LETTERS TO THE EDITOR
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Eosinophilia with *STAT5B*N642H Mutation: A Heterogeneous Entity with Overlapping Morphological Features and Poor Outcome

Shashidhar V. et al.: *STAT5B N642H* Positive Eosinophilia

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Dear Editor,

Loss of function *STAT5B* mutations can lead to immunodeficiency and autoimmune diseases, and activating mutations are associated with large granular lymphocytic leukemia (LGL), T-cell lymphomas, rarely myeloid malignancies[1–6]; and are potential candidates for targeted therapeutic intervention[6]. We report three cases of eosinophilia with *STAT5B*N642H mutation that underscore several critical insights into this category (Table 1 and supplementary figure S1).

Case-1: 63-year-old female presented with transfusion-dependent anemia, generalized-itching, and hepatosplenomegaly for five months. Investigations revealed anemia, leukocytosis, eosinophilia (absolute eosinophil count/AEC 4.7x10^9/L), thrombocytopenia, and a leucoerythroblastic picture in the peripheral blood film(PBF). Bone marrow examination(BME) showed eosinophil hyperplasia and patchy myelofibrosis. Flow-cytometry(FCM) identified clonal T-cells (3.8%) with CD7dim, CD5dim, CD8pos immunophenotype, confirmed by T-cell receptor beta gene rearrangement assay. FISH testing using eosinophilia-panel showed no rearrangement, leading to a diagnosis of a lymphocytic variant of hypereosinophilic syndrome(L-HES). The patient did not respond to steroids or imatinib. A repeat BME after five months showed diffuse myelofibrosis. NGS revealed a *STAT5B*N642H mutation(variant allele frequency/VAF-24%). The diagnosis was revised to chronic eosinophilic syndrome (CEL) with *STAT5B*N642H mutation. The patient had chronic refractory cytopenias and succumbed to pneumonia two years after diagnosis.

Case-2: 13-year-old male presented with cytopenias requiring transfusions, cervical lymphadenopathy, and hepatosplenomegaly. Investigations showed anemia, thrombocytopenia, normal leukocyte count, and mild eosinophilia(AEC 0.9x10^9/L). BME revealed 21% eosinophils, suboptimal erythroid response(10%), and mild myelodysplasia. Cytogenetic testing showed 7q deletion and trisomy 8 in 60% of cells, but no tyrosine-kinase-domain rearrangements on FISH testing for the eosinophilia panel. Diagnosed with myelodysplastic syndrome (MDS), he was treated with azacytidine, eltrombopag, and steroids without response. A repeat BME showed extensive myelofibrosis, mastocytosis, and dysplastic megakaryocytes. NGS revealed a *STAT5B*N642H mutation(VAF-45%). The patient had a refractory course and succumbed to infection-related complications after 14 months.

Case-3: 25-year-old female presented with fatigue and massive splenomegaly. The hemogram showed anemia, leukocytosis, eosinophilia(AEC 63x10^9/L), normal platelet counts, and a leucoerythroblastic picture resembling chronic myeloid leukemia-chronic phase(CML-CP). BME revealed hypercellular marrow with eosinophilia but no dysplasia or myelofibrosis. FISH and molecular studies were negative for *BCR::ABL1, JAK2V617F, CALR* mutations, and other tyrosine kinase fusions. There was no response to imatinib. NGS

revealed a S*TAT5B*N642H mutation(VAF 81%). She was lost to follow-up and expired within a year of diagnosis(cause not known).

Our patients' ages ranged from 13 to 63 years. Although the disease is more commonly reported in the elderly with a median age of 70-74 years[3–5], it has also been documented in the pediatric population[4]. While *STAT5B*N642H mutations are more frequently reported in males[3,4], two of our patients were females, similar to the slight female preponderance in one of the series[5].

All our patients exhibited refractory anemia, with or without accompanying thrombocytopenia, and moderate to severe splenomegaly. The eosinophilia varied(0.9 to 63x10^9/L), suggesting that hypereosinophilia (defined as >1.5x10^9/L) is not a definitive requirement to suspect this condition. Mild myelodysplasia was present in only one case(case 2), and in two cases, developed myelofibrosis(cases 1 and 2), which steadily progressed during the disease course. The second case morphologically and cytogenetically(with 7q deletion and trisomy 8) resembled MDS/MPN, and the third case morphologically resembled CML-CP. This suggests an overlapping spectrum of *STAT5B*N642H positive myeloid neoplasms with MDS, MDS/MPN, and MPNs [3].

The first case in our series also had clonal T cells, leading to a misdiagnosis of L-HES. While *STAT5B* mutations are described in L-HES, a high VAF of mutation(24% in case 1), which is disproportionate to the proportion of T cells, lack of response to steroids, and presence of fibrosis, should raise suspicion of myeloid lineage involvement. The immunophenotype of clonal T cells in L-HES[7] also resembles other T cell non-Hodgkin lymphomas (NHLs) like AITL. Therefore, it is crucial to rule out significant hematological disorders like lymphomas or co-existing myeloid neoplasms before diagnosing L-HES. The documentation of non-canonical mutations like *STAT5B* T628S and its restriction to the lymphocyte compartment will also help to diagnose L-HES[5]. While the VAF of *STAT5B* mutations can range from 1-80%, most cases of eosinophilia show VAF >40%, indicating its potential role as a driver mutation.

In our experience, all patients had a refractory course and died within two years of diagnosis without any response to steroids or imatinib, like most of the previous reports. This underscores the importance of diagnosing this condition and the need for a consensus guideline to treat these patients. Though occasional reports have shown the efficacy of JAK inhibitors in patients with gain of function mutations in *STAT5B* [8], its utility in *STAT5B* mutated eosinophilia needs to be clarified. Other agents that need further evaluation in *STAT5B*-mutated eosinophilia with organ dysfunction include anti-IL-5 pathway antagonists like mepolizumab, benralizumab, reslizumab, depemokimab, and newer therapeutics like dexpramipexole and lirentilimab, as they have been found effective in eosinophil-mediated conditions like asthma, esophagitis, and gastroenteritis [9,10]. Considering that there is no globally accepted list of genes tested in myeloid mutation panels, it is essential to ensure the testing of this gene, especially in patients with myeloid neoplasm, irrespective of the morphological diagnosis of AML, MDS, MDS/MPN, or MPN, but having eosinophilia.

Keywords: Hypereosinophilia; *STAT5B*N642H; Myelofibrosis, Myelodysplastic syndrome, Myeloproliferative neoplasm

Contributor's statement –

SS designed the study and obtained funding. SS and VS wrote the manuscript. AB performed next-generation sequencing. AK, PM, JA, and RD were involved in patient care. All authors agree to the content of the manuscript and its interpretations.

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Table 1: Summary of clinical and laboratory findings in patients with *STAT5B* mutation.

Parameter	**Case 1**	**Case 2**[11]	**Case 3**
Age/ sex	63/Female	13 years/Male	25/Female
Clinical presentation	Deep vein thrombosis, transfusion-dependent anemia, generalized itching, hepatosplenomegaly – 5 months	Cytopenias requiring transfusions and hepatosplenomegaly	Fatigue and abdominal discomfort due to massive splenomegaly
Haemoglobin, leukocyte count, platelet count	4.5 g/dl, 33.8x10^9/L, 53x10^9/L	5.1 g/dl, 4.9x10^9/L, 100x10^9/L	7.3 g/dl, 131.4x10^9/L, 176x10^9/L
Absolute eosinophil count	4732/µl	910/µl	63072/µl
Other investigations	Biochemistry: normal; Autoimmune workup: Normal	Biochemistry: elevated serum B12 (1225pg/ml); Autoimmune workup: Normal	Biochemistry: elevated uric acid (9.9 mg/dl)
Peripheral blood	Leucoerythroblastic picture, eosinophilia	Eosinophilia (14-26%)	Leukoerythroblastic picture, eosinophilia, basophilia (3%) - CML-like picture
Bone marrow findings	Hypercellular bone marrow with patchy fibrosis at diagnosis and diffuse fibrosis at follow-up. (Figure S1)	Eosinophilia, sub-optimal erythroid response (10%), and mild myelodysplasia (5% hypogranulated myelocytes and monolobated eosinophils) abd diffuse myelofibrosis at follow-up	Hypercellular bone marrow with eosinophilia.

Flow cytometry	3.8% abnormal T cells (CD7dim, CD5dim, CD8pos)	Normal	Not done
T Cell receptor rearrangement assay/FISH	Clonal TCR beta rearrangement/Normal FISH (eosinophilia panel)	Polyclonal TCR gamma rearrangement FISH: 7q deletion and Trisomy 8 in 60% cells. Normal FISH (eosinophilia panel)	Normal FISH (eosinophilia panel)
Initial diagnosis	Lymphocytic variant of hypereosinophilic syndrome (L-HES)	MDS with eosinophilia	Chronic Myeloid Leukemia with eosinophilia by morphology
Treatment	No response to steroid (1mg/kg), imatinib (400mg)	No response to azacytidine, eltrombopag, Imatinib, or steroids	No response to steroid, Imatinib
Next generation sequencing (44 gene panel)	*STAT5B* (N642H) VAF of 24%	*STAT5B* (N642H) VAF of 45%	*STAT5B* (N642H) VAF of 81%.
Follow up	Dead at 26 months	Dead at 14 months	Dead at 12 months
Revised diagnosis	Chronic eosinophilic syndrome (CEL) with *STAT5B*N642H mutation	Myeloid neoplasm or Chronic eosinophilic syndrome (CEL) with *STAT5B*N642H mutation	CEL with with *STAT5B*N642H mutation
Learning points	**The case highlights the co-existence of clonal T cells in *STAT5B* (N642H) positive chronic eosinophilic leukemia.**	***STAT5B* (N642H) positive CEL can resemble MDS/MPN**	***STAT5B* (N642H) positive CEL can mimic CML and can have massive splenomegaly**
Eosinophilia panel tested in fluorescence in-situ hybridization includes *FIP1L1::PDGFA* (tri-color probe), *PDGFRB*, *FGFR1, JAK2, ABL1,* and *ETV6* (break-apart probes; Metasystems, Germany). Clonality testing was performed using kit from Invivoscribe,USA. Case 2 was previously published[11]			