Expression of *TP53* deletion and Clinical Presentations of Nigerians with Chronic Lymphocytic Leukaemia: A Preliminary Report

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Background:

The prevalence of chronic lymphocytic leukaemia (CLL) among Africans is unknown (WHO 2024) [1, 2], but the mean incidence of CLL in Southwestern Nigeria was estimated to be 0.24/100,000 over a five-year period from 2014 to 2018 (iwCLL 2019; abstract 2013). [3] In Nigeria, the diagnosis of CLL has been largely dependent on peripheral blood film morphology of lymphocytes and histology of lymph nodes and/or bone marrow biopsies. Immunophenotypical profiling may be obtained with the use of immunohistochemical (IHC) tests, which are expensive and not readily available. A fluorescence *in-situ* hybridisation (FISH) laboratory was established to bridge the gap due to inequity in the availability of prognostication tools for CLL in Nigeria. This study was also aimed at determining the expression of *TP53* deletion in this small cohort of patients, its relationship to the clinical features at enrolment and response to chemotherapy.

Method:

Sixteen (16) patients with CLL from four centres were investigated over a period of 16 months between 2022 and 2023. Clinical data were collected using clinical report forms (CRF) on REDcap database, venous blood samples were obtained for full blood count (FBC) and cytogenetics analysis. The FBC were obtained with the use of a 5-part Mindray auto-analyser. A fluorescence-*in-situ* hybridization (FISH) laboratory was established through a National Research Fund (NRF) grant from the Tertiary Education Trust Fund (TETfund), Nigeria. Chromosomes of interphase mononuclear cells were harvested from peripheral blood within 6-10 hours of collection. The probe of interest, *TP53* deletion was hybridized to complementary DNA sequences using standard operating procedures for FISH. A total of 100 nuclei were scored from each slide and reported with the use of 4',6-diamidino-2-phenylindole (DAPI)/fluorescein isothiocyanate (FITC)/Texas Red filters in a fluorescence microscope (KFL 40LED, EPI-Fluorescent Microscope). Positive slides were confirmed by experts at OGT, Cambridge, UK, and doubtful slides were repeated. An expected abnormal signal pattern was defined as at least 10% of 1R2G and/or 1R1G signals. Data was analysed using descriptive and inferential statistics; $p < 0.05$ defined the level of significance.

Results:

The majority (68.7%) of patients were under the age of 65 years (range - 49 to 80 years). Males were not significantly more than females (M: F ratio of 1.67: 1 ($p = 0.317$), but females were significantly older than males [74.67 (±4.27) versus 64.50 (±10.6); p=0.044]. Half (8) of the patients enrolled were treatment naïve, and the majority (87.5%) were symptomatic in Binet Stage C. The common presenting clinical features were pallor in 11 (68.7%); splenomegaly in 11 (68.8%); and lymph node enlargement in 9 (56.2%); and fatigue in 8 (50.0%; Figure 2). Two (12.5%) patients had WHO performance status of 0, while 8 (50%) had a score of 1; and 6 (37.5%) had a score of 2. Males had a significantly higher performance status score than females ($p = 0.024$). Binet Stage A was seen in 2 (12.5) patients; while 14 (87.5%) patients were in Binet Stage C of whom eight (57.1%) had fatigue, six (37.5%) had unplanned weight loss, and four (28.6%) had fever. The white cell count ranged from 5,600/ µL to 164200/ µL. Anaemia (Hb less than 10g/dL) occurred in 9/16 (56.2%) and thrombocytopaenia occurred in four patients (25%; Figure 4). Two (12.5%) patients had *TP53* deletion, and one of them had two subclones of nuclei identified (Figure 6). There were no significant differences in the haematology profile between patients with or without *TP53* deletion and between males and females. The 14 (87.5%) patients who were treated received chemotherapy as none could afford immunotherapy (Figure 7). The median overall survival at 38 months after commencing the study was short (32 ± 10.7 months), and it was not influenced by the *TP53* deletion status (p=0.738)., the Binet Stage at diagnosis (p=0.053)., previous history of treatment (p=0.680; Figure 8), or type of chemotherapy (p=0.393).

Discussion:

This study investigated 16 patients with CLL for the expression of *TP53* deletion using the FISH procedure for the first time in Nigeria. The demographic data of the patients were similar to those previously published in Nigeria, but females were significantly older than males (p=0.044; Figure 2). [3] Most patients in this region are symptomatic at diagnosis and investigations of the patients in this cohort showed that pallor, splenomegaly, lymph node enlargement, and fatigue (Figure 3) were more common. Constitutional B symptoms are not often reported in literature for CLL in this region, however, it is not surprising that fatigue occurred in up to 50% of the patients in this study, since the majority presented late in Binet Stage C (as previously reported), but unplanned weight loss and fever were not as common. The WHO performance status was worse among males (1 to 2) than females (0 to1; p = 0.024), and different from those reported by Eek *et al.*, 2021 [4]. Rai Stages 3 (anaemia) and/or 4 (thrombocytopenia), i.e. Binet Stage C, were not as common in this small cohort of patients as previously reported in this region but they are rarely observed in data from resource rich settings [5, 6]. This preliminary study reports that *TP53* deletion occurred in 12.5% of patients with CLL similar to reports from non-African populations, but the population size of this study is small. The lack of a relationship between the *TP53* deletion status and the clinical and laboratory features may also be due to the population size of this study in addition to the presence of other features, such as, IGHV gene, *TP53,* and NOTCH-3 mutations, etc., which were not investigated, and could have had a significant influence on the relationships between these parameters. [7, 8] Less than one fifth of the patients could afford fludarabine-based regimens in this cohort and the chemotherapy regimens administered did not influence the outcome of treatment. The overall survival of these patients was not influenced by the Binet Stage or treatment exposure at diagnosis, neither the *TP53* deletion status (Figure 8) which are at variance to reports from elsewhere. [7] This may probably be due to other factors or markers that were not investigated in this study as stated earlier. The small sample size is a limitation of this preliminary study, which has been expanded to include the other biomarkers of CLL in a larger on-going study.

Conclusion:

The establishment of the first FISH laboratory in Nigeria has made it possible to detect the presence of *TP53* deletion in two of the 16 patients investigated. The clinical features of this small cohort were similar to those previously reported in the region, but had no relationship with *TP53* deletion status, probably because the numbers were few. This study shows that the response to chemotherapy and median overall survival were short and independent of the *TP53* deletion status, thus suggesting that other prognostic biomarkers, such as unmutated IGHV gene and *TP53* mutation(s) that were not investigated, might have influenced this result. The availability of *TP53* deletion test would reduce the inequity in prognostication of CLL in this region and detect patients who would benefit from targeted-pathway therapy and improve the quality of life and median overall survival of these patients. The result of a larger cohort of patients in this setting investigating these observations further is being awaited.

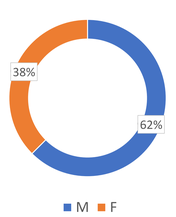

Figure 1: Sex distribution of the cohort of patients

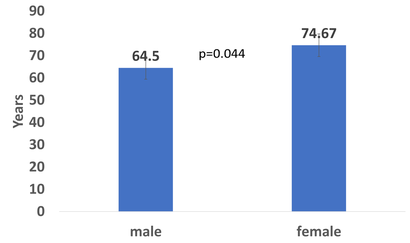

Figure 2: Mean ages of male and female patients

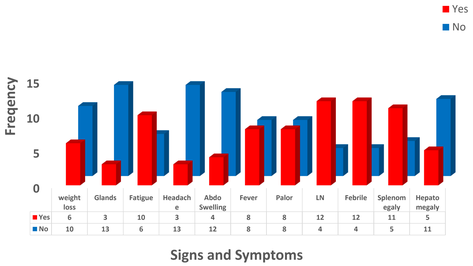

Figure 3: Clinical Features of Patients Enrolled

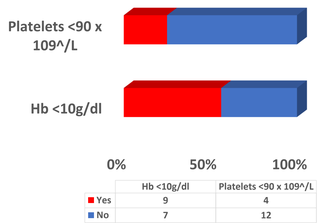

Figure 4: Percentages of patients with or without haemoglobin less than 10g/dl or platelet count less than 90 x 10^9/L

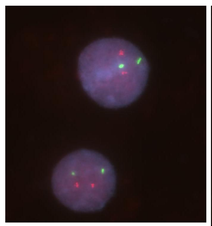

Figure 5: Expected 2R2G signals

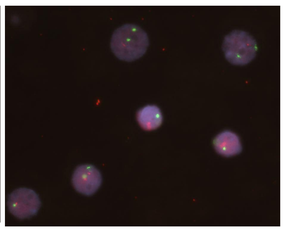

Figure 6: Two subclones of nuclei signalling *TP53* deletion in a patient

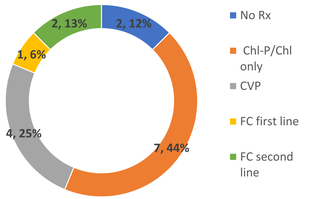

Figure 7: Treatment Regimens Available

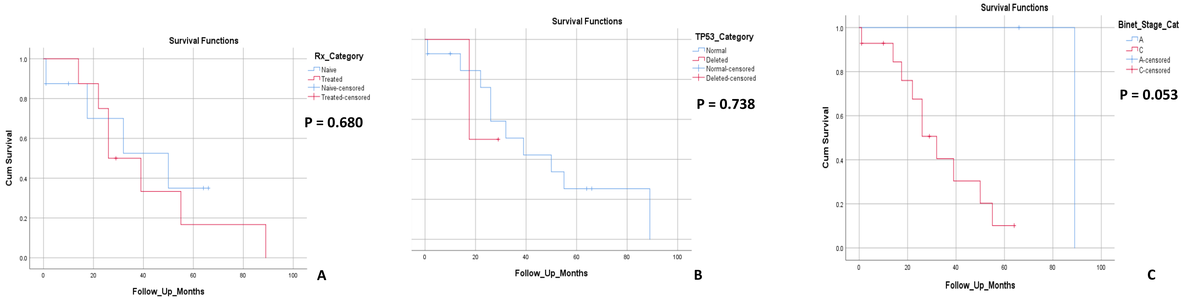

Figure 8: Survival curves for patients who were treatment naïve or pretreated (A), with or without *TP53* deletion (B), and with Binet Stage A or C (C) , at 38 months of the study

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