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CSF3R T618I mutated chronic myelomonocytic leukemia: A proliferative subtype with a distinct mutational profile

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ABSTRACT

Chronic myelomonocytic leukemia (CMML) is a clonal myeloid neoplasm characterized by sustained monocytosis and mutations in *TET2, ASXL1, SRSF2, SETBP1, NRAS*, and *KRAS*. We describe a rare case of *CSF3R* T618I mutated CMML that has a proliferative phenotype, myelodysplasia, and additional mutations in *ASXL1, SETBP1, KRAS,* and *PTPN11*. Comparing the clinicopathologic features of this case to previously reported cases of *CSF3R* T618I mutated CMML and *CSF3R* non-T618I mutated CMML, *CSF3R* T618I seems to define a unique proliferative subtype of CMML with a distinct mutational profile. The diagnostic challenges and molecular pathogenesis associated with this case are also briefly discussed.

1. Introduction

Chronic myelomonocytic leukemia (CMML) is a clonal myeloid neoplasm characterized by persistent absolute ($>1 \times 10^9$/L) and relative (>10% of leukocytes) monocytosis in peripheral blood (PB), the presence of myelodysplasia or acquired clonal genetic abnormalities, and no morphologic or cytogenetic/genetic features suggestive of another myeloid malignancy [1, 2]. The neoplasm is subdivided into a dysplastic subtype with white blood cell count (WBC) $<13 \times 10^9$/L and a proliferative subtype with WBC $>13 \times 10^9$/L. Patients with a dysplastic subtype are often cytopenic and thus more likely to present with symptoms such as bleeding, infection, and fatigue, whereas patients with a proliferative subtype more often present with hepatosplenomegaly, fever, and weight loss. The typical mutational profile for CMML involves *TET2, ASXL1, SRSF2, SETBP1,* and genes in the RAS signaling pathway [1, 2].

Colony-stimulating factor 3 receptor gene (*CSF3R*) mutation is rare in CMML and is more often associated with chronic neutrophilic leukemia (CNL), a neoplasm characterized by sustained neutrophilia and hypercellular bone marrow with a predominance of neutrophilic granulocyte forms [3]. *CSF3R* mutation is detected in ~80–90% of CNL cases (90% of mutated cases with *CSF3R* T618I mutation) and is highly sensitive and characteristic of CNL [4, 5]. It is also detected in ~30% of atypical chronic myeloid leukemia cases (aCML, ~60% of mutated cases with *CSF3R* T618I mutation) [6–8].

We describe a rare case of *CSF3R* T618I mutated CMML and compare the clinicopathologic features of this case with previously reported *CSF3R* T618I mutated CMML and *CSF3R* non-T618I mutated CMML. The diagnostic challenges and molecular pathogenesis associated with this case are also briefly discussed.

2. Case report

A 27-year-old woman was incidentally noted to have leukocytosis and macrocytic anemia during her pregnancy. She later presented for sustained leukocytosis, monocytosis for about one year, macrocytic anemia, and hepatosplenomegaly. There was no family history of cancer or personal evidence of a syndrome. PB, bone marrow (BM) aspirate, and core biopsy were submitted for morphology, flow cytometric immunophenotyping, cytogenetics, and molecular studies.

The PB smear (Fig. 1A) demonstrated granulocytic leukocytosis (WBC 35×10^9/L) with left-shifted neutrophils, including myelocytes and metamyelocytes [5%, green arrow; mature neutrophils (segmented/banded) 62%] and occasional dysplastic neutrophils, markedly relative and absolute monocytosis (29%, 7×10^9/L, blue arrow), severe anemia (hemoglobin 4.3 g/dL) with occasional coarse basophilic stippling

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(orange arrow), and mild thrombocytopenia (platelets 146 × 10^9/L). BM aspirate smear demonstrated left-shifted granulopoiesis with 3% myeloblasts (Fig. 1B, red arrow), scattered atypical/dysplastic neutrophils, increased monocytic cells (10%, blue arrow), dysplastic erythroids with ring sideroblasts (10% of erythroids, Fig. 1C, orange arrow) and nuclear irregularity (Fig. 1D, orange arrow), increased myeloid: erythroid ratio (7.9:1), and dysplastic hypolobated and small megakaryocytes (Fig. 1E, violet arrow). Core biopsy and clot sections (Fig. 1F) showed a hypercellular BM (cellularity ~100%) with increased left-shifted granulocytic and monocytic cells and occasional hypolobated megakaryocytes.

Flow cytometric analysis on the BM aspirate (Fig. 2) was performed on 10-color BD FACSCanto flow cytometer (Becton Dickinson, San Jose, CA) and analyzed using cluster analysis with Cytopaint Classic Software (Leukobyte, CA). Analysis revealed a 0.88% population of CD34 (+)/CD117(+) myeloblasts (in red) with immunophenotypic aberration [CD56(+), CD5(dim+), CD33(dim+), CD11b (few +)], abnormal maturation pattern in neutrophils (in green) on the CD11b/CD13 plot, and increased immunophenotypically aberrant monocytes (in blue, 11% with a nearly uniform expression of CD56) and a high fraction of CD14 (+)/CD16(-) classical monocytes (estimated at 96% of total monocytes).

The cytogenetic study revealed a normal female karyotype. Next Generation Sequencing (NGS) by Foundation Heme panel revealed mutations in *CSF3R* T618I [VAF (variant allele frequency) 11.2%)], *KRAS* G12D (5.8%), *ASXL1* S1014fs*10 (48.2%), *BCORL1* R1149W (51.5%), *PTPN11* E76Q (2.0%), *SETBP1* D868N (3.8%), and *SETBP1* G870S (5.1%); there was no evidence of mutations in *JAK2, MPL, CARL, TET2,* or *SRSF2*, or fusion of *BCR-ABL1*. *PTPN11* E76Q has been reported in hematologic malignancies [9] and is likely a somatic mutation given its low VAF (2.0%). Overall, there are no-known risk factors for inherited predisposition syndromes.

The constellation of morphologic and immunophenotypic findings in conjunction with molecular abnormalities led to a diagnosis of CMML-1 with an unusual *CSF3R* T618I mutation. The follow-up clinical information regarding treatment and prognosis is not available.

3. Discussion

The presence of the *CSF3R* T618I mutation along with leukocytosis, sustained monocytosis, and myelodysplasia in our case raises differential diagnoses that include CNL, aCML, and CMML. Key diagnostic criteria for CNL include peripheral leukocytosis (WBC count >25 × 10^9/L) with a neutrophilic predominance (>80% segmented/banded neutrophils), no dysgranulopoiesis or monocytosis (<1 × 10^9/L monocytes in PB), and either presence of an activating *CSF3R* mutation (most commonly T618I) or persistent (>3 months) neutrophilia and splenomegaly with no other identifiable causes. Key diagnostic criteria for aCML include peripheral leukocytosis (WBC count >13 × 10^9/L) with neutrophilic precursors accounting for >10% of the WBC, dysplastic neutrophils, hypercellular BM with granulocytic/neutrophilic proliferation and dysplasia, and no or minimal PB basophilia and monocytosis (basophils <2% of WBC, monocytes <10% of WBC).

In our case, the findings of sustained absolute and relative monocytosis, multilineage dysplasia, and lack of neutrophilic predominance in PB (<80% segmented/banded neutrophils) argue against CNL despite the *CSF3R* T618I mutation. Additionally, while both aCML and CMML are classified as myelodysplastic/myeloproliferative neoplasms and share many overlapping morphologic and genetic features, including myelodysplasia and recurrent mutations in *KRAS, ASXL1,* and *SETBP1* (as seen in our case) [10], significant relative and absolute monocytosis with immunophenotypically aberrant monocytes (uniform expression of CD56) and a high fraction of classical monocytes (96% of total monocytes, higher than the cut-off of 94%) argues for CMML with an unusual *CSF3R* T618I mutation over aCML. Specifically, these immunophenotypic findings support the neoplastic nature of monocyte proliferation and help to distinguish CMML from myelodysplasic/myeloproliferative neoplasms with associated monocytosis [11, 12].

One unusual finding in our case is the presence of ring sideroblasts without mutation in spliceosome-related genes. Ring sideroblast status has not yet been reported in *CSF3R* mutated CMML. While the mutation

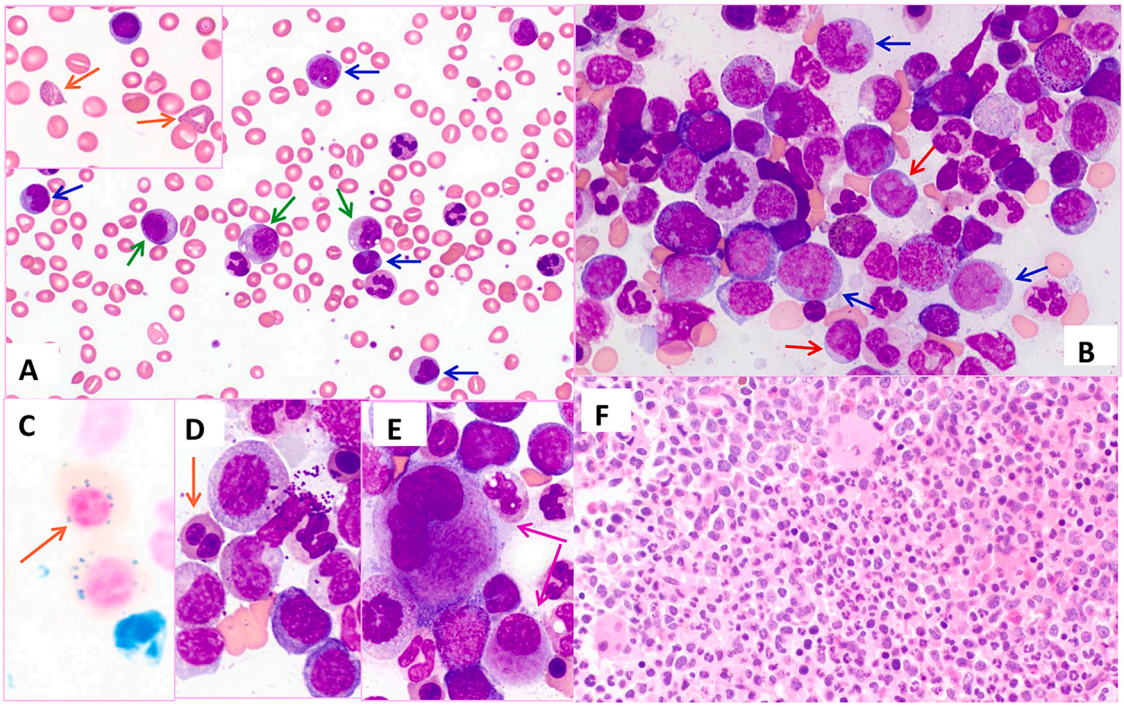

Fig. 1. Morphologic findings in peripheral blood and bone marrow. Peripheral blood smear (A) shows left-shifted neutrophils (green arrow), monocytosis (blue arrow), and coarse basophilic stippling (orange arrow). Bone marrow aspirate shows scattered myeloblasts (red arrow, B), monocytic cells (blue arrow, B), ring sideroblasts (C), nuclear irregularity in erythroids (D), and dysplastic megakaryocytes (E). Clot section shows hypercellularity, atypical megakaryocytes, and left-shifted granulocytic and monocytic cells (F). [Wright-Giemsa stains in images A, B, D, and E (100x objective), iron stain in C (100x objective), and hematoxylin and eosin stain in F (20x objective)].

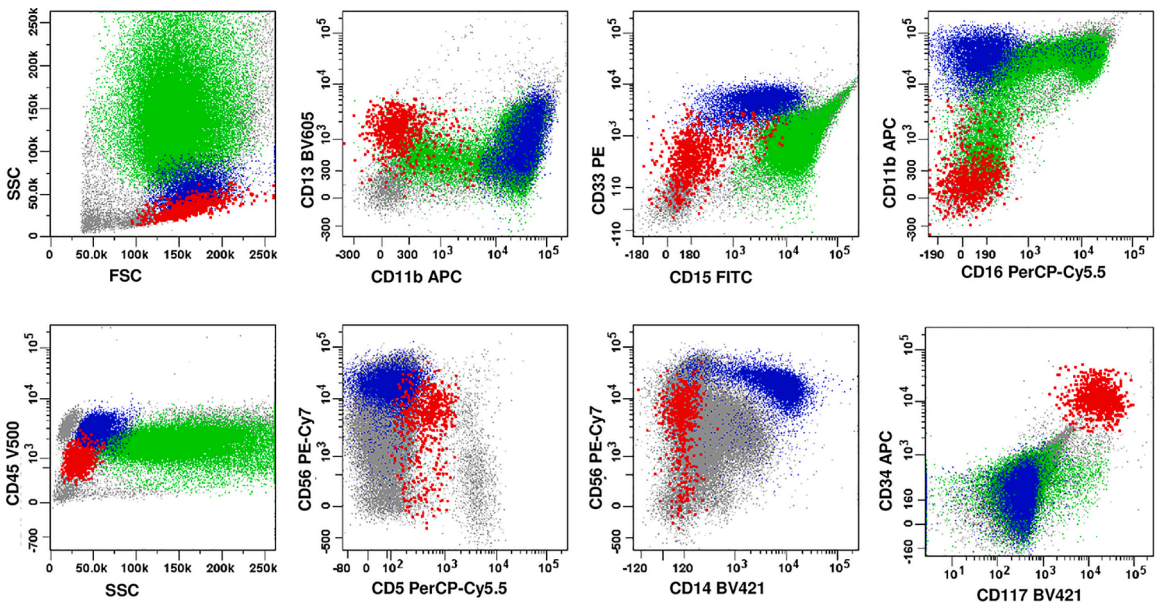

Fig. 2. Immunophenotypic findings in bone marrow aspirate by 10-color flow cytometry: There are immunophenotypic aberrancy on CD34(+)/CD117(+) myeloblasts (in red, 0.88% population, aberrantly expressing CD5, CD56, and dimly expressing CD33), increased monocytes [(in blue, 11%, with a nearly uniform expression of CD56 and a high fraction of CD14(+)/CD16(-) classical monocytes (estimated at 96% of total monocytes)], and neutrophils (in green) with abnormal maturation pattern on the CD11b/CD13 plot. The fractions of CD14(+)/CD16(+) intermediate and CD14(-)/CD16(+) non-classical monocytes could not be estimated due to the panel design.

in spliceosome-related genes (such as *SF3B1* and *SRSF2*) plays a role in the formation of ring sideroblasts, the mechanisms responsible for mitochondrial iron accumulation are not completely understood. It is possible that ring sideroblasts in this case resulted from alteration(s) in the genes involved in iron (heme and iron-sulfur cluster biosynthesis) and mitochondrial protein metabolism [13, 14].

Notably, the *CSF3R* mutation is rare in CMML, with only ~40 cases being reported (~30 cases with *CSF3R* non-T618I and ~10 cases with *CSF3R* T618I, accounting for ~4% and ~1% of CMML, respectively) [4, 8, 15–17]; our case is thus unusual. Although the mechanism by which the *CSF3R* mutation contributes to pathogenesis in CMML is largely unknown, it seems that the *CSF3R* T618I mutation, a membrane-proximal mutation, results in ligand-independent receptor activation and constitutive downstream signaling through JAK2, whereas the other membrane-distal *CSF3R* mutations may lead to ligand hypersensitivity and increased cell surface CSF3R expression [6, 18]. This proposed mechanism may explain why leukocytosis (proliferative subtype) is commonly associated with *CSF3R* T618I mutated CMML (a higher median WBC of 38 × 10^9/L in 6 cases [16] and WBC of 35 × 10^9/L in our case), whereas the non-proliferative subtype is commonly associated with *CSF3R* non-T618I mutated CMML (a median WBC of 11.3 × 10^9/L in 6 cases [15]).

Moreover, the mutational profile in *CSF3R* T618I mutated CMML also appears to be distinct from *CSF3R* non-T618I mutated CMML and *CSF3R* unmutated CMML. CMML is characterized by recurrent mutations in *TET2* (~60%), *ASXL1* (~40%), *SRSF2* (~50%), *SETBP1* (~15%), and genes in the RAS signaling pathway (~30%) [1, 2], characteristic of an aging-associated disease with mutations affecting epigenetic (DNA methylation and histone modification), splicing, and signaling pathways. Compared to *CSF3R* unmutated CMML, *CSF3R* T618I mutated CMML (including our case) seems to have more frequent mutations in *ASXL1* (~80%) and infrequent mutations in *TET2* (0/8, 0%) or *SRSF2* (1/8, ~14%), a feature suggestive of minimal disturbance of DNA methylation and splicing pathways [16, 17]. This contrasts with the mutational profile in *CSF3R* non-T618I mutated CMML, with the frequency of *TET2* and *SRSF2* mutations (~30% for each) similar to *CSF3R* unmutated CMML [4, 8, 15]. Our case additionally has mutations in genes in the RAS signaling pathway (*KRAS* and *PTPN11)* and *SETBP1*; the presence of multiple mutated genes in the signaling pathways (*KRAS* and *CSF3R*) may contribute to the proliferative phenotype of CMML. The mutation status in *KRAS, PTPN1,* and *SETBP1* in *CSF3R* mutated CMML was not systematically reported. It would be interesting to evaluate any changes in the mutational patterns over the course of the disease.

Similarly, *CSF3R* T618I mutated CNL seems to have a mutational profile different from *CSF3R* non-T618I mutated CNL regarding the *SETBP1* mutation; this mutation is more frequent in *CSF3R* T618I mutated CNL (~50%) than in *CSF3R* non-T618I mutated CNL (~5–10%). The mutational frequency of *ASXL1* is similar between these two subtypes of CNL [4, 5, 19–21].

Finally, the proposed mechanism of *CSF3R* T618I pathogenesis may provide a rationale for molecularly-directed targeting of *CSF3R* T618I mutated CMML with JAK2 kinase inhibitors such as ruxolitinib [6, 21]. Encouragingly, the clinical benefit of ruxolitinib was recently demonstrated in a subset of CNL patients (with lower-risk features) harboring *CSF3R* T618I mutation with hematologic responses and allele burden reduction [22]. However, given the complex mutational profile in our CMML patient, including *ASXL1* and *SETBP1,* therapies including hypomethylating agents, hydroxyurea in addition to supportive care with packed red cell transfusions to alleviate severe anemia were considered. Additionally, JAK2 inhibitors are increasingly recognized as potential therapies for *CSF3R* T618I mutated myeloproliferative neoplasms, however the effects may be transient and are not well established [23]. Given the patient's overall fitness, young age, and desire for curative-intent, allogenic hematopoietic cell transplantation with a matched unrelated donor was ultimately successfully pursued.

4. Conclusion

CSF3R mutations in a myeloid neoplasm with monocytosis may pose a diagnostic challenge. A comprehensive morphologic, immunophenotypic, and molecular study is essential to arrive at the correct diagnosis. *CSF3R* T618I mutation is rare in CMML and seems to define a unique proliferative subtype of CMML with a mutational profile distinct from both *CSF3R* non-T618I mutated CMML and *CSF3R* unmutated CMML. However, additional cases of CMML with this unusual mutation need to be studied to further characterize genotype-phenotype associations and

better understand clinical and therapeutic outcomes.

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Informed consent

Not Applicable (The samples were collected only for diagnosis and a general IRB rule was applied in this study.)

Declaration of Conflict of Interests

None

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