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

*STAT5B*N642H Mutasyonu ile Birlikte Eozinofili: Çakışan Morfolojik Özelliklere ve Kötü Prognoza Sahip Heterojen Bir Durum

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To the Editor,

Loss-of-function *STAT5B* mutations can lead to immunodeficiency and autoimmune diseases, while activating mutations are associated with large granular lymphocytic leukemia, T-cell lymphomas, and, more rarely, myeloid malignancies [1,2,3,4,5,6]. These mutations are potential candidates for targeted therapeutic intervention [6]. We report three cases of eosinophilia with the *STAT5B*N642H mutation that underscore several critical insights into this mutation category (Table 1; Figure 1).

Case 1 involved a 63-year-old woman who presented with transfusion-dependent anemia, generalized pruritus, and hepatosplenomegaly for 5 months. Investigations revealed anemia, leukocytosis, eosinophilia with an absolute eosinophil count (AEC) of $4.7x10^9$/L, thrombocytopenia, and a leukoerythroblastic picture in the peripheral blood film. Bone marrow examination (BME) revealed eosinophil hyperplasia and patchy myelofibrosis. Flow cytometry identified clonal T-cells (3.8%) with the $CD7^{dim}CD5^{dim}CD8^{+}$ immunophenotype, confirmed by T-cell receptor beta gene rearrangement assay. Fluorescence in situ hybridization (FISH) testing using an eosinophilia panel showed no rearrangements, leading to the diagnosis of a lymphocytic variant of hypereosinophilic syndrome (L-HES). The patient did not respond to steroids or imatinib. Five months later, BME showed diffuse myelofibrosis. Next-generation sequencing revealed a *STAT5B*N642H mutation with variant allele frequency (VAF) of 24%. The diagnosis was subsequently revised to chronic eosinophilic syndrome with *STAT5B*N642H mutation. The patient had chronic refractory cytopenia and died of pneumonia 2 years after the diagnosis.

Case 2 involved a 13-year-old boy who presented with cytopenia requiring transfusions, cervical lymphadenopathy, and hepatosplenomegaly. Investigations showed anemia, thrombocytopenia, normal leukocyte count, and mild eosinophilia with AEC of $0.9x10^9$/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 were observed by FISH testing for the eosinophilia panel. Diagnosed with myelodysplastic syndrome (MDS), this patient was treated with azacytidine, eltrombopag, and steroids without response. A subsequent BME showed extensive myelofibrosis, mastocytosis, and dysplastic megakaryocytes. Next-generation sequencing revealed the *STAT5B*N642H mutation (VAF: 45%). The patient had a refractory course and died of infection-related complications after 14 months.

Case 3 involved a 25-year-old woman who presented with fatigue and massive splenomegaly. Her complete blood count showed anemia, leukocytosis, eosinophilia (AEC: $63x10^9$/L), normal platelet count, and a leukoerythroblastic picture resembling chronic myeloid leukemia in the chronic phase (CML-CP). BME revealed hypercellular marrow with eosinophilia but no dysplasia or myelofibrosis. FISH and molecular studies were negative for *BCR::ABL1*, *JAK2*V617F, and *CALR* mutations and other tyrosine kinase fusions. There was no response to imatinib. Next-generation sequencing revealed the S*TAT5B*N642H mutation (VAF: 81%). She was lost to follow-up and died within a year of the diagnosis with the cause of death unknown.

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

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

Parameter	Case 1	Case 2 [11]	Case 3
Age (years) and sex	63/Female	13/Male	25/Female
Clinical presentation	Deep vein thrombosis, transfusion-dependent anemia, generalized pruritus, and hepatosplenomegaly for 5 months	Cytopenia requiring transfusions and hepatosplenomegaly	Fatigue and abdominal discomfort due to massive splenomegaly
Hemoglobin, 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 (1225 pg/mL); Autoimmune workup: normal	Biochemistry: elevated uric acid (9.9 mg/dL)
Peripheral blood	Leukoerythroblastic 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 1)	Eosinophilia, suboptimal erythroid response (10%), and mild myelodysplasia (5% hypogranulated myelocytes and monolobated eosinophils) with diffuse myelofibrosis at follow-up	Hypercellular bone marrow with eosinophilia
Flow cytometry	3.8% abnormal T-cells ($CD7^{dim}$, $CD5^{dim}$, $CD8^+$)	Normal	Not done
T-cell receptor rearrangement assay/FISH	Clonal T-cell receptor beta rearrangement/normal FISH (eosinophilia panel)	Polyclonal T-cell receptor gamma rearrangement FISH: 7q deletion and trisomy 8 in 60% cells Normal FISH (eosinophilia panel)	Normal FISH (eosinophilia panel)
Initial diagnosis	L-HES	MDS with eosinophilia	CML with eosinophilia by morphology
Treatment	No response to steroids (1 mg/kg), imatinib (400 mg)	No response to azacytidine, eltrombopag, imatinib, or steroids	No response to steroids, 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	CEL with *STAT5B*N642H mutation	Myeloid neoplasm or CEL with *STAT5B*N642H mutation	CEL with *STAT5B*N642H mutation
Learning points	This 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 may present with massive splenomegaly

Eosinophilia panel tested in FISH included *FIP1L1::PDGFA* (tri-color probe), *PDGFRB*, *FGFR1*, *JAK2*, *ABL1*, and *ETV6* (break-apart probes; Metasystems, Altlussheim, Germany). Clonality testing was performed using a kit from Invivoscribe (San Diego, CA, USA). Case 2 was previously published [11]. CML: Chronic myeloid leukemia; FISH: fluorescence in situ hybridization; L-HES: lymphocytic variant of hypereosinophilic syndrome; MDS: myelodysplastic syndrome; VAF: variant allele frequency; CEL: chronic eosinophilic syndrome; MPN: myeloproliferative neoplasm.

All of our patients had refractory anemia, with or without accompanying thrombocytopenia, and moderate to severe splenomegaly. Eosinophilia varied from 0.9x10^9/L to 63x10^9/L, suggesting that hypereosinophilia, defined as >1.5x10^9/L, is not a definitive requirement for suspecting this condition. Mild myelodysplasia was present in only one case (case 2), and two of the patients developed myelofibrosis (cases 1 and 2), which steadily progressed during the course of the disease.

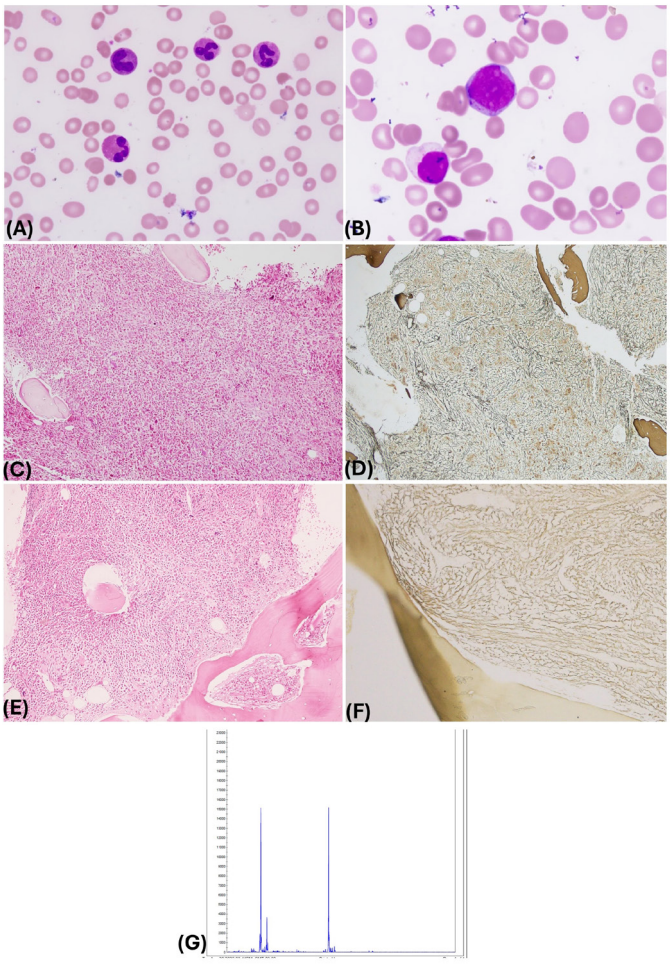

Figure 1. Representative images from the first case of our series. A, B) Peripheral blood film showed eosinophilia (40^{x}) and occasional blasts (100^{x}) (May-Grunwald-Giemsa). C) Bone marrow biopsy showed hyperplasia of eosinophils and precursors, with a few megakaryocytes with hyperchromatic nucleus and D) patchy myelofibrosis (reticulin stain). E, F) Bone marrow biopsy performed during follow-up showed diffuse myelofibrosis (H&E and reticulin stain, respectively; 20^{x}). G) T-cell receptor beta gene rearrangement assay showed monoclonality.

The second case resembled MDS/myeloproliferative neoplasms (MPNs) overlap morphologically and cytogenetically (with 7q deletion and trisomy 8), while the third case morphologically resembled CML-CP. This suggests a spectrum of overlap between *STAT5B*N642H-positive myeloid neoplasms and MDS, MDS/MPN, and MPNs [3].

The first patient in our series also had clonal T-cells, leading to an initial misdiagnosis of L-HES. While *STAT5B* mutations are described in cases of L-HES, a high VAF for the mutation (24% in case 1) disproportionate to the proportion of T-cells, a lack of response to steroids, and the 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 such as angioimmunoblastic T-cell lymphoma. 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 in diagnosing L-HES [5]. While the VAF of *STAT5B* mutations can range from 1% to 80%, most cases of eosinophilia show VAF of >40%, indicating their potential roles as driver mutations.

The patients presented here all had a refractory course and died within 2 years of diagnosis without any response to steroids or imatinib, similar to most previous reports. This underscores the importance of diagnosing this condition and the need for consensus guidelines to treat these patients. Though occasional reports have shown the efficacy of Janus kinase inhibitors in patients with gain-of-function mutations in *STAT5B* [8], their utility in *STAT5B*-mutated eosinophilia needs to be clarified. Other agents that need further evaluation in *STAT5B*-mutated eosinophilia with organ dysfunction include anti-interleukin-5 pathway antagonists like mepolizumab, benralizumab, reslizumab, and depemokimab and newer therapeutics like dexpramipexole and lirentelimab, as they have been found effective in eosinophil-mediated conditions such as 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 *STAT5B*, especially in patients with myeloid neoplasms, irrespective of a morphological diagnosis of acute myeloid leukemia, MDS, MDS/MPN, or MPN in the presence of eosinophilia.

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

Anahtar Sözcükler: Hipereozinofili, *STAT5B*N642H, Miyelofibrozis, Miyelodisplastik sendrom, Miyeloproliferatif neoplazi

Ethics

Informed Consent: Written informed consent was obtained from all patients in this study.

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Footnotes

Authorship Contributions

Surgical and Medical Practices: V.S., J.A., R.D., P.M., S.S.; Concept: V.S., S.S.; Design: S.S.; Data Collection or Processing: V.S., A.K., A.B., S.R., J.A., R.D., P.M., S.S.; Analysis or Interpretation: V.S.,

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