Serum vascular endothelial growth factor is associated with cardiovascular involvement and response to therapy in Erdheim-Chester disease

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Abstract

Erdheim-Chester disease (ECD) is a rare histiocytosis, considered to be an inflammatory myeloid neoplasm. Tropism for specific involvements of the disease remains unexplained. Vascular endothelial growth factor-A (VEGF) is implicated in cancer pathophysiology and mutations of the *RAS* oncogene have been shown to induce upregulation of *VEGF* gene expression. We therefore hypothesized that VEGF might play a particular role in ECD pathophysiology. We conducted a retrospective, single-center study to assess serum VEGF (sVEGF) concentrations and determine whether they were associated with the characteristics of ECD patients, and to determine whether VEGF was expressed by histiocytes. We evaluated 247 ECD patients, 53.4% of whom had sVEGF levels above the normal range (>500 pg/mL). Patients with high sVEGF levels more frequently had cardiac and vascular involvement (58.3% *vs.* 41.4%, *P*=0.008 and 70.5% *vs*. 48.3%, *P*=0.0004, respectively). In treatment-naïve patients (n=135), the association of C-reactive protein >5 mg/L and sVEGF >500 pg/mL was strongly associated with vascular involvement (odds ratio=5.54 [95% confidence interval: 2.39-13.62], *P*<0.001), and independently associated with cardiac involvement (odds ratio=3.18 [95% confidence interval: 1.34-7.83], *P*=0.010) after adjustment for the presence of the *BRAF* V600E mutation. Changes in sVEGF concentration on treatment were associated with a response of cardiac involvement on consecutive cardiac magnetic resonance images. All histological samples analyzed (n=24) displayed histiocytes with intracytoplasmic expression of VEGF, which was moderate to high in more than 90% of cases. Our study suggests a role for VEGF in cardiac and vascular involvement in ECD.

Introduction

Erdheim-Chester disease (ECD) is a rare histiocytosis characterized by tissue infiltration with $CD68^+$ $CD1a^-$ histiocytes derived from mononuclear phagocytes harboring recurrent mutations of MAPK-signaling pathway genes.[1] Angiogenesis plays an important role in neoplastic and inflammatory processes. Vascular endothelial growth factor-A, commonly referred to simply as VEGF, is the main pro-angiogenic isoform, and a key regulator of blood vessel growth.[2] It is secreted by immune cells, smooth muscle cells, and many tumor cells. VEGF interacts with immune cells in the tumor microenvironment, generating a pro-tumor microenvironment.[3]

Activation of the *RAS* oncogene has been shown to induce upregulation of VEGF production.[4] VEGF is expressed by histiocytes in 70% of Langerhans cell histiocytosis (LCH) lesions,[5] but little is known about the expression of this

factor in ECD. Serum VEGF (sVEGF) levels in a series of 24 ECD patients were reported to be higher than those in healthy individuals.[6] Polyneuropathy, organomegaly, endocrinopathy, monoclonal gammapathy, and skin changes (POEMS) syndrome is associated with high sVEGF concentrations, which are thought to underlie increased microvascular permeability and other disease manifestations in this syndrome.[7]

We hypothesized that VEGF plays a role in the pathophysiology of ECD. The aim of this study was to assess sVEGF concentrations in ECD patients, and their association with the characteristics of the patients. The secondary objective was to determine whether VEGF is produced by histiocytes in ECD lesions.

Methods

Patients and methods

We conducted a retrospective study of all patients with ECD seen at the French National Reference Center for Histiocytoses at Pitié-Salpêtrière Hospital. All patients had a clinical and radiological presentation consistent with ECD, and tissue samples were centrally reviewed for pathological confirmation. Patients were included if they had at least one sVEGF determination between January 1, 2009 and December 31, 2019. Previously reported patients were not included in this analysis.[6] We searched for the *BRAF* V600E mutation as previously described.[8] sVEGF concentration was assessed by enzyme-linked immunosorbent assay and considered high if it exceeded 500 pg/mL. Interleukin (IL)-6 levels were determined with an immunoenzyme assay (Lumipulse G1200 Fujirebio). Cardiac and vascular involvement (coating of the aorta and/or its branches) was assessed in paraclinical examinations prescribed by the physician. Cardiac magnetic resonance imaging (MRI) scans were reviewed centrally for the purposes of this study by an expert in cardiac imaging. Targeted treatments with BRAF inhibitors (vemurafenib and dabrafenib) and MEK inhibitors (cobimetinib and trametinib) were prescribed at the discretion of the doctors, generally when required due to disease severity. Follow-up ended in May 2022.

Histological samples from patients with extreme (high and low) sVEGF concentrations were reviewed centrally. The immunostaining procedure for VEGF was performed on formalin-fixed, deparaffinized, 3 µm-thick sections with the Ventana Benchmark Ultra platform (Roche Diagnostics, France) and the Optiview visualization system (Roche Diagnostics), according to the manufacturer's instructions. Two different primary antibodies were used: F/PU483-UP (polyclonal, rabbit, Biogenex, USA; dilution 1/20, incubation 32 min at 37°C), and SC-53462 (monoclonal, VG-1, mouse, Santa Cruz, USA; dilution 1/25, incubation 44 min at 37°C). Staining was analyzed by two pathologists. Staining intensity was evaluated with a semi-quantitative score from 0 (no staining) to 3 (intense staining). Endothelial cells were used as an internal positive control. A placental sample was used as an external positive control.

Ethical considerations

This study was performed in accordance with French data protection regulations (CNIL) reference methodology and the Declaration of Helsinki. Demographic, clinical, biological and imaging data were extracted from medical records, together with treatment information. The study was approved by the ethic committee Ile de France III (#2011-A00447–34).

Statistics

We used χ^2 tests, Fisher exact tests, Student *t* tests, Wilcoxon matched-pairs signed-rank tests and log-rank tests for comparisons, as appropriate. Multiple logistic regression models were built to evaluate the predictive value of biological parameters and their association with disease characteristics. A sensitivity analysis was performed to evaluate the impact of the treatment previously received on these biological parameters. Survival curves were generated by the Kaplan-Meier method, taking into account the time from histological documentation of ECD to death or last follow-up. All the tests performed were two-tailed, with *P* values <0.05 considered statistically significant. Statistical analyses were performed with R-Studio open-source software version 1.1.456, R open-source software version 3.5.1 (2018-07-02, "Feather Spray") and GraphPad Prism V 8.3 (GraphPad software, La Jolla, CA, USA).

Results

We checked the inclusion criteria for 288 patients. We excluded 30 patients for whom no sVEGF determinations were available, three due to missing data in the medical records and eight for whom the diagnosis was uncertain. sVEGF concentration was high (>500 pg/mL) at the first determination in 132 of the 247 patients (53.4%), and >1,000 pg/mL in 47 (19.0%) (Figure 1). The median sVEGF concentration was 847 pg/mL (interquartile range [IQR]: 627-1,245 pg/mL) in patients with high sVEGF concentrations, and 286 pg/mL (IQR: 194-387 pg/mL) in patients with low sVEGF concentrations. At the time of the first sVEGF determination, 135 of the 245 (55.1%) patients had not yet received any treatment for ECD.

Sex, age, *BRAF* status and prior medication did not differ significantly between patients with and without initially high sVEGF concentrations (Table 1). The proportion of ECD patients with vascular involvement was significantly

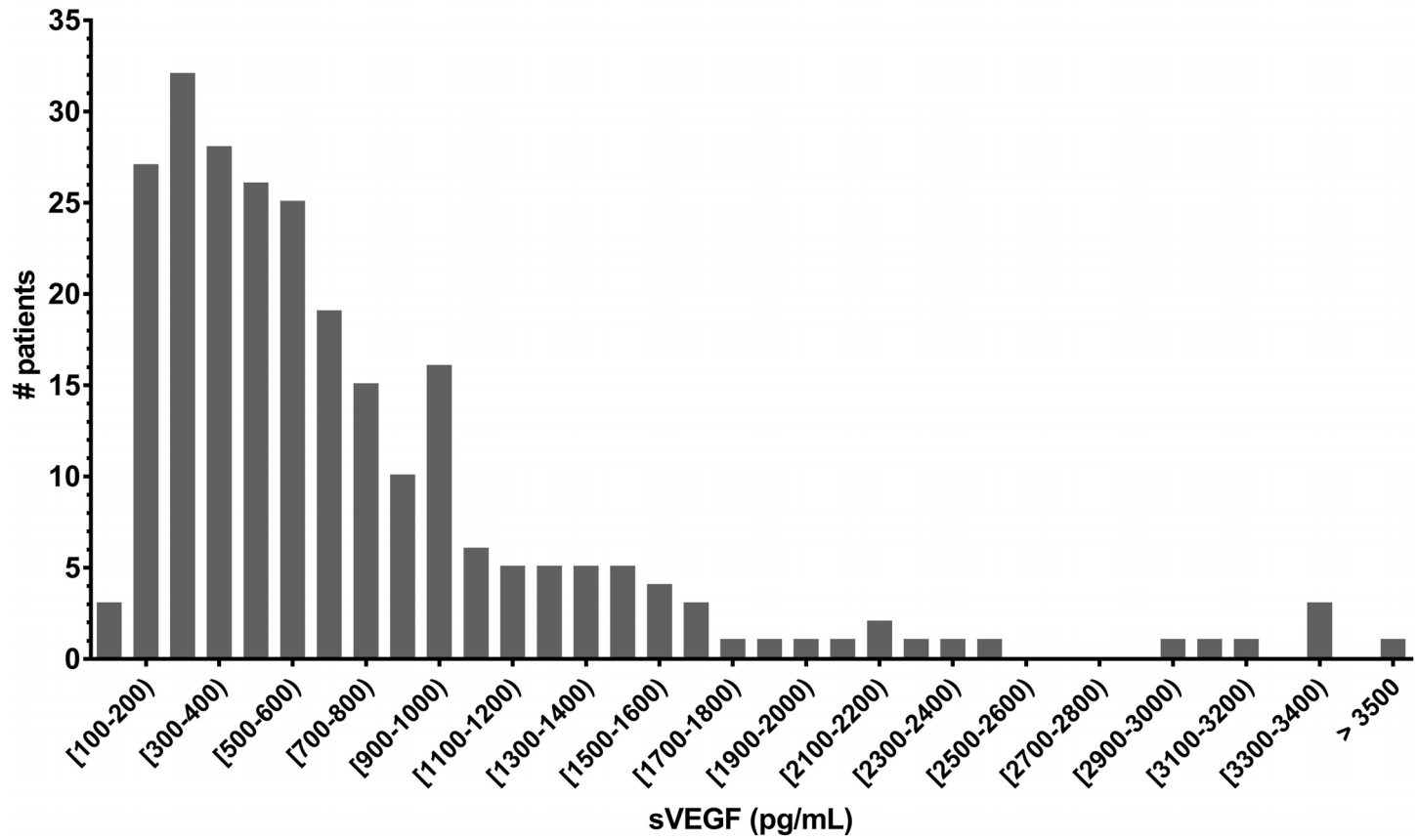

Figure 1. Distribution of serum vascular endothelial growth factor levels at initial determination. sVEGF: serum vascular endothelial growth factor.

higher in the group of patients with high sVEGF concentrations (70.5% *vs*. 48.3%, *P*=0.0004), especially for "coated aorta" (50% *vs.* 33.6%, *P*=0.009), and retroperitoneum (70.5% *vs*. 55.1%, *P*=0.006). Cardiac involvement was also more frequent in the high sVEGF group (58.3% *vs*. 41.4%, *P*=0.008). Coronary arteries, in particular, were affected twice as frequently (27.2% *vs.* 14.7%, *P*=0.016). Right atrium pseudotumors were also more frequent in the high sVEGF group (41.7% *vs*. 29.3%, *P*=0.043). In 67 patients with cardiac involvement evaluated on centrally reviewed cardiac MRI, late gadolinium enhancement did not differentiate between patients with high and low sVEGF concentrations (31/41 *vs*. 18/26, *P*=0.57). Other types of involvement, such as diabetes insipidus, central nervous system involvement, and xanthelasma, were distributed equally between the two groups.

In a logistic regression model, high sVEGF concentration (>500 pg/mL) at the initial measurement (M0) was associated with vascular involvement (odds ratio [OR]=2.56, 95% confidence interval [95% CI]: 1.51-4.38; *P*=0.001), and, after adjustment for the presence of the *BRAF* V600E mutation, with cardiac involvement (OR=1.95, 95% CI: 1.09-3.53; *P*=0.026). The performance of sVEGF concentration for predicting cardiac or vascular involvement, evaluated on a receiver operating characteristic curve (area under the curve: 0.6397), identified a threshold sVEGF concentration at 486.5 pg/mL (sensitivity 61.85%, specificity 64%) for the detection of cardiac or vascular involvement (*Online Supplementary Figure S1*). A sVEGF concentration threshold of 1,001 pg/mL had a specificity of 89.3% (95% CI: 80.3-94.5) and a sensitivity of 22.5% (95% CI: 17.0-29.3) for cardiac or vascular involvement, and a specificity of 86.2% (95% CI: 79.0-91.2) and sensitivity of 24% (95% CI: 17.4-32.2) for cardiac involvement alone.

We evaluated the specificity of the correlation observed between sVEGF concentration and cardiovascular involvement by studying other inflammatory biomarkers. C-reactive protein (CRP) concentration at M0, which was available for 167 patients, was high (>5 mg/L) in 116/167 (69.5%) (median CRP: 16.0 mg/L; IQR=3.0-26.06). IL-6 concentration could be determined for 36 patients with high sVEGF concentrations at M0: it was high (>6.5 pg/mL) in 28/36 (77.8%) (median IL-6 level: 14.10 pg/mL; IQR=10.7-22.0). We observed a significant correlation between sVEGF and CRP concentrations at M0 (Pearson: 0.201, *P*=0.009), and between sVEGF and IL-6 concentrations (Pearson: 0.381, *P*=0.002).

Cardiac involvement was more frequent in patients with a high CRP concentration (> 5 mg/L) at M0 (70/116 [60.3%] *vs*. 16/51 [31.4%], *P*=0.0006), as was vascular involvement (77/116 [66.4%] *vs.* 22/51 [43.1%], *P*=0.0049). The performance of CRP concentration for predicting cardiac or vascular involvement was evaluated on a receiving operating characteristic curve (area under the curve: 0.6561). A threshold CRP concentration of 40.5 mg/L had a specificity of 88.4% (95% CI: 74.9-96.1) and a sensitivity of 28.2% (95% CI: 20.5-37.1).

In a multiple logistic regression model, high CRP (>5 mg/L) and sVEGF (>500 pg/mL) concentrations were independently associated with vascular involvement (OR=2.09

Table 1. Characteristics of Erdheim-Chester disease patients with and without high serum vascular endothelial growth factor concentrations.

	All (N=247)	High serum VEGF concentration (N=132)	Low serum VEGF concentration (N=115)	*P*
Male/female, N/N	167/80	93/39	74/41	0.306
Age at diagnosis in years, mean (SD)	58 (14)	61 (13)	56 (16)	
BRAF V600E, n/N (%)	142/221 (64.3)	82/122 (67.2)	60/100 (60.0)	0.265
Mixed histiocytosis, N (%)	44 (17.8)	25 (18.9)	19 (16.4)	0.598
Langerhans cell histiocytosis	37 (14.9)	21 (15.9)	16 (13.8)	-
Rosaï Dorfman disease	6 (2.5)	3 (2.2)	3 (2.6)	-
ECD involvements, N (%)				
Cardiac involvement	125 (50.6)	77 (58.3)	48 (41.4)	**0.008**
Pericardium	71 (28.7)	41 (31.1)	30 (25.9)	0.366
Right atrium pseudotumor	89 (36.0)	55 (41.7)	34 (29.3)	**0.043**
Atria-ventricular septum	37 (15.0)	22 (16.7)	15 (12.9)	0.410
Coronary artery	53 (21.5)	36 (27.2)	17 (14.7)	**0.016**
Cardiac dysfunction	25 (10.1)	15 (11.4)	10 (8.6)	0.474
Vascular involvement	149 (60.3)	93 (70.5)	56 (48.3)	**0.0004**
Coated aorta	105 (42.5)	66 (50.0)	39 (33.6)	**0.009**
Mesenteric artery	40 (16.2)	22 (16.7)	18 (15.5)	0.806
Renal artery	48 (19.4)	28 (21.2)	20 (17.2)	0.429
Xanthelasma	53 (21.5)	30 (22.7)	23 (20.7)	0.602
Diabetes insipidus	58 (23.5)	28 (21.2)	30 (25.9)	0.388
CNS involvement	90 (36.4)	51 (37.9)	39 (32.8)	0.442
Retro-orbital involvement	45 (18.2)	28 (21.2)	17 (14.7)	0.181
Retroperitoneal involvement	157 (63.6)	93 (70.5)	62 (55.1)	**0.006**
Deaths, N (%)	66 (26.6)	40 (30.3)	26 (22.4)	0.161
Treatment-naïve, n/N (%)	125/216 (57.9)	69/114 (60.5)	56/102 (54.9)	0.403
Treatments previously received, N (%)				
Corticosteroids	34 (13.8)	19 (14.4)	15 (13.0)	0.758
IFN-α or PEG-IFN-α	20 (8.1)	9 (3.6)	11 (9.6)	0.430
Anakinra	6 (2.4)	5 (3.8)	1 (0.9)	0.220
Vinblastine	4 (1.6)	1 (0.8)	3 (2.6)	0.340
Cladribine	4 (1.6)	1 (0.8)	3 (2.6)	0.340
BRAF inhibitor	8 (3.2)	2 (1.5)	6 (5.2)	0.150
MEK inhibitor	2 (0.8)	2 (1.5)	0 (0)	0.500

n: number; N: number for whom information available; VEGF: vascular endothelial growth factor; SD: standard deviation; ECD: Erdheim-Chester disease; CNS: central nervous system; IFN: interferon; PEG-IFN: pegylated interferon. Statistically significant differences are shown in bold.

[95% CI: 1.02-4.29]; *P*=0.043 and 2.11 [95% CI: 1.08-4.15], *P*=0.029; respectively). The presence of a *BRAF* V600E mutation and high CRP concentration (>5 mg/L) were independently associated with cardiac involvement (OR=5.51 [95% CI: 2.55-12.59]; *P*<0.001 and 2.58 [95% CI: 1.15-5.93]; *P*=0.023, respectively), whereas high sVEGF concentration (>500 pg/mL) was not (OR=1.84 [95% CI: 0.88-3.90]; *P*=0.106).

We performed a sensitivity analysis in the subgroup of treatment-naïve patients (n=135). In this subgroup, high CRP (>5 mg/L) and sVEGF (>500 pg/mL) concentrations were independently associated with vascular involvement (OR=2.92 [95% CI: 1.14-7.74]; *P*=0.027 and 2.99 [95% CI: 1.28-7.13]; *P*=0.012, respectively). The *BRAF* V600E mutation, high CRP concentration and high sVEGF concentration were independent predictive factors for cardiac involvement (OR=3.97 [95% CI: 1.48-11.46]; *P*=0.008, 3.80 [95% CI: 1.26-13.16]; *P*=0.023 and 2.62 [95% CI: 1.03-6.96]; *P*=0.047, respectively). The combination of CRP concentration >5 mg/L and sVEGF concentration >500 pg/mL was strongly associated with vascular involvement (OR=5.54 [95% CI: 2.39-13.62]; *P*<0.001), and independently associated with cardiac involvement (OR=3.18 [95% CI: 1.34-7.83]; *P*=0.010) after adjustment for the presence of the *BRAF* V600E mutation.

Consecutive determinations of sVEGF were available for 183 patients with at least two sVEGF determinations. The median time between the first and last determinations

was 24 months (standard deviation [SD]: 31.86 months). A significant decrease in sVEGF concentration was observed between the first and last visits (P<0.0001), with a median change in sVEGF concentration (ΔsVEGF) of -153 (IQR: -376; -8) (Figure 2A). Early changes in sVEGF concentration were evaluated for 139 patients for whom sVEGF determinations had been obtained 6 months (M6) after the initial determination (M0). We observed a significant decrease in sVEGF concentration at M6 (Wilcoxon matched-pairs signed-rank test, P<0.0001; mean sVEGF concentrations: 700.12 pg/mL at M0 and 527.98 pg/mL at M6) (Figure 2B).

At least two cardiac MRI scans had been obtained, separated by an interval of at least 9 months, in 45 patients. The median time between the first and last MRI evaluations was 1,464 days (SD: 1,098.4 days; range: 358-4,506 days). Thoracic aorta coating was observed in 31 patients (68.9 %) and persisted in all cases, whereas some of the cardiac abnormalities observed regressed: a complete response with the disappearance of all abnormalities was noted in six patients, a partial response in 22 patients, stable cardiac lesions in nine patients, and a progression of cardiac involvement was observed in two patients (Table 2). The mean ΔsVEGF was -591.3 pg/mL (SD: 356.4) for patients with a complete response, -163.9 pg/mL (SD: 258.4) for those with a partial response, -239.6 pg/mL (SD: 242.5) for patients with stable disease and +555.5 pg/mL (SD: 219.9) for those displaying disease progression (Figure 3, Table 2). In patients with an initial contrast enhancement on cardiac MRI, ΔsVEGF did not differ significantly between patients with regression or persistence of contrast enhancement (mean difference 67.4 pg/mL, P=0.64). In univariate analysis, survival differed significantly between patients with a complete response, a partial response, stable disease and progression on cardiac imaging (median survival: undefined for complete responders, 11.5 years for partial responders, 9.2 years for patients with stable disease, and 5.4 years for patients in progression; log-rank test for trend, P=0.0248) (*Online Supplementary Figure S2*). No significant difference in survival was observed between patients with high and low initial sVEGF concentrations (median survival 10 years *vs.* 16 years, log-rank test, P=0.087) (*Online Supplementary Figure S2*), or between patients with ΔsVEGF < -500 pg/mL [-500;0), [0; 500) and ≥500 pg/mL (P=0.54).

No patients in our cohort received systemic anti-VEGF therapy for ECD or any other indication.

We analyzed 26 histological samples from ECD patients with extreme (13 high and 13 low) sVEGF concentrations. Biopsies were performed on perirenal infiltrate in 13 patients (50%), skin in seven (26.9%), bone in three (11.5%) and specimens from other sites in three patients. The median sVEGF levels for the patients for whom biopsy specimens were analyzed was 678.5 pg/mL (IQR: 181-2,011). A *BRAF* V600E mutation was found in 14 cases (58.3%), a *MAP2K1* mutation in three, and a *NRAS* mutation in one. Two samples could not be evaluated, due to a lack of material for one sample and failure of staining of decalcified bone for the other. The Kendall correlation coefficient between the two clones was 0.597. In all 24 samples analyzed, ECD histiocytes displayed intracytoplasmic VEGF staining, classified as low (grade 1) in two cases (8.3 %) and one (4.2%) case, moderate (grade 2) in

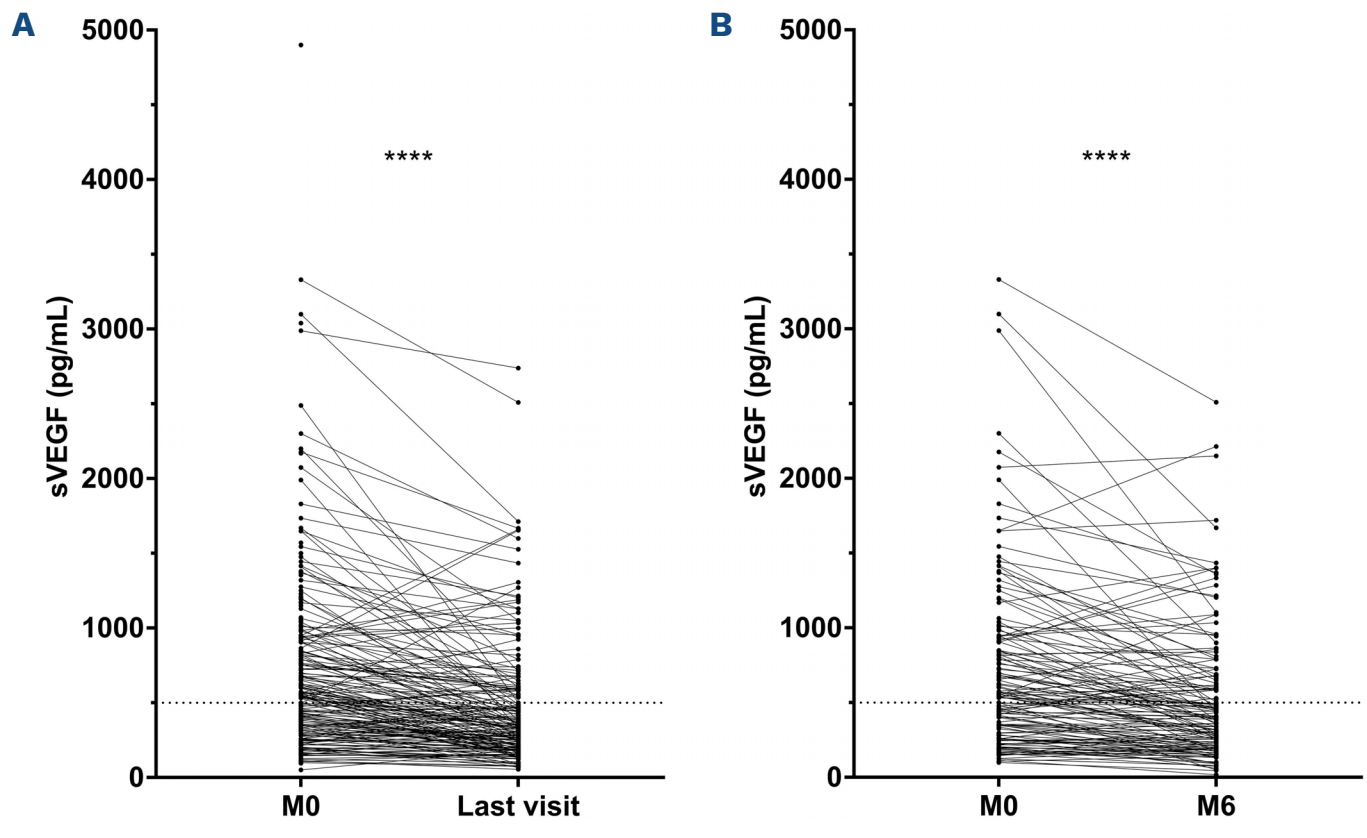

Figure 2. Change in serum vascular endothelial growth factor concentration in Erdheim-Chester disease patients over time. Change in serum vascular endothelial growth factor (sVEGF) concentration between the initial determination (M0) and determinations at (A) last visit, and (B) first follow-up visit at 6 months (M6).

Table 2. Characteristics of patients with cardiac involvement in Erdheim-Chester disease and different responses to treatment on follow-up cardiac imaging

	All (N=45)	Complete response (N=6)	Partial response (N=25)	Stability (N=12)	Progression (N=2)	*P*
Male/female, N/N	28/17	2/4	16/9	8/4	0/2	0.954
Age at diagnosis in years, mean (SD)	57.9 (13.9)	53 (17.3)	57.3 (13.7)	58.4 (13.1)	68.5 (10.6)	0.440
BRAF V600E, N (%)	41 (91.1)	6 (100)	22 (88)	11 (91.7)	2 (100)	1.00
Mixed histiocytosis, N (%) Langerhans cell histiocytosis Rosaï Dorfman disease	9 (20) 9 (20) 0 (0)	1 (16.7) 1 (16.7) 0 (0)	3 (12) 3 (12) 0 (0)	3 (25) 3 (25) 0 (0)	2 (100) 2 (100) 0 (0)	0.111 - -
Deaths, N (%) Median survival, years	15 (33.3) 10.86	0 (0) Undefined	6 (24) 11.51	8 (66.7) 9.24	1 (50) 5.39	**0.025**
Treatments, N (%) Corticosteroids IFN-α or PEG-IFN-α Anakinra Infliximab Cladribine Imatinib Targeted therapy Vemurafenib Dabrafenib Cobimetinib Trametinib	 15 (30) 36 (80) 8 (17.8) 2 (4.4) 0 (0) 1 (2.2) 29 (64.4) 28 (62.2) 2 (4.4) 6 (13.3) 1 (2.2)	 0 (0) 4 (66.7) 0 (0) 0 (0) 0 (0) 0 (0) 4 (66.7) 4 (66.7) 0 (0) 0 (0) 0 (0)	 9 (36) 18 (72) 6 (24) 1 (4) 0 (0) 0 (0) 18 (72) 17 (68) 1 (4) 5 (20) 1 (4)	 5 (41.7) 12 (100) 2 (16.7) 1 (8.3) 0 (0) 1 (8.3) 5 (41.7) 5 (41.7) 0 (0) 1 (8.3) 0 (0)	 1 (50) 2 (100) 0 (0) 0 (0) 0 (0) 0 (0) 2 (100) 2 (100) 1 (50) 0 (0) 0 (0)	 0.497 0.039 1.00 0.530 - - 0.197 0.255 0.530 0.648 1.00
ΔsVEGF in pg/mL, mean (SD)	-210.2 (350.0)	-591.3 (356.4)	-163.9 (258.3)	-239.5 (242.5)	+555.5 (219.9)	

VEGF: vascular endothelial growth factor; SD: standard deviation; IFN: interferon; PEG-IFN: pegylated interferon. ΔsVEGF: difference in serum vascular endothelial growth factor concentration. Statistically significant difference shown in bold.

five cases (20.8%) and one (4.2%) case, and intense (grade 3) in 17 (70.8 %) and 22 (91.7 %) cases, based on the F/PU483-UP and VG-1 clones, respectively (Figure 4). None of the biopsies revealed a total absence (grade 0) of histiocytic VEGF staining. Staining of one sample obtained from the decalcified tibia of a patient with a high sVEGF level (1,477 pg/mL) was classified as grade 1 with both clones. No association was found between VEGF expression and high or low sVEGF levels (Wilcoxon signed rank, *P*=0.714), mutational status, or organ involvement. As controls, samples from four cases of reactional sinusal histiocytosis were stained; none of the histiocytes in these cases expressed VEGF.

Finally, we investigated whether the observed high levels of sVEGF and histiocytic VEGF expression were ECD-specific or common to other histiocytoses, by analyzing a cohort of patients with Rosai-Dorfman disease (RDD) or LCH. We found that 11 of 31 LCH patients (35.5%) and 12 of 26 of those with RDD (46.1%) had high sVEGF levels (>500 pg/mL). Immunohistochemical analysis of five biopsy specimens from LCH patients revealed grade 3 histiocytic VEGF staining in all five samples after staining with F/PU483-UP and in three of five samples after staining with SC-53462 antibodies; the other two samples displaying no staining (grade 0) with this second antibody. Two

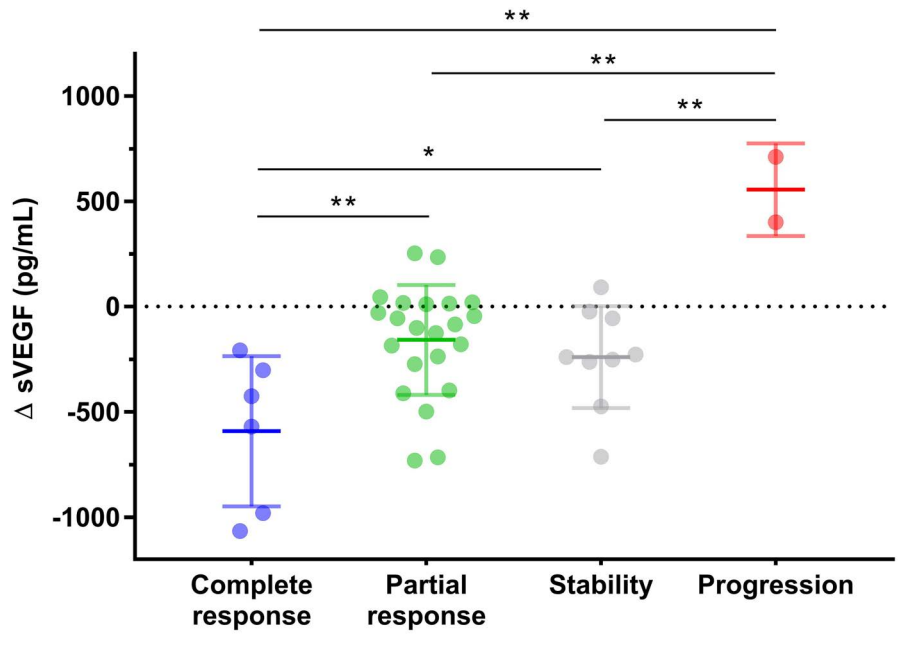

Figure 3. Change in serum vascular endothelial growth factor concentration in patients with Erdheim-Chester disease and cardiac involvement with a complete response, partial response, stability or progression on follow-up cardiac magnetic resonance imaging. ΔsVEGF: change in serum vascular endothelial growth factor. **P* value <0.05; ***P* value <0.01

of the five biopsy specimens from RDD patients presented grade 3 histiocytic VEGF staining with the F/PU483-UP antibody, and three displayed grade 3 histiocytic VEGF staining with the SC-53462 antibody. Three and two samples, respectively, presented grade 2 staining.

Discussion

In this study we found that sVEGF concentrations were high in 53.4% of ECD patients, and that ECD histiocytes express VEGF. Cardiac and vascular involvements are frequent in ECD, and prognostically relevant.[9] However, routine examinations, such as echocardiography or cardiac computed tomography (CT) scans, may underestimate the prevalence of such involvement. We identified an association between high sVEGF and CRP concentrations and cardiac and vascular involvements. This association was confirmed in the subset of treatment-naïve patients, in whom high sVEGF and CRP concentrations were predictive factors independently of each other and of the main factor known to be associated with cardiac involvement, the *BRAF* V600E mutation. The change in sVEGF concentration was correlated with the response of cardiac involvement to treatment. sVEGF determinations, combined with determinations of CRP concentration and *BRAF* status, could help to identify patients likely to benefit from cardiac MRI and vascular imaging, and contribute to monitoring cardiac involvement during treatment. Plasma chromogranin A, which is produced by cardiomyocytes, has been proposed as a biomarker of cardiac involvement in ECD.[10] VEGF, at least some of which is produced by histiocytes, may reflect disease activity rather than myocardial injury. Tropism for the heart and blood vessels in ECD has yet to be explained. In mycobacterium-associated granulomas, macrophages produce VEGF, which then triggers monocyte recruitment.[11] The VEGF produced by the vascular smooth muscle cells may induce the recruitment of pathological mononuclear cells and inflammatory cells. However, in our study, VEGF expression by histiocytes and increases in sVEGF concentration were not a hallmark of ECD, but a pattern shared with LCH and RDD patients, who have no cardiovascular involvement. Other factors may explain the tissue tropism of ECD. *VEGF* gene poly-

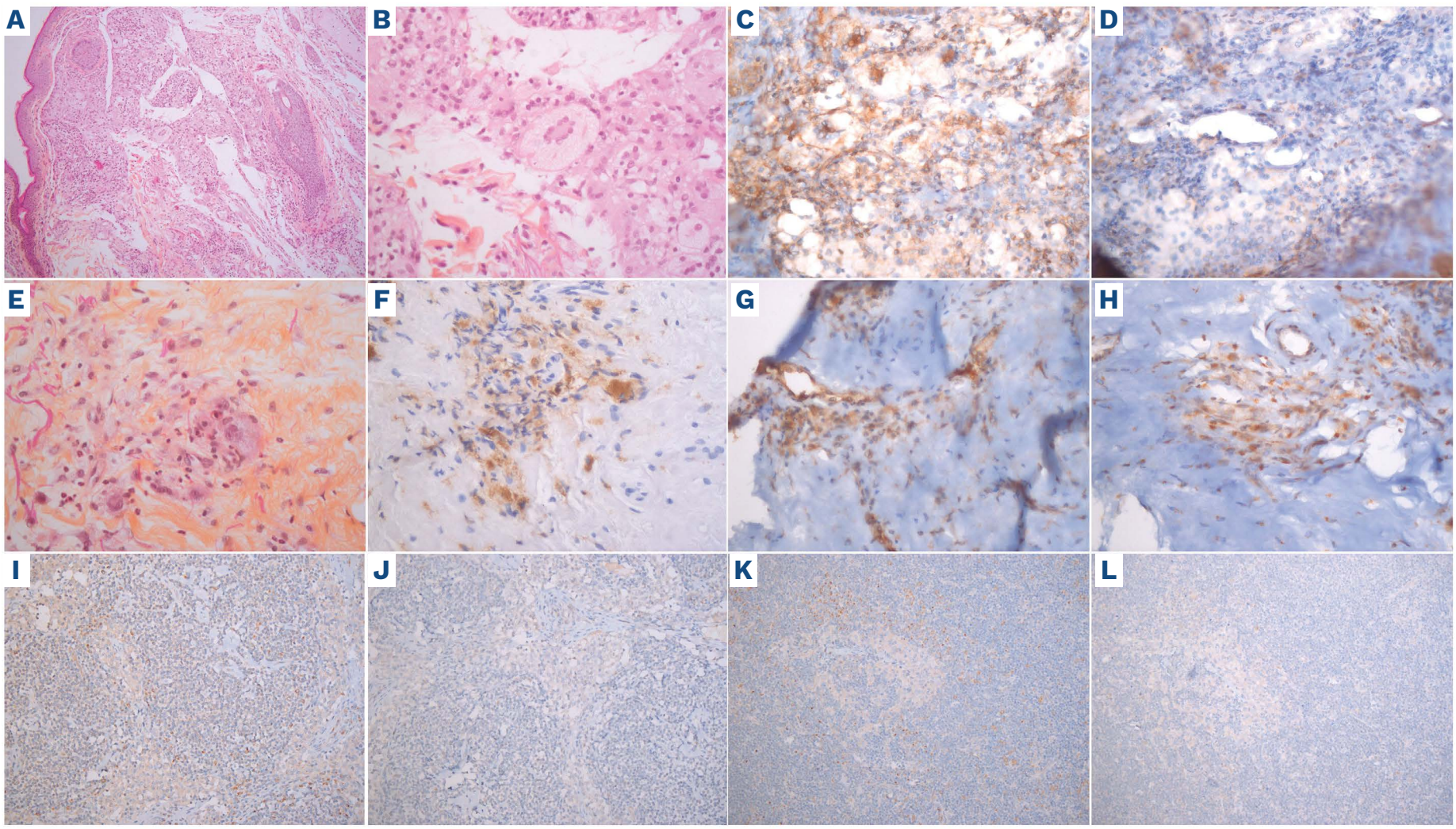

Figure 4. Vascular endothelial growth factor staining on Erdheim-Chester disease lesions and reactional sinusal histiocytosis. Top. Erdheim-Chester disease (ECD) xanthelasma: (A) infiltration of the dermis with foamy histiocytes (HES x10); (B) Touton giant cell (HEX x40); (C) intense cytoplasmic VEGF staining of histiocytes (VEGF VG1 x40); (D) moderate cytoplasmic VEGF staining of histiocytes (VEGF F/PU483-UP x40). Middle. ECD perirenal infiltrate: (E) histiocyte aggregates embedded in fibrosis (HES x40); (F) cytoplasmic CD68 staining of histiocytes; (G) intense cytoplasmic VEGF staining of histiocytes (VEGF VG1 x40); (H) intense cytoplasmic VEGF staining of histiocytes (VEGF F/PU483-UP x40). Bottom. Reactional sinusal histiocytosis: (I, K) VEGF VG1 x 20; (J, L) VEGF F/PU483 x20.

morphisms have been associated with susceptibility to various types of vasculitis, including giant cell arteritis, Behçet disease, Kawasaki disease[12] and chronic peri-aortitis,[13] and further studies will be needed to determine their role in ECD.

Systemic anti-VEGF therapy has not yet been evaluated in ECD. Three patients with intraocular manifestations of ECD and one case of multisystem LCH with uveal involvement and neovascular glaucoma have been successfully treated with intracameral bevacizumab, in association with systemic treatments, photodynamic therapy, or radiotherapy.[14,15] Two cases of juvenile intraocular xanthogranuloma have been treated with intravitreal bevacizumab, leading to a complete or partial regression of lesions.[16] Of note, anti-fibrotic tyrosine kinase inhibitors, such as nintedanib, target VEGF receptor, and may be able to decrease the inflammatory and fibrosing effects of VEGF. ERK inhibitors can decrease *VEGF* gene transcription *in vitro*.[17] Further studies are warranted to assess the potential utility of systemic therapies targeting VEGF in combination with standard therapy or in refractory cases.

Our study has several limitations. First, it was a retrospective study, and the evaluation of organ involvement was not standardized. However, CT angiography of the aorta and cardiac MRI or cardiac CT scans were routinely performed at diagnosis at our center, and cardiac MRI scans were reviewed centrally. Immunohistochemical analyses of VEGF expression were performed on a sample of histological specimens from our cohort. There is no consensual VEGF staining protocol. We used a polyclonal rabbit antibody and a monoclonal mouse antibody described in previous immunohistochemical studies.[18,19] The correlation between the results obtained with the two antibodies was limited (Kendall coefficient, 0.597). Previous studies have reported poor inter-observer correlation for VEGF staining (k=0.57 in a study on ductal carcinomas of the breast).[20] In our study, intensity scores were determined by consensus between two pathologists. sVEGF is a nonspecific marker that may reach high levels in several diseases, including POEMS syndrome, cancer,[21-23] anemia with iron deficiency and chronic obstructive pulmonary disease with exacerbations.[24] In our study, none of the patients met POEMS criteria, only seven patients had a medical history of prior chronic obstructive pulmonary disease, and eight had a history of solid neoplasm either still active or within the preceding 5 years.

In conclusion, high sVEGF concentrations were frequently observed in ECD patients and were associated with cardiac and vascular involvement. VEGF was at least partly produced by ECD histiocytes. Our results suggest a role for VEGF in the pathogenesis of ECD. sVEGF concentration may reflect cardiovascular involvement, and its determination could potentially help to improve the detection and follow-up of such involvement in patients with ECD. Further studies will be necessary to validate its use as a biomarker.

Disclosures

No conflicts of interest to disclose.

Contributions

AR and JH designed the study; AR, JH, and FCo collected the data; MB centrally reviewed cardiac magnetic resonance imaging; LD and FCh performed the histological analysis; MM and PG-D determined serum VEGF levels; HO and J-ML determined serum IL-6 levels; J-FE analyzed MAPK pathway mutations; AR performed the statistical analysis, interpreted the data and wrote the original draft of the manuscript; and AR, JH, L-DA, J-FE, and FCh reviewed and edited the manuscript. All the authors critically reviewed and approved the final version of the manuscript.

Data-sharing statement

The datasets used and/or analyzed during the current study are available from the corresponding author (JH) on reasonable request.

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