A Study of 8 Patients with Polycythaemia Vera: The Impact of JAK2 (V617F) Mutation on Diagnosis

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Abstract: *Background:* The discovery of the Janus Kinase 2 (JAK2) gene mutation provides a new molecular approach for the diagnosis of polycythaemia vera (PV).

We studied retrospectively patients with a diagnosis of PV at initial presentation to the hospital. They were followed up for an initial period of 2 months.

Objective: The objectives were to access the clinicopathological characteristics, impact of identification of JAK2 mutation on diagnosis and treatment strategies.

Result: Of the 6 patients with complete medical records, 3 were males (50%). The mean haematocrit values were 59.7%, males had a value of 60.3% while females a values of 59.0%. The total WBC count was 8.8 x 10^9/L while the platelet count was 463.5 x 10^9/L. None of the patients' had splenomegaly, all had pruritus. During follow up the mean haematocrit values were 52.3%, males had a value of 55.8% while females a values of 48.7%. The total WBC count was 11.1 x 10^9/L while the platelet count was 652.1 x 10^9/L. There was a reduction in the haematocrit value but the white blood cell and platelet counts remained high.

Of the 5 patients who had molecular analysis done, 4(80%) had the JAK2 mutation while in 1 (20%) the mutation was not detected. The results of the molecular analysis done in a regional laboratory were received after an average of 21 days.

The patients were treated with phlebotomy and hydroxyurea. One patient with increasing thrombocytosis had clopidogrel initiated. 3 patients were lost in the follow-up period once there was clinical improvement, at 6 months and 1 year only 1 patient was left in follow-up.

Conclusion: The lag time for obtaining results of molecular analysis was long, which made diagnosis and management on clinicalbasis.

This highlights the need for the upgrading of more laboratories to acquire molecular technology for improved patients care and diagnostic accuracy.

Keywords: Polycythaemia vera, JAK2 mutation, Clinicopathological diagnosis, UUTH, Nigeria.

INTRODUCTION

Chronic myeloproliferative disorders polycythaemia vera (PV), essential thrombocythaemia (ET) and primary myelofibrosis (PMF) are now called myeloproliferative neoplasm's (MPNs) by the 4th edition of the WHO classification of haemopoietic tumors [1]. They are disorders due to the clonal proliferation of a multipotential haemopoietic stem cell with normal maturation and a tendency for extramedullary haemopoiesis. Beyond these characteristics, PV, ET and PMF share more in common genotypically and phenotypically with each other than with the other MPNs [2-5]. The tendency for each to acquire the phenotypic characteristics of the other makes it worth asking whether they are separate disorders, different manifestation of the same disease or combination of both. The current molecular evidence supports the last possibility [6]. Their initial clinical presentations are also highly variable and are subject to change overtime.

In PV also termed polycythaemia rubra vera, there is increased erythrocyte mass, the blood, bone marrow spleen and liver are variably involved during progression through 2 disease phase, a proliferative or polycythaemic phase with increased red cells and a post polycythaemic or spent phase with marked cytopenias, extramedullary haemopoiesis, hyper-splenism and myelofibrosis. A subset may transform to acute leukemia.

Haemorrhage, thrombosis and hyperviscosity represent the primary clinical manifestations. Thrombotic events include stroke, Deep vein thrombosis, Myocardial infarction, Budd - Chiari syndrome [7]. Bleeding complications include epistaxis, oral mucosal bleeding, gastrointestinal haemorrhages, non- specific ecchymoses. Hyperviscosity syndromes

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from sludging of blood flow and micro thrombi may cause hypertension, headache, dizziness, visual disturbances vertigo, tinnitus, claudication and erythromelagia. Pruritus classically described following exposure to warm water results from histamine release from activated basophils and mast cells.

The eligibility criteria for PV were originally created by the Polycythaemia Vera Study Group known as the PVSG diagnostic criteria. The combination of their recommended major and minor criteria (Table **1**) remained the gold standard for several decades [8]. In 2001 the WHO classification of PV was published (Table **2**). This classification included a modernized approach to diagnosis by adding several new laboratory studies, the classification also aimed at applying the Revised European – American lymphoma (REAL) classification which was successfully used for lymphoid and myeloid neoplasm's to MPDs [9].

The discovery of JAK2 and MPL genetic mutations provided new insights into the molecular basis of MPDs [10-13]. In 2007 there was a revision in the 4th edition of the WHO diagnostic criteria for MPDS to reflect this [6, 14]. The objective of identification of JAK 2 mutation in patients with clinical manifestation suggestive of PV was aimed at diagnostic accuracy, to facilitate targeted pharmacotherapeutics and prevent disease progression.

PATIENTS AND METHODS

Study Population

The data used were from the medical records of the 8 patients that a diagnosis of PV was made by Consultant Haematologists in the University of Uyo Teaching Hospital after reviewing the history, physical signs and laboratory investigations in accordance to the PVSG diagnostic criteria, from January 2008 – December 2015. The follow-up period was two, six months and 1year. Two patient's record were incomplete therefore were excluded.

Permission was sought and obtained in written form from the Head of Medical Records Department after obtaining Ethical clearance from the Hospital's Ethical committee.

Table 1: PVSG Diagnostic Criteria for PV

Major Criteria	1. Total red blood cell mass- In males, ≥36ml/kg; in females ≥32ml/kg. 2. Arterial oxygen saturation ≥ 92%. 3. Splenomegaly.
Minor Criteria	1. Thrombocytosis with platelet count >400,000/*u*L. 2. Leukocytosis with a white blood cell count >12,000/*u*L. 3. Increased leukocyte alkaline phosphatase > 100U/L. 4. Serum vit. B12 concentration > 900 pg/ml or binding capacity > 2200pg/ml.

*Diagnosis of PV is established with all major criteria or first 2 major criteria plus any 2 minor criteria.

Table 2: WHO Diagnostic Criteria for PV

A1	Elevated RBC mass > 25% above mean normal predicted value, or Hb> 18.5g/dl in men, 16.5g/dl in women.
A2	No cause of secondary erythrocytosis, including absence of familial erythrocytosis; no elevated erythropoietin due to hypoxia (arterial PO_2<92%), high oxygen affinity haemoglobin, truncated erythropoietin receptor, inappropriate erythropoietin production by tumor.
A3	Splenomegaly.
A4	Clonal genetic abnormality other than Ph chromosome or BCL/ABL fusion gene in marrow cells.
A5	Endogenous erythroid colony formation *in vitro*.
B1	Thrombocytosis >400 x 10^9/L.
B2	WBC count >12 x10^9/L.
B3	Bone marrow biopsy showing pan myelosis with prominent erythroid and megakaryocytic proliferation.
B4	Low serum EPO levels

*PV is diagnosed when A1+A2 +any other A or A1 +A2 + any 2B. RBC indicates red blood cell, Hb, haemoglobin; Ph, Philadelphia; WBC, white blood cell; EPO, erythropoietin.

Records from the Haematology clinic and Haematology Laboratory registers were used to authenticate clinic attendance and the laboratory results obtained. The JAK2 mutation was analyzed at Safety Molecular Laboratory, Owerri, Nigeria, a regional reference laboratory using allele specific Polymerase chain reaction (PCR).

RESULTS

Table **3**. shows the demographic and clinical characteristics of the patients at diagnosis and during the 2 months follow up period. There were 8 patients at entry, 2 patients had to be excluded due to poor records during follow up. 3 (50%) patients were males while 3 (50%) were females. The mean age at diagnosis was 57.5 years, 3(50%) were above 60 years. The mean haematocrit was 59.0% and 52.3% at diagnosis and follow up respectively. For males it was 60.3% and 55.8% at diagnosis and follow up while females had 59.0% and 48.7% respectively.

The total WBC counts 8.8 x 10 ^{9}/L and 11.1 x 10^{9}/l at diagnosis and follow up with 5(83.3%) patients having values below 11 x 10^{9}/L. The mean platelet count was 463.5 x 10^{9}/L at diagnosis rising to 652.1 x 10^{9}/L during follow up. 4 (66.7%) patients had platelets > 400 x 10^{9}/L at diagnosis while 5 (83.3%) had during follow up.

There was no splenomegaly in any of the patients while all had pruritus and conjunctival suffusion. There was no thrombotic event at diagnosis; one patient had a persistent rising thrombocytosis. 3 (50%) patients defaulted once there was clinical improvement. At 6 months and 1 year there was only one patient left in

Table 3: Demographic and Clinical Characteristics of Patients at Diagnosis and during Follow up

Variables	Patients at Diagnosis	Patients In Follow Up
No of patients No (%)	6 (100)	6 (100)
Male sex No (%)	3 (50)	3 (50)
Female sex No (%)	3 (50)	3 (50)
Mean Age at diagnosis (yrs)	57.5	-
≤ 60	3 (50)	-
≤ 60	3 (50)	-
Mean Haematocrit (%)	59.7	52.3
Male	60.3	55.8
Female	59.0	48.7
Mean WBC count x 10^{9}/ L	8.8	11.1
≤ 11	5 (83.3)	5 (83.3)
≥ 11	1 (16.7)	1 (16.7)
Mean Platelet x 10^{9}/L	463.5	652.1
≤ 400	2 (33.3)	1 (16.7)
≥ 400	4 (66.7)	5 (83.3)
Splenomegaly	Nil	Nil
Thrombotic event	Nil	1 (16.7)
Pruritus	6 (100)	Nil
Conjunctival suffusion	6 (100)	Nil
JAK2 Mutation	4 (66.7)	
Time for obtaining Result in days	21	
Disease Outcome		
Left treatment facility		3 (50)
Still in follow up		3 (50)
Death		Nil

follow up. The average time it took for results of JAK2 analysis to arrive at treatment facility was 21 days.

DISCUSSION

The prevalence of PV in the study area was 6.3% [15]. In the study in Benin, Nigeria with a larger population size, the prevalence was 1.4% [16]. There was none recorded in the study done in Ilorin, Nigeria [17]. Due to the lack of large population based studies in a disease of low frequency [18], the prevalence of PV is not well documented. In a unique study in Connecticut, USA using health claim records and applying it to the entire population of the state, the age-standardized prevalence of 22 per 100,000 was obtained, and is similar to the Italian and Swedish studies [19]. The lower frequencies in the studies in developing countries needs to be further analyzed in a larger multi-centered study program. In this study there are 3 males (50%) and 3 females (50%) at presentation, the mean haematocrit value was 59.7%. The males had a value of 60.3% while in females it was 59%. There was no statistical significant difference. This absence of statistical difference may be age related as most of the females were above 60 years.

In the follow up patients there was a significant reduction ($P<0.01$) in the haematocrit value to 52.3%. The males and females had a value of 55.8% and 48.7% respectively. This was in response to phlebotomy.

The tWBC at presentation was 8.8 x 10^9/L and there was a progressive increase to 11.1 x10^9/L in the follow up period despite initiation of hydroxurea a cytoreductive therapy.

The platelet count at presentation was 463.5 x10^9/L with a statistical significant increase to 652.1 x10^9/L in the follow up ($P< 0.001$) despite the cytoreductive therapy. The persistence and rising thrombocytosis necessitated the addition of clopidogrel an anti-platelet drugs in one of the patient. Acetylsalicylic acid was used for the other patients in the follow-up period.

The average time for obtaining the results of the JAK2 analysis was 21 days; this is outside the time it takes to educate and counsel the patient's family to bear the cost of the test. Applied in this manner the identification of JAK2 mutation may only improve diagnostic accuracy. The initiation of targeted pharmacotherapeutics with anti-JAK2 properties would have been of benefit to the patient's outcome as rising WBC counts and platelet counts are risk factors for thrombotic events [20].

CONCLUSION

The impact of the identification of JAK2 mutation in patients with PV- like symptoms offers diagnostic accuracy but needs to be readily available and affordable to improve the quality of management of cancer patients in developing countries.

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