



A case of chronic eosinophilic leukemia with secondary transformation to acute myeloid leukemia



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ABSTRACT

The natural history of primary eosinophilia remains highly variable and is characterized by underlying disease heterogeneity. Chronic eosinophilic leukemia, not otherwise specified (CEL-NOS) is a rare and aggressive disease characterized by non-specific cytogenetic abnormalities or elevated blasts, with high risk of transformation to acute leukemia. We describe a case of CEL-NOS with two hierarchically related non-specific cytogenetic rearrangements, associated with an *NPM1* mutation and followed by evolution to secondary AML. *NPM1* mutations are not previously described in CEL-NOS.

1. Introduction

Hypereosinophilia (HE) ($> 1.5 \times 10^3/\mu\text{L}$) can be observed in a wide range of diseases and can lead to severe organ damage, as a result of eosinophilic infiltration in peripheral tissues and release of granules. Most eosinophilia is reactive, secondary to atopic conditions, infections, medication, auto-immune disorders and/or malignancies and is caused by overproduction of eosinophilopoietic cytokines such as interleukin 3 (IL-3), interleukin 5 (IL-5) or granulocyte-macrophage colony-stimulating factor (GM-CSF) [1].

Primary HE is caused by clonal proliferation of eosinophils and is associated with myeloid or lymphoid neoplasms [2]. Its natural history remains highly variable, given the underlying disease heterogeneity. Eosinophilia associated with non-specific clonal or molecular abnormalities and/or increased marrow blasts (between 5% and 20%), termed chronic eosinophilic leukemia, not otherwise specified (CEL-NOS) should be distinguished from myeloid/lymphoid neoplasms associated with eosinophilia and rearrangement of *PDGFRA*, *PDGFRB*, or *FGFR1* or with a *PCMI-JAK2* fusion. In cases without evidence for clonal proliferation, a diagnosis of idiopathic hypereosinophilic syndrome (HES) is made, which is a diagnosis of exclusion [3].

Primary HE has an age-adjusted incidence rate of approximately 0.036 per 100.000 [4]. Eosinophilia's with recurrent genetic

abnormalities comprise only a minority of these patients (*FIP1L1-PDGFR* fusion has a median frequency of 23%). Thus, in the majority of patients no cytogenetic aberration can be found, demonstrating the need for molecular markers to further delineate this category [3].

We present here a case of CEL-NOS, associated with two rare, non-specific clonal rearrangements and rapid evolution to acute myeloid leukemia (AML).

2. Case report

A 68-year old female patient was referred to a tertiary haematology department with unexplained eosinophilia. Only a recurrent fever and subjective impression of weight loss was present. The patient had no history of allergies nor drug use. Clinical examination showed morbid obesity, without significant other findings. Repeated fecal exams excluded parasitic infections and further examinations ruled out other organ involvement.

Complete blood count (CBC) showed leukocytosis ($43.2 \times 10^3/\mu\text{L}$) consisting mainly of eosinophils (82%; $35424/\mu\text{L}$) with aberrant granulation (Fig. 1a) and thrombocytopenia ($34 \times 10^3/\mu\text{L}$). Also, a slight increase in basophils and monocytes and a left-shift with the presence of 0.5% myeloblasts was noted. Biochemistry showed elevated vitamin B12 (1790 ng/L [197–866]) and LDH (689 U/L [105–233]) levels,

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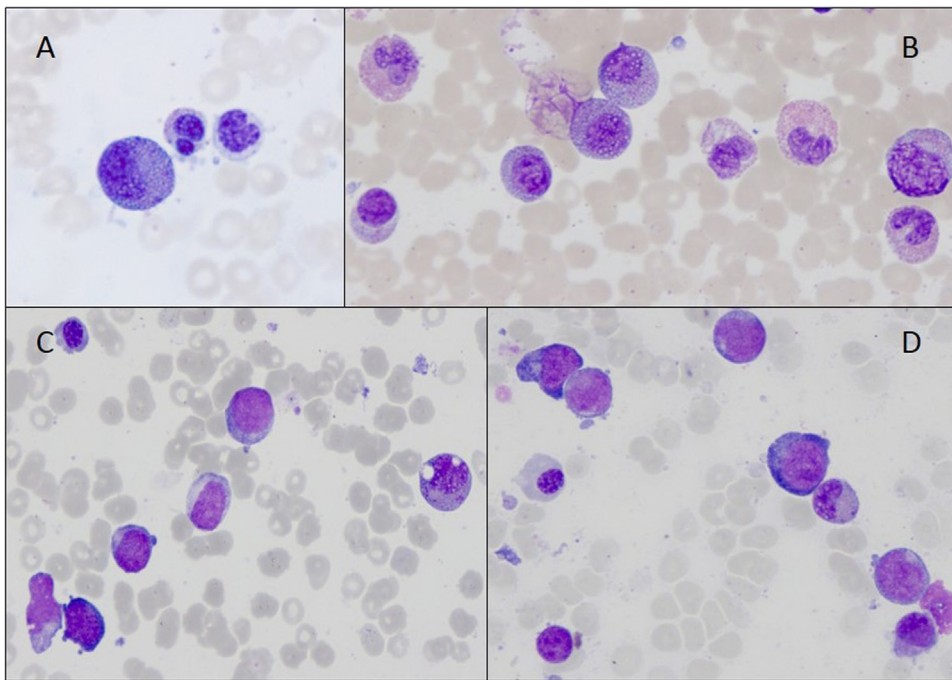


Fig. 1. (a) Peripheral blood smear showing eosinophilia with eosinophilic precursors, aberrant granulation, and vacuolisation (May-Grunwald-Giemsa staining, 500×). (b) Bone marrow aspirate showing hypercellular marrow with striking presence of eosinophils and eosinophil precursors with dysplastic features, abnormal nuclei and aberrant mixed granulation (May-Grunwald-Giemsa staining, 500×). (c and d) Bone marrow aspirate five months after the initial diagnosis of CEL showing increased number of myeloblasts with presence of Auer rods and mild dysplasia in the erythroid lineage (May-Grunwald-Giemsa staining, 500×).

whereas serum tryptase (9.73 $\mu\text{g/L}$ [< 11.4]) and IgE (14.23 kU/L [0–100]) levels were normal.

Bone marrow (BM) findings included a hypercellular marrow with striking presence of eosinophils and eosinophilic precursors with aberrant (mixed) granulation (Fig. 1b). Also an increased number of myeloblasts was observed (9%). Cytogenetic analysis revealed an abnormal karyotype (46,XX,t(7;15)(p22;q22) [4] /46,XX,idem,t(5;12)(q31;p11.2) [6]) with two hierarchically related abnormal rearrangements. As *PDGFRB* is located on the long arm of chromosome 5, a *PDGFRB* rearrangement was suspected and therapy with imatinib was started. However, fluorescence in situ hybridization (FISH) using break-apart probes (Leica Microsystems) excluded both *FIP1L1-PDGFRB* and *PDGFRB* rearrangements. To exclude rare *PDGFRB* translocations, we analyzed the *PDGFRB* expression level, which serves as a generic marker for the presence of *PDGFRB* gene fusions [5], but no significant overexpression was observed. In a further search for the genes involved in the t(5;12) translocation, the patient also tested negative for the presence of *ACSL6-ETV6* fusions, previously described in patients with AML with eosinophilia harboring a t(5;12) translocation [6]. We used the same primers as described in [7], so only the presence of those fusions could be excluded in this patient. Therapy with imatinib showed no effect and the patient was switched to hydroxyurea and later interferon- α in combination with corticosteroids.

Five months after the initial diagnosis of CEL-NOS, the patient was re-hospitalized, due to rapid deterioration and generalized weakness. BM aspirate confirmed eosinophilia and revealed mild dysplasia in the erythroid lineage together with an elevated number of myeloblasts (21%) with Auer rods, leading to a diagnosis of secondary AML (Fig. 1c-d). Cytogenetic analysis revealed the same abnormal karyotype. Next-generation sequencing (NGS) with an in-house developed myeloid panel of 15 genes showed a type A mutation in *NPM1* (c.860_863dup) with a high variant allele frequency (VAF) of 47%, a missense mutation in the conserved domain 1 of *TET2* (c.4075C > T) with a VAF corresponding to a heterozygous mutation in the myeloid blasts (VAF: 9%) and a *FLT3-TKD* (tyrosine kinase domain) mutation (c.2505T > A; VAF: 3%), present in a minor clone. Therapy with Decitabine was started.

The patient completed 4 cycles of Decitabine, during which the pancytopenia continued, with persistent eosinophilia (500–1000/ μL) and intermittent appearance of myeloblasts, suggesting only minor

effects from this hypomethylating therapy. Unfortunately, no formal re-evaluation could be performed as the patient passed away due to head trauma and progressive subdural hematoma.

3. Discussion

The diagnosis of primary HE relies on the combined evaluation of blood cell counts and morphology, biochemistry, BM morphology, immunophenotyping, genetic and molecular analyses. Elevated serum tryptase (30 ng/mL on average) and vitamin B12 (often > 2000 pg/mL) with normal IgE can help to distinguish it from reactive causes [7]. Morphologic and cytogenetic analyses of BM aspirate are warranted to diagnose genetically-defined eosinophilic neoplasms. Rapid identification of myeloid neoplasms with *PDGFRA* or *PDGFRB* rearrangement using both FISH and PCR is needed to timely initiate therapy with imatinib [7,8].

The diagnostic criteria of CEL-NOS are: persistent eosinophilia greater than 1500/ μL , no definitive features of other myeloid neoplasms, and the presence of either increased blasts or a non-specific clonal genetic abnormality. In this case, two hierarchically related non-specific genetic clones could be identified. The t(7;15)(p22;q22) is present in all mitoses and can therefore be a de novo balanced constitutional translocation. Due to insufficient patient material, this could not be confirmed. Translocations between chromosomes 7 and 15 are rare in cancer, but the presence of *PDGFA* on chromosome 7p22, encoding the ligand of the *PDGFRA* receptor, is intriguing. Theoretically, the translocation could increase *PDGFA* expression and lead to an activation of this signaling pathway, through an alternative mechanism as compared to the classical rearrangements of the *PDGFRA* and *PDGFRB* receptors.

It was first thought that the additional t(5;12)(q31;p11.2) led to a *PDGFRB* fusion, as this gene is located close to the breakpoint on 5q. However, this could not be confirmed, neither by FISH nor by PCR. Using PCR, we also excluded the presence of an *ACSL6-ETV6* fusion, previously described in patients with AML and eosinophilia harboring a t(5;12) translocation. Several genes encoding eosinophilopoietic cytokines (GM-CSF, IL-3, IL-4 and IL-5) also reside in this genomic region (5q31–33), which could be affected by this translocation. Previous studies showed IL-3 to be ectopically expressed in leukemic cells with a

t(5;12)(q31;p13), suggesting that the expression of IL-3 was deregulated by this translocation [9]. Interestingly, this region has been linked to familial eosinophilia [10], but the responsible genes are not yet identified. At the time of transformation to secondary AML, the sample contained a *NPM1* mutation at a high frequency, suggestive of a heterozygous mutation in all cells. Retrospective analysis of the sample from the initial CEL-NOS diagnosis also showed the presence of the *NPM1* mutation at a high level. A review of the literature could not detect a clear link between *NPM1* mutation and eosinophilia, so the role of this mutation remains puzzling, especially as *NPM1* is located distal to the breakpoint on 5q. In contrast, mutations in *TET2* are more common in CEL-NOS, although their relevance to the disease is unclear.

In general, patients with CEL-NOS have a poor prognosis. One small case series of 10 patients showed a median overall survival of 22 months with five patients transforming to acute leukemia [11]. Transformation occurred both to myeloid and lymphoblastic acute leukemia and the median time from acute transformation to death was only two months (range, 1.0–6.1) [11].

No standard of care exists for the treatment of CEL-NOS. Response to imatinib is uncommon but reported. Hydroxyurea may be useful in selected patients to control hyperleukocytosis, eosinophilia and splenomegaly. In therapy refractory patients, interferon- α has been reported to induce remission in cases with translocations with a 5q31–33 breakpoint. In some cases allogeneic hematopoietic stem cell transplantation induced long-term remission [7].

As the patient was not fit enough to undergo classical induction chemotherapy after transformation to AML, we started treatment with hypomethylating agents. To our knowledge, this is the first report of treatment with a hypomethylating agent for AML arising from CEL-NOS. Unfortunately, the patient died from head trauma four months after the start of treatment, before formal re-evaluation could be performed.

4. Conclusion

Accurate diagnosis is critical for effective management of eosinophilia and consists of peripheral blood and bone marrow examination. Rapid identification of *PDGFRA* or *PDGFRB* rearrangement using both FISH and PCR is warranted to timely initiate therapy with imatinib. CEL-NOS is a rare and aggressive disease with high rate of transformation to acute leukemia and resistance to conventional treatment. Whereas hydroxyurea may be useful to control hyperleukocytosis and splenomegaly, allogeneic stem-cell transplantation remains the only curative option, but is only available for younger and fit patients.

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Declarations of interest

None.

References

- [1] M.E. Rothenberg, Eosinophilia, *N. Engl. J. Med.* 338 (22) (1998) 1592–1600.
- [2] D.A. Arber, A. Orazi, R. Hasserjian, J. Thiele, M.J. Borowitz, M.M. Le Beau, C.D. Bloomfield, M. Cazzola, J.W. Vardiman, The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia, *Blood* 127 (20) (2016) 2391–2405.
- [3] J. Gotlib, J. Cools, Five years since the discovery of FIP1L1-PDGFR α : what we have learned about the fusion and other molecularly defined eosinophilias, *Leukemia* 22 (11) (2008) 1999–2010.
- [4] M.M. Crane, C.M. Chang, M.G. Kobayashi, P.F. Weller, Incidence of myeloproliferative hypereosinophilic syndrome in the United States and an estimate of all hypereosinophilic syndrome incidence, *J. Allergy Clin. Immunol.* 126 (1) (2010) 179–181.
- [5] P. Erben, D. Gosenca, M.C. Muller, J. Reinhard, J. Score, F. Del Valle, C. Walz, J. Mix, G. Metzgeroth, T. Ernst, C. Haferlach, N.C. Cross, A. Hochhaus, A. Reiter, Screening for diverse PDGFRA or PDGFRB fusion genes is facilitated by generic quantitative reverse transcriptase polymerase chain reaction analysis, *Haematologica* 95 (5) (2010) 738–744.
- [6] F. Yagasaki, I. Jinnai, S. Yoshida, Y. Yokoyama, A. Matsuda, S. Kusumoto, H. Kobayashi, H. Terasaki, K. Ohyashiki, N. Asou, I. Murohashi, M. Bessho, K. Hirashima, Fusion of TEL/ETV6 to a novel ACS2 in myelodysplastic syndrome and acute myelogenous leukemia with t(5;12)(q31; p13), *Genes Chromosomes Cancer* 26 (3) (1999) 192–202.
- [7] J. Gotlib, World Health Organization-defined eosinophilic disorders: 2017 update on diagnosis, risk stratification, and management, *Am. J. Hematol.* 92 (11) (2017) 1243–1259.
- [8] A. Tefferi, J. Gotlib, A. Pardanani, Hypereosinophilic syndrome and clonal eosinophilia: point-of-care diagnostic algorithm and treatment update, *Mayo Clin. Proc.* 85 (2) (2010) 158–164.
- [9] J. Cools, N. Mentens, M.D. Odero, P. Peeters, I. Wlodarska, M. Delforge, A. Hagemeijer, P. Marynen, Evidence for position effects as a variant ETV6-mediated leukemogenic mechanism in myeloid leukemias with a t(4;12)(q11-q12; p13) or t(5;12)(q31;p13), *Blood* 99 (5) (2002) 1776–1784.
- [10] J.D. Rioux, V.A. Stone, M.J. Daly, M. Cargill, T. Green, H. Nguyen, T. Nutman, P.A. Zimmerman, M.A. Tucker, T. Hudson, A.M. Goldstein, E. Lander, A.Y. Lin, Familial eosinophilia maps to the cytokine gene cluster on human chromosomal region 5q31-q33, *Am. J. Hum. Genet.* 63 (4) (1998) 1086–1094.
- [11] G. Helbig, A. Soja, A. Bartkowska-Chrobok, S. Kyrz-Krzemien, Chronic eosinophilic leukemia-not otherwise specified has a poor prognosis with unresponsiveness to conventional treatment and high risk of acute transformation, *Am. J. Hematol.* 87 (6) (2012) 643–645.