

Evaluation of Clinical and Laboratory Features of Newly Diagnosed Chronic Myeloid Leukaemia Patients Seen in Enugu, South-East Nigeria over a 2-Year Period Using Gen Expert Technology

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Abstract

Background: Chronic Myeloid Leukaemia (CML) is a myeloproliferative neoplasm (MPN) of abnormal marrow stem cell with *BCR-ABL1* fusion gene as a pathognomonic feature. In resource-poor countries, clinical and laboratory parameters were invaluable tools used in the diagnosis. However, the advent of GeneXpert technology was a paradigm shift in the detection of the fusion gene. **Aim of the study:** To highlight the clinical usefulness of geneXpert in the evaluation of clinical and laboratory features of newly diagnosed patients. **Patients and Methods:** A cross-sectional descriptive study was conducted at University of Nigeria Teaching Hospital (UNTH) Enugu. Sixty-five *BCR-ABL 1* positive consenting patients were evaluated using demographic, clinical and laboratory data. Data was analyzed using GraphPad Prism software version 9 with probability <0.05 as statistically significant. **Results:** A total of 65 patients with mean age 45.22 ± 15.44 years were studied. Age group 39 – 49 were mostly affected 20 (30.8%) with Enugu State having the highest disease burden 20 (30.8%). Abdominal swelling was the most common presenting feature 57 (87.7%) while splenomegaly was the most common finding on imaging 60 (92.3%). Mean WBC and *BCR-ABL1* were $207.37 \pm 87.57 \times 10^9/L$ and $41.02 \pm 24.53\%$ (IS) respectively ($r = -180$, $P = 0.015$). Majority of the cases were in chronic phase 56 (86.2%). Risk assessment of cases on presentation using Sokal and Hasford scores were statistically significant ($t = 20.23$, $P < 0.0001$), [CI = 1429 to 1739]. **Conclusion:** There was improved diagnostic yield of cases of CML using the molecular diagnostic technique.

Keywords: *BCR-ABL1*, fusion gene, GeneXpert, Sokal, Hasford score, CML.

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INTRODUCTION

CML is a myeloproliferative neoplasm (MPN) which originated in abnormal bone marrow pluripotent stem cell. Its characteristic feature is the presence of *BCR-ABL1* fusion gene that is in the Philadelphia (Ph[']) chromosome Elias J *et al.* (2018), David G *et al.* (1997). The Ph['] is a derivative chromosome 22 resulting from translocation between long arm of chromosomes 9 and 22, designated as t (9; 22) (q34.12; q11.23) Elias J *et al.* (2018), David G *et al.* (1997). The protein product of this fusion gene includes an enzymatic domain from the normal *ABL1* with tightly regulated tyrosine kinase catalytic activity Elias J *et al.* (2018), David G *et al.* (1997). The kinase activity of *BCR-ABL1* is enhanced and is continuously produced at a constant rate because of the fusion with a portion of *BCR*. The deregulated tyrosine kinase activity is therefore implicated in the uncontrolled proliferation of the cells of the granulocytic series as seen in patients with chronic myelogenous leukaemia Elias J *et al.* (2018), David G *et al.* (1997).

Each year, 1.0-1.5 of newly diagnosed patients with CML is identified per 100,000 individuals Elias J *et al.* (2018), David G *et al.* (1997), Van Etten R (2003). Its prevalence is estimated to increase at a yearly rate of 4%, and the number of individuals living with this disease will likely double by 2030. It has slight male predominance and accounts for about 15 to 20% of leukaemias in adults [Huang X *et al.*, (2012), Siegel R *et al.*, (2017), Sant M *et al.* (2010), Smith A *et al.*, (2011), Chen Y *et al.*, (2013). At presentation, median age is approximately 50 years in clinical trial patients, while the actual median age from data obtained from cancer registry may be 10 years older. Ionizing radiation exposure has remained the only known risk factor Moloney W (1987), Hoffman W *et al.*, (1931). However, there is no known familial disposition to the disease Björkholm M *et al.*, (2013). At diagnosis, the clinical findings vary and depend mainly on the stage of the disease. Twenty to 50% of the patients are asymptomatic, with the first suspicion usually occurring during routine blood tests Kamada, N *et al.*, (1978). Among symptomatic patients, common systemic symptoms include malaise (general feeling of unwell), weight loss, abdominal fullness, fatigue, excessive sweating, and bleeding episodes due to dysfunctional platelet Rowe, J.M. (1983).

Abdominal pain and discomfort may include left upper quadrant pain and easy fullness, usually due to an enlarged spleen and/or splenic infarction. Tenderness may be seen over the lower sternum, due to bone marrow expansion. Gouty arthritis of sudden onset may also present at this time, due to overproduction of uric acid Faderl S *et al.*, (1999).

Other findings that are frequent include splenomegaly, anaemia, white blood cell count above

100 x 10⁹/L, and platelet count above 600 to 700 x 10⁹/L Faderl S *et al.*, (1999), Thompson, R *et al.*, (1982). Extramedullary tissue involvement such as the skin, lymph nodes, and soft tissues is generally confined to patients who are in blast crisis. This disease clinical hallmark is the uncontrolled proliferation of transformed mature and maturing granulocytes, predominantly neutrophils and to some extent basophils and eosinophils. This is enhanced through escape from apoptosis, altered interaction with the cellular matrix and discordant maturation Thompson, R *et al.*, (1982), Shimkin, M *et al.*, (1951). Without treatment, the disease has a triphasic or biphasic clinical course as it can progress from a chronic phase which is present at the time of diagnosis in approximately 85% of patients, to an accelerated phase in which neutrophil differentiation becomes progressively impaired and leucocyte counts are more difficult to control with treatment and finally to a terminal blast crisis a condition resembling acute leukemia in which myeloid or lymphoid blasts proliferate in an uncontrolled manner Lichtman, M. (1973). At times, it can transform directly from chronic phase to blast crisis Preston, F *et al.*, (1978), Scott, R (1957), Swerdlow S *et al.*, (2008).

The peripheral smear typically demonstrates a leucocytosis with a median white cell count of approximately 100 x 10⁹/L (range 12 to 1,000 x 10⁹/L) Spiers A *et al.*, (1977). The differential count typically shows all cells of the granulocytic series, starting from myeloblasts to mature neutrophils with peaks in the myelocytes and segmented neutrophils. Myeloblasts typically account for <2 %. The classic findings in CML are the presence of greater percent of myelocytes than the more mature metamyelocytes Melo J *et al.*, (1994). The granulocytes present in the chronic phase of the disease are morphologically normal and have no evidence of dysplasia. In more advanced disease, especially in accelerated phase, dysplasia can develop. The platelet count may be normal or raised. Platelet counts above 600 x 10⁹/L are seen in 15 to 30 percent of patients with the disease Swerdlow S *et al.*, (2008), Spiers A *et al.*, (1977), Melo J *et al.*, (1994). Blood film shows normochromic, normocytic anemia in 45 to 60 percent of the patients. Absolute basophilia and eosinophilia are universal findings in the blood smears of patients with CML. Absolute monocytosis (>1 x 10⁹/L) is common, though the percentage of monocytes is usually low (<3 percent) Ravandi F *et al.*, (1999), Goldman J. (2008).

Before the development of molecular techniques in the diagnosis of CML as was the case in resource-poor countries like Nigeria with less availability of such techniques, the clinical and laboratory parameters like total white blood cell (WBC) count, spleen size, peripheral and bone marrow smears were invaluable tools used in the diagnosis of the disease. However, the advent of Cepheid GeneXpert technology has helped developing countries immensely

in the detection of the presence of the fusion gene *BCR-ABL* 1 transcript which is the molecular hallmark of the disease.

Xpert *BCR-ABL* Ultra is a quantitative test for *BCR-ABL* 1 major breakpoint (p210) transcripts that provides extremely sensitive and on-demand molecular results Beillard E *et al.*, (2003), Branford S *et al.*, (2006). Based on this innovation, it automates the entire test processes which include isolation of RNA, stage of reverse transcription, fully nested real-time Polymerase Chain Reaction of target *BCR-ABL* 1 gene and *ABL* reference gene; all are done in one fully automated cartridge Branford S *et al.*, (2006), Cepheid Xpert *BCR-ABL* Ultra Package Insert]. The system is sensitive and valuable in the quantitative monitoring of *BCR-ABL* 1 mRNA in patients with chronic myelogenous leukemia (CML) Branford S *et al.*, (2006), Cepheid Xpert *BCR-ABL* Ultra Package Insert.

On presentation, following the detection of *BCR-ABL* 1 transcript, the patient's risk and prognosis were assessed using the Sokal, Hasford and EUTOS risk assessment scoring systems. The scores were based on age in years, spleen size, blast %, platelet, eosinophil, and basophil counts. The scores obtained were used to categorize the patients into low, intermediate, and high-risk groups Sokal J *et al.*, (1985), Hasford J *et al.*, (1998) and thereafter they were referred to Obafemi Awolowo University Teaching Hospital, (OAUTH) Ile-Ife Nigeria, for enrollment into the free Glivec International Patient Assisted Programme (GIPAP). The enhanced tyrosine kinase activity associated with *BCR-ABL* 1 fusion gene remains the reason why tyrosine kinase inhibitors (TKIs) are used for the treatment of CML Hochhaus A *et al.*, (2020). However, our patients did not receive a monitored treatment because upon confirmation of diagnosis on presentation they were referred to OAUTH. In resource-poor countries, the high cost and unavailability of TKIs have made it difficult for our patients to be able to access the drug as such most patients depended on hydroxyurea and or GIPAP for those that had the logistic means.

OBJECTIVES

To highlight the clinical usefulness of molecular diagnostic technique in the evaluation of the clinical and laboratory features of newly diagnosed CML patients.

PATIENTS AND METHODS

This was a cross-sectional descriptive study carried out at the department of Haematology and Immunology of the University of Nigeria Teaching Hospital, Ituku-Ozalla Enugu, South-East Nigeria over a period of two years, between April 2017 to July 2019.

Before we commenced the study, we sought and obtained ethical approval from the UNTH Health Research Ethics Committee with protocol number UNTH/CSA/329/VOL.5/012.

Sixty-five (65) consenting patients with features of myeloproliferative neoplasms based on full blood count and blood film review were evaluated. The full blood count was carried out using Mythic 22 automated blood cell analyzer, (Orphee, Geneva, Switzerland). Patients fresh whole blood collected in an Ethylene diamine tetra-acetic acid (EDTA) bottle was used for the detection the presence of *BCR-ABL* 1 transcript using a four-module Cepheid GeneXpert machine. Demographic, clinical and laboratory data were collected upon detection of the transcript in their venous blood. Chemical pathology, microbiological and imaging tests were also performed as a baseline work-up.

A 100 - 500µl of fresh whole blood collected in an edta bottle was used for the analysis based on total wbc count of the patient on presentation. Sample was prepared on the working bench which involved bringing the reagents to room temperature, vortex of proteinase k reagent mixture and incubation. The mixture was then transferred into the genexpert cartridge using a micropipette.

The GeneXpert systems automate and integrate sample purification, nucleic acid amplification, and detection of the target sequence once the cycle threshold was reached.

A cycle usually involves three-step processes, namely:

- 1). The Denaturation step which divides the DNA strands at high temperature.
- 2). An Annealing step in which a primer was needed by the polymerase to amplify the DNA. The primer will bind to the DNA or cDNA sequence if complementary.
- 3). The Extension step, where the DNA strands will be extended.

STATISTICS

Data obtained were analyzed using GraphPad Prism software version 9. We did descriptive statistics, used student t-test to compare frequencies of occurrences, and Pearson's correlation coefficient to analyze correlation. Statistical significance was set at $P = 0.05$, and results were presented in tables and figures.

RESULTS

Of the 65 patients studied, 37 were males (56.9%) and 28 females (43.1%) with male to female ratio of 1.3:1. The mean age was 45.22 ± 15.44 with a range of 17-92 years. The age groups mainly affected were 39 – 49 years with 20 patients (30.8%) while civil servants were the most affected occupation with 22

patients (33.8%). The religious group affected most were Christians 61 (93.8%). Married patients constitute most of the cases 47 (72.3%). Igbo tribes were

predominantly affected by the disease 50 (76.9%) while Enugu State has the highest incidence 20 (30.8%) as shown in table 1.

Table-1: Demographic characteristics of the patients

	Frequency	Percent
Age group (years)		
≤27	9	13.8
28 - 38	14	21.5
39 - 49	20	30.8
50 - 60	11	16.9
61 - 71	8	12.3
>71	3	4.6
Gender		
Female	28	43.1
Male	37	56.9
Marital Status		
Married	47	72.3
Single	18	27.7
Occupation		
Civil servant	22	33.8
Farmer	4	6.2
Trader	15	23.1
Student	14	21.5
Artisan	1	1.5
Driver	9	13.8
Religion		
Christian	61	93.8
Moslem	4	6.2
Tribe		
Igbo	50	76.9
Hausa	2	3.1
Ikwere	5	7.7
Efik	2	3.1
Kalabari	2	3.1
Idoma	1	1.5
Berom	3	4.6
State of Origin		
Abia	3	4.6
Rivers	7	10.8
Akwa Ibom	2	3.1
Benue	1	1.5
Ebonyi	2	3.1
Enugu	20	30.8
Anambra	12	18.5
Imo	13	20.0
Nasarawa	2	3.1
Plateau	3	4.6

The commonest presenting complaint was abdominal swelling 57 (87.7%) followed by weight loss

53 (81.5%), easy fullness/satiety 32 (49.2%) and headache 15 (23.1%) as shown in figure 1. below.

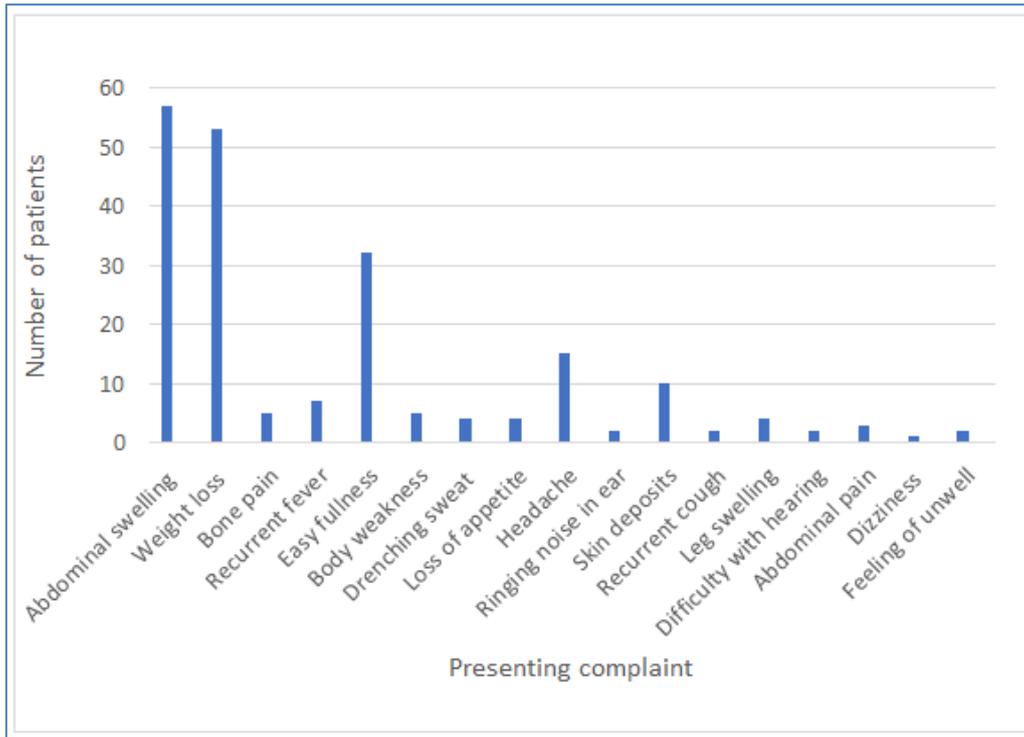


Fig-1: Distribution of presenting complaints

Haematological profile showed mostly normochromic normocytic anaemia on blood film, mean PCV of $30.12 \pm 4.07\%$, mean total WBC $207.37 \pm 87.57 \times 10^9/L$, mean absolute neutrophil count of $100.82 \pm 53.58 \times 10^9/L$, mean absolute eosinophil count

of $6.09 \pm 5.56 \times 10^9/L$ and mean platelet count of $458.60 \pm 133.77 \times 10^9/L$ while the *BCR-ABL 1* transcript has a mean of $41.02 \pm 24.54\%$ (IS) as shown in table 2 below.

Table-2: The Descriptive Statistics of the haematological and BCR-ABL 1 parameters

	N	Minimum	Maximum	Mean	Std. Deviation
Packed Cell Volume in %	65	18	41	30.12	4.075
Total White Cell x $10^9/L$	65	102	585	207.37	87.570
Absolute Neutrophil x $10^9/L$	65	7	322	100.82	53.584
Absolute Lymphocyte	65	0	164	50.12	33.707
Absolute Monocyte	65	0	23	4.43	4.179
Absolute Eosinophil	65	0	39	6.09	5.564
Absolute Basophil	65	0	22	5.85	4.922
Myeloblast	65	0	50	4.35	7.759
Promyelocyte	65	0	65	6.09	9.799
Myelocyte	65	0	100	10.66	14.846
Metamyelocyte	65	0	32	7.37	6.820
Band	65	0	58	8.62	12.350
Total Platelet x $10^9/L$	65	66	879	458.60	133.773
BCR-ABL1 in % (IS)	65	0.01	90.0	41.02	24.54

A significant correlation was observed between the total WBC count and *BCR-ABL1* transcript level $r = -180$, $P = 0.015$.

Chronic phase was the predominant form of the disease 56 (86.2%) followed by accelerated phase which was seen in 5 (7.7%) patients. Refer to figure 2.

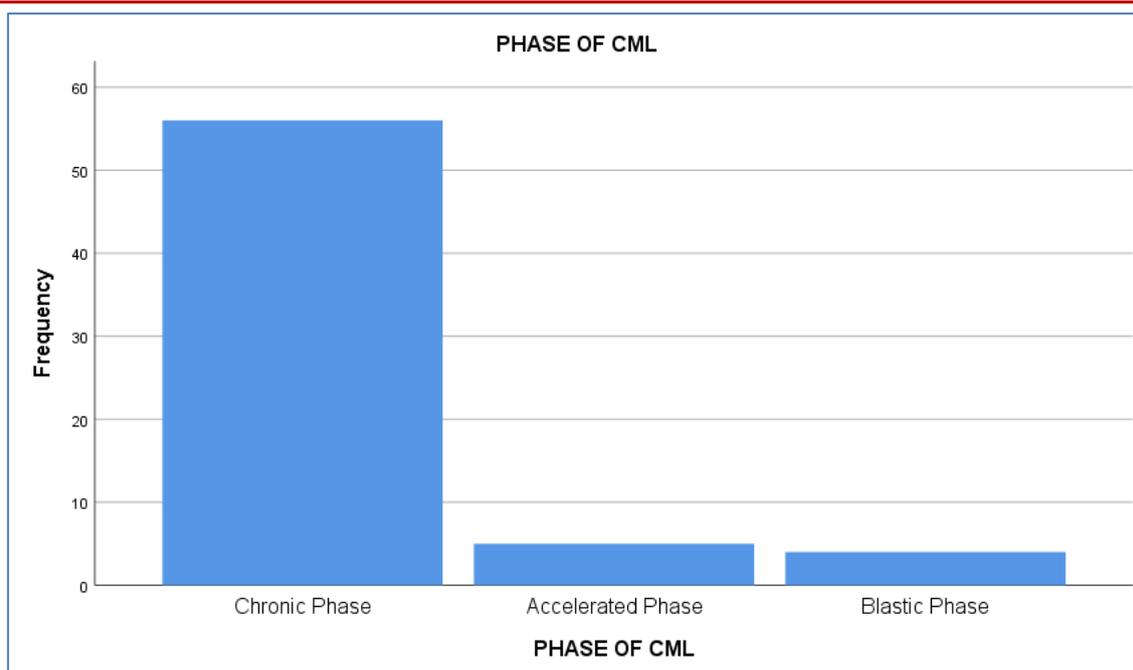


Fig-2: Bar chart showing the distribution of the triphasic state of CML in the patients.

Renal and Liver assessments showed raised mean uric acid level of 1.99 ± 1.18 mmol/L and raised mean alkaline phosphatase, aspartate and alanine

transaminases as shown in tables 3a and 3b respectively.

Table-3a: Descriptive statistics of renal assessment in the study cohort

	N	Minimum	Maximum	Mean	Std. Deviation
Sodium	65	127.0	152.0	142.446	4.5209
Potassium	65	2.3	5.9	4.258	.6405
Bicarbonate	65	16.0	35.0	25.738	3.9302
Chloride	65	94.0	113.0	102.954	4.2110
Urea	65	2.5	14.5	5.420	1.6421
Creatinine	65	64.0	339.0	105.092	36.1286
Uric Acid	65	.2	7.5	1.997	1.1825
Inorganic Phosphate	65	1.0	1.9	1.329	.2044
Valid N (listwise)	65				

Table-3b: Descriptive statistics of Liver assessment in the study cohort

	N	Minimum	Maximum	Mean	Std. Deviation
Total Bilirubin	65	3.2	20.0	12.488	4.1441
Conjugated Bilirubin	65	1.5	8.5	6.166	1.7408
Alkaline Phosphatase	65	19.0	246.0	68.446	39.3057
Aspartate Transaminase	65	4.0	45.0	16.746	9.3936
Alanine Transaminase	65	4.0	33.0	13.094	7.1332
Total Protein	65	65.0	85.0	77.646	4.7085
Albumin	65	40.0	54.0	49.015	3.3236
Globulin	65	20.0	40.0	28.785	3.4255
Fasting Blood Glucose	65	3.0	7.2	4.403	.7374
Valid N (listwise)	65				

Viral screen showed that 3 (4.62%) of the cases were positive for Hepatitis B and C while 1(1.54%) was positive for retroviral disease respectively. Imaging studies revealed that massive splenomegaly was the commonest finding 60 (92.3%) patients followed by cardiomegaly 12 (18.5%) patients

respectively. Significant correlation was observed between the age of patients and *BCR-ABL 1* transcript level $r = -296$, $P = 0.017$. No relationship existed between spleen size and *BCR-ABL 1* transcript level $r = 106$, $P = 0.399$.

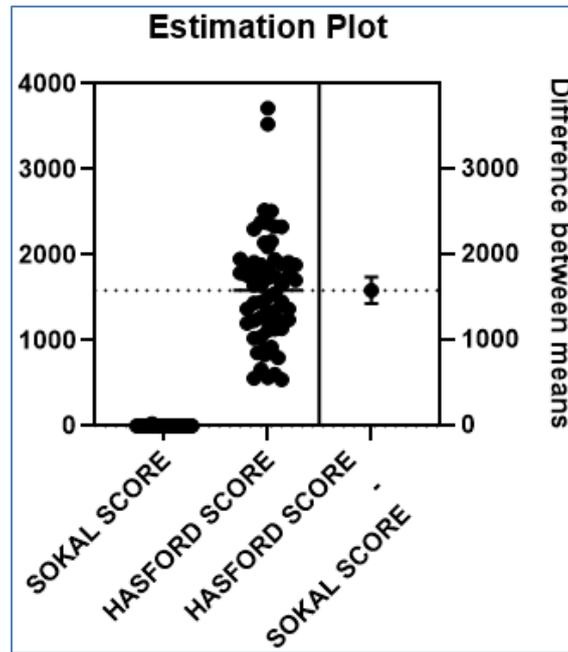


Fig-3: Forest plot showing comparison between risk assessments of cases on presentation across the two categories of scoring system

Risk assessment of cases on presentation using Sokal and Hasford scores were statistically significant (t

$= 20.23$, $P < 0.0001$), [CI = 1429 to 1739] as shown in figure 3.

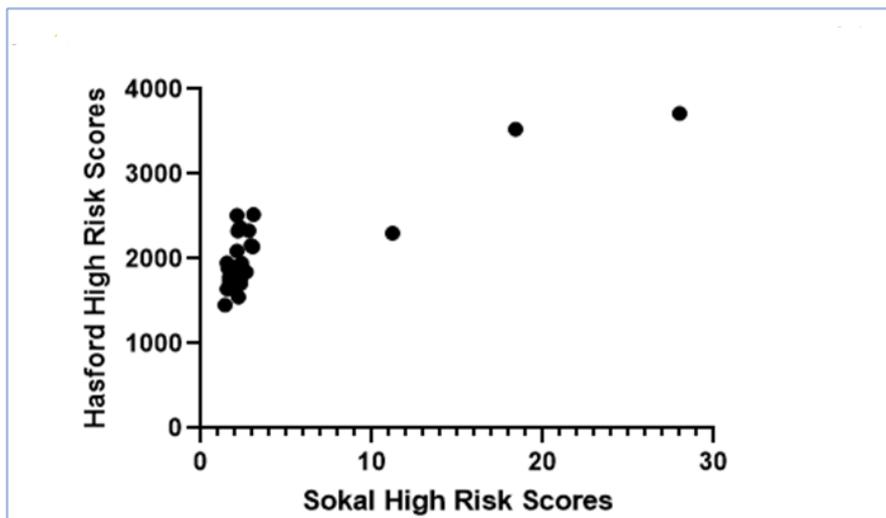


Fig-4: SCAtter plot showing relationship of high risk scores on presentation

On presentation, the mean Sokal score was 2.49 ± 4.03 in 48 cases (73.8%), and mean Hasford score was 1586.23 ± 631.20 in 36 (55.38%) respectively. There was significant correlation between Sokal high risk and Hasford high risk scores on presentation of the study cohort $r = 0.8470$, $P < 0.0001$. CI = 0.7131 to 0.9213 as shown in figure 4.

DISCUSSION

Results obtained from this study showed that of the 65 subjects with chronic myeloid leukaemia evidenced by detection of *BCR-ABL1* transcript in their whole blood, 37 were males (56.9%) and 28 females

(43.1%) with male to female ratio of 1.3:1 as shown in table 1. This finding was consistent with works done by Huang *et al.* [4], Siegel *et al.* [5], Sant *et al.* [6], Smith *et al.* [7] and Chen *et al.* [8]. The mean age was 45.22 ± 15.44 with a range of 17-92 years. The age groups mainly affected were 39 – 49 years with 20 patients (30.8%). These support the works done by Chen *et al.* [8] and Kamada *et al.* [12] and it may be because the risk of getting CML increases with age as it has been observed that the more patient gets older the more the transcript copies.

Significant correlation therefore was observed between the age of patients and their *BCR-ABL 1* transcript level $r = -296$, $P = 0.017$.

Civil servants were the most affected occupation with 22 patients (33.8%). This finding may support the works done by Moloney *et al.* [9] and Hoffman *et al.* [10] in which they stated that exposure to ionizing radiation has remained the only risk factor to the development of the disease. In line with this thought, it is worthy to note that the bulk of workers in the radiation department of most medical facilities are civil servants in the rank of Consultant Radiologist, Consultant Radiotherapist, Radiographers and X-ray technicians hence the risk of exposure may be high. The religious group affected most were Christians 61 (93.8%). This finding could be attributed to the fact that the location of this study was inhabited predominantly by Christians. Married patients constitute most of the cases 47 (72.3%). This can be because most married individuals are within 39 to 49 years age group. This finding also did not corroborate with works done by Björkholm *et al.* [11] in which they stated that there is no known familial disposition to the disease. Igbo as a tribe being predominantly affected by the disease 50 (76.9%) may be attributed to the location of the study which is an Igbo-dominated region. The latter may also explain why Enugu State has the highest incidence 20 (30.8%). These observations are clearly shown in table 1.

The commonest presenting complaint was abdominal swelling 57 (87.7%) followed by weight loss 53 (81.5%), easy fullness/satiety 32 (49.2%) and headache 15 (23.1%) as shown in figure 1. These findings are in keeping with works done by Kamada *et al.* [12], Rowe *et al.* [13], Faderl *et al.* [14] and Thompson *et al.* [15]. However, it is important to know that at diagnosis, the clinical findings vary and depend mainly on the stage of the disease.

The haematology profile and *BCR-ABL1* transcript levels showed evidence of normochromic normocytic anaemia predominantly which supported the work done by Spiers *et al.* [21], hyperleukocytosis, raised absolute white cell differential counts, thrombocytosis on peripheral blood film examination, and elevated transcript levels respectively. A significant correlation was observed between the total WBC count and *BCR-ABL1* transcript level $r = -180$, $P = 0.015$. These findings are corroborated with works done by Faderl *et al.* [14], Thompson *et al.* [15] and Shimkin *et al.* [16]. This is because the protein product of this *BCR-ABL1* fusion gene involved the enzymatic domain from the normal *ABL1* that has tightly regulated tyrosine kinase catalytic activity. However, with the formation of the fusion gene the kinase activity of *BCR-ABL1* is enhanced due to the fusion with a portion of *BCR*. The deregulated tyrosine kinase activity therefore drives the uncontrolled proliferation of the cells of the

granulocytic series as seen in patients with CML. The major factors that drive this uncontrolled proliferation are the ability of the cells to escape apoptosis, their altered interaction with the cellular matrix and the consequent discordant maturation.

Chronic phase was the predominant form of the disease 56 (86.2%) as was observed in this study (figure 2). This finding was consistent with works done by Lichtman *et al.* [17], Preston *et al.* [18] and Scott *et al.* [19]. This finding is because chronic phase is usually a smoldering stage of the disease in which most patients look apparently healthy, asymptomatic or has mild symptoms. This is the reason most findings are incidental during routine medical checks or following fitness test for employment, with low proportions of blasts (less than 10%) in blood and bone marrow.

Assessment of the patients' renal function on presentation showed some evidence of dys-electrolyteamia and raised urea level (table 3a). Renal dysfunction attributable to leukemic infiltration is usually rare. However, when it does occur, suggestions are that the diffuse infiltration compresses the microvasculature and renal tubules thereby causing intrarenal obstruction and tissue ischemia. The raised plasma uric acid level observed in the study cohort on presentation may be due to overproduction from excessive blood cell breakdown (table 3a).

Liver derangement was also observed on patients' presentation (table 3b). This may be because three of the study cohort was positive to Hepatitis B and C on presentation and these may have caused significant damage to the liver parenchyma.

Imaging studies done on the patients on presentation showed that massive splenomegaly was the commonest finding, and these were consistent with works done by Kamada *et al.* [12] and Rowe *et al.* [13] and Thompson *et al.* [15]. This was a consequence of extramedullary haemopoiesis.

On presentation, following the detection of *BCR-ABL 1* transcript, the patient's risk and prognosis were assessed using the Sokal, Hasford and EUTOS risk assessment scoring systems. The scores which were based on age in years, spleen size, blast %, platelet, eosinophil, and basophil counts were used to categorize the patients into different risk groups according to works done by Sokal *et al.* [28] and Hasford *et al.* [29]. The enhanced tyrosine kinase activity associated with *BCR-ABL 1* fusion gene remains the reason why tyrosine kinase inhibitors (TKIs) are used for the treatment of CML Hochhaus *et al.* [30]. However, our patients did not receive a monitored treatment because upon confirmation of diagnosis on presentation they were referred to Obafemi Awolowo University Teaching Hospital, (OAUTH) Ile-Ife Nigeria, for enrollment into the free Glivec International Patient

Assisted Programme (GIPAP). In resource-poor countries, the high cost and unavailability of TKIs have made it difficult for most of our patients to be able to access the drugs as such most patients depended on hydroxyurea and or GIPAP for those that had the logistic means.

CONCLUSION

There was improved diagnostic yield of cases of CML using the molecular diagnostic technique. The data obtained in this study can help physicians make better and quicker therapeutic decisions while managing this disease.

RECOMMENDATION

We recommend the establishment of a second center in the Southern Nigeria for the free Glivec International Patient Assisted Programme because of the financial and logistics challenges our patients go through to access the drug. It will also help us to monitor closely the patients clinical and laboratory outcomes while on treatment. We strongly recommend further research on chronic myeloid leukaemia to elucidate more facts that will guide haematologists in the treatment of the disease. Financial constraint was a major limitation encountered during this study.

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DECLARATION

Authors have declared that no competing interests exist.

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