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# Hypereosinophilic syndrome due to ETV6/PDGFR-beta gene translocation - a diagnostic and therapeutic challenge

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## KEY WORDS

*Eosinophilia; PDGFR gene translocation; Hypereosinophilic syndrome*

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## Summary

**Background.** Hypereosinophilic syndromes are characterized by sustained overproduction of eosinophils, leading to eosinophilic infiltration, mediator release and multi-organ damage. **Case report.** A 67 year old male was referred to our Department for investigation of a persistent mild-to-moderate eosinophilia, identified 10 years previously and unresponsive to corticosteroid treatment. No other alterations were present in his differential blood count and physical examination was unremarkable. Allergic, rheumatologic and iatrogenic causes of eosinophilia were excluded by clinical history, skin-prick tests and blood and stool analysis. Iliac crest bone marrow aspiration and biopsy were performed, revealing normal cellularity with an increased eosinophil count (6%). RT-PCR of the aspirate revealed the presence of transcripts of ETV6/PDGFR-beta t(5;12) gene fusion. Karyotype analysis was normal and no mutation in PDGFR-alpha was identified. There was no evidence in analytic or imaging studies of cardiac, skin, neurologic, pulmonary or splenic involvement. A skin biopsy showed no evidence of pathologic infiltration. Initially the patient was treated with a 100 mg daily dose of imatinib mesylate, a specific inhibitor of the tyrosine-kinase domain of PDGFR. Subsequently, the daily dosage was increased to 200 mg/day to obtain eosinophil count normalization. Currently, he is under monthly hematologic and hepatic function screening. No drug side effects have been reported. **Conclusion.** This patient was diagnosed with a rare myeloproliferative variant of hypereosinophilic syndrome due to a t(5;12) ETV6/PDGFR-beta translocation. Imatinib mesylate, previously used successfully in syndromes associated with PDGFR-alpha mutations, showed efficacy in the context of this mutation as well.

## Background

Eosinophilia remains relatively common in the Western world. Its etiology is not always clear: a broad variety of allergic, infectious, inflammatory, neoplastic, and idiopathic diseases are associated with increased blood and/or tissue eosinophilia and range in severity from self-limiting conditions to life-threatening disorders (1).

Persistently elevated levels of blood eosinophilia should prompt ongoing pursuit of the underlying etiology, and monitoring for

organ-associated damage. Blood eosinophil values do not necessarily indicate the extent of eosinophil involvement in affected tissues, because these cells are primarily tissue-dwelling, being several hundredfold more abundant in tissues than in blood (2). Moreover, case-reports show that eosinophil-mediated damage occurred without elevation of peripheral blood eosinophils (3). Although accepted upper limits of normal blood eosinophil numbers vary somewhat, a value above 500 eosinophils/ $\mu$ l of blood is considered abnormal in the vast majority of cases (4). Traditionally, degrees of eosinophilia have been categorized as

mild (500-1500 cells/ $\mu$ l), moderate (1500-5000 cells/ $\mu$ l), and severe (> 5000 cells/ $\mu$ l). The term "hypereosinophilia" refers to eosinophil levels > 1500/ $\mu$ l, regardless of the underlying cause - primary, secondary or idiopathic.

A thorough investigation of a patient with eosinophilia requires consideration of their clinical history, physical examination, and information from laboratory and imaging studies. When a cause for secondary eosinophilia is not readily apparent, it is reasonable to make a working diagnosis of primary or idiopathic eosinophilia, and pursue specific diagnosis in this regard.

In primary eosinophilia, there is evidence of clonal expansion of eosinophils. It can accompany any of the myeloid malignancies defined by the World Health Organization (WHO) classification system for hematologic malignancies (5), usually acute leukemia or chronic myeloid disorders.

Idiopathic eosinophilia implies that both secondary and clonal eosinophilia have been ruled out as possible diagnoses. Hypereosinophilic syndrome (HES) is a subcategory of idiopathic eosinophilia and, as such, remains an exclusion diagnosis whose criteria have evolved over time, as more of its pathophysiology has been discovered, and additional investigative methods have been made available (6).

Classic criteria of 1) blood eosinophilia > 1500/ $\mu$ l for longer than 6 months, 2) lack of secondary causes of eosinophilia, and 3) presumptive signs and symptoms of eosinophilia-associated organ involvement have been largely abandoned as treatment options for these patients became available, with the aim of preventing tissue damage before it develops. Currently, patients with markedly increased blood eosinophilia and obvious tissue dysfunction should start the appropriate treatment before irreversible damage occurs, and no longer need to be observed for a six-month period (2).

According to the revised WHO Classification of Tumours of Hematopoietic and Lymphoid Tissues (7), patients who meet HES criteria fall into 2 different categories:

1) myeloproliferative neoplasms including hypereosinophilic syndrome (M-HES) or chronic eosinophilic leukemia not otherwise specified (CEL-NOS); 2) myeloid and lymphoid neoplasms with eosinophilia and abnormalities of platelet-derived growth factor  $\alpha$  (PDGFRA), platelet-derived growth factor  $\beta$  (PDGFRB), and fibroblast growth factor receptor 1 (FGFR1). Unfortunately, in most cases, HES either presents with overlapping features, or fails to meet any of the above criteria. A 2005 international consensus workshop on HES treatment provided an alternative classification system, subdividing patients into six clinical subgroups:

1) myeloproliferative HES; 2) lymphocytic HES; 3) familial eosinophilia; 4) undefined HES (idiopathic HES with or without symptoms, including episodic variants); 5) overlap HES (eosinophilic disease restricted to a single organ system accompanied

by peripheral eosinophilia) and 6) associated HES (eosinophilia in the setting of another diagnosis such as sarcoidosis or inflammatory bowel disease) (8).

Diagnostic evaluation relies on a combination of morphologic review of the blood and marrow, standard cytogenetics, fluorescent *in-situ* hybridization, flow cytometry and assessment of T-cell clonality, to detect histopathologic or clonal evidence for acute or chronic myeloid or lymphoproliferative disorders.

In this clinical case, we try to emphasize the most important diagnostic procedures associated with the investigation of a patient that presented with eosinophilia, and its treatment after diagnosis has been established.

### Case presentation

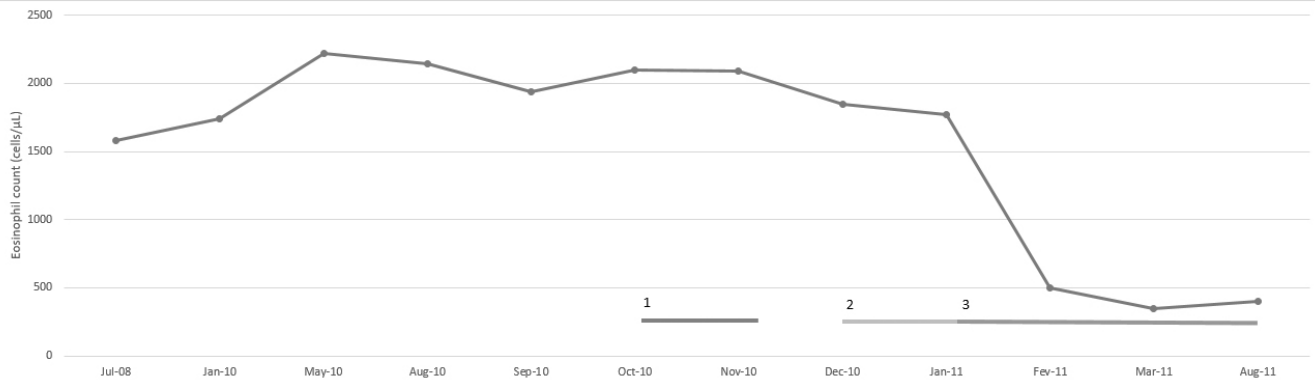
A 67 year old male of Indian descent living in Lisbon, was referred to our out-patient clinic in September 2010. He had been recently diagnosed with persistent eosinophilia by his assistant nephrologist. Eosinophil counts ranged from 1500 to 2300/ml in several blood counts, with no evidence of other differential blood count abnormalities. The patient had a history of chronic renal disease, currently in NKF-KDOQI stage 4, with evidence of renal osteodystrophy associated with type 2 diabetes mellitus. He also had a history of nonallergic rhinitis and elevated, controlled blood pressure.

His medication list included nifedipine, furosemide, irbesartan, gliclazide, atorvastatine, clopidogrel, bisoprolol, and insulin. Although theoretically any medication may potentially be the cause of a hematologic alteration, none of the above are usually considered to be associated with hypereosinophilia.

A retrospective evaluation of past blood counts revealed that eosinophilia was present as early as May 2000 (age: 57 years) and similar, persistently elevated counts were identified over the following years. No records were available prior to the 2000. The evolution of eosinophil counts is presented in **figure 1**.

The patient had no symptoms directly attributable to eosinophilia. He denied recent or long-standing respiratory or gastrointestinal symptoms, as well as rheumatologic or constitutional symptoms, namely arthralgia, myalgia, fatigue or weight loss. He had no history of smoking or drug abuse, and did not consume alcohol regularly. He complained of occasional episodes of runny, itchy nose that were not associated with contact with potential allergens or specific seasons of the year. He had no history of exposure to toxic substances or pesticides. The patient lived in an urban setting, in conditions of good hygiene, with little contact with animals. He had not travelled abroad during the previous 10 years.

Physical examination was unspecific. He had dry and scaly skin on the trunk and inferior limbs. There were no changes in auscultation, or evidence of hepato/splenomegaly. Other blood count parameters were normal, including hemoglobin,

**Figure 1** - Evolution of eosinophil count between 2008 and 2011.**Graph 1:** Evolution of eosinophil count between 2008 and 2011.

Eosinophil values were unaltered after treatment with daily prednisolone 1mg/kg (1) and only a slight decrease was observed with a 100mg dose of imatinib mesilate (2). In contrast, a good response was obtained with a 200mg dose of imatinib mesilate (3).

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leukocyte and platelet count. A peripheral blood film showed normal eosinophils, red blood cells and platelets. There were no circulating blast cells. Routine laboratory testing, including serum tryptase, troponin, angiotensin-converting enzyme, immunoglobulin and vitamin B12 levels, were within the normal range. Total IgE value was 62 UI/ml. Eosinophil cationic protein values were elevated at 89 μg/l. Stool samples were collected on three different occasions, with no evidence of eggs and/or cysts. Specific blood antibodies for *Schistosoma*, *Hidatide*, *Fasciola*, *Strongyloides*, *Echinococcus*, *Toxocara* and *Aspergillus*, as well as anti-thyroid or anti-nuclear antibodies, were not detected. HIV 1/2 tests were negative. Skin prick tests were negative, both to an aeroallergen and a food allergen battery. An electrocardiogram, echocardiogram and pulmonary function tests were performed and were normal.

An iliac crest bone marrow aspiration and biopsy were performed, revealing normal cellularity with an increased eosinophil count (6%) without increased myeloblasts. Subsequent reverse transcription polymerase chain reaction (RT-PCR) of the aspirate revealed the presence of transcripts of a ETV6/PDGFRB t(5;12) gene fusion. Karyotype analysis was normal and no mutation in PDGFRA or Ph chromosome t(9;22) were identified.

Systemic glucocorticoid was chosen as the first line of therapy. Prior to referral, for a period of approximately 3 weeks, the patient had received a daily dose of 20mg prednisolone. A re-introduction of prednisolone was made, with a higher dose of 1 mg/kg daily. However, after 4 weeks, eosinophil values were unaltered. Higher glucocorticoid doses and/or longer treatment regimens were considered, but dismissed, due to the patient's co-morbidities.

Prednisolone was reduced and subsequently stopped, and an alternative steroid-sparing regimen planned.

The patient was started on imatinib mesylate, a specific inhibitor of the tyrosine-kinase domain of ABL, c-kit and PDGF-receptor genes, initially at a daily dose of 100 mg. A slight improvement was seen, although eosinophil levels were still above the desired threshold. The dose was increased to 200 mg, resulting in a marked reduction in the peripheral blood eosinophil count within three weeks.

During daily imatinib mesylate administration, regular evaluations of heart and liver function were performed and no alterations were observed. RT-PCR was performed on a new bone marrow aspirate obtained at beginning of 2014, and did not detect the presence of ETV6/PDGFRB t(5;12) transcripts (total treatment time: 37 months). Imatinib was subsequently stopped and the patient remains under clinical and analytical observation. Eosinophil levels have remained stable at less than 500 cells/μl during the follow-up period. No organ-specific alterations have so far become evident.

## Discussion / Conclusions

The lack of other identified reasons for secondary eosinophilia, the presence of a mutation known to cause eosinophilia, and the good treatment response, all constitute strong arguments for the cause of the high eosinophil count in this patient being due to a specific, imatinib-responsive, mutation in the PDGFRB gene.

Hypereosinophilic syndromes associated with PDGFRB mutations seem to be less frequent than their PDGFRA counterparts (5,9).

The PDGF receptors belong to the receptor tyrosine kinase family, more precisely to the type III group, which also includes c-KIT, FLT3 and the macrophage-colony-stimulating factor receptor. Two highly homologous receptor genes have been cloned: PDGFRA and PDGFRB (10).

The first genetic alteration in PDGFRB receptors was reported in 1994 by Golub and Gilliland in patients with chronic myelomonocytic leukemia, as a result of a t(5;12) translocation, leading to the fusion of TEL (now renamed ETV6) with PDGFRB (11). This hybrid oncogene consists of the in-frame fusion of the N-terminal portion of the transcription repressor ETV6, including its pointed domain, with the kinase domain of PDGFRB (12). When introduced in mouse bone marrow cells, ETV6-PDGFRB induces a fatal myeloproliferative disorder. Noticeably, eosinophilia is not observed in this model, in contrast to the human disease (13).

The discovery that imatinib, a molecule approved for the treatment of BCR-ABL-positive chronic myelogenous leukemia, also blocks PDGF receptors when used at an even lower concentration, was a major breakthrough. Indeed, most patients with myeloproliferative neoplasms harbouring a PDGF receptor fusion respond well to low dose imatinib, despite rare resistant mutations having been described (14,15).

Previously described cases of ETV6-PDGFRB mutations have presented with more severe manifestations than those observed in this case (16,17,18); typically, they have featured myeloblast marrow infiltration and, thus, a chronic eosinophilic leukemia diagnosis.

A favourable response to imatinib mesylate was, in part, expected. The above-described cases had mostly good responses to this drug in doses ranging from 100 to 400 mg. A 2007 case series evaluated 12 patients (17) with BCR-ABL-negative chronic myeloproliferative disorders and reciprocal translocations involving PDGFRB, and receiving imatinib. Eleven had prompt responses featuring normalization of peripheral-blood cell counts and disappearance of eosinophilia, 10 with complete resolution of cytogenetic abnormalities and decrease or disappearance of fusion transcripts. Similarly good results were described in 4 patients in 2002 (19).

Despite the reassuring data, the prognosis for these patients is still, to a degree, uncertain. A French study of 40 patients in 1989, prior to the imatinib era, noted an 80% survival at five years and a 42% survival at 15 years (20). Several authors have reported slow progression towards full-blown T cell lymphoma (21). Malignant progression in such patients may be heralded by the appearance of cytogenetic changes (19). Drug-associated cardiomyopathy has also been reported as a rare complication of imatinib in patients being treated for CML, which should warrant additional attention in a patient with other significant co-morbidities.

In our case, the absence of findings associated with myeloproliferative disorders, such as elevated serum vitamin B12, abnormal leukocyte alkaline phosphatase scores, splenomegaly, cytogenetic abnormalities, myelofibrosis, and myeloid dysplasia, as indicated by the quarterly examinations performed, are likely a sign of a good prognosis. The absence of end-organ eosinophilic infiltration is also an important factor, and underlines the importance of an early intervention in idiopathic hypereosinophilic syndromes.

Monitoring of patients with HES must be individualized. This patient was assessed clinically every month at the beginning of treatment, and at increasing intervals when the disease stabilized. Monthly eosinophil counts were performed, coinciding with the provision of imatinib in our hospital. PCR testing for the ETV6-PDBFRB transcript was only repeated once, approximately 36 months after the beginning of treatment. Unfortunately, it is not possible to assess exactly when the transcripts became undetectable, but previous studies with variable doses of imatinib have shown cytogenetic transformation in as little as 9 months. Treatment has currently been stopped and the patient's eosinophil levels have remained within the normal range during the 3 months post treatment cessation.

The patient is currently evaluated at regular intervals in our department. His disease showcases the complex interactions in the regulation of normal eosinophil genesis and the differing etiologies associated with similar genetic abnormalities.

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### References

1. Mejia R, Nutman TB. Evaluation and differential diagnosis of marked, persistent eosinophilia. *Semin Hematol.* 2012;49:149-59.
2. Weller PF. The immunobiology of eosinophils. *N Engl J Med.* 1991;324:1110.
3. Blauwet LA, Breen JF, Edwards WD, Klarich KW. Atypical presentation of eosinophilic endomyocardial disease. *Mayo Clin Proc.* 2005;80:1078.
4. Roufosse E, Weller PF. Practical approach to the patient with hypereosinophilia. *J Allergy Clin Immunol.* 2010;126:39.
5. Gotlib J. World Health Organization-defined eosinophilic disorders: 2011 update on diagnosis, risk stratification, and management. *Am J Hematol.* 2011;86:677-88.
6. Tefferi A, Patnaik MM, Pardanani A. Eosinophilia: secondary, clonal and idiopathic. *Br J Haematol.* 2006;133:468-92.
7. Swerdlow SH, Campo E, Harris NL, et al., editors. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Lyon, France: IARC;2008.
8. Klion AD, Bochner BS, Gleich GJ. Approaches to the treatment of hypereosinophilic syndromes: a workshop summary report. *J Allergy Clin Immunol.* 2006;117(6):1292-302.

9. Demoulin JB, Montano-Almendras CP. Platelet-derived growth factors and their receptors in normal and malignant hematopoiesis. *Am J Blood Res.* 2012;2(1):44-56.
10. Toffalini F, Demoulin JB. New insights into the mechanisms of hematopoietic cell transformation by activated receptor tyrosine kinases. *Blood.* 2010;116:2429-37.
11. Golub TR, Barker GF, Lovett M and Gilliland DG. Fusion of PDGF receptor beta to a novel ETS-like gene, TEL, in chronic myelomonocytic leukemia with t(5;12) chromosomal translocation. *Cell.* 1994;77:307-16.
12. Carroll M, Tomasson MH, Barker GF, Golub TR and Gilliland DG. The TEL/platelet-derived growth factor  $\beta$  receptor (PDGF $\beta$  R) fusion in chronic myelomonocytic leukemia is a transforming protein that self-associates and activates PDGF $\beta$  R kinase-dependent signalling pathways. *Proc Natl Acad Sci USA.* 1996;93:14845-50.
13. Tomasson MH, Sternberg DW, Williams IR, Carroll M, Cain D, Aster JC, Ilaria RL Jr, Van Etten RA and Gilliland DG. Fatal myeloproliferation, induced in mice by TEL/PDGFB R expression, depends on PDGF $\beta$  R tyrosines 579/581. *J Clin Invest.* 2000;105:423-32.
14. David M, Cross NC, Burgstaller S, Chase A, Curtis C, Dang R, Gardembas M, Goldman JM, Grand F, Hughes G, Huguet F, Lavender L, McArthur GA, Mahon FX, Massimini G, Melo J, Rouselot P, Russell-Jones RJ, Seymour JF, Smith G, Stark A, Waghorn K, Nikolova Z and Apperley JF. Durable responses to imatinib in patients with PDGFRB fusion gene-positive and BCR-ABL-negative chronic myeloproliferative disorders. *Blood* 2007;109:61-4.
15. Lierman E, Michaux L, Beullens E, Pierre P, Marynen P, Cools J and Vandenberghe P. FIP1L1-PDGFRalpha D842V, a novel pan-resistant mutant, emerging after treatment of FIP1L1-PDGFR T674I eosinophilic leukemia with single agent sorafenib. *Leukemia.* 2009;23:845-51.
16. Tipirneni E, Buttar A, Brettler D, Miron P, Bathini V. Chronic myeloproliferative disorder with ETV6-PDGFR $\beta$  fusion gene. *Ann Hematol.* 2012;91(4):637-8.
17. Wälz C, Haferlach C, Hänel A, Metzgeroth G, Erben P, Gosencica D, Hochhaus A, Cross NC, Reiter A. Identification of a MYO18A-PDGFRB fusion gene in an eosinophilia-associated atypical myeloproliferative neoplasm with a t(5;17)(q33-34;q11.2). *Genes Chromosomes Cancer.* 2009;48(2):179-83.
18. David M, Cross NC, Burgstaller S, Chase A, Curtis C, Dang R, Gardembas M, Goldman JM, Grand F, Hughes G, Huguet F, Lavender L, McArthur GA, Mahon FX, Massimini G, Melo J, Rouselot P, Russell-Jones RJ, Seymour JF, Smith G, Stark A, Waghorn K, Nikolova Z, Apperley JF. Durable responses to imatinib in patients with PDGFRB fusion gene-positive and BCR-ABL-negative chronic myeloproliferative disorders. *Blood.* 2007;1;109(1):61-4.
19. Apperley JF, Gardembas M, Melo JV, Russell-Jones R, Bain BJ, Baxter EJ, Chase A, Chessells JM, Colombat M, Dearden CE, Dimitrijevic S, Mahon FX, Marin D, Nikolova Z, Olavarria E, Silberman S, Schultheis B, Cross NC, Goldman JM. Response to imatinib mesylate in patients with chronic myeloproliferative diseases with rearrangements of the platelet-derived growth factor receptor beta. *N Engl J Med.* 2002 15;347(7):481-7.
20. Lefebvre C, Bletry O, Degoulet P, et al. Prognostic factors of hypereosinophilic syndrome. Study of 40 cases. *Ann Med Interne (Paris).* 1989;140:253.
21. Roufosse F, Cogan E, Goldman M. The hypereosinophilic syndrome revisited. *Annu Rev Med.* 2003;54:169.