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Triple mutation Negative Thrombocytosis: A Diagnostic Conundrum and DNMT3A Mutation may be an Early Event Indicating Essential Thrombocythemia

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ABSTRACT

Essential thrombocythemia (ET) is a type of myeloproliferative neoplasm (MPN) characterized by stem cell-derived clonal myeloproliferative and presence of somatic mutations affecting in JAK2V617F, Cal-reticulin (CAL-R) and Myeloproliferative Leukemia Protein (MPL) in majority of patients. However triple negative ET exists and further molecular studies are warranted to look for other mutations of pathologic significance and also to establish mutational order.

KEYWORDS

Essential thrombocythemia; DNMT3A; Myeloproliferative neoplasm; JAK2; MPL; CAL-R

1. INTRODUCTION

ET is a haematological malignancy under the umbrella term of MPNs as per the revised 2016 The World Health Organization (WHO) classification system for hematopoietic tumors [1,2]. ET is characterized by stem cell-derived clonal myeloproliferative and most of the cases are caused by somatic mutations affecting in exon 14 (JAK2V617F) 55%, CAL-R (19p13.2) 15% - 24% and MPL in Exon 10 (1p34) in approximately 4% of ET patients [3-9]. However, we are reporting a triple negative ET case with DNMT3A mutation which raises questions about mutation order of these patients.

2. METHODS & MATERIALS

An old female of 77 years was seen in hematology department with 9 months history of slowly progressive thrombocytosis. She gave no history of bleeding; also she has no history of chronic infection or inflammation. Her C-reactive protein is normal, ferritin level is normal. Apart from recurrent venous thrombosis without any precipitating factors she has no other medical co-morbidities. She is never smoked and he is not obese. He does not have relevant family history.

Her investigations are shown in the below (Table 1). She was tested negative for mutations in JAK2 (exon 12+14), CAL-R (exon 9) and MPL (exon 10). But DNMT3A mutation was detected on myeloid gene panel analysis with significant Allele burden.

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Most recent blood results Hemoglobin 136 g/L, WBC 9.7 × 10^9/L, normal differential WBC count, platelet count 695 × 10^9/L.
Radiology Plain chest X-Ray: was reported to be normal. Ultrasound scan of abdomen and renal tract: did not show hepato-splenomegaly, normal kidneys, no abnormal mass identified.
Bone Marrow Investigation **Aspirate**: Hyper-cellular particles and trails Present, megakaryocytes are hyperlobulated and occasional clustering micro-megakaryocytes seen, some platelet lakes seen. No excess of blasts. Normal iron stain, no evidence of ring sideroblasts. **Comment**: hyper-cellular for age with evidence of myeloproliferative neoplasm. **Trephine biopsy:** A 17mm core, hyper-cellular for age. Tri-lineage haematopoiesis seen, megakaryocytes appear increased with significant clustering, no micro-megakaryocytes on CD61 staining. CD34+ in <5% of cells, CD117+ in <5% of cells. Decreased erythropoiesis. Reticulin-grade 1 and focally 2/3. **Comment**: Consistent with MPN/ET
Cytogenetic analysis on bone marrow sample: 20 G-banded metaphase showed normal male karyotype of 46XX.
Molecular studies on blood sample **Myeloid Gene Panel [MGP]** Report Combination: Mutated Gene: DNMT3A NM_022552.4 Variant: p.Arg899Pro Allele Burden: 24% Classification: Likely pathogenic **Test Overview:** 33 genes or gene mutation hotspots sequenced on an Illumina MiSeq using TruSeq Custom Amplicon reagents. Mutations/variants below 10% frequency are generally NOT reported unless they are known pathogenic variants. All variants of unknown significance have been excluded. **Genes in the Panel:** ASXL1 (exon 12). BCOR (all exons). CALR (exon 9). CBL (exons 7-9). CEBPA (all exons). CSF3R (exons 14-17). DNMT3A (all exons). ETV6 (all exons). EZH2 (all exons). FLT3 (exons 14-15+20). GATA2 (all exons). GNAS (exons 8+9). IDH1 (exon 4). IDH2 (exon 4). IKZF1 (all exons). JAK2 (exons 12+14). KIT (exons 2, 8-11, 13+17). KRAS (exons 2-3). MPL (10). NPM1 (exon 12). NRAS (exons 2-3). PDGFRA (exons 12, 14, 18). PHF6 (all exons). PTPN11 (exons 3+13). RUNX1 (all exons). SETBP1 (exon 4). SF3B1 (exons 12-16). SRSF2 (exon 1). TET2 (all exons). TP53 (all exons). U2AF1 (exons 2+6). WT1 (exons 7+9). ZRSR2 (all exons).
MPN molecular testing on bone marrow sample **Final Report** Normal result/s Jak2 V617F Assay Result: Not Detected Jak2 V617F Assay Sensitivity: 0.2% MPL W515 Mutation was Not Detected, as shown by Allele specific PCR (Assay Sensitivity 1%). CALR exon 9 Mutation was Not Detected, as shown by PCR fragment length analysis (Assay Sensitivity 5%).

Table 1: Investigations.

3. DISCUSSION

Majority of patients with ET a somatic mutation either in JAK2 V617F, CAL-R or MPL as described above [3-9]. As there are many alternative causes of thrombocytosis; the diagnosis sometimes remains in doubt when these mutations are not identified. These genetic alterations represent a key feature, and are very useful for diagnostic, prognostic and therapeutic approaches for MPN like ET. Molecular biology tests are now widely available with different specificity and sensitivity. Even though JAK2/CAL-R &MPL mutations are found in the vast majority of ET patients, cases with a diagnosis of ET from bone marrow investigation has been described in patients who are lacking these mutations, raising the question of other mutations causing this phenotype. Although somatic mutations in JAK2 V617F, CAL-R and MPL are found most of the ET patients; there are many other patients also harbor somatic mutations in epigenetic regulators of DNA methylation (TET2, DNMT3A and IDH1/2) or chromatin structure (ASXL1 and EZH2) [10-12].

In MPN patients, mutations in TET2, ASXL1 and EZH2 can occur either prior to or following the acquisition of JAK2V617F [13] and recently the order of mutation acquisition for JAK2V617F and TET2 has been shown to influence hematopoietic stem/progenitor cell biology and clinical presentation [14]. DNMT3A is frequently mutated gene in MPN after TET2 mutation, affecting 7% - 10% of patients [15,16]. However in contrast to other mutations, DNMT3A mutations have only been reported to occur either early or late in myeloid disease: prior to acquisition of JAK2V617F or in a separate clone in MPN [13,17,18]. A recent study showed that in MPN, DNMT3A mutation can either precede or follow JAK2 V617F and MPL mutations, and that JAK2/MPL single-mutant sub-clones have a competitive disadvantage in vivo compared with DNMT3A mutation sub-clones. This study also showed that mutation order of JAK2V617F and DNMT3A mutation is associated with differences in MPN phenotype as DNMT3A mutation seems be an earlier event that JAK2V617F mutation in many of the ET patients [18]. This concept is consistent with observations that DNMT3A and TET2 mutations confer an advantage to hematopoietic stem/progenitor cells [19-21].

4. CONCLUSION

Triple negative ET can pose a diagnostic challenge and further molecular studies to look for new mutation of pathological significance should be undertaken and also should aim to establish mutational orders for this group of ET patients.

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6. CONFLICT OF INTEREST

Nothing to disclose

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