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ORIGINAL ARTICLE

Evaluation of clinicopathologic characteristics and the BRAF V600E mutation in Erdheim-Chester disease among Chinese adults

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Abstract Erdheim-Chester disease (ECD) is a rare form of histiocytosis with a broad, non-specific clinical spectrum. Here, we retrospectively evaluated the clinical and pathologic characteristics, presence of the BRAF V600E mutation, treatment options, and outcomes of Chinese patients diagnosed with ECD at our center. Patients diagnosed with ECD between January 2010 and April 2015 at Peking Union Medical College Hospital were included for study. We evaluated baseline characteristics, reviewed histological material, and tested for the presence of the BRAF V600E mutation using immunohistochemistry and polymerase chain reaction (PCR). Sixteen patients were diagnosed with ECD. Median disease duration (from the first symptom to diagnosis) was 22.5 months (range, 3–100 months). The main sites of involvement included bone (93.8 %), cardiovascular region (43.8 %), skin (31.3 %), central nervous system (25 %), and "hairy kidney" (25 %). The BRAF V600E mutation was detected in 68.8 % patients using PCR and 50 % patients with immunohistochemistry. Three patients could not be diagnosed using histological analysis owing to similarities with Rosai-Dorfman disease, and diagnosis in these cases was confirmed based on the BRAF V600E mutation status. Ten patients (62.5 %) received IFN-α as first-line treatment. Thirteen patients (81.3 %) were still alive at median follow-up of 14.5 months. ECD remains a largely overlooked disease, and increased recognition by clinicians and pathologists is necessary for effective diagnosis and treatment. The presence of the BRAF V600E mutation may facilitate discrimination of ECD from other non-Langerhans cell histiocytoses.

Keywords Erdheim-Chester disease · BRAF V600E mutation · Histiocytosis

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Introduction

Histiocytoses encompass a wide range of rare and heterogeneous diseases characterized by accumulation of histiocytes within various tissues. Classification of histiocytoses is based on Langerhans or non-Langerhans cell origin [1], determined according to the presence of Birbeck granules and CD1α expression in formalin-fixed, paraffin-embedded samples [2]. Non-Langerhans cell histiocytoses include Rosai-Dorfman disease, Erdheim-Chester disease (ECD), dendritic cell tumor/sarcoma, and histiocytic sarcoma [3, 4].

ECD is a multisystemic form of non-Langerhans cell histiocytoses characterized by infiltration of different tissues by lipid-laden foamy macrophages. The clinical spectrum of ECD is broad, as pathologic histiocytes can infiltrate virtually every organ and tissue, and diagnosis relies on histological criteria. Historically, ECD is difficult to diagnose due to its rarity and histological similarities with other subtypes of non-Langerhans cell histiocytoses. Recent studies have

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demonstrated that infiltrating histiocytes from a significant proportion of patients with ECD bear an activating mutation in the BRAF oncogene [5, 6]. Since the BRAF V600E is absent in the setting of Rosai-Dorfman disease [7], the status of this mutation in patients might aid in differentiating between the two entities in extremely rare cases with equivocal immunohistochemical staining and morphological findings. In the current study, we examined the clinical and pathological features, treatment outcomes, and BRAF V600E mutation status of ECD cases diagnosed at our center over the last 5 years.

Methods

Patients

Patients diagnosed with ECD between January 2010 and April 2015 at Peking Union Medical College Hospital were included in this retrospective study. Clinical data, including age, sex, lesion location, physical examination, routine biologic analysis, treatment, and survival data, were collected. Routine biologic analyses included evaluation of C-reactive protein (CRP) or high sensitive CRP (hsCRP), erythrocyte sedimentation rate (ESR), platelet count, and fibrinogen level. Cytokine levels, including interleukin (IL)-6, IL-8, IL-10, and tumor necrosis factor (TNF)-α, were measured in two patients. ^{18F}Fluorodeoxyglucose (FDG) positron emission tomography (PET) scan, thoracic and abdominal computed tomography (CT) scan, cardiac magnetic resonance imaging (MRI), and cerebral MRI results were collected. Informed consent was obtained from all patients and the study was approved by Peking Union Medical College Hospital Ethics Committee. The study has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Histological and immunohistochemical analyses

Histological materials were reviewed independently by two academic pathologists. BRAF immunohistochemistry was performed using the anti-BRAF V600E (VE1) antibody (Ventana Medical Systems) on the Benchmark XT platform. Visualization of V600E mutant-specific BRAF protein was accomplished using the OptiView DAB IHC Detection Kit (Ventana Medical Systems), according to the manufacturer's instructions. Pre-validated BRAF V600E-positive and -negative specimens were used as positive and negative controls, respectively. Immunoreactivity of mutant-specific BRAF was evaluated based on cytoplasmic staining of tumor cells using the anti-BRAF V600E (VE1) antibody. As expected, no staining observed with the negative control.

DNA extraction

DNA of the collected tissue samples was extracted using the QIAGEN QIAamp DNA FFPE Tissue Kit (56404, QIAGEN) following the manufacturer's protocol. DNA from each sample was eluted in 50 μl ATE (included in the kit). Absorbance was measured using a spectrophotometer, and DNA diluted to approximately 2–3 ng/μl with distilled water for subsequent experiments.

Detection of BRAF V600E

The BRAF V600E mutation was detected using the China Food and Drug Administration-approved human BRAF V600E ARMS-PCR kit (Amoy Diagnostics Co. Ltd, Xiamen, China). The quality of extracted DNA was evaluated by amplification of a housekeeping gene following instructions in the HEX channel. Amplification was performed for 47 cycles. FAM and HEX signals were obtained during the third stage. Run files were analyzed and interpreted as specified by the manufacturer.

Follow-up

All patients were followed up every 3 to 6 months, depending on disease activity. Informed consent was obtained from all patients. ECD activity was evaluated based on association with clinical symptoms (fatigue, pain, fever, and physical signs of histiocytic infiltration), biology (CRP or hsCRP blood level, ESR, platelet count, fibrinogen level), specific morphologic changes at various sites of involvement and PET scan, if available.

Data analysis

Overall survival (OS) was defined as the time from diagnosis to the date of death or last follow-up.

Results

General characteristics

The study included a total of 16 (nine men and seven women) newly diagnosed ECD patients between January 2010 and April 2015 at our center. Histologic analyses confirmed infiltration of CD68 + CD1α– histiocytes in all patients. General characteristics of patients and treatments are outlined in Table 1. Median age at diagnosis was 47 years (range, 22–61 years). The presenting symptoms included bone pain, headache, skin rash, polydipsia/polyuria, lymph node enlargement, shortness of breath, edema, fever, and weakness. Median disease duration (from the first symptom to diagnosis)

Table 1 Characteristics and treatment of 16 patients with ECD

Patient	Sex/ age, years	Disease duration, mo	Main sites of involvement	Biopsy site	Typical clinical findings	Type of bone	BRAF IH	BRAF PCR	Therapy	Vital status	OS, mo
1	M/33	5	B	B	B	TBS, BP	N/A	−	IFN-6 MIU 3/week	Alive	15
2	M/22	43	S, B	B	B	TBS, BP	−	−	IFN-3 MIU 3/week	Alive	11
3	M/25	18	B, LN, CNS	LN	B	TBS	−	+	Pred	Dead	13
4	F/28	3	S, B	S	B	BL	+	+	None	Alive	16
5	M/60	27	B, PIT	PIT	B	TBS	+	+	Surgery	Alive	15
6	F/61	5	B, H, LV, R, CNS, MS, S	S	CA, HK, B, RAM	TBS, BP	N/A	+	IFN-6 MIU 3/week	Dead	25
7	F/23	67	S, H, LV, B	S	B	TBS	−	−	IFN-3 MIU 3/week	Alive	19
8	M/60	43	B, P, LV, R	B	B, CA, HK	TBS, BP	N/A	+	IFN-6 MIU 3/week	Alive	14
9	M/46	84	CNS, B	CNS	None	BL	+	+	IFN-6 MIU 3/week	Alive	22
10	F/51	7	PIT	CNS	None	N/A	+	+	Surgery	Alive	6
11	F/36	72	PIT, B	PIT	B	TBS	+	+	Surgery	Alive	30
12	M/55	100	B, S, CNS, PIT	S	B	TBS, BP	−	+	IFN-6 MIU 3/week	Alive	3
13	F/50	11	B, H	H	B, RAM	TBS	N/A	+	IFN-6 MIU 3/week	Alive	5
14	F/46	8	B, LV, P	LV	B	BL	+	+	IFN-6 MIU 3/week	Alive	1
15	M/52	30	B, LV, R, P, E	B	B, CA, HK	TBS, BP	−	−	IFN-6 MIU 3/week	Alive	1
16	M/47	4	B, LV, R, LN	B	B, CA, HK	TBS, BP	−	−	None	Dead	36

Age is at diagnosis; disease duration is from the first symptom to diagnosis

IH immunohisochemistry, *B* long bones, *LN* lymph nodes, *LV* large vessels, *H* heart, *S* skin, *CNS* central nervous system, *MS* maxillary sinus, *PIT* pituitary gland, *R* retroperitoneal, *P* pericardial effusion, *E* exophthalmos, *MIU* million international units, *N/A* not available, *TBS* typical bone scan, *BL* bone lesion, *BP* bone pain, *CA* coated aorta, *HK* hairy kidney, *RAM* right atrium mass

was 22.5 months (range 3–100). The main sites of involvement included bone (15/16), large vessels (6/16), skin (5/16), central nervous system (4/16), retroperitoneal (4/16), pituitary gland (4/16), heart (3/16), pericardial effusion (3/16), lymph nodes (2/16), exophthalmos (1/16), and maxillary sinus (1/16). Six cases had fever, weakness, or weight loss at diagnosis. The typical clinical findings included long bone involvement (14/16), circumferential soft-tissue sheathing of the thoracic and abdominal aorta, the so-called coated aorta (4/16), infiltration of perinephric tissues leading to the "hairy kidney" (4/16) and mural pseudo-tumoral infiltration of the right atrium (2/16). The bone involvement were divided to typical bone scan (12/15) and bone lesion (3/15), and seven patients had bone pain.

Pathologic findings

Thirteen patients displayed characteristic histological features, including foamy histiocyte infiltration of polymorphic granuloma and fibrosis or xanthogranulomatosis, with CD68-positive and CD1-α-negative immunostaining. Three patients (designated 3, 5, and 11) displayed CD68-positive and CD1α-negative histiocyte infiltration, but not the above histological characteristics. Lesional tissue demonstrates infiltration of histiocytes, a few of which present showing emperipolesis in extranodal tissue. Focal area showed foamy histiocyte and fibrosis. S100 were dim positive. They were thus initially misdiagnosed as Rosai-Dorfman disease. All three cases were BRAFV600E mutation-positive, leading to revision of diagnosis as ECD. Diagnosis of ECD in each case was additionally supported by typical radiographic findings of bone. Six patients were positive for S100. The results of the BRAF V600E mutation status are shown in Table 1. BRAF V600E mutations were detected in 11/16 patients using PCR (68.8 %). Immunohistochemistry analysis revealed positive staining for BRAF V600 in 6/12 cases (50.0 %). Sufficient tissue specimens for testing were not obtained from the four remaining patients.

Laboratory evaluation

Platelet count was elevated in 4/16 patients (range, 16–454), the hsCRP level in 7/10 patients (range, 0.16–39.3), ESR level in 11/14 patients (range, 1–106), and fibrinogen level in 8/12 patients (range, 1.7–6.7). Nearly normal laboratory results were obtained for three patients (5, 10, and 11). Cytokine levels of two patients (14 and 15) were elevated, specifically, 26.2–27.6 pg/ml IL-6 (normal <5.9 pg/ml), 230–831 pg/ml IL-8 (normal <62 pg/ml), and 25.5–150 pg/ml TNF-α (normal <8.1 pg/ml). However, IL-10 levels remained normal in both patients.

Treatment and follow-up

Ten patients received IFN-α as first-line treatment. Three patients (5, 10, and 11) underwent transsphenoidal pituitary lesion surgery but were not subjected to systemic treatment, owing to the absence of symptoms and disease activity post-surgery. One patient received prednisone as first-line treatment. Two patients (4 and 16) refused further treatment after diagnosis.

The median follow-up time was 14.5 months (range, 1–30 months). Three patients died 13, 25, and 36 months from diagnosis. Among the ten patients receiving IFN-α, three showed improvement (symptoms disappeared, CRP or hsCRP, ESR, platelet count and fibrinogen levels returned to normal, PET scan, and other radiologic results showed decreased lesion numbers and activity), three remained stable, three were too early for evaluation and one died. Three patients without systemic treatment had stable disease after a median of 16 months (range, 6–30 months) from diagnosis.

Discussion

ECD, an extremely rare non-Langerhans cell histiocytosis, remains a largely overlooked disease. The clinical spectrum at presentation is broad, as infiltration of different organs leads to the development of protean manifestation, including the "coated aorta", right atrium mass, "hairy kidney", central nervous system involvement, and skin involvement [8, 9]. Since the clinical picture at the time of onset may be different and not specific for ECD, early diagnosis is particularly challenging. In the current study, we investigated a series of 16 patients with histologically proven ECD. Disease diagnosis took a median of 22.5 months, with confirmation of diagnosis in patient 12 taking more than 8 years. This prolonged diagnosis may be attributed to the fact that disease is a slowly evolving histiocytosis and successive manifestation may occur over years. More importantly, increased recognition of the disease by clinicians is necessary for effective diagnosis and treatment, in view of its rarity and non-specific clinical manifestations.

Another reason for delayed diagnosis is the histological similarities of ECD with other subtypes of non-Langerhans cell histiocytoses. Immunohistochemical results revealed CD68-positive and CD1α-negative immunostaining in all non-Langerhans cell histiocytoses, which did not facilitate differential diagnosis. Depending on the series and methods used, 50–100 % patients with ECD carry the BRAF V600E mutation [5, 10], in contrast to other non-Langerhans cell histiocytoses in which this mutation is absent [7]. Accordingly, determination of the BRAF V600E mutation status may aid in differentiation. In our study, three patients could not be diagnosed using histological analysis owing to

similarities with Rosai-Dorfman disease, and diagnosis in these cases was confirmed based on the BRAF V600E mutation status. The results of immunohistochemical analyses and PCR detection of BRAF V600E were comparable (50.0 vs 68.8 %). In some rare cases, patients could have mixed histiocytosis (ECD and LCH) [11]. We did not find the occurrence of both diseases in the same patient.

Multisystemic histiocyte infiltration with sclerotic lesions were observed in 15 patients (93.8 %), cardiovascular involvement in seven patients (43.8 %), cutaneous rash or plaque in five patients (31.3 %), central nervous system involvement in four patients (25.0 %), and "hairy kidney" in four patients (25 %), in keeping with earlier reports [8, 9].

ECD is a disease that can be considered at the crossroad between inflammation and oncogenic mutations. The majority of ECD patients are diagnosed between the ages of 40 and 70 years. The median age of patients in our series was 47. And in parallel to the high frequency of BRAF V600E mutation supporting the clonal nature of ECD, ECD histiocytes express a pattern of proinflammatory cytokines and chemokines responsible for local activation and recruitment of these cells [12]. The serum of ECD patients is reported to contain elevated levels of interferon (IFN)-α, IL-12, monocyte chemotactic protein-1, and decreased levels of IL-4 and IL-7 [13]. Two of our patients had elevated IL-6, IL-8, and TNF-α, but normal IL-10 levels. Further long-term studies are required to establish whether or not these results may be applicable in the monitoring of disease activity.

The clinical course of ECD is largely dependent on the extent and distribution of the disease. Some patients may be asymptomatic or exhibit only mild and limited symptoms, while others may have a more aggressive form of the disease with poor prognosis. Three patients in our series did not receive systemic treatment, since they showed no symptoms or disease activity after local treatment. After 6 to 30 months follow-up, patients remained stable, suggesting very slow disease progression in some cases.

The first-line treatment for ECD is IFN-α and pegylated IFN-α [8]. In our study, IFN-α was employed as first-line therapy in ten patients (62.5 %), among which three (30 %) responded to therapy, three (30 %) remained stable, three were to early for evaluation, and one died. Since ECD is a slowly progressing disease, treatment usually leads only to partial remission [14, 15], rather than complete recovery. Thus, long-term follow-up studies on larger patient cohorts with ECD are warranted.

Therapeutic strategies now available have improved prognosis of ECD patients. The overall 5-year survival of 68 % in patients subjected to interferon therapy [16]. In our series, three patients (18.7 %) died during a median follow-up of 14.5 months. Thanks to the discovery of BRAFV600E mutation from a significant proportion of ECD patients, BRAF inhibitor vemurafenib has been used to patients of ECD refractory to first-line treatments (IFN-α and/or anakinra). To date, treatment of eight patients who were refractory to first-line treatment and harbored a BRAF V600E mutation with an inhibitor of BRAF (vemurafenib) has led to dramatic and unprecedented clinical and radiographic improvements [17], indicating potential enhancement of long-term survival with emerging new drugs.

In conclusion, ECD, an extremely rare non-Langerhans cell histiocytosis, remains a largely overlooked disease, and increased recognition by clinicians and pathologists is necessary for effective diagnosis and treatment. The clinical spectrum of ECD is broad, with IFN-α and pegylated IFN- α currently used as first-line treatment. The presence of the specific BRAF V600E mutation might aid in distinguishing ECD from other non-Langerhans cell histiocytoses. Further research on large patient cohorts with long-term follow-up is needed to substantiate the sensitivity and specificity of the finding of BRAF V600E mutation for sub-classifying histiocytoses and further therapeutic progress.

Compliance with ethical standards Informed consent was obtained from all patients and the study was approved by Peking Union Medical College Hospital Ethics Committee. The study has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Conflict of interest The authors declare that they have no competing interests.

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