Isolated ten-eleven translocation 2 positive in triple negative essential thrombocythemia: Case report and literature review

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Abstract
Essential thrombocythemia is one of the famous diseases under the category of myeloproliferative disorder. It is an end result of a genetic mutation of one or more of the freuest oncogenes such as Janos kinase 2 (JAK2), MPL proto-oncogene, thrombopoietin receptor (MPL), and calreticulin (CALR). However, negative genetic markers, so-called (triple negative disease), can happen in the presence of other uncommon types of mutation. TET2 (ten-eleven translocation 2) positive as isolated genetic marker in triple negative essential thrombocythemia is uncommon genetic presentation. For that, we are reporting a 22-year-old lady who presented with a feature of dyspepsia and accidentally found to have persistently high platelet count, even after treating her mild iron deficiency anemia with no other secondary causes. Further investigations and bone marrow biopsy supported the diagnosis of isolated TET2 positive in triple negative essential thrombocythemia. We treated her conservatively with good hydration and low dose of aspirin. In conclusion, isolated TET2 positive in triple negative essential thrombocythemia at presentation is uncommon with no clear management or risk stratification guideline. However, it is hypothesized that TET2 mutation precedes JAK2; therefore, the detection of isolated TET2 in a triple negative essential thrombocythemia case should be closely followed for clonal evolution in long term. Further study and guidelines required in this area.

Keywords
Essential thrombocythemia, myeloproliferative disorder, TET2 positive, triple negative, gene

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Learning points
• Isolated TET2 positivity in triple negative essential thrombocythemia at presentation is an uncommon presentation.
• There are no guidelines on treatment as well as no clear significance of TET2 in essential thrombocythemia, the area needs further study and research works.

Introduction
Essential thrombocythemia (ET) is one of the hematological diseases under the classification of myeloproliferative disease.¹ It is predominantly acquired genetic disease that occurs due to somatic gene mutations of the stem cells. Janos kinase 2 (JAK2) (V617F), MPL, and calreticulin (CALR) (that account for 60%, 8%, and 30%–35%, respectively) were incorporated by the World Health Organization (WHO) as major diagnostic criteria to diagnose ET. Up to 20% of ET case have no mutations of those common oncogenes called triple negative disease.²,³ Less commonly, ET can be

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inherited as an autosomal dominant familial ET. Damla Olcaydu et al. in a study of the role of genetic mutation in familial type of myeloproliferative neoplasm stated that, the germline mutations of ten-eleven translocation 2 (TET2) do not explain familial type of myeloproliferative disorder.

Case report

A 22-year-old female is not known to have chronic medical illnesses. Presented to the emergency department with epigastric pain and vomiting for a couple of days with no similar presentation before. Thoroughly investigated by gastroenterologist and labeled as dyspepsia after gastroscopy. Found to have persistently high platelet count ranging between 600 and 900 × 10^9/L level for 4 years duration. Referral to hematology service was done for further investigation.

Patient had thrombocytosis since 2015, had no history of thrombosis or bleeding, and no history of significant weight loss. She has no risk factors for Coronary Vascular Disease. Has positive family history of myeloproliferative disease in her aunt who was diagnosed recently to have high-risk ET with positive CALR gene mutation.

On examination, the patient looks well, not pale, jaundiced, or cyanosed. Blood pressure was 113/53 mm Hg, temperature was 36.6°C, pulse was 85 beats per minute, respiratory rate was 19 per minute, and oxygen saturation was 100% on room air. There was no lymphadenopathy. Respiratory system and cardiovascular system examination was unremarkable. Abdominal examination was negative for any masses or organomegaly. Grossly intact neurological examination was carried out. In lower limbs, there is no edema or evidence of deep veins thrombosis clinically.

Initial investigations showed hemoglobin of 12.7 g/L, white blood cell (WBC) of 6.08 × 10^9/L, platelets of 680 × 10^9/L, with normal mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH). Reticulocytes count was 1.39%. Liver panel and renal panel were within normal, lactate dehydrogenase (LDH) was 163 U/L, aspartate aminotransferase (AST) was 15.2 U/L, total bilirubin was 6 umol/L, and direct bilirubin was 2 umol/L. Partial thromboplastin time (PTT) was 31.9 and international normalized ratio (INR) was 1.13. Ferritin was 20.8, iron was 10 which is the borderline level, as per our reference range, and total iron binding capacity (TIBC) was 66. Peripheral Blood Film showed normochromic normocytic red blood cells picture with normal weight blood cells count and morphology, platelets were increased in distribution, and no blast or abnormal cell was seen. Ultrasound abdomen showed no organomegaly, spleen measures were 9.5 cm, and liver measures were 12.6 cm, otherwise unremarkable scan.

Bone marrow examination showed increased megakaryocytes with no increased in blast cells or dysmorphic cell and no fibrosis (Figure 1). Flow cytometry showed normal study with no signaling that indicated the presence of any active hematological malignancy. Cytogenetic study for BCR/ABL gene mutation showed negative test for any mutation, and no mutation was detected in JAK2 gene, Exon 12 and 14, as well as CALR and MPL genes. Extended cytogenetic and molecular study showed TET2 (c.3813C>T) mutational burden 45%. Other genes such as ASXL1, CBL, DNMT3A, EZH2, IDH1, RUNX1, SF3B1, SRSF, TP53, UAF1, and ZRSR2 were not mutated.

Hence, the patient was labeled as very low-risk triple negative—TET2 positive—ET and she was advised to increase fluid intake, her iron deficiency was treated, and to maintain on prophylaxis, aspirin 81 mg was given per oral once daily. Since then, she is on follow-up with hematologist for any progress in her condition. She had no further admissions for any reason since then.

Discussion

In 2016, WHO updated the diagnostic criteria for ET. The diagnosis requires meeting either all four major criteria or first three major criteria and one minor criterion. The major criteria are as follows (1) platelet count ≥450 × 10^9/L, (2) bone marrow (BM) biopsy showing proliferation mainly of the megakaryocyte lineage, (3) not meeting WHO criteria for chronic myeloid leukemia (CML), myelodysplastic syndrome (MDS), or other myeloproliferative or myeloid neoplasms, and (4) the presence of JAK2, CALR, or MPL mutation. The minor criteria were the presence of a clonal marker or the absence of evidence for reactive thrombocytosis. Based on the above criteria, our case was matching first three major criteria and one minor criterion to diagnose ET. TET2 mutation is not involved as criteria neither major nor minor to diagnose ET. As well, it is not considered as a marker for familial ET which was expected in our case scenario with positive family history of ET. The importance of the presence of TET2 mutation and its prognostic impact in ET was of major concern in such a case, especially with the absence of major oncopathes that have prognostic and diagnostic value in ET. For that, we are reporting this case with further review of the literature to clarify how isolated TET2 will play a role in the management and outcome of patients with ET.

What is TET2?

The TET2 is a gene that encodes in epigenetic regulator which is important for myeloid cell function. It is one of the genetic variants which has diagnostic and prognostic impact in several hematological diseases such as myeloid malignancy. Although it accounts for 5%–10% of genetic changes that occurs in normal population especially in above 65 years old group of age, TET2 mutation is also detected in chronic myelomonocytic leukemia (CMML) by around 40%–50% of positive cases, 20%–30% in MDS, and 10%–15% of acute
myeloid leukemia (AML) cases.\textsuperscript{7,8} \textit{TET2} mutation plays an important role in myeloid cell function, DNA demethylation of hematopoietic cell which affects the myeloid malignancy such as MDS and tumor progression as well as the patient outcome and reduces the overall survival in AML of intermediate risk group of patients.\textsuperscript{9,10} Moreover, \textit{TET2} mutations have been found in lymphoid malignancies as in some T cell lymphomas, as well and to a less extent in B cell lymphoma as in mantle cell lymphomas and diffuse large B cell lymphoma.\textsuperscript{11} Even more, \textit{TET2} mutation was also reported in benign cases as in patients with bronchial asthma and chronic obstructive pulmonary disease (COPD) patients with no clear relationship.\textsuperscript{12}

\textbf{Frequency of TET2 positive in ET?}

The incidence of \textit{TET2} mutations in myeloproliferative disorders in general ranges between 0% and 17%.\textsuperscript{13} Luz Martinez-Aviles et al.\textsuperscript{14} reviewed 62 cases with myeloproliferative disorder those are negative for \textit{JAK2} and \textit{MPL} gene mutations, 52 cases were diagnosed as ET, and three of them were isolated \textit{TET2} gene mutation positive. Julia Asp et al.\textsuperscript{2} studied the genetic mutation detected by TruSight Myeloid Sequencing Panel in 129 patients diagnosed with ET between 2008 and 2013, and they found that 6% were triple negative and extended genetic panel showed the expression of \textit{TET2}, \textit{SRSF2}, or \textit{ASXL1}, around 2% of them were isolated \textit{TET2} positive. Recent genetic study revealed that around 50% of patients with ET have types of mutations other than \textit{JAK2}, \textit{MPL}, or \textit{CALR} mutation such as \textit{TET2}, \textit{ASXL1}, \textit{DNMT3A}, and \textit{SF3B1} with frequency of 16%, 11%, 6%, and 5%, respectively.\textsuperscript{15}

\textbf{Significance and prognosis of TET2 in ET}

Patients with ET are at risk of both thrombosis and bleeding which account for the major cause of morbidity and mortality. However, less than 5% of cases with ET will end by transformation to myelofibrosis or acute leukemia especially in high-risk group stratification. The presence of \textit{JAK2} or \textit{MPL} mutations in ET patients has been associated with higher risk of arterial thrombosis. Moreover, the presence of at least one of the following mutations \textit{SH2B3}, \textit{SF3B1}, \textit{U2AF1}, \textit{TP53}, \textit{IDH2}, and \textit{EZH2} is associated with inferior overall survival in ET population. Julia Asp et al.\textsuperscript{2} stated that \textit{TET2} mutations have different types and cannot use it as diagnostic or prognostic marker with no effect in overall survival. This fact is not applicable to \textit{SRSF2} and \textit{ASXL1} as both were found to have worse overall survival.

In experimental mice, \textit{TET2} mutation and deficiency were found to play a role in the pathogenesis of cardiovascular events and atherosclerosis as well it increases lung inflammation and increases pulmonary arterial pressure.\textsuperscript{16} There is no study about cardiovascular events and atherosclerosis in

\begin{figure}[h]
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\caption{Real bone marrow picture for the same patient showed (a) trephine biopsy with increase cellularity and no blast cell seen, (b) trephine biopsy with increase megakaryocyte distribution, and (c) morphology from aspiration with healthy appearing megakaryocyte (arrow).}
\end{figure}
human being affected by TET2 positive ET. However, some studies showed that TET2 mutations may be an independent risk factor for thrombosis in patients with polycythemia vera but not in ET. Recently, one publication by Ayalew Tefefi et al.\textsuperscript{15} presented the analytic data of deep sequencing in ET, and it highlights the association between positive TET2 mutations and thrombosis in ET independent to the presence of famous oncogenes or age.

TET2 has more prevalence in old age population affected by ET in comparison to younger age group. In the study by Tefefi et al.,\textsuperscript{17} three out of fifty ET patients who had TET2 mutations were senior age and followed up for several months showed no leukemic transformation, as well as, there was no correlation between the presence of mutant TET2 and survival or leukemic transformation in other types of myeloproliferative disorder.

TET2 proteins play a significant role in epigenetic regulation which affects immune responses and regulation. Furthermore, TET2 mediated the oxidation of 5-methlycytosine (5mc) and regulated DNA methylation as well as demethylation which by itself affect differentiation and function of lymphoid and myeloid. In case of such gene mutation, function of TET2 will be impaired, oxidation to 5mc will be affected and DNA methylation will be altered. Alteration of DNA methylation plays an important role in pathogenesis of myeloproliferative disorder (MPD) and other hematological malignancy. Furthermore, the possibility exists that TET2 mutations at the level of the stem cell might result in increase malignancy. Furthermore, the possibility exists that TET2 mutations at the level of the stem cell might result in increase malignancy. Furthermore, the possibility exists that TET2 mutations at the level of the stem cell might result in increase malignancy.

As TET2 mutation plays a dynamic role in alteration of DNA methylation and development as well as progression of hematological malignancy, consideration of hypomethylating agent as a treatment option could carry significant value. Studies showed that clonal TET2 mutations predicted response to hypomethylating agents such as azacitidine (AZA) in MDS and improved survival rate in promyelocytic leukemia (PML).\textsuperscript{22,23} Although the alteration of DNA methylation plays an important role in pathogenesis of MPD, there is no report or study supports the role of hypomethylating agent in MPD with TET2 positive mutation.

Whether TET2 is positive or not, triple negative ET seems to have risk of complications almost similar to ET with JAK/MPL or CALR mutation. In case series of 22 triple negative ET, the rate of thrombosis and symptom burden is similar to other ET patient with positive famous genes mutation in low-risk group.\textsuperscript{24} Although we risk stratify and manage our patient as very low-risk group based on NCCN guideline, yet quarterly a year is desired for follow-up and assessing for any complications or transformation.

Since there is no specific guideline for management of TET2 positive ET, the follow-up of the patient will be as other ET patient which is every 3–6 month and as per need to monitor for active symptoms, complications, Von Willebrand disease (VWD), and any transformation. In general, our case scenario is considered as series to what was reported previously rather than reporting for novelty and the aim of such report is to promote further research to help in more understanding of the importance of such finding.

**Conclusion**

Isolated TET2 positive mutation in ET is an uncommon genetic presentation of the disease. It could carry some significant prognosis and outcome that requires attention and lifelong follow-up. There is no guideline to classify the patient with isolated TET2 positive ET to specific risk group stratification. Moreover, there are no guidelines in how to manage this group of patients and whether cytoreduction agent is indicated or not. However, it is hypothesized that TET2 mutation...
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