



## HYPEREOSINOPHILIC SYNDROME AND CHRONIC EOSINOPHILIC LEUKAEMIA: AN EXPERIENCE OF TERTIARY TEACHING CENTRES

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### ABSTRACT

**Introduction:** Eosinophilia has been defined as an absolute eosinophil count of more than 500 eosinophils/mm<sup>3</sup> of venous blood. In patients with marked eosinophilia (>1,500 eosinophils/mm<sup>3</sup>) which persisted for more than six months, hypereosinophilia (HE) was classified as familial, underdetermined significance, primary clonal or neoplastic and reactive. It should be classified as Hypereosinophilic Syndrome (HES) when associated with end organ damage. The majority of cases are secondary or reactive, in developing countries most commonly due to infections, especially tissue invasive parasites. Reactive eosinophilia to other tumors, allergy or collagen vascular disease needs to be excluded. The age adjusted incidence for HES is of the order of 0.036 cases/100,000 population, of which Chronic Eosinophilic Leukemia (CEL) comprises a minority of these patients. Methods and Materials: Clinical, epidemiological and hematological findings of 9 cases of Hypereosinophilic syndrome and one case of chronic eosinophilic leukemia were noted retrospectively. CBC, PBS and Bone marrow study was done in all cases. **Result:** Absolute eosinophilic count was >1500/ $\mu$ l in each case, peripheral blast was <2% in 9 cases.

One patient of eosinophilic leukemia had absolute eosinophilic count >1500/ $\mu$ l and blasts 5%. male female ratio was 4:1. Mean age was 31.6 years. In this retrospective study, all patients had detected positive for FIP1L1 PDGFRA. All responded with imatinib however higher dose was needed in chronic eosinophilic leukaemia. **Conclusion:** reactive eosinophilia was commoner disease and primary neoplastic eosinophilia was uncommon disease so early diagnosis with CBC, PBS, BONE MARROW and molecular findings are important to initiate early treatment to prevent end organ damage.

**KEYWORDS :** Chronic Eosinophilic Leukemia, Hypereosinophilic syndrome, FIP1L1PDGFRA, Imatinib

### INTRODUCTION:

In 2008, the WHO revised the classification of eosinophilia in myeloid neoplasms, creating a new category; myeloid and lymphoid neoplasms with eosinophilia and abnormalities of Platelet Derived Growth Factor Receptor Alpha (PDGFRA), Platelet Derived Growth Factor Receptor Beta (PDGFRB) or Fibroblast Growth Factor Receptor 1 (FGFR1) (1).

There are two forms of Chronic Eosinophilic Leukemia (CEL), the CEL FIP1L1-PDGFRA rearrangement positive, which is part of the new subcategory of the WHO classification and the CEL-NOS (not otherwise specified) which forms part of the myeloproliferative neoplasms of the WHO classification [6]. CEL is a chronic myeloproliferative disease with a clonal proliferation of eosinophilic precursors. This results in a persistently elevated number of eosinophils in the blood, bone marrow and peripheral tissues (2). The WHO proposed diagnostic criteria for CEL include: a persistent eosinophilia of  $\geq 1.5 \times 10^9/L$  in peripheral blood persisting for more than 6 months and increased bone marrow eosinophilia with between 5-19% of myeloblasts in bone marrow samples or >2% in the peripheral blood and clonality of the myeloid cells should be demonstrated (3). The etiology of CEL is unknown. The available evidence suggests that its incidence is highest in the fourth decade. Differing from other causes of hypereosinophilia where there is no clear gender difference, CEL NOS is more common in males with a ratio of 1.47, whereas CEL FIP1L1-PDGFRA patients are in the majority of cases male and in almost all cases there is clinically palpable splenomegaly (4).

The disease has been characterized by a chronic phase that may progress to a blast crisis. Historical case series reported a high mortality, with a 12 year survival of only 12%, the majority presenting in advanced stages and the dying of cardiac disease caused by the hypereosinophilia (5). However in the era of molecular diagnosis, earlier diagnosis, and the use of

targeted therapy and cardiovascular surgery, the survival has improved (6).

The neoplastic nature of CEL has been substantiated by a number of chromosomal abnormalities, the most frequent being trisomy 15, trisomy 8, isochromosome 17, translocations t(2;5)(p23;q31) and translocations t(5;12)(q33;p13) (7-9). At the molecular level CEL has been divided into two entities, which has important therapeutic implications.

The FIP1L1-PDGFRA rearrangement is caused by a cryptic deletion of 800kb on chromosome 4q12 and contains the CHIC2 gene (10), the incidence of this entity as part of the HES is not known, those with fusion protein rearrangements are very rare, estimated to be <1/100,000 persons with HES. The original work of Cools et al., (11) reported 56% of patients were FIP1L1-PDGFRA positive, however in those series with more than 10 patients with HES, the incidence of CEL FIP1L1-PDGFRA ranges from 3% to 56% (12). Although these studies have selection bias, the incidence of patients positive for FIP1L1-PDGFRA is probably between 10-20% of patients presenting with HES in developed countries.

Fusion of FIP1L1 to the PDGFRA protein yields a constitutive active tyrosine kinase which stimulates the proliferation and mediates survival of the eosinophils in CEL patients, through activation of several signaling pathways including phosphoinositol3-kinase, ERK 1/2 and STAT5 [13,14]. The exact mechanism, however, by which FIP1L1-PDGFRA preferentially affects eosinophils, remains unclear.

FIP1L1-PDGFRA is a clonal marker associated with the myeloproliferative variant of hypereosinophilia (15,16). These patients often present with organomegaly, a hypercellular bone marrow with increased mast cells and/or myelofibrosis and increased serum tryptase levels.

The use of low dose imatinib (100-400mg/day) produces complete and rapid hematological remissions and normalization of peripheral blood and bone marrow eosinophilia, with a median time to response of four weeks [20]. Molecular remissions, with patients becoming RT-PCR negative for FIP1L1-PDGFR $\alpha$  occurring after a median of 3 months [20,21]. However, discontinuation of imatinib can rapidly lead to relapse (17-19), with molecular remission being achieved following its reintroduction. A single weekly dose of 100-200mg of imatinib may be sufficient to maintain remission [18].

Acquired resistance to imatinib is much less frequent in patients with CEL FIP1L1-PDGFR $\alpha$  than that observed in CML patients. It is associated with the T674I mutation within the ATP binding domain of PDGFR $\alpha$  and may be seen in patients developing a blast crisis. The T674I confers broad spectrum resistance to tyrosine kinase inhibitors nilotinib, sorafenib (20), and dasatinib (20), ponatinib a pan-FIP1L1-PDGFR $\alpha$  inhibitor has shown *in vitro* activity in cells expressing the T674I mutation (21). CEL-NOS is operationally defined by; an absolute peripheral eosinophilia count of  $>1.5 \times 10^9/L$ ; there is no philadelphia chromosome or BCR-ABL fusion gene detectable, is not part of another myeloproliferative neoplasms (polycythemia vera, essential thrombocythosis; myelofibrosis or systemic mastocytosis) or a myelodysplasia/myeloproliferation syndrome (CMML or atypical CML); there is no t(5;12) or other rearrangement of PDGFR $\beta$ ; there is no FIP1L1-PDGFR $\alpha$  fusion gene or other rearrangement of PDGFR $\alpha$ ; there is no rearrangement of FGFR1 and finally the blast cell count in the peripheral blood is less than 2% and in the bone marrow bone marrow is between 5% and 20% and there is no inv(16)(p13;q22) or t(16;16)(p13;q22) or other feature diagnostic of AML (10). In cases of CEL where the RT-PCR for FIP1L1-PDGFR $\alpha$  is negative or unknown, the

prognosis is poor, being unresponsive or short lived to imatinib (19), conventional therapy [26] and has a high rate of acute transformation (20). However, it has been reported that approximately 40% of patients who response to imatinib are negative for the FIP1L1-PDGFR $\alpha$  fusion protein, although doses as high as 600mg/day may not be effective (18-21). The complete response rate has been reported as 24% compared with 98% of cases which were FIP1L1-PDGFR $\alpha$  positive [(19). However, hematological responses are often partial and short lived (20). However, high doses of imatinib may not be tolerated, with grade 3 or 4 leukopenia, neutropenia, lymphopenia, thrombocytopenia and anemia being reported in between 3 and 8% (21). Fluid retention may require the use of diuretics and nausea and diarrhea may be sufficient to withhold therapy in 4% of patients [28].

Nevertheless the prognosis of CEL-NOS is poor in a cohort of 10 patients; the median survival was 22.2 months, with acute transformation occurring in 5 of the 10 patients (20).

#### METHODS AND MATERIALS:

Clinical, epidemiological and hematological findings of 9 cases of Hypereosinophilic syndrome and one case of chronic eosinophilic leukemia were noted retrospectively. CBC, PBS and Bone marrow study was done in all cases. Echocardiography, Chest X-ray and HRCT were done to know evidence of fibrosis. Absolute eosinophilic count, blasts%, and bone marrow blasts and eosinophilic count were also done. There were divided into Hypereosinophilic syndrome and chronic eosinophilic leukemia on the basis of blasts percentage in peripheral blood and bone marrow. Fluorescence *in situ* hybridization (FISH) and/or molecular genetic methods for detecting BCR-ABL1, PDGFR $\alpha$ , PDGFR $\beta$ , and FGFR1 were performed at the NABL accredited laboratories as outsourced sample.

#### RESULT:

**Table .1 Hypereosinophilic syndrome and chronic eosinophilic leukemia patients**

| CASE NO | AGE | GENDER | TLC | AEC           | BLASTS | BM | MOLECULAR        | DIAGNOSIS |
|---------|-----|--------|-----|---------------|--------|----|------------------|-----------|
| 1       | 55  | M      | 20  | $>1500/\mu l$ | $<2\%$ |    | PDGFR $\alpha$ + | HES       |
| 2       | 35  | M      | 12  | $>1500/\mu l$ | $<2\%$ |    | PDGFR $\alpha$ + | HES       |
| 3       | 20  | M      | 30  | $>1500/\mu l$ | $<2\%$ |    | PDGFR $\alpha$ + | HES       |
| 4       | 30  | F      | 25  | $>1500/\mu l$ | $<2\%$ |    | PDGFR $\alpha$ + | HES       |
| 5       | 18  | M      | 25  | $>1500/\mu l$ | $<2\%$ |    | PDGFR $\alpha$ + | HES       |
| 6       | 20  | F      | 18  | $>1500/\mu l$ | $<2\%$ |    | PDGFR $\alpha$ + | HES       |
| 7       | 38  | M      | 22  | $>1500/\mu l$ | $<2\%$ |    | PDGFR $\alpha$ + | HES       |
| 8       | 19  | M      | 20  | $>1500/\mu l$ | $<2\%$ |    | PDGFR $\alpha$ + | HES       |
| 9       | 45  | M      | 22  | $>1500/\mu l$ | $<2\%$ |    | PDGFR $\alpha$ + | HES       |
| 10      | 36  | M      | 19  | $>1500/\mu l$ | 5%     | 8% | PDGFR $\alpha$ + | CEL       |

Absolute eosinophilic count was  $>1500/\mu l$  in each case, peripheral blast was  $<2\%$  in 9 cases (Table 1). BM was assessed for the following morphological parameters : cellularity, megakaryocyte numbers, morphology and distribution; fibrosis; dysgranulopoiesis; dyserythropoiesis; myeloid:erythroid (M:E) ratio; and eosinophil morphology. PB smears were also reviewed for eosinophil morphology and evidence of dysgranulopoiesis. Hypercellularity was defined by a cellularity at least 20% higher than the age-appropriate cellularity, and overall  $\geq 70\%$  in patients age 50–60 years;  $\geq 60\%$  in patients  $>60$  years; and  $\geq 90\%$  in patients  $<30$  years of age. Megakaryocyte morphology was recorded as predominantly MDS-like (small with hypolobated/non-lobated nuclei or separated nuclear lobes), MPN-like (medium-sized to large megakaryocytes with hyperlobulated, hyperchromatic, or bulbous nuclei, often with clustering and increase in numbers), mixed MDS and MPN-like, or within normal limits (WNL). In order to define dysgranulopoiesis and dyserythropoiesis strictly, the features had to be seen in  $\geq 20\%$  of cells of the assessed lineage. Myelofibrosis grade was assessed according to the European Bone Marrow Fibrosis Consensus criteria. For eosinophil morphology, abnormal

features were markedly abnormal granulation (hypogranulation or uneven granulation), cytoplasmic vacuoles, abnormal nuclear lobation (non-lobated or multilobated), unusually large size or markedly increased immature forms.

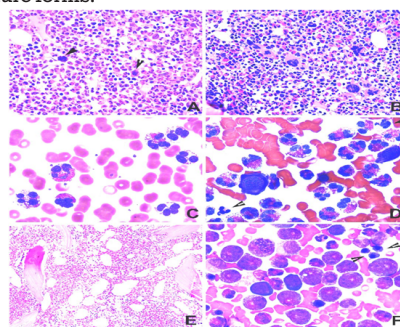


**FIGURE1.**

Bone marrow (BM) cellularity is either normal [(A) patient aged 40 years] or only mildly increased [(B) patient aged 50 years], with increased BM eosinophils and normal-

appearing megakaryocytes. (C) Eosinophils in peripheral blood (PB) may show mild uneven granulation (D) but are unremarkable on BM smear. No dysgranulopoiesis or dyserythropoiesis (BM biopsy, hematoxylin & eosin,  $\times 400$ ; PB and BM smears Leishmans  $\times 1000$ ).

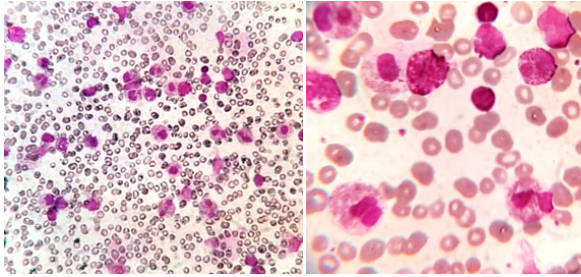


**FIGURE 2.**

Peripheral blood (PB) 10x shows abnormal eosinophils with multiple lobes and marked hypo granulation or agranulation in few and same in 100x reveals two myeloblasts (CEL)

Fluorescence *in situ* hybridization (FISH) and/or molecular genetic methods for detecting *BCR-ABL1* and *PDGFRA* were done in NABL accredited laboratories. 8 of total 10 patients revealed positivity for

**PDGFRA.** All patients were negative for *BCR:ABL* translocation.

## DISCUSSION:

Hypereosinophilia has generally been defined as a peripheral blood eosinophils count greater than  $1500/\text{mm}^3$  and may be associated with tissue damage(2). After exclusion of secondary causes of eosinophilia, diagnostic evaluation of primary eosinophilia relies on a combination of morphologic review of the blood and marrow, standard cytogenetics, fluorescent *in situ*-hybridization, flowcytometry, and T-cell clonality assessment to detect histopathologic or clonal evidence for an acute or chronic myeloid or lymphoproliferative disorder(3-7).

Disease prognosis relies on identifying the subtype of eosinophilia. After evaluation of secondary causes of eosinophilia, the 2016 World Health Organization endorses a semi-molecular classification scheme of disease subtypes which includes the major category "myeloid/lymphoid neoplasms with eosinophilia and rearrangement of *PDGFRA*, *PDGFRB*, or *FGFR1* or with *PCM1-JAK2*," and the "MPN subtype, chronic eosinophilic leukemia, not otherwise specified" (CEL, NOS). Lymphocyte-variant hypereosinophilia is an aberrant T-cell clone-driven reactive eosinophilia, and idiopathic hypereosinophilic syndrome (HES) is a diagnosis of exclusion.

The goal of therapy is to mitigate eosinophil-mediated organ damage. For patients with milder forms of eosinophilia (e.g.,  $< 1500/\text{mm}^3$ ) without symptoms or signs of organ involvement, a watch and wait approach with close-follow-up may be undertaken (10-13). Identification of rearranged *PDGFRA* or *PDGFRB* is critical because of the exquisite responsiveness of these diseases to imatinib. Corticosteroids are first-line therapy for patients with lymphocyte-variant hypereosinophilia and HES. Hydroxyurea and interferon-alpha have demonstrated efficacy as initial treatment and steroid-refractory cases of HES. In addition to hydroxyurea, second line cytotoxic chemotherapy agents and hematopoietic cell transplant have been used for aggressive forms of HES and CEL with outcomes reported for limited numbers of patients. The use of antibodies against interleukin-5 (IL-5) (mepolizumab), the IL-5 receptor (benralizumab), and CD52 (alemtuzumab), as well as other targets on eosinophils remains an active area of investigation (20,21).

## CONCLUSION:

In patients with eosinophilia, the presence of the *FIP1L1-PDGFR* mutation is an indication for low dose imatinib and implies an excellent prognosis, though long term therapy is required. Those complying with the diagnostic criteria of CEL but are *FIP1L1-PDGFR* negative, may warrant a trial of higher dose imatinib. These are rare entities, especially CEL. Late diagnosis of these patients has risk of mortality due to organ fibrosis, especially affecting lung and heart. Thus early diagnosis and treatment with imatinib and in some cases with steroid was life saving, curative and at least progression free survival in majority. Bone Marrow Morphology Is A Strong Discriminator Between Chronic Eosinophilic Leukemia, Not Otherwise Specified And Reactive Idiopathic Hypereosinophilic Syndrome.

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