Diagnostic Challenges during Pretreatment Long-term Follow-up in a Patient with FIP1L1-PDGFRA-positive Eosinophilia

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Abstract

Obtaining a precise characterization of eosinophilia is crucial, as successful treatment relies on the underlying etiology of the disease. Platelet-derived growth factor receptor alpha-related disorders were first specified in 2008 as a distinct group of clonal eosinophilic disorders with exceptional responsiveness to imatinib. We herein present the case of a man with myeloid neoplasm and eosinophilia in whom a definitive diagnosis could not be adequately made based on histopathological features who was ultimately diagnosed only after extensive molecular analyses and successfully treated with imatinib. In addition, we discuss the diagnostic and therapeutic approaches to treating patients presenting with eosinophilia.

Key words: eosinophilia, PDGFRA, imatinib

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Introduction

Establishing the etiology of blood eosinophilia, which is frequently encountered by hematologists, can be a diagnostic challenge (1). Eosinophilia may be an incidental finding and, in some cases, a transient disorder, as well as a chronic condition with various organ system involvement. Until recently, most cases of blood eosinophilia >1.5×10⁹/L lasting more than six months with signs and symptoms of organ damage were grouped under the term hypereosinophilic syndrome (HES) (2). However, recent developments in molecular genetics have revolutionized the diagnosis, classification and treatment of eosinophilic disorders (3).

We herein report the clinical course of a man with a myeloid neoplasm and eosinophilia. After five years of follow-up, the presence of the FIP1L1-PDGFRA (F/P) fusion gene was confirmed. At the same time, the patient’s condition deteriorated, possibly due to the administration of corticosteroids. Subsequently, he was successfully treated with imatinib. In light of its increasingly complex clinical presentation, we review the current stratification of eosinophilia, with a special emphasis on the diagnostic criteria and treatment for F/P-positive eosinophilia (4, 5).

Case Report

A 40-year-old Caucasian man was referred to a hematologist due to splenomegaly, without other complaints, in April 2007. He was an agricultural engineer and in regular contact with animals. A physical examination revealed splenomegaly (+9 cm), which was confirmed on ultrasonography (21 cm). The initial complete blood cell (CBC) count showed a white blood cell (WBC) level of 10×10⁹/L with 52% neutrophils, 22% lymphocytes, 20% eosinophils, 3% monocytes and 3% basophils in formula, as well as an absolute eosinophilic count (AEC) of 2×10⁹/L, hemoglobin (Hb) level of 110 g/L and platelet (Plt) count of 110×10⁹/L. The results of biochemical analyses were normal. The leukocyte alkaline phosphatase score was very low, at 0 IU/L (normal range: 20-80). Parasitic infestations (strongyloidiasis, schistosomiasis, filariasis, toxocariasis), allergic reactions and other causes of common eosinophilia were excluded. A bone marrow (BM) biopsy revealed sparse megakaryocytic and

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erythroid lineages with granulocyte hyperplasia and marrow fibrosis of grade I/II (Fig. 1). Histologically, the morphological findings of the BM were consistent with a diagnosis of chronic myeloid leukemia (CML). Spontaneous erythroid colony formation in vitro [Burst Forming Unit-Erythroid (BFU-E)] from the bone marrow was positive. Conventional cytogenetics showed a normal male karyotype (Fig. 2). Reverse transcription polymerase chain reaction (RT-PCR) for BCR-ABL (p190, p210) was negative. Consequently, the diagnosis of Philadelphia (Ph)-negative CML was made, and no treatment was initiated. The patient remained well and checked his CBC values without further follow-up by a hematologist for the next five years (Fig. 3).

In April 2012, the patient felt discomfort below the left costal margin. He also became tired easily, without any other signs of disease progression. A physical examination revealed marked splenomegaly (+13 cm) with non-itchy skin changes (Fig. 4). There was no significant dyspnea or signs of myocardial involvement. Abdominal ultrasound confirmed substantial splenomegaly (25 cm), and a further increase was observed in the WBC count (23.7×10^9/L, AEC 7.6×10^9/L) in association with a decrease in the Plt count (87×10^9/L). Biochemical analyses showed an elevated uric acid level (564 μmol/L) and lactate dehydrogenase activity (592 U/L). In addition, the levels of vitamin B12 (>2,000 pmol/L) and IgE (650 IU/mL) were increased. According to the patient’s dermatologist, the skin changes were caused by eosinophilic vasculitis with recurrent episodes of erythematous purpuric papules, although, at the time of the examination, the lesions were in regression and unsuitable for biopsy. A repeated BM biopsy revealed individual, polymorphic, largely hypolobulated megakaryocytes (Fig. 5a). The granulocytic lineage was hyperplastic, with a slight shift to the left. There was no excess of blasts, whereas the level of eosinophils in the BM was moderately increased and immunostaining showed non-cohesive clusters of small tryptase-positive mast cells (Fig. 5b). A conventional cytogenetics analysis was normal. Neither the BCR-ABL fusion or JAK2V617F mutation were found. A further evaluation leading to a diagnosis of clonal eosinophilic disorder was performed. In November 2012, a fluorescent in situ hybridization (FISH) analysis was conducted (Labordiagnostik, Vienna), which demonstrated CHIC2 deletion [CHIC2+FIP1L1/PDGFRα(4q12) dual-color probe, Kreatech diagnostic, Amsterdam, Netherlands] in the majority of BM (93.5%) and peripheral blood (91.5%) interphase nuclei (Fig. 6a). This deletion is a surrogate marker of F/P fusion (6). A repeated hematological work-up resulted in a diagnosis consistent with World Health Organization (WHO)-based myeloid neoplasm with eosinophilia and abnormalities of PDGFRα.

During the work-up, the patient developed lassitude and night sweating at the end of December 2012. The CBC count demonstrated a further increase in WBCs (64.5×10^9/L, 32% eosinophils, AEC 20.6×10^9/L) and the persistence of moderate thrombocytopenia (71×10^9/L). Pending approval for imatinib therapy, treatment with hydroxyurea (500 mg/day) was started. A few days later, the patient developed back pain that radiated down to the right knee. His general practitioner started him on parenteral steroid treatment (Lemod Solu 40 mg i.v. and Diprofos i.m.) without consulting the hematologist. After 10 days, significant increases in the WBC count (375×10^9/L) and AEC level (75×10^9/L) were noted with a shift to myelocytes without blasts (40% neutrophils, 3% lymphocytes, 20% eosinophils, 3% monocytes, 7% basophils, 17% bands, 4% metamyelocytes, 6% myelocytes) in formula, with a subsequent drop in the Hb level (100 g/L) and Plt count (40×10^9/L). Due to the patient’s precarious condition, as well as additional chest discomfort and dyspnea, without electrocardiogram (ECG) changes, urgent leukapheresis was performed. The dose of hydroxyurea was increased to 1,500 mg/day, and treatment with imatinib was started (100 mg/day). After five days of treatment, the patient’s condition further deteriorated, and he was hospitalized with malaise, weakness and a fever of >38°C in poor general condition. His Eastern Cooperative Oncology Group (ECOG) score was 3. Treatment subsequently decreased the WBC count to 44×10^9/L, with consequent worsening of thrombocytopenia (14×10^9/L) and the development of a skin hematoma and epistaxis. Even after the cessation of all treatment, he went on to develop severe pancytopenia (Hb 58 g/L, WBC 1.2×10^9/L, Plt 19×10^9/L) that lasted for almost three weeks and required supportive therapy. Further examinations, including a chest X-ray and echocardiogram, were normal. Abdominal ultrasound revealed hepatomegaly (17 cm) and splenomegaly (25 cm). A fibroscan demonstrated initial fibrosis of the liver and absolute fibrosis of the spleen (75 KPa). Repeated BM trepanobiopsies showed granulocytic hyperplasia with increased eosinophils and fibrosis of grade I/II, focal grade III/IV (Fig. 7), matching the features of a myeloproliferative neoplasm. Following the recovery of the CBC count, the imatinib treatment was continued at a dose of 100 mg/day. The patient’s clinical condition quickly improved, and, after
six months, he was found to be in good condition without symptoms and an ECOG score of 0. There was no hepatomegaly, although the spleen was slightly palpable (+2 cm). In addition, the blood cell count was normal, without eosinophilia (approximately 1% eosinophils), and a new FISH analysis revealed no CHIC2 deletion in the BM or peripheral blood interphase nuclei (Fig. 6b).

Discussion

Making a differential diagnosis of eosinophilia can be difficult. Properly characterizing the disease is important, as the success of treatment depends on the underlying etiology. Currently, eosinophilia can be divided into three types: secondary, clonal and idiopathic (1).

Secondary (reactive) eosinophilia is a consequence of a cytokine-induced phenomenon that most often results from an upsurge in interleukin-5 secretion, leading to the proliferation of eosinophils and their precursors (7). The main causes include parasitic infections, allergic conditions or vasculitis, drug reactions and non-myeloid malignancies. The management of reactive eosinophilia is based on treating the underlying condition.

In last several years, the number of patients with a diagnosis of HES has decreased due to an increase in the proportion of cases classified as clonal eosinophilia. The diagnosis of HES requires the exclusion of all primary (clonal) and secondary causes of eosinophilia and is based on the presence of an absolute eosinophil count over 1.5×10^9/L with signs of tissue damage. Corticosteroid therapy is generally sufficient to achieve remission in patients with HES. In contrast to HES, the term idiopathic hypereosinophilia is used in cases in which organ damage is absent (8). On the other hand, in clonal eosinophilia, eosinophils and their precursors are components of the malignant clone. The establishment of myeloid malignancy requires histology, cytogenetics and/or molecular tests (9).

In the present case, it was difficult to define the precise type of myeloproliferative neoplasm in 2007 based on the patient’s findings at that time. In order to obtain an accurate diagnosis, it was necessary to perform a range of molecular analyses, including RT-PCR for JAK2 and BCR-ABL fusion genes. Finally, FISH confirmed the presence of F/P rearrangement encoding an oncoprotein with an increased and aberrant tyrosine kinase activity (10).

In the new classification of tumors of hematopoietic and lymphoid tissues issued in 2008, the WHO Working group defined myeloid neoplasms with eosinophilia and abnormalities of PDGFRA as belonging to a separate entity of clonal eosinophilia distinct from chronic eosinophilic leukemia (CEL) (6). This entity is characterized by a cryptic deletion on chromosome 4 (del(4)(q12;q12)), which results in the formation of a chimeric gene composed of fused PDGFRA and FIP1L1 genes (11). Del(4) cannot be detected using conventional cytogenetic analyses.

F/P-positive eosinophilia is rare. This entity is present in 4% of all cases of eosinophilia (11), although in select groups it can rise up to 18% (12). It is more common in men, with a male:female ratio of 17:1 and a peak incidence between 25 and 55 years of age. The etiology is unknown, although several cases have been reported following cytotoxic chemotherapy (13-15). Our patient had not received cytotoxic therapy or had past contact with mutagenic chemical compounds.

The present patient experienced an unusually extended and stable clinical phase lasting more than five years with-
out any symptoms of disease. Otherwise, his clinical and hematological presentations were similar to those of previously described patients (12, 16). In a Belgium cohort of patients with F/P-positive eosinophilia, 63% (5/8) of the patients had splenomegaly, while 50% (4/8) were anemic with a moderately increased WBC count and AEC (18.8×10^9/L, 15.2×10^9/L, respectively) (16).

In the diagnostic work-up, the possibility of systemic mastocytosis, primary myelofibrosis and CEL not otherwise classified (CEL-NOS) should be excluded. The diagnosis of systemic mastocytosis requires the presence of dense clusters of spindle-shaped mast cells with CD25 antigen positivity, an elevated serum tryptase level and/or the presence of the KITD816V mutation (17). The diagnosis of primary myelofibrosis is made based on the presence of atypical and clustered megakaryocytes, BM fibrosis, leukoerythroblastosis and/or the JAK2V617F mutation (18). CEL-NOS may be considered if the level of blood eosinophilia is ≥1.5×10^9/L, accompanied by cytogenetic or molecular genetic abnormalities other than the BCR-ABL and PDGFRα/B rearrangements, or the proportion of blast cells is either >2% in the peripheral blood or >5% in the BM (8).

The clinical importance of recognizing specific mutations directs targeted therapy. JAK inhibitors are used to treat primary myelofibrosis, while Kit inhibitors are administered in cases of mastocytosis, and tyrosine-kinase inhibitors are applied in cases of PDGFRα-related disorders. The tyrosine kinase activity of the F/P fusion is 100 times more sensitive to inhibition by imatinib mesylate than the BCR-ABL1 kinase activity detected in CML (10). According to current views, the appropriate dose of imatinib for induction and maintenance therapy is 100 mg daily (12, 19). Resistance to imatinib is rare. Our patient was treated with the recommended dose of imatinib and achieved a complete hematological and almost complete clinical response, retaining minor splenomegaly, which can be explained by the long duration of disease prior to the commencement of imatinib treatment with the subsequent development of splenic fibrosis, as confirmed on a fibroscan.

It has also been suggested that further molecular monitoring of the depth of response is not always necessary in many patients (19). Moreover, the leukemic transformation of PDGFRα-related eosinophilia is rare. Two of 22 patients (9%) in a series by Pardanani et al. either presented with

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**Figure 4.** Skin and mucosal changes.

**Figure 5.** a: Bone marrow biopsy (2012)/Hematoxylin and Eosin staining ×200: Polymorphic, frequently hypolobulated megakaryocytes of low ploidy. Granulocytic hyperplasia with maturation and a slight shift to the left. No excess of blasts. b: Tryptase immunostaining of the bone marrow biopsy specimen: Non-cohesive clusters of small tryptase-positive mast cells.
Figure 6. a: Interphase fluorescent in situ hybridization: Identification of CHIC2 deletion [CHIC2+FIP1L1/PDGFRA(4q12) probe] in the majority of BM (93.5%) and peripheral blood (91.5%) nuclei. b: Interphase fluorescent in situ hybridization: All of the analyzed nuclei were negative for CHIC2 deletion in both the peripheral blood and bone marrow samples.

Figure 7. Bone marrow biopsy (2013)/Hematoxylin and Eosin staining ×200: Granulocytic hyperplasia with eosinophilia, fibrosis grade I/II and foci grade III/IV.

acute disease (CD34+) or progressed to a similar leukemic transformation during their clinical course (19). Even in cases of leukemic transformation, imatinib treatment is effective, eliminating the need for prompt hematopoietic stem cell transplantation (20). In resistant patients, especially those with a confirmed T674I mutation, further treatment with sorafenib is suggested (21).

The diagnosis of PDGFRA-related disorders should be considered in Ph-negative male patients with hematological features of CEL and associated splenomegaly. This disorder does not have any specific cytological or histopathological bone marrow features that distinguishes it from reactive eosinophilia. Therefore, molecular genetic tests are an important tool for reaching the diagnosis of clonal eosinophilia. In addition, the early introduction of targeted therapy is of great value, allowing for prompt achievement of remission and averting subsequent evolution into aggressive disease associated with severe organ dysfunction.

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