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# Prevalence and Clinical Significance of SF3B1 Mutation in Sudanese Patients with Chronic Lymphocytic Leukemia

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## Abstract

**Background**: The *SF3B1* gene encodes a key component of the spliceosome and is recurrently mutated in chronic lymphocytic leukemia (CLL). These mutations have been linked to adverse prognosis and advanced disease stages in Western populations, but data from African cohorts remain scarce.

**Objectives**: To determine the prevalence of *SF3B1* mutations in Sudanese patients with CLL and assess their associations with demographic, clinical, hematologic, and morphological features.

**Methods**: A cross-sectional study was conducted on 100 treatment-naïve CLL patients. Clinical staging was evaluated using Rai and Binet systems. Mutation detection was performed by allele-specific PCR. Hematologic indices and morphology parameters, including smudge cells, prolymphocytes, and composite morphology index (CMI), were compared between mutated and wild-type cases.

**Results**: *SF3B1* mutations were detected in 14% of patients. Mutated cases had significantly higher white blood cell counts (86.1 vs. 69.1 $\times 10^9$/L, p=0.027) and were more likely to present with advanced Rai stage (adjusted OR 2.58, p=0.031). Morphologically, *SF3B1* mutations were associated with prolymphocytic features (35.7% vs. 8.1%, p=0.006) and high CMI scores (42.9% vs. 18.6%, p=0.039). No significant differences in hemoglobin or platelet counts were observed.

**Conclusion**: *SF3B1* mutations occur in 14% of Sudanese CLL patients and are independently associated with leukocytosis, prolymphocytic morphology, and advanced Rai stage. These findings reinforce *SF3B1* as an adverse biomarker and support its integration into baseline prognostic evaluation in CLL.

**Keywords:** Chronic lymphocytic leukemia; Mutation; Africa

## 1. Introduction

Chronic lymphocytic leukemia (CLL) is a common hematologic malignancy of mature B cells, characterized by progressive lymphocytosis and accumulation of $CD5^+$ $CD19^+$ lymphocytes in the peripheral blood, bone marrow, and lymphoid tissues [1]. It is the most frequently diagnosed adult leukemia in Western countries and exhibits a strikingly heterogeneous clinical course [2,3]. While some patients remain asymptomatic for years, others progress rapidly with systemic symptoms, immune dysfunction, or cytopenias [4].

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The Rai and Binet staging systems have long served as the cornerstone of CLL risk stratification, classifying patients based on the extent of lymphadenopathy, organomegaly, anemia, and thrombocytopenia [5,6]. Although widely used, these clinical staging frameworks fall short in accounting for underlying molecular heterogeneity, which significantly influences disease progression and therapeutic response [7].

Cytogenetic lesions such as del(13q14), del(11q), trisomy 12, and del(17p) are well-established prognostic markers [8]. Beyond these, next-generation sequencing has identified recurrent somatic mutations—including *SF3B1*, *NOTCH1*, and *BIRC3*—which contribute to disease aggressiveness and therapeutic resistance [9–11]. Among these, *SF3B1* mutations, particularly the hotspot variant K700E, disrupt normal mRNA splicing and are present in 10–15% of CLL cases [12]. These mutations are consistently associated with shorter progression-free survival and increased risk of Richter transformation [13–15].

Despite extensive genomic profiling in Western populations, data from African and Middle Eastern cohorts remain limited. In sub-Saharan Africa, including Sudan, resource constraints and underdiagnosis have impeded the integration of molecular diagnostics into routine care [16]. Local data on *SF3B1* prevalence and its prognostic impact are critically lacking.

This study aimed to estimate the prevalence of *SF3B1* gene mutations among newly diagnosed Sudanese CLL patients and evaluate their association with Rai and Binet clinical staging.

## 2. Materials and Methods

### 2.1. Study Design and Population

A hospital-based, retrospective cohort study was conducted at the Radiation and Isotope Centre Khartoum (RICK) between January and December 2021. Adult patients (≥18 years) newly diagnosed with CLL were included. Diagnosis was based on sustained absolute lymphocytosis and characteristic morphology. Due to resource constraints, flow cytometry was not systematically available. Patients were excluded if they had received prior CLL therapy, had active severe infections, or concurrent malignancies.

### 2.2. Clinical and Laboratory Data

Patient demographics, clinical presentation, and physical examination findings were collected using a structured questionnaire. Clinical staging was assessed using both Rai and Binet systems. Routine investigations included complete blood count (CBC), differential, and peripheral smear morphology. Smear findings (e.g., smudge cells, prolymphocytes, nuclear abnormalities) were aggregated into a Composite Morphology Index (CMI). A subset of smears was double-read by independent evaluators to assess inter-rater reliability.

### 2.3. Sample Collection and DNA Extraction

Peripheral venous blood (7.5 mL) was collected into $K_2$-EDTA tubes. Samples were processed within 6 hours of collection; 2.5 mL was used for CBC and smears, while ~5 mL was reserved for DNA extraction. Genomic DNA was isolated using the QIAamp DNA Blood Mini Kit (Qiagen, Germany), with elution in 100 µL AE buffer. Purity was verified using a NanoDrop ND-1000 spectrophotometer (A260/A280 ratio of 1.8–2.0 considered acceptable).

### 2.4. Molecular Mutation Detection

Mutation analysis targeted four *SF3B1* hotspots: K700E, G742D, K666N, and H662Q, using allele-specific quantitative real-time PCR (qRT-PCR) with TaqMan chemistry. The internal control gene was GAPDH. Assays were performed on an ABI 7500 platform (Applied Biosystems, USA) under the following cycling conditions: 50 °C for 2 min (UNG activation), 95 °C for 10 min, followed by 50 cycles of 95 °C for 15 s and 60 °C for 60 s.

### 2.5. Primer and Probe Sequences

Ct values <35 were interpreted as positive. Ambiguous results were repeated in duplicate. A 20% randomly selected subset underwent Sanger sequencing for validation. For this, *SF3B1* exons 14–16 were PCR amplified and sequenced using BigDye Terminator v3.1 (Applied Biosystems) on an ABI 3500 platform.

**Table Provide caption to the table**

| Mutation | Primer/Probe | Sequence (5′→3′) | Amplicon Size | Reference |
|---|---|---|---|---|
| SF3B1 K700E | Forward | CAGCAGCTCTGGCATCTTCT | 120 bp | [12] |
| | Reverse | TTGAGGACAGGTCAGGAGGA | | |
| | Probe | FAM-TGGTGAGGCTGAGGAGCTGAGG-BHQ1 | | |
| SF3B1 G742D | Forward | AAGGACCTCTGGGAGGAAGA | 110 bp | [13] |
| | Reverse | CCAGCTTCTTGGTGCTGTCT | | |
| | Probe | FAM-CAGCAGGTTGCTGAGCTGGT-BHQ1 | | |
| GAPDH (Control) | Forward | GAAGGTGAAGGTCGGAGTCA | 140 bp | [14] |
| | Reverse | GACAAGCTTCCCGTTCTCAG | | |
| | Probe | FAM-CCAGCCTGCACCACCAACTGCTT-BHQ1 | | |

### 2.6. Statistical Analysis

Data were analyzed using SPSS v25 (IBM Corp., USA) and GraphPad Prism v8. Continuous variables were expressed as mean ± standard deviation (SD) or median (interquartile range, IQR); categorical variables as frequencies or percentages. Comparisons used Welch t-test or Mann–Whitney U test for continuous variables and chi-square or Fisher's exact test for categorical data. Logistic regression assessed associations with advanced-stage disease (Rai III–IV), adjusting for age, sex, hemoglobin, WBC count, *SF3B1* status, and prolymphocyte scores.

Model performance was evaluated using ROC curve analysis (AUC), calibration slope, Brier score, and Hosmer–Lemeshow test. Internal validation was performed via 1,000-bootstrap resampling.

### 2.7. Quality Control and Ethics

All qPCR assays included positive/negative controls and duplicate runs for quality control. The study was approved by the RICK Ethics Committee (Ref. 2021-CLL-01), and written informed consent was obtained from all participants, in accordance with the Declaration of Helsinki.

## 3. Results

### 3.1. Characteristics of study participants

A total of 100 treatment-naive Sudanese patients with CLL were included. The mean age was 59.0 ± 11.2 years (range 33–85), with a male predominance (62%). Two-thirds of patients resided in urban areas, and occupational status was heterogeneous. Table 1 summarizes the baseline demographics of the cohort.

**Table 1** Baseline cohort characteristics (N=100)

| Variable | Value |
|---|---|
| Age, mean ± SD (range) | 59.0 ± 11.2 (33–85) |
| Male sex, n (%) | 62 (62.0) |
| Female sex, n (%) | 38 (38.0) |
| Urban residence, n (%) | 67 (67.0) |
| Rural residence, n (%) | 33 (33.0) |
| Employment status | Employed 38 Other/ Unemployed 62 |

### 3.2. Clinical Staging

According to Rai staging, 73% of patients were stage 0–II and 27% were stage III–IV. By Binet classification, 52% were stage A, 31% stage B, and 17% stage C. The presence of *SF3B1* mutation was associated with a significantly higher likelihood of advanced Rai stage (III–IV) (P = 0.01). This association remained significant after multivariable adjustment (P = 0.03). No other significant associations detected. Table 2 details the clinical stage distribution.

**Table 2** Clinical staging distribution of study participants

| Staging system | Distribution |
|---|---|
| Rai stage 0–II | 73 (73.0%) |
| Rai stage III–IV | 27 (27.0%) |
| Binet A | 52 (52.0%) |
| Binet B | 31 (31.0%) |
| Binet C | 17 (17.0%) |

### 3.3. *SF3B1* Mutation Prevalence

*SF3B1* mutation was identified in 14 (14%) of patients. qPCR median Ct value was (28.1 (26.9–29.4). Figure 1 summarize the mutation frequencies.

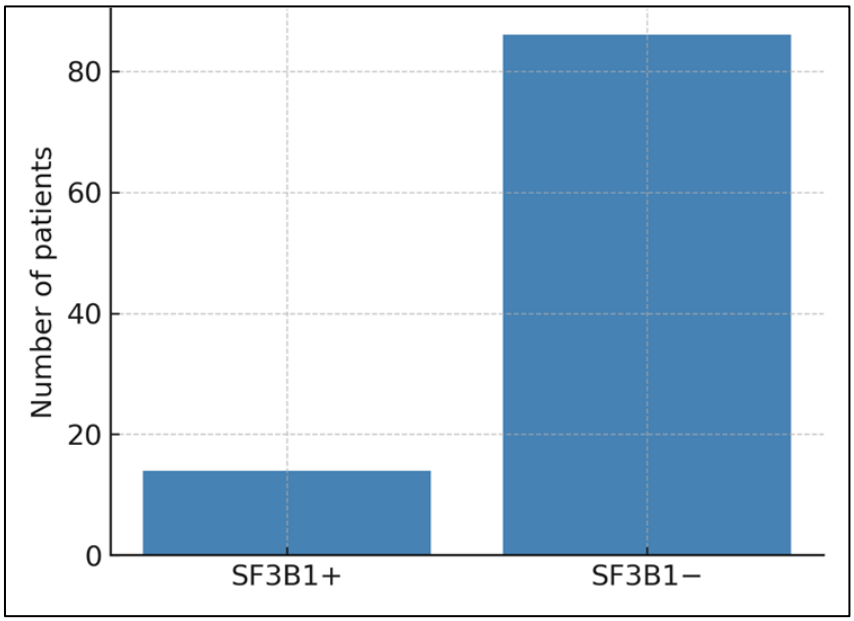


**Figure 1** Prevalence of SF3B1 gene mutation among participants

### 3.4. CBC Indices

Patients with *SF3B1* mutations had significantly higher WBC counts compared to wild-type cases (86.1 vs 69.1 $\times 10^9$/L, p=0.027). Hemoglobin levels tended to be lower (10.8 vs 11.7 g/dL, p=0.076) and platelets trended lower, though not statistically significant. Table 3 & Figure 2 provide the detailed CBC comparisons.

**Table 3** CBC indices by SF3B1 mutation status among participants

| Parameter | SF3B1+ mean ± SD | SF3B1− mean ± SD | Δ (95% CI) | p-value |
|---|---|---|---|---|
| WBC ($\times 10^9$/L) | 86.1 ± 31.2 | 69.1 ± 27.9 | +17.0 (2.0–32.0) | 0.027 |
| Hb (g/dL) | 10.8 ± 1.9 | 11.7 ± 2.1 | −0.9 (−1.9 to 0.1) | 0.076 |
| Platelets ($\times 10^9$/L) | 151 ± 68 | 171 ± 75 | −20 (−58 to 18) | 0.29 |

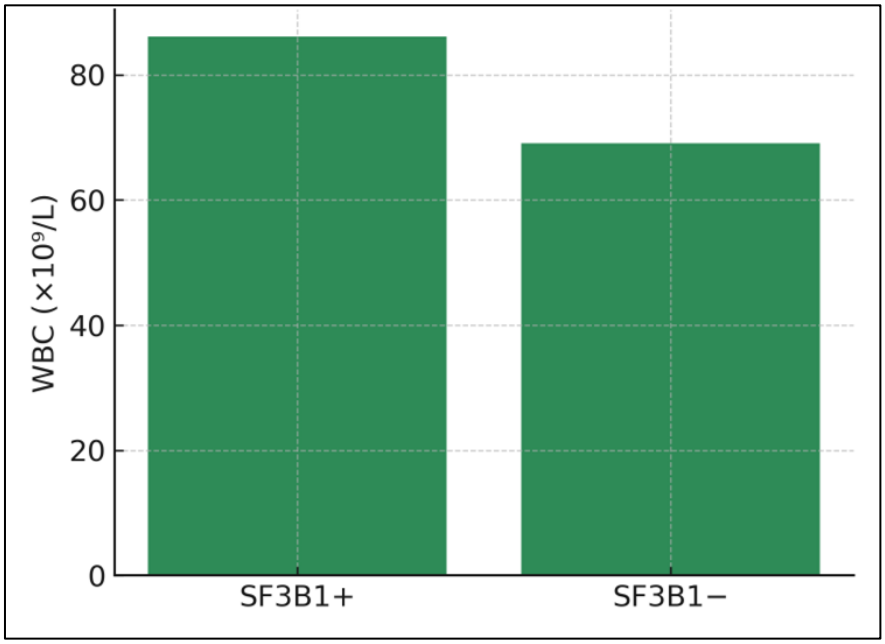


**Figure 2** WBCs count according to SF3B1 gene mutation status among participants

### 3.5. Morphology

Severe smudge-cell fields (Score 2) were frequent (44%), whereas marked prolymphocytic change (Score 2) was less common (12%). A composite morphology index (CMI) classified 22% as high aberrancy, 36% intermediate, and 42% low. Inter-rater agreement was substantial in a blinded subset: κ = 0.78 (95% CI 0.60–0.96) for smudge Score 2 and κ = 0.71 (0.50–0.92) for prolymphocytes Score 2, supporting the internal validity of morphology endpoints. Compared with Rai 0–II, advanced Rai III–IV showed more marked prolymphocytic change (Score 2: OR 4.77, 95% CI 1.45–16.0; Fisher p = 0.006) and more high-aberrancy CMI (OR 2.94, 1.12–7.70; p = 0.023), alongside fewer severe smudge fields (OR 0.36, 0.14–0.90; p = 0.024).

*SF3B1* mutations were strongly enriched in patients with prolymphocytic features and high composite morphology index (CMI). Smudge-cell distribution did not differ significantly. Table 4 summarizes the morphology associations.

**Table 4** Morphology features by SF3B1 status among participants

| Feature | SF3B1+ (%) | SF3B1− (%) | OR (95% CI) | p-value |
|---|---|---|---|---|
| Smudge cells score-2 | 28.6 | 46.5 | 0.47 (0.14–1.56) | 0.25 |
| Prolymphocytes score-2 | 35.7 | 8.1 | 6.25 (1.83–21.3) | 0.006 |
| High CMI (≥3 Score-2) | 42.9 | 18.6 | 3.21 (1.05–9.86) | 0.039 |

## 4. Discussion

In this Sudanese cohort of treatment-naïve CLL patients, *SF3B1* mutations were detected in 14% of cases, which is within the range (10–20%) reported globally [17]. Our findings support the growing evidence that *SF3B1* mutations contribute to adverse biological features and disease progression in CLL.

The significant association between *SF3B1* mutations and advanced Rai stage in our cohort is consistent with large international series that describe *SF3B1* as a high-risk molecular biomarker [18]. Even after multivariable adjustment for blood counts and morphology, the mutation retained independent prognostic value. This underlines its importance alongside TP53 and NOTCH1 as drivers of poor outcomes [19].

Recent meta-analyses have confirmed that *SF3B1* mutations shorten progression-free survival and time to first therapy, independent of IGHV mutation status. The enrichment of advanced Rai stages in *SF3B1*-mutated patients in our study supports these findings and highlights the utility of incorporating *SF3B1* into risk stratification models, especially in regions where access to more comprehensive genomic profiling is limited [20].

In the current study, *SF3B1*-mutated patients had higher leukocyte counts and more frequent prolymphocytic morphology, aligning with the concept that aberrant splicing drives proliferative signaling in malignant B cells. High CMI scores were also enriched in the mutated group, suggesting that *SF3B1* influences cytologic aberrancy beyond traditional staging. Our results echo recent functional studies demonstrating how mutant *SF3B1* generates aberrant RNA isoforms that support cell survival [21,22].

While hemoglobin and platelet counts were lower among mutated patients, these differences did not reach statistical significance, possibly due to limited sample size. Nevertheless, anemia and thrombocytopenia remain clinically relevant endpoints in *SF3B1*-mutated CLL, as shown in larger trials [18].

Therapeutically, *SF3B1* mutations are associated with reduced sensitivity to fludarabine and conventional chemoimmunotherapy. However, novel agents such as BTK and BCL2 inhibitors may mitigate this risk, and real-world data suggest no difference in response rates to venetoclax-based regimens. Still, recent studies highlight that *SF3B1*-associated clonal hematopoiesis can evolve under continuous BTK inhibition, raising concerns about long-term genomic instability.

Emerging approaches aim to exploit the splicing vulnerability of *SF3B1*-mutant cells. Preclinical evidence suggests that splicing modulators and synthetic lethal partners may provide therapeutic opportunities. Integrating such strategies with standard targeted therapy could improve outcomes for this high-risk subset [23-25].

### 4.1. Limitations and strengths

The main limitation of the current study is the modest sample size, which may have reduced statistical power to detect subtle hematologic differences. Nonetheless, strengths include the use of uniform methodology, complete staging data, and internal validation of morphology scoring, which enhance the reliability of associations observed. Importantly, this is the first study to report *SF3B1* mutation prevalence and correlates in Sudanese CLL patients, filling a regional knowledge gap.

## 5. Conclusion

Overall, our findings confirm that *SF3B1* mutations are enriched in advanced-stage disease and correlate with proliferative and prolymphocytic features. These results reinforce the need to integrate *SF3B1* testing into baseline diagnostic workup in CLL, particularly as genomic-guided precision medicine expands in low- and middle-income settings.

## Compliance with ethical standards

### *Disclosure of conflict of interest*

No conflict of interest to be disclosed.

*Statement of informed consent*

Informed consent was obtained from all individual participants included in the study.

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