Polyneuropathy in POEMS syndrome: role of angiogenic factors in the pathogenesis

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In order to clarify the role of angiogenic factors in polyneuropathy of POEMS (polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, skin changes) syndrome, we measured the serum concentrations of vascular endothelial growth factor (VEGF) and erythropoietin (EPO) in 11 patients and correlated these with VEGF and EPO peripheral nerve expression and the degree of endoneurial vessel involvement. We found that POEMS syndrome was associated with high levels of serum VEGF and, conversely, low levels of serum EPO. Similarly, in POEMS nerves VEGF was highly expressed in blood vessels and some non-myelin-forming Schwann cells. In contrast, the expression of VEGF receptor 2 was down-regulated compared with that in normal nerves. Both EPO and EPO receptor were localized to the nerve vasculature and were expressed to similar extents in normal and POEMS nerves. The inverse correlation between VEGF and EPO serum levels was maintained during the clinical course; however, both levels returned to normal when there was a response to therapy. High serum VEGF, low serum EPO and high peripheral nerve VEGF were all associated with more severe endoneurial vessel involvement and nerve damage. Light microscopy showed an increased thickness of the basal lamina and a narrowing of the lumina of endoneurial vessels in POEMS samples, while proliferation of endothelial cells and opening of tight junctions were observed by electron microscopy. The present data support the role of angiogenic factors as diagnostic and prognostic markers of POEMS syndrome. They also suggest that VEGF and EPO are involved in the pathogenesis of polyneuropathy. In conclusion, establishing the role of angiogenic factors in polyneuropathy may lead to a better understanding of the effects of VEGF and EPO on microangiopathy and Schwann cell function.

Keywords: endoneurial vessels; EPO; nerve; POEMS; VEGF

Abbreviations: EPO = erythropoietin; EPO-R = EPO receptor; HIF-1 = hypoxia-inducible factor; IVIg = intravenous immunoglobulins; MGUS = monoclonal gammopathy of undetermined significance; POEMS = polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, skin changes; VEGF = vascular endothelial growth factor; VEGFR2 = VEGF receptor 2


Introduction

POEMS (polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, skin changes) syndrome is a rare multisystemic disease that occurs in the setting of plasma cell dyscrasia and is characterized by an elevation of serum vascular endothelial growth factor (VEGF) levels (Miralles et al., 1992; Watanabe et al., 1996; Hashiguchi et al., 2000; Michizono et al., 2001). The clinical spectrum of POEMS syndrome is quite broad, and since not all the typical features may be present at onset, it is crucial to establish minimal criteria for diagnosis. Peripheral nerves are one of the principal targets in POEMS syndrome (Vital et al., 2003). In fact, the presence of a chronic progressive, distal, sensorimotor polyneuropathy is mandatory in establishing diagnosis along with monoclonal plasmaproliferative disorder.
VEGF and erythropoietin (EPO) were first characterized, respectively, as angiogenic and haematopoietic growth factors. VEGF acts as a potent, multifunctional cytokine inducing angiogenesis and microvascular hyperpermeability via action on endothelial cells, while EPO regulates proliferation and differentiation of red blood cells. However, growing evidence suggests that they have other biological roles. The production of VEGF and EPO is under the control of a labile transcription factor called hypoxia-inducible factor (HIF-1). HIF-1 is a heterodimer consisting of an inducible α- and a constitutive β-subunit and acts as a DNA-binding factor. Under hypoxic conditions the HIF-1α subunit is induced and interacts with the hypoxia response elements (HREs) of target genes to determine transcriptional activity (Pugh and Ratcliffe, 2003). The human VEGF gene gives rise to six different protein isoforms (Ferrara et al., 2003). A number of polymorphisms localized in the promoter region of the VEGF gene, outside the HRE site, have been described and associated with several diseases (Del Bo et al., 2005) and modification of transduction levels. VEGF exerts its action via high-affinity binding to two types of phosphotyrosine kinase receptors: VEGF receptor 1 (VEGFR1; also known as FLT-1) and VEGF receptor 2 (VEGFR2; also known as KDR and FLK-1). Both receptors are essential for the development and organization of endothelial cells and, interestingly, VEGFR2 has been shown to be present on neurons and Schwann cells in mice (Sondell et al., 1999). Similarly, EPO receptor (EPO-R), a class 1 cytokine receptor, has been identified in several different tissue types including neurons, dorsal root ganglia and Schwann cells.

Recent studies have revealed that VEGF and EPO are involved in neuroprotection and have neurotrophic activity (Sakanaka et al., 1998; Rosenstein and Krum, 2004). In fact, EPO has been shown to prevent and even reverse diabetic neuropathy in rats (Bianchi et al., 2004). A lack of VEGF activity has been implicated in many diseases, including amyotrophic lateral sclerosis (ALS) (Lambrechts et al., 2003) and diabetes (Kakizawa et al., 2004), and a lack of EPO activity has been described in some renal disorders (Hassan et al., 2003). In contrast, there are few conditions associated with increased levels of VEGF and EPO; these are usually related to inherited (Krieg et al., 1998) or sporadic tumours. In fact, tumour cells under active replication are frequently hypoxic and, accordingly, this can lead to increased levels of VEGF and sometimes EPO, inducing a paraneoplastic erythrocytosis.

In order to provide new insights into the pathogenetic role of angiogenic factors in POEMS syndrome, we evaluated longitudinally VEGF and EPO serum levels in 11 POEMS patients and correlated these with the clinical course, response to therapy and neuropathological findings. We also investigated the expression and localization of HIF-1α, VEGF, VEGFR2, EPO and EPO-R in peripheral nerves.

Our data suggest that polyneuropathy in POEMS syndrome might be caused by the direct or indirect effect of angiogenic factors. VEGF and EPO serum changes therefore have diagnostic, prognostic and pathogenetic implications.

Material and methods

Patients: clinical and laboratory data

Between 1990 and 2003, we recruited 11 patients (10 males and one female) affected with POEMS syndrome, from three hospitals in Northern Italy (Table 1). All the patients initially presented with a chronic progressive sensorimotor polyneuropathy that began 8–14 months before the diagnosis was made. Deep sensation was generally affected more than cutaneous sensation and the sensory disturbances were reported as being as severe as the motor symptoms. Duration of follow-up, defined as the interval between the time of the first clinical examination, and collection of the first serum sample, and the last visit, ranged from 3 to 30 months. The presence of polyneuropathy was diagnosed on the basis of clinical symptoms and signs (distal sensory disturbances at onset with progressive weakness in the lower and, less extensively, in the upper limbs, with reduced or absent deep tendon reflexes) and electrophysiological findings (slowing of motor and sensory nerve conduction velocities with reduction of compound muscle action and sensory nerve action potentials) (data not shown). For serial evaluation of neurological impairment, we used the Rankin scale score, which ranges from 0 (no deficit) to 5 (maximal deficit). CSF protein levels were elevated in all 11 subjects. Seven patients underwent sural nerve biopsy, after giving informed consent. Regarding the systemic features, the most frequent endocrinopathies present were asymptomatic reduction of thyroid function (six out of 11) and hypogonadism (seven out of 11) with or without gynaecomastia. Three patients (cases 2, 5 and 11) also had Castelman’s disease diagnosed by lymph node biopsy. Bone lesions of a sclerotic type were present in four patients. Kidney function was normal in all of the patients.

Standard approaches to the treatment of POEMS syndrome (Dispensieri and Gertz, 2004), including different combinations of corticosteroid therapy, plasma exchange, intravenous immunoglobulins (IVIg), radiation, alkylator-based chemotherapy and peripheral blood stem cell transplant, were provided. All patients received at least one combined treatment. A positive response was defined as improvement in the Rankin score during follow-up. Six patients died of complications of congestive stroke, cardiorespiratory or renal failure.

As controls for VEGF and EPO measurements, we selected 16 patients with monoclonal gammapathy of undetermined significance (MGUS), eight of whom had a neuropathy, as well as sixty healthy controls.

This retrospective and ongoing study was approved by the local ethics committee.
Table 1  Clinical features of patients with POEMS syndrome

<table>
<thead>
<tr>
<th></th>
<th>Therapy responders</th>
<th>Therapy non-responders</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Case 1</td>
<td>Case 5</td>
</tr>
<tr>
<td>Age at onset (years)/sex</td>
<td>56/M</td>
<td>33/M</td>
</tr>
<tr>
<td>Polineuropathy</td>
<td>± ±</td>
<td>± ±</td>
</tr>
<tr>
<td>Rankin score pre-therapy</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Organomegaly</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Endocrinology</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Bone lesions</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Hyperpigmentation</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hypertrichosis</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Oedema/ascites</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Papilloedema</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>First VEGF (pg/ml)†</td>
<td>1487.2</td>
<td>1216.7</td>
</tr>
<tr>
<td>First EPO (mIU/ml)†</td>
<td>1.06</td>
<td>1.42</td>
</tr>
<tr>
<td>Therapy treatment†</td>
<td>I</td>
<td>III+IVlg</td>
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<tr>
<td>Rankin score post-therapy</td>
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<td>0</td>
</tr>
<tr>
<td>Last VEGF (pg/ml)‡</td>
<td>781.35</td>
<td>576.9</td>
</tr>
<tr>
<td>Last EPO (mIU/ml)‡</td>
<td>5.8</td>
<td>5.4</td>
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<tr>
<td>Follow-up (months)</td>
<td>36</td>
<td>32</td>
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<tr>
<td>Deceased</td>
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<tr>
<td>Platelet count (×10³/dl)§</td>
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<td>230</td>
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<td>Red blood cells count (×10⁶/dl)§</td>
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<td>5.17</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)§</td>
<td>13.8</td>
<td>14.9</td>
</tr>
<tr>
<td>Creatinine (mg/dl)§</td>
<td>NV</td>
<td>NV</td>
</tr>
<tr>
<td>Sural nerve biopsy</td>
<td>+</td>
<td>–</td>
</tr>
</tbody>
</table>

VEGF and EPO measurements were done on the same serum samples. †Therapy combination: I, corticosteroids and alkylators; II, corticosteroids, plasma exchange and IVlg; III, corticosteroids, plasma exchange and radiation; IV, corticosteroids, alkylators and peripheral stem cell transplant. §Platelet and red blood cells count, haemoglobin and creatinine serum level were those pre-therapy. M = male; F = female; NE = not examined; NV = normal value; S = sclerotic bone lesion.

Measurement of serum VEGF and EPO concentrations

To detect serum VEGF and EPO concentrations, blood samples were allowed to clot at room temperature for 2 h, then centrifuged for 5 min at 2500 rpm. Aliquotted serum was stored at −20 °C until analysis. VEGF and EPO serum levels were assessed in duplicate by an enzyme-linked immunosorbent assay (ELISA) kit (Quantikine®; R&D Systems, Minneapolis, MN, USA) according to the manufacturer’s protocol. VEGF and EPO concentrations were determined by linear regression from a standard curve using the VEGF and EPO standards supplied with the kit. One-way analysis of variance and Pearson’s correlation test were used where appropriate. In all statistical tests, a P value of <0.05 was considered to be significant.

Morphological and immunofluorescence studies of peripheral nerves

For cryosection, a nerve segment was embedded in tissue-TEK O.C.T.™ compound (Sakura-Finetek) and snap-frozen in liquid nitrogen. For haematoxylin and eosin analysis, another portion of each biopsy sample was fixed in 10% formalin and embedded in paraffin. For semi-thin section analysis, a portion was fixed in 2% buffered glutaraldehyde, and then post-fixed in 1% osmium tetroxide. Following alcohol dehydration, the samples were embedded in Epon (Fluka; Sigma, Cologno Monzese, Italy). Transverse sections (0.5–1 μm thick) were stained with Toluidine Blue and examined by light microscopy. Ultra-thin sections were stained with uran acetate and lead citrate and examined by electron microscopy.

For quantitative analysis of endoneurial microvessels, three POEMS nerves (cases 1, 6 and 8) and a nerve biopsy of a 54-year-old patient with ALS were studied. Photographs of the nerve sections were enlarged to 4500× and measurements were made using the Image Analyzer (Leica QWin Software; Leica, Germany). Vessels were considered obstructed, narrowed or opened based on the difference between the vessel diameter (the diameter of a circle of equivalent area), excluding the basement membrane, and the luminal diameter (the diameter of a circle of equivalent area). Vessels were considered closed when the lumen was completely obstructed, narrowed when the shorter luminal diameter was approximately less than one-third of the vessel diameter and opened in all the other cases (by Saida et al., 1997 and Giannini and Dyck, 1993; modified).

Indirect immunofluorescence was performed on transverse cryosections of human nerve prepared as described previously (Quattrini et al., 1996). Sections were obtained from four patients with POEMS syndrome (cases 1, 6, 8 and 10). As a control, we used a portion of sural nerve from young healthy patients who had had amputations following traffic accidents; sections were processed and immunostained along with the experimental nerves. The following antibodies recognizing specific antigens (species/clone) were used for the study: VEGF (rat/1A-20), EPO (rabbit/H-162) and EPO-R (rabbit/M-20) from Santa Cruz Biotechnology (Santa Cruz, CA); VEGFR2 (mouse/ab-9530) from Abcam Limited (Cambridge, UK); HIF-1α (mouse/ab-9530) from Abcam Limited (Cambridge, UK).
(mouse/ESEE122) and CD31 (PECAM-1) (mouse/ab8166) from Novus Biologicals (Littleton, CO, USA); GFAP (mouse/G-A-5) from Chemicon (Temecula, CA, USA); GFAP (rabbit) from Sigma; and MBP (rat) was a gift from V. Lee. Fluorescein isothiocyanate (FITC)-conjugated or tetramethylrhodamine isothiocyanate (TRITC)-conjugated, affinity-purified antibody cocktails were used to recognize rabbit or mouse IgG (Southern Biotechnology Association, Birmingham, AL, USA). Ten micrometre thick sections of nerve were placed on poly-L-lysine coated glass slides, fixed in cold acetone, blocked with 10% (w/v) normal goat serum, and then incubated with primary antibody. Double staining was performed by consecutively applying on the same section antibodies raised in different species. Positive staining was revealed with FITC- and/or TRITC-conjugated secondary antibodies and examined with an Olympus BX 51 microscope or a Bio-Rad confocal microscope. Control experiments included the omission of the primary antiserum.

Immunofluorescence and electron microscopy sections were observed by blinded evaluators (M.S., S.C.P. and A.Q.).

**Results**

**Inverse correlation between VEGF and EPO at the time of diagnosis**

Serum levels of VEGF were analysed in 11 patients (Table 1) with POEMS syndrome at the time of diagnosis, in 16 patients with MGUS and in 60 healthy controls. In POEMS patients, levels of serum VEGF (median 2230.62 pg/ml; range 1216.65–3179.4) were higher than in patients affected by MGUS (median 299.59 pg/ml; range 91.58–576.94; \( P < 0.01 \)) or healthy subjects (median 395.14 pg/ml; range 64.76–858.28; \( P < 0.01 \)) (Fig. 1A). Serum EPO levels were measured in 24 controls (CTR), 11 POEMS and 16 MGUS patients. (Fig. 1B) Serum EPO in 24 controls (CTR), 11 POEMS and 16 MGUS patients. (Fig. 1B)

Since VEGF is released from platelets under physiological conditions, and serum levels of VEGF are markedly higher than the corresponding plasma levels (Hashiguchi et al., 2000), we compared serum VEGF concentrations and platelet counts (PLT) among the patients. There was a positive correlation (\( r = 0.53 \)) but it did not reach significance (\( P = 0.092 \)). In fact, the three patients with VEGF levels <1500 pg/ml (mean 1318.8 pg/ml) presented with PLT lower than the eight patients with VEGF levels >1500 pg/ml (mean 2604.4 pg/ml) [PLT, mean 321 (\( \times 10^{3}/dl \)) ± 124.5 SD versus 495 (\( \times 10^{3}/dl \)) ± 118 SD].

**Localization of VEGF, VEGFR2, EPO, EPO-R and HIF-1α in the nerve**

To verify whether the VEGF/EPO balance was also altered in the peripheral nerve of POEMS patients, we used immunohistochemistry to compare the expression and localization of VEGF, EPO, their receptors and the modulatory molecule HIF-1α in the sural nerve of four patients affected by POEMS and two control samples (Fig. 2). We observed VEGF staining in the epineurial and endoneurial blood vessels in both controls and POEMS sural nerves. Interestingly, this immunoreactivity was consistently higher in blood vessels of the POEMS specimens when compared with controls. In POEMS nerves, VEGF was also expressed in some non-myelin-forming Schwann cells identified as GFAP-positive cells (Fig. 2A and B).

Conversely, VEGFR2 staining was only detectable in some endoneurial blood vessels of control patients, whereas it was completely absent in POEMS nerves (Fig. 2C and D).

We then investigated the expression of EPO and EPO-R in the contiguous sections. EPO staining was equally expressed in the epineurial and endoneurial blood vessels in both normal and POEMS sural nerves, as was the EPO receptor staining, although this latter one was mainly restricted to endothelial cells (Fig. 2E–H).

HIF-1α staining showed a similar distribution of immunoreactivity in both POEMS and control nerves. HIF-1α was expressed in myelin-forming Schwann cells, generally with ad-axonal localization as identified by an external ring to MBP staining (Fig. 2, I4-6 and J4-6). Similarly, HIF-1α

![Fig. 1](https://example.com/figure1.png)  
**Fig. 1** Box plots showing VEGF and EPO serum levels at the time of the diagnosis. (A) Serum VEGF in 60 controls (CTR), 11 POEMS and 16 MGUS patients. (B) Serum EPO in 24 controls (CTR), 11 POEMS and 16 MGUS patients.
was present in non-myelin-forming Schwann cells, identified as GFAP-positive cells (Fig. 2, I1-3 and J1-3) and in endoneurial vessels (data not shown). When observed by confocal microscopy using identical settings, the staining appeared higher in POEMS than in control nerves.

Neuropathology of POEMS: endoneurial vessel involvement

To evaluate whether the VEGF and EPO relationship and the high VEGF expression in peripheral nerve was correlated with the severity of endoneurial vessel involvement, seven sural nerve biopsies of POEMS patients were analysed. Light microscopic examination of paraffin-embedded sections did not reveal any inflammatory infiltrate. Endoneurial oedema was reported in two cases. In transverse semi-thin sections, myelinated nerve fibre density was variably diminished and all patients had a variable degree of endoneurial fibrosis. Myelin ovoids, indicating acute axonal degeneration, were observed in cases 2, 3 and 8. Clusters of thinly myelinated fibres, a sign of regeneration after axon degeneration, were observed in case 1. In case 8, some myelinated fibres were

Fig. 2 Expression of VEGF, VEGFR2, EPO, EPO-R and HIF-1α in POEMS nerves. Cryosections of sural nerve biopsies from control patients (A, C, E, G, I) and two POEMS patients, cases 10 (B and D) and 3 (F, H, J). The VEGF expression was very mild in blood vessels (identified by CD31 staining) of control nerves (A1–A3), whereas non-myelin-forming Schwann cells (identified by GFAP) were unstained (A4–A6). In POEMS patients, the VEGF expression was very high in the blood vessel wall (B1–B3) and presented also in non-myelin-forming Schwann cells (B4–B6). The VEGFR2 expression was mild in blood vessels (identified on a consecutive section by CD31) of control nerves (C1 and C2), but always absent in the blood vessels of POEMS nerves (D1 and D2). The EPO expression was similar and restricted to the blood vessel wall in control and POEMS nerves (E1–E3 and F1–F3, respectively). Regarding the EPO-R expression, there was no difference between controls and POEMS nerves (G and H, respectively) and the staining was restricted to the blood vessels, especially the endothelium (G1–G3 and H1–H3, respectively). The presence of HIF-1α was diffuse and identical in both control and POEMS nerves (I and J), including non-myelin-forming Schwann cells (identified by GFAP, respectively I1–I3 and J1–J3) and myelin-forming Schwann cells (identified by MBP, respectively, I4–I6 and J4–J6). Scale bar = 7 μm in all panels except I1–I6 and J1–J6, where scale bar = 20 μm.
demylinating. We observed a trend of correlation between VEGF serum levels and the degree of endoneurial vessels damage, confirmed by quantitative study (Table 2). In fact, the luminal diameters of POEMS nerves were significantly smaller ($P < 0.001$) than those of control nerve. Moreover, patients with VEGF levels $>2500$ pg/ml presented more compromised endoneurial vessels, with a higher percentage of narrowed or closed vessels (cases 6 and 8; Table 2) compared with a POEMS patient with VEGF level $<1500$ pg/ml (case 1; Table 2). The basal lamina of endoneurial vessels was often increased in thickness. Electron microscopy confirmed the presence of ultrastructural alterations in capillaries and small endoneurial vessels. The endothelial cells were hypertrophied, with processes that almost completely occluded the lumen, some with long branched cytoplasmic projections that were often vacuolated. Other vascular abnormalities, such as hyperplasia of endothelial cell layers, gaps between endothelial cells and subendothelial amorphous material deposition, were noted.

**Inverse correlation between VEGF and EPO during the clinical course and response to therapy**

VEGF and EPO serum concentrations were evaluated longitudinally in POEMS patients. For all but one there was more than one time point available (mean of three serum samples for each patient; range one to 10) covering a follow-up period ranging from 3 to 50 months. All four patients that showed improvement in their clinical condition (from Rankin score 2 pre-therapy to 0–1 post-therapy) during treatment, presented a marked reduction of serum VEGF (from a pre-therapy median of 1369 pg/ml to a post-therapy median of 397.34 pg/ml; $P < 0.01$) and a concomitant increase of EPO serum concentration, with the levels returning to normality (from a pre-therapy median of 1.23 mIU/ml to a post-therapy median of 5.60 mIU/ml; $P < 0.05$). Patients that did not respond to therapy (from Rankin score 3 pre-therapy to 4 post-therapy) had almost no modification in their VEGF and EPO serum levels (VEGF, from a pre-therapy median of 2845.82 pg/ml to a post-therapy median of 2816.97 pg/ml; EPO, from a pre-therapy median of 1.75 mIU/ml to a post-therapy median of 1.95 mIU/ml) (Fig. 3A and B). The two groups of patients, responders and non-responders, differed in pre-treatment VEGF levels; in particular, three patients out of four who improved after therapy had a pre-therapy VEGF level $<1500$ pg/ml.

Two representative patients of the different clinical courses, responses to therapy and grades of endoneurial vessel involvement are briefly summarized here.

**Case 1**

Case 1 presented with a predominantly sensory neuropathy with mild motor impairment (grade 2 according to Rankin scale); in $<1$ year, his condition had worsened to moderate motor involvement of the lower limbs (reaching grade 3 according to Rankin scale). At this point a sural nerve biopsy was performed (Fig. 4A), showing a uniform reduction in the number of myelinated nerve fibres. No inflammatory infiltrates were observed. Small clusters of regenerating axons were observed. There was a mild thickening of the endoneurial vessels. Electron microscopy showed a slight microangiopathy with masses of intracytoplasmic filaments within the capillary endothelium. The patient was treated with six courses of corticosteroids in association with alkylators every 6 weeks. Clinical improvement started after the second course of therapy with a reduction of the skin changes, peripheral oedema and the neuropathic disturbances, and was associated with a marked reduction of serum VEGF and a concomitant increase of serum EPO concentration (Fig. 3C).

**Case 6**

Case 6 presented with a sensorimotor neuropathy (grade 3 according to Rankin scale) with absence of peroneal CMAP and sural SAP amplitudes. The sural nerve biopsy (Fig. 4B–E) showed extensive loss of myelinated fibres, with prominent and thick-walled vasa nervorum. Axonal degeneration was observed. Electron microscopy showed endothelial cytoplasmic enlargement, accumulation of intracytoplasmatic filaments, inclusion and pinocytotic vesicles adjacent to the cell membranes. Amorphous material in the subendothelial space was observed. Many of the tight junctions between endothelial cells of the endoneurial microvessels had disappeared, and obvious gaps were observed between endothelial cells. The diagnosis was made within 6 months and IVIg

### Table 2: Quantitative study of endoneurial microvessels

<table>
<thead>
<tr>
<th>Case</th>
<th>Vessel density (No./mm$^2$)</th>
<th>No. vessels</th>
<th>Vessel diameter ($\mu$m)</th>
<th>Luminal diameter ($\mu$m)</th>
<th>No. open (%)</th>
<th>No. narrowed (%)</th>
<th>No. closed (%)</th>
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<tbody>
<tr>
<td>1</td>
<td>44</td>
<td>9</td>
<td>12.71 ± 3.2</td>
<td>5.54 ± 1.86$^a$</td>
<td>2 (22.3)</td>
<td>6 (66.6)</td>
<td>1 (11.04)</td>
</tr>
<tr>
<td>6</td>
<td>37</td>
<td>12</td>
<td>10.18 ± 2.14</td>
<td>2.63 ± 1.96$^a$</td>
<td>1 (8.3)</td>
<td>6 (50)</td>
<td>5 (41.7)</td>
</tr>
<tr>
<td>8</td>
<td>47</td>
<td>8</td>
<td>10.08 ± 2.8</td>
<td>3.8 ± 2.11$^a$</td>
<td>2 (25)</td>
<td>4 (50)</td>
<td>2 (25)</td>
</tr>
<tr>
<td>Control</td>
<td>49</td>
<td>9</td>
<td>10.98 ± 3.92</td>
<td>6.79 ± 2.91</td>
<td>8 (88.9)</td>
<td>1 (11.1)</td>
<td>0</td>
</tr>
</tbody>
</table>

Vessels in the endoneurium were measured. Cases 1, 6 and 8 were POEMS nerves; control, ALS patient’s nerve. Vessel diameter (basement membrane was not included) = mean ± SD. Luminal diameter = mean ± SD. $^a$Statistically significant data as determined by two-tailed paired $t$-test.
Fig. 3 (A and B) VEGF and EPO serum levels before and after therapy; for each patient, first and last sample of the follow-up. Dashed lines indicate patients that did not respond to therapy while continuous lines indicate responders to therapy. (A) VEGF serum concentration in 11 POEMS patients. (B) EPO serum concentration in 11 POEMS patients. (C) Longitudinal evaluation of VEGF (top) and EPO (bottom) serum concentrations of case 1. Time points refer to the follow-up period (months). The mark indicates when the sural nerve biopsy was done, the arrow when the therapy was started. (D) Longitudinal evaluation of VEGF (top) and EPO (bottom) serum concentrations of case 6. Time points refer to the follow-up period (months). The mark indicates when the sural nerve biopsy was done, the arrow when the therapy was started.
therapy was started with no benefit. The general conditions of the patient worsened (grade 4 according to Rankin scale) despite 4 months of therapy with corticosteroids and plasma exchange. The patient is now undergoing autologous stem-cell bone marrow transplant. VEGF serum levels remained constantly high (>2500 pg/ml) and EPO serum levels low (median 1.83 mIU/ml; range 1.1–4.52) (Fig. 3D).

Discussion

We have shown that the angiogenic factors VEGF and EPO are inversely correlated during the clinical course of POEMS syndrome and in terms of their nerve expression. VEGF is already known to be a useful marker of the disease, but it has not previously been shown how well it correlates with the clinical course, the response to therapy and the severity of endoneurial vessel involvement. We have also demonstrated for the first time that a decrease in EPO serum concentration, with a normal red blood cell count and kidney function, is related to POEMS syndrome.

In all patients, polyneuropathy was the presenting symptom and reason for consultation. In our population the neuropathic symptoms improved in four out of 11 patients treated with standard combined therapy (from Rankin score 2 to 0–1). We observed a better prognosis in patients with VEGF serum levels <1500 pg/ml before therapy. Those patients tended to have a better response to therapy, with resolution of the skin changes, improvement of the neuropathic disturbances and reduction all of the features assumed to be related to increased permeability, such as papilloedema and organomegaly. These data, while confirming VEGF as a diagnostic marker of the disease, also support VEGF as a prognostic marker for POEMS syndrome, strongly predictive of response to therapy. Conversely, EPO levels pre-therapy do not differ significantly between responders and non-responders to treatment. The efficacy of the therapies might well be related to their ability to interfere with VEGF production and action. In fact, according to our experience, IVIg are not effective in modifying the disease course, plasma exchange provides only temporary benefit, while radiation therapy and alkylators are more successful, probably because they affect the source of VEGF. In fact, VEGF production has been shown to be stimulated by oestrogen hormones and improvement of POEMS syndrome symptoms has been reported after treatment with tamoxifen (Enevoldson and Harding, 1992), a drug with strong anti-oestrogen action. In addition, thalidomide (Sinisalo et al., 2004), a drug with anti-angiogenic action, has been reported to be effective in treating the symptoms.

The correlation between VEGF serum concentration and severity of POEMS syndrome suggests that VEGF may be a causative agent in the disease. Moreover, the neuropathological data of POEMS nerve biopsies, showing involvement of vasa nervorum, strongly support a role of angiogenic factors in the pathogenesis of polyneuropathy. Saida et al. (1997)
previously reported vascular abnormalities in the nerve of POEMS patients giving rise to the possibility of a chronically accelerated coagulation due to alterations in the serum coagulation factors. In fact, VEGF can also act as activator of the coagulation pathway (Senger et al., 1996). Adams and Said (1998) instead suggested a direct role of the M component in the lesion of nerve fibres having observed M deposit in the endoneurium. However, it could be a consequence of microvascular hyperpermeability induced by VEGF with a secondary opening of the blood–nerve barrier, which is less tight than the blood–brain barrier (Kanda et al., 2000). No deposition of Ig or amyloid was detected in our cases. On the contrary, we found a considerable involvement of the nerve vasculature and a direct correlation with the VEGF serum levels. We propose that the endothelial injury is directly or indirectly caused by an abnormal activation of endothelial cells by VEGF, which is overexpressed in the nerves of patients with POEMS syndrome. Elevated systemic levels of VEGF probably determine hypertrophy and proliferation of the endothelial cells with a secondary microangiopathy. The consequent reduction of oxygen supply induces a robust expression of HIF-1α by all of the constituents of the nerve, with a secondary increase in local VEGF expression causing a self-perpetuating VEGF toxic gain of function. At the same time, since physiological activities of VEGF include induction of platelet aggregation and promotion of vascular permeability, VEGF also indirectly sustains its release and induction. The biological activity of VEGF can also be modulated indirectly, as happens with receptor transcription. The absence of VEGFR2 staining in POEMS nerve biopsies could, in fact, be due to receptor down-regulation.

Microvascular changes have been reported in vasa nervorum of patients with peripheral neuropathy associated with dysglobulinaemia (Powell et al., 1984). In a series of 11 nerve biopsies, eight with MGUS, two with polyclonal gammopathy and one with Waldenström’s macroglobulinaemia, electron microscopy showed abnormal accumulation of masses of intracytoplasmic filaments accompanied by endothelial proliferation. Our ultrastructural analysis of POEMS nerves revealed instead endothelial cytoplasmic enlargement, opening of the tight junctions between endothelial cells and presence of many pinocytic vesicles adjacent to the cell membranes, suggesting a permeability alteration of endoneurial vessels.

The low EPO concentration in POEMS serum may reflect a compensatory consequence of the high levels of VEGF, since they share some angiogenic properties. Low EPO may also be due to renal dysfunction. Although blood urea and creatinine levels in the patients were within the normal range, we cannot exclude involvement of the kidney as the result of high VEGF circulating levels, especially in patients that did not respond to therapy.

The role of EPO in nerve damage during POEMS syndrome is more difficult to define. We found similar EPO expression in POEMS and control nerves, although the immunofluorescence studies were not quantitative. It has been reported that EPO effects include prevention of neuronal apoptosis and that it has protective effects against diabetic neuropathy in rats (Bianchi et al., 2004). Interestingly, diabetic neuropathy is characterized by a microangiopathy and a direct correlation between hyperglycaemia and VEGF has been found (Kakizawa et al., 2004). It is therefore conceivable that the reduction of EPO could be responsible for the increased susceptibility of endothelial cells to VEGF-induced damage. An imbalance between these two angiogenic factors in the local setting of the nerve might alter their neurotrophic activities.

The absence of different distribution of the four polymorphisms or macrodeletions in the VEGF gene promoter region analysed in five POEMS patients (unpublished observations) leaves open the question about the cause of VEGF over-production. Identification of the source and mechanism behind this increase will lead to a more effective therapeutic approach.

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