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Editorial

Triple Negative Polycythemia Vera: A Diagnostic Challenge and DNMT3A Mutation may be an Early Event

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Editorial

Polycythemia Vera (PV) is a Myeloproliferative Neoplasm (MPN) responsible for increased hematopoiesis. PV is a clonal disorders of the hematopoietic stem cell caused by somatic mutations. PV is molecularly well characterized with 96% and 3% of patients exhibiting Jenas Kinase 2 (JAK2) JAK2V617F in exon 14 and JAK2 exon 12 mutations, respectively [1-3]. The hallmark of BCR-ABL1-negative MPNs is the presence of a driver mutation in JAK2, Calreticulin (CAL-R) [4] or Myeloproliferative Leukemia Protein (MPL) gene. In most of the cases, mutational genes are identified as either JAK2, exon 9 of CAL-R [4] or exon 10 of MPL [5].

67 years old male was seen in hematology department with 5 years history of raised Hemoglobin (Hb) and raised Hematocrit (HCT). His Hb runs between 178-185 g/L and HCT runs between 52.6% to 56%. Apart from type 2 diabetes for which he takes Metformin he does not have any other medical co-morbidity. He is never smoked and he is not obese. He does not have relevant family history and does not live in high altitude. His investigations are shown in the below (Table 1). Bone marrow trephine biopsy, Red Cell Mass study (RCM) and persistent high Hb and HCT without any obvious causes is consistent with a diagnosis of true erythrocytosis but he was tested negative for mutations in JAK2 (exon 12 + 14), CAL-R (exon 9) and MPL (exon 10). But DNMT3A mutation was detected with significant Allele burden.

Most recent blood results

Hb 185 g/L, HCT 548%, WBC 7.8 x 10^9/L, Platelet 233 x10^9/L, Erythropoietin level 9 i.u/L

Radiology

Plain chest X-Ray: was reported to be normal.

Ultra-sound scan of abdomen and renal tract: did not show hepato-splenomegaly, normal kidneys, no abnormal mass identified.

Bone Marrow Trephine

Hypercellular marrow, no increase in reticulin staining (WHO grade 0). Tri-lineage maturation, erythroid predominance, megakaryocytes showed normal morphology, CD117, CD34, CD14 staining showed no excess immature precursors. Features are suggestive of MPN-favours Polycythemia Vera.

Cytogenetic analysis and molecular studies on bone marrow sample

Myeloid gene panel study: 38 genes were analysed for mutations and abnormalities using Next Generation Sequencing (NGS) showed mutation in DNMT3A with allele burden of 23% which was thought to be pathogenic. ASXL1 (exon 12), CAL-R (exon 9), EZH2 (all exons), JAK2 (exons 12 +14), MPL (exon 10), TET2 (all exons) TP53 (all exons) did not show any mutations.

Cytogenetic karyotype: 20 G-banded metaphase showed normal male karyotype of 46XY.

Table 1: Investigations.

Most Recent Blood results:
Hb 185 g/L, HCT 548%, WBC 7.8 x 10^9/L, Platelet 233 x10^9/L, Erythropoietin level 9 i.u/L
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Cytogenetic karyotype: 20 G-banded metaphase showed normal male karyotype of 46XY.
Red Cell Mass Study (RCM)
Nuclear medicine RCM showed increased RCM (predicted RCM-2419 mls, Measured RCM 3071 mls, variance 27%. **Conclusion:** increased RCM estimation).

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Red Cell Mass Study (RCM)

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In patients with PV a somatic mutation in exon 14 of JAK2 involving the pseudokinase domain (c.1849G>T; p.V617F) is found in ~96% of, and is a major diagnostic criterion in the 2008 World Health Organization (WHO) classification [6] and its proposed forthcoming revision [7]. A further 2–3% of PV patients have mutations in exon 12 of JAK2, so that very few PV patients are 'JAK2-negative', lacking a somatic mutation in that gene. As there are many alternative causes of absolute or relative erythrocytosis the diagnosis sometimes remains in doubt when no JAK2 mutation is identified. These genetic alterations represent a key feature, useful for diagnostic, prognostic and therapeutic approaches. Molecular biology tests are now widely available with different specificity and sensitivity. Even though JAK2 mutations are found in the vast majority of PV patients, "true" PV has been described in patients lacking mutations in the exon 12 or 14 of JAK2, raising the question of other mutations causing this phenotype.

Although somatic mutations in JAK2, CALR and MPL are found in the majority of MPN and PV but many patients also harbor somatic mutations in epigenetic regulators of DNA methylation (TET2, DNMT3A and IDH1/2) or chromatin structure (ASXL1 and EZH2). In MPN patients, mutations in TET2, ASXL1 and EZH2 occur either prior to or following the acquisition of JAK2V617F [8] and, recently, the order of mutation acquisition for JAK2V617F and TET2 has been shown to influence hematopoietic stem/progenitor cell biology and clinical presentation [9]. DNMT3A is frequently mutated gene in MPN after TET2, affecting 7–10% of patients [10,11]. 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 [12-14].

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