	10	−1	253	495	7,600	+
			OS	0.2	23.5	13	−0.5	247	528		−
12	61	M	OD	0.8	24	20	0	249	581	8,970	−
			OS	1.2	23.9	16	−1	271	467		−
13	55	M	OD	1.2	22.9	10	−0.25	240	416	11,200	+
			OS	1.2	22.9	9	−0.25	239	484		+
14	45	M	OD	1.2	25.7	11	−4.75	266	332	5,360	+
			OS	1.2	25.3	13	−3.5	265	318		+
15	61	M	OD	1.2	23.9	16	0.75	225	430	11,900	+
			OS	1.2	23.8	15	0.75	220	425		+
16	36	F	OD	1.2	24.2	9	−0.5	249	475	6,420	+
			OS		24.1	9	−0.75	240	554		+
Mean	56.3				23.6	12.3	−0.4	243.8	417.9	6,314	
SD	11.4				1	2.8	1.55	35.2	73.5	3,648	

BCVA best-corrected visual acuity, *AL* axial length, *IOP* intraocular pressure, *SE* spherical equivalent, *FT* foveal thickness, *SCT* subfoveal choroidal thickness, *OCT* optical coherence tomography, *VEGF* vascular endothelial growth factor, *ODE* optic disc edema

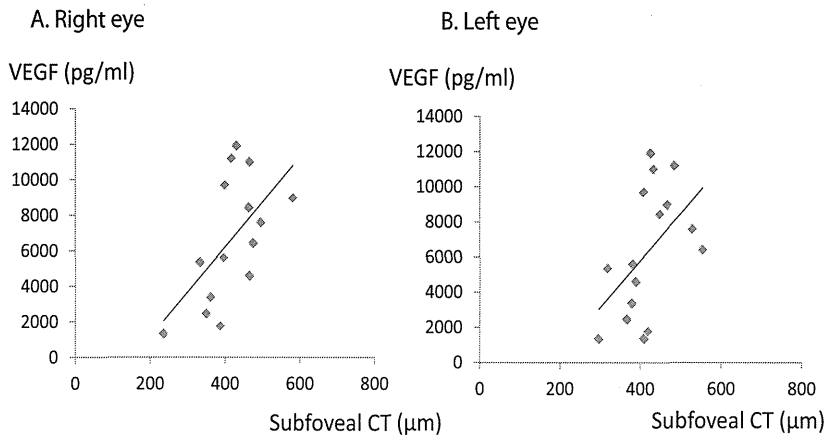

Fig. 2 Correlation between subfoveal choroidal thickness (CT) of both eyes and serum VEGF levels in patients with POEMS syndrome. There was a significant correlation between subfoveal CT and serum VEGF in patients with POEMS syndrome. (**a**) right eye, r=0.58, p=0.021, (**b**) left eye, r=0.60, p=0.012, Spearman's rank correlation coefficient

was 243.8±35.2 μm for both eyes; it was 248.8±22.0 μm in the right eyes and 239.1±44.6 μm in the left eyes. The mean serum VEGF level among patients was 6,314±3,648 pg/ml, with a range of 1,330 to 11,900 pg/ml, which is almost 30-fold higher than that of normal subjects (219 pg/ml) [16].

There was a significant positive correlation between the serum VEGF level and subfoveal CT (right eye, r=0.58, p=0.021; left eye, r=0.60, p=0.012; Fig. 2). In addition, there was a strong positive correlation between the subfoveal CT of right eyes and left eyes (r=0.77, P=0.00034). On the other hand, the correlation between the serum VEGF levels and FT was not significant for the right eyes (r=0.007, p >0.05) or left eyes (r=0.25, p>0.05; Fig. 3.).

Discussion

The pathogenesis of POEMS syndrome is complex, and several systemic factors are thought to be involved. The results of several studies have suggested that hyperproduction of VEGF by abnormal plasma cells is the major contributor to the development of POEMS syndrome [1, 2]. We found bilateral optic disc edema in seven patients (43.7 %), which is consistent with reports that bilateral optic disc edema was the most common (30–70 %) sign associated with POEMS syndrome [5–7].

The difference in serum VEGF concentrations between patients with and without optic disc edema was not significant (p=0.35). It has been reported that both elevated intracranial pressure (ICP) [2, 17] and elevated VEGF concentration [6] are causes of optic disc edema in patients with POEMS syndrome. The difference in serum VEGF concentration between patients with and without optic disc edema was not considered significant, as elevated ICP may have affected optic disc edema in our patients.

Subfoveal CT in normal eyes, as determined by EDI-OCT, has been reported from 254 to 354 μm [9–11, 18–20]. Studies have also shown that age [10, 14], intraocular pressure [21, 22], refractive error [14], and axial length [21, 22] can affect CT. In addition, the choroid in highly myopic eyes is very thin and undergoes further thinning with increasing age and degree of myopia [14]. An increase in the IOP is associated with choroidal thinning and elongation of the axial length [21]. The mean age of our patients was 56.3±11.4 years (range from 36 to 75 years), the mean intraocular pressure was 12.3± 2.82 mmHg (range, 9 to 20 mmHg), and the mean axial length was 23.6±1.0 mm (range, 21.8 to 25.7 mm). The correlation between age and the subfoveal CT was not significant (r= 0.20, p=0.26). There was also no significant correlation

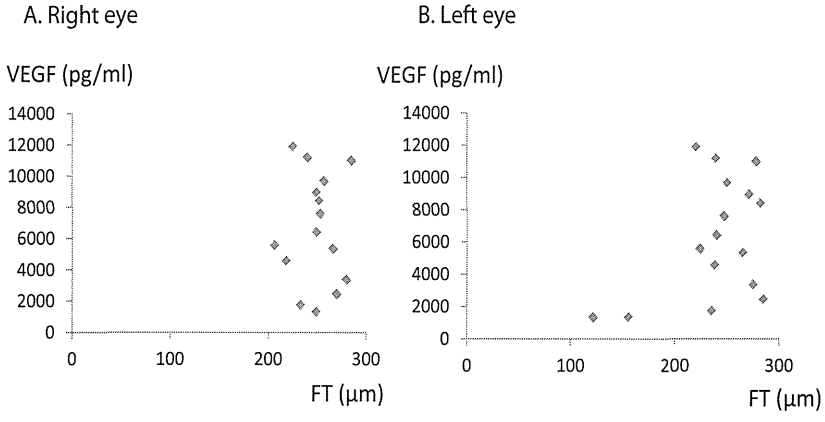

Fig. 3 Correlation between foveal thickness (FT) of both eyes and serum VEGF levels in patients with POEMS syndrome. The correlation between the FT and serum VEGF in patients with POEMS syndrome was not significant. (**a**) right eye, r= 0.007, p>0.05, (**b**) left eye, r= 0.25, p>0.05, Spearman's rank correlation coefficient

between intraocular pressure and subfoveal CT (r=0.14, P=0.44). Likewise, the correlation between axial length and the subfoveal CT was not significant (r=0.15, p=0.40). Thus, the effects of age, intraocular pressure, and axial length were most likely minimal in our cases, and the mean subfoveal CT (417.9±73.5 μm) was thicker than that reported among studies for normal eyes (254 to 354 μm) [9–11, 18–20].

The increased choroidal thickness may be due to increased choroidal vascular permeability caused by the higher levels of VEGF. VEGF is a cytokine that targets endothelial cells, inducing neovascularization and enhancing vascular permeability [23, 24]. An increase in microvascular permeability is supported by the presence of edema elsewhere in the body—e.g., lower extremities, abdomen, pleura, and pericardium—in patients with POEMS syndrome [25]. In this study, edema was present elsewhere—e.g., lower extremities, abdomen, pleura, and pericardium—in most patients.

Alterations in the function and structure of the choroid are known to play a role in the pathogenesis of several ocular disorders. Recent studies have shown that eyes with central serous chorioretinopathy (CSC) [12] and Harada disease [13] have greater subfoveal choroidal thickness. The thickened choroid in CSC may be due to increased vascular permeability, and in Harada disease it may be due to inflammation of the choroid.

Cystoid macular edema (CME) and serous retinal detachment (SRD) have been reported in eyes of patients with POEMS syndrome [8, 26]. Imai et al. [26] suggested that CME in these cases was due to elevated serum VEGF and not to the VEGF secreted from retinal tissues. It has been reported that patients with POEMS syndrome can develop SRD or macular edema (ME) during the course of the disease process [6, 8, 26]. In our cases, the foveal thickness of the left eyes in Cases 5 and 7 were very thin, although we could not find a history of retinal and choroidal diseases. Because these patients had a longer duration of POEMS syndrome, the optic disc edema, SRD, and ME might have developed unknowingly during the course of the disease. Thus, we believe that these disease processes may have caused the reduction in retinal thickness, although we did not detect signs of these diseases in either case during the study. In Case 7, the right eye was phthisic due to trauma that occurred in childhood. Thus, data of the right eye in Case 7 are not known.

In contrast, the correlation between the serum levels of VEGF and FT was not significant. It is well known that VEGF is a cytokine that can affect vascular permeability via intravascular compartments [23, 24]. The reason why such high serum VEGF did not cause retinal edema is that the increased levels of serum VEGF in the choroidal vasculature may be due to the larger caliber and greater volume of flow in comparison with the retinal circulation. Thus, high serum VEGF may not cause retinal edema, and the serum levels of VEGF may have a greater effect on choroidal than on retinal tissues.

Our study had several limitations. First, the possible effects of other factors such as systemic or topical medications, diurnal variations, and nutrition on choroidal thickness must be taken into consideration. Second, our results cannot answer the question of a cause–effect relationship between increased serum levels of VEGF and increased choroidal thickness. Further studies are needed to determine whether treatment of POEMS syndrome such as high-dose chemotherapy with autologous peripheral blood stem-cell transplantation (auto-PBSCT), anti-VEGF monoclonal antibody (bevacizumab) therapy, and thalidomide therapy can lead to a decrease in choroidal thickness due to a reduction in serum VEGF level. Positive results would support the idea that the higher levels of serum VEGF were the cause of the thickened choroid in patients with POEMS syndrome. Finally, we are not able to conclude that serum levels of VEGF influence choroidal thickness in normal eyes. Further studies with a large sample size may help to explain the role of serum VEGF in the choroid of normal eyes.

In conclusion, we showed that a significant correlation between serum level of VEGF and subfoveal CT was present in patients with POEMS syndrome. These results not only aid in understanding the pathogenesis of ocular changes in patients with POEMS syndrome, but also offer clues on the pathogenesis of other choroidal diseases.

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Conflict of interest No conflicting relationship exists for any author.

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Altered axonal excitability properties and nerve edema in POEMS sydnrome

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Altered axonal excitability properties and nerve edema in POEMS syndrome

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HIGHLIGHTS

- Axonal excitability and its correlation with serum VEGF and nerve edema detected by ultrasound were studied in POEMS syndrome.
- Excitability testing suggested possibly altered sodium, potassium, and inward rectifying currents, some of which were correlated with VEGF levels and nerve edema.
- In addition to structural changes (demyelination), nerve edema induced by upregulated VEGF, and upregulated inflammatory cytokines can modulate profiles of POEMS neuropathy.

ABSTRACT

Objective: POEMS (polyneuropathy, organomegaly, endocrinopathy, M-protein, and skin changes) syndrome is a rare cause of demyelinating neuropathy with upregulation of vascular endothelial growth factor (VEGF). This study aimed to elucidate axonal excitability properties and their relation to VEGF levels and nerve edema in POEMS neuropathy.
Methods: Axonal excitability measurement and nerve ultrasound were performed in the median nerve of 33 patients with POEMS syndrome. Serum VEGF levels were measured by ELISA.
Results: Compared with normal subjects ($n = 87$), POEMS patients showed longer strength-duration time constant, fanning-out of threshold electrotonus curves, and greater threshold changes in a hyperpolarizing current–threshold relationship. Nerve ultrasound showed significant enlargement in POEMS patients. Serum VEGF levels and the extent of nerve edema partly correlated with nerve conduction slowing, as well as persistent sodium currents and inward rectification.
Conclusions: In POEMS syndrome, patterns of changes in excitability properties could suggest increased persistent sodium currents, and impaired potassium and inward rectifying channels. The findings were not consistent with depolarization due to nerve edema and compression ischemia.
Significance: In addition to demyelination, nerve edema induced by upregulated VEGF, and upregulated inflammatory cytokines could modulate profiles of POEMS neuropathy.

1. Introduction

POEMS (polyneuropathy, organomegaly, endocrinopathy, M-protein, and skin changes) syndrome is a rare cause of demyelinating polyneuropathy associated with monoclonal plasma cell proliferation and multi-organ involvement (Bardwick et al., 1980; Kuwabara et al., 2008a; Dispenzieri, 2014). Serum levels of vascular endothelial growth factor (VEGF) are markedly increased in POEMS syndrome, and increased vascular permeability and neovascularization mediated by VEGF are likely to cause characteristic symptoms such as edema, pleural effusion/ascites, organomegaly and skin angiomata (Watanabe et al., 1996). However, mechanisms for neuropathy in POEMS syndrome have not yet been elucidated,

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whereas pathological studies have shown perineurial edema, and segmental demyelination with uncompacted myelin and secondary axonal degeneration (Kanda, 2013).

The proposed mechanisms for POEMS neuropathy include that the combination of blood-nerve barrier breakdown by VEGF and following invasion of other inflammatory cytokines such as interleukin-12, and tumor necrosis-α causes nerve demyelination (Kanai et al., 2012), and that nerve edema mediated by VEGF leads to compression ischemia and axonal depolarization (Kanda, 2013), but these hypotheses still need confirmation.

Axonal excitability testing using threshold tracking was developed to investigate ion channel function, membrane potential, and passive membrane properties of human axons *in vivo* (Bostock et al., 1998; Burke et al., 2001), and over the past two decades, the technique has been extensively applied to the study of the biophysical properties of human peripheral nerves and have provided important insights into axonal ion channel function in health and disease (Nodera and Kaji, 2006; Sawai et al., 2008).

Separately, nerve ultrasound is also becoming increasingly important in the diagnosis and evaluation of peripheral neuropathies particularly in the 2000's (Padua et al., 2012) providing new insights into macroscopic nerve morphology. In this study, we aimed to elucidate axonal excitability properties and their relation to nerve morphology and serum VEGF levels in patients with POEMS syndrome.

2. Methods

2.1. Subjects

This study prospectively enrolled 33 consecutive patients (25 men; age range 36–75 years, mean 55 years) with newly diagnosed POEMS syndrome, who fulfilled published criteria (Kuwabara et al., 2008a) seen at a single tertiary hospital (Chiba University Hospital) in Japan from January 2012 to September 2014. We excluded patients with renal failure because serum K^+ levels can significantly alter membrane potential and axonal excitability properties (Kiernan et al., 2000).

Normal control data for axonal excitability testing were obtained from 87 age-matched healthy subjects (49 men; age range 38–76 years, mean 56 years). Serum VEGF levels were measured by ELISA commercially (Special Reference Laboratory Co. Ltd., Tokyo, Japan). All the patients and normal control subjects gave informed consent to the study procedures, which was approved by the Ethics Committee of Chiba University Graduate School of Medicine.

2.2. Neurophysiological assessment

Neurophysiological evaluation was performed before thalidomide treatment, and in 9 patients, follow-up studies were done 3 months later. Nerve conduction studies were conducted using conventional procedures and a standard electromyography machine (Viking 4, Nicolet Biomedical Japan, Tokyo, Japan). Nerve excitability testing was performed on the median nerve at the wrist (3 cm proximal to the wrist crease) using a computerized program (QTRAC® with multiple excitability protocol, TRONDNF, Institute of Neurology, London, UK) as described previously (Kiernan et al., 2000; Nasu et al., 2014). Compound muscle action potentials (CMAPs) were recorded from the abductor pollicis brevis. Skin temperature was measured near the stimulating site and maintained above 32.0 °C (using extra heating, if necessary). Excitability indices included strength-duration time constant, threshold electrotonus, and refractoriness, supernormality, and late subnormality of the recovery cycle of axonal excitability with a single supramaximal conditioning stimulus, and a current–threshold relationship.

2.3. Nerve ultrasound

Ultrasound examination was performed with Logiq E9 (GE Healthcare Japan, Tokyo, Japan) with a 6–12 MHz electronic linear array probe at the wrist, forearm, and elbow portion of the median nerve trunk. Cross-sectional area were measured at the inner border of the thin hyperechoic epineural rim by the continuous tracing technique and the average values were calculated after serially measuring three times (Kerasnoudis et al., 2014). No additional force was applied other than the weight of the transducer and the extremities were kept in the neutral position to avoid causing any artificial nerve deformity.

2.4. Statistical analysis

All statistical tests were two-sided. The comparison of paired parameters of nerve conduction studies or excitability testing between baseline and the second examination was evaluated via the paired *t*-test with Bonferroni's correction for multiple testing. Regression analysis was performed by Pearson's correlation coefficient test. All statistical analyses were performed using JMP software, version 5.1.1 (SAS Institute, Inc., Cary, NC, USA).

3. Results

3.1. Nerve excitability testing and ultrasound

Table 1 shows results of nerve conduction studies, and excitability testing. Nerve conduction velocities were significantly slowed consistent with primary demyelinating neuropathy. In excitability testing, strength-duration time constant was significantly longer and current required for produce 50% of the maximal CMAP was greater for POEMS patients than for normal controls. In the recovery cycle of axonal excitability, POEMS patients had greater superexcitability and smaller late subexcitability. POEMS patients showed fanning-out in threshold electrotonus particularly in the hyperpolarizing direction, and greater threshold changes to hyperpolarizing currents in current–threshold relationships (Fig. 1).

Nerve ultrasonography showed significantly greater cross-sectional area at the wrist, forearm, and elbow portion of the median nerve in patients with POEMS syndrome than in normal subjects.

3.2. Correlation with serum VEGF levels and nerve enlargement

Before treatment, serum VEGF levels were greatly increased in all patients with POEMS syndrome; the mean value was 5143 pg/ml (normal < 1000 pg/ml), ranging from 1080 to 16,400 pg/ml. Table 2 shows correlation of electrophysiological indices with serum VEGF levels and cross-sectional area at the elbow. Higher serum VEGF levels were associated with longer distal motor latency, and smaller CMAP amplitude in nerve conduction studies, and greater threshold changes to long hyperpolarizing conditioning currents.

Separately larger cross-sectional area on nerve ultrasound was associated with slower nerve conduction velocity, smaller CMAP amplitude, and loner strength-duration time constant. Serum VEGF levels and nerve cross-sectional area at the time of examination did not show significant correlation.