original reports abstract

Feasibility of Cell-Free DNA Collection and Clonal Immunoglobulin Sequencing in South African Patients With HIV-Associated Lymphoma

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PURPOSE Diagnosis of AIDS lymphoma in low-resource settings, like South Africa, is often delayed, leaving patients with limited treatment options. In tuberculosis (TB) endemic regions, overlapping signs and symptoms often lead to diagnostic delays. Assessment of plasma cell-free DNA (cfDNA) by next-generation sequencing (NGS) may expedite the diagnosis of lymphoma but requires high-quality cfDNA.

METHODS People living with HIV with newly diagnosed aggressive B-cell lymphoma and those with newly diagnosed TB seeking care at Chris Hani Baragwanath Academic Hospital and its surrounding clinics, in Soweto, South Africa, were enrolled in this study. Each participant provided a whole blood specimen collected in cell-stabilizing tubes. Quantity and quality of plasma cfDNA were assessed. NGS of the immunoglobulin heavy chain was performed.

RESULTS Nine HIV+ patients with untreated lymphoma and eight HIV+ patients with TB, but without lymphoma, were enrolled. All cfDNA quantity and quality metrics were similar between the two groups, except that cfDNA accounted for a larger fraction of recovered plasma DNA in patients with lymphoma. The concentration of cfDNA in plasma also trended higher in patients with lymphoma. NGS of immunoglobulin heavy chain showed robust amplification of DNA, with large amplicons (> 250 bp) being more readily detected in patients with lymphoma. Clonal sequences were detected in five of nine patients with lymphoma, and none of the patients with TB.

CONCLUSION This proof-of-principle study demonstrates that whole blood collected for cfDNA in a low-resource setting is suitable for sophisticated sequencing analyses, including clonal immunoglobulin NGS. The detection of clonal sequences in more than half of patients with lymphoma shows promise as a diagnostic marker that may be explored in future studies.

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INTRODUCTION

Nearly 75% of non-Hodgkin lymphomas are diagnosed at advanced stage in sub-Saharan Africa (SSA) with two thirds of patients presenting with poor performance status (≥ 2) and 80% presenting with B-symptoms.[1] Reports from South Africa (SA) suggest that advanced stage, poor performance status, and B-symptoms are more common in people living with HIV (PLWH).[2,3] In 2018, HIV prevalence in adults of age 15-49 years in SA was 20.4%,[4] representing a major public health burden. Despite the introduction of antiretroviral therapy, the incidence of HIV-associated B-cell lymphomas has increased, in part due to improved survival of PLWH.[3,5] Yet, many patients are too sick at the time of diagnosis to receive curative therapy.[6] Delayed diagnosis contributes to advanced disease.

The diagnosis of lymphoma requires a team of specialists including radiologists, surgeons, pathologists, and laboratory personnel to obtain a biopsy specimen and render a diagnosis. In SA, this infrastructure exists, but is grossly overburdened. Additionally, the diagnostic evaluation is often confounded by infections, especially in PLWH. Tuberculosis (TB) is the leading cause of death in PLWH in SA.[7-9] Symptoms of TB, including fever, night sweats, weight loss, and lymphadenopathy, overlap with those of lymphoma. The empiric treatment of TB in PLWH and possible misdiagnosis of TB in patients with lymphoma have been recognized as an important problem in SSA.[6,10-12] Thus, improved understanding of diagnostic delays may help guide strategies to improve outcomes. To that end, a recent review of time to diagnosis in SA found that the longest period of delay occurred between initial presentation to the healthcare

ASSOCIATED CONTENT

Appendix

Author affiliations and support information (if applicable) appear at the end of this article.

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CONTEXT

Key Objective

Can high-quality DNA, suitable for next-generation sequencing, be collected in a low-resource setting using cell-stabilizing tubes?

Knowledge Generated

Whole blood samples collected from patients with HIV-associated lymphoma and HIV patients with tuberculosis displayed similarly high quantity and quality of cell-free DNA. Clonal immunoglobulin was detected in more than half of the patients with lymphoma and none of the patients with tuberculosis.

Relevance

Whole blood collected and processed in a low-resource setting can yield high-quality plasma DNA suitable for sophisticated molecular analysis. Clonal immunoglobulin detection by next-generation sequencing holds promise as a diagnostic marker for lymphoma in this setting that is worthy of further study.

center and until a diagnosis was pathologically confirmed, termed the healthcare practitioner interval; when this interval exceeded 6 weeks, patients were more likely to be diagnosed with late-stage disease.[10] In PLWH diagnosed with lymphoma in SA, the median healthcare practitioner interval was 8-11 weeks.[10,13] One way to prioritize patients presenting with suspicious symptoms for biopsy might involve molecular analysis of plasma cell-free DNA (cfDNA), rationales for which were described in earlier work.[14] Clonal immunoglobulin (cIg) gene rearrangements can be detected in cfDNA in patients with either non-Hodgkin lymphoma or Hodgkin lymphoma (HL),[15-19] a finding that is recapitulated in PLWH diagnosed with lymphoma.[20] The fact that cIg in plasma correlates with lymphoma disease burden[21] and treatment response[18,22] suggests that it may be a sensitive and specific marker.

Over the past several years, a variety of massively parallel sequencing techniques have been applied to the study of cfDNA, particularly tumor-derived circulating tumor DNA. Although the importance of preanalytical variables and processing techniques to ensure high-quantity and high-quality cfDNA is evident,[23,24] there is yet to be such a study focused on immunoglobulin sequencing or PLWH from a low-resource setting. In this pilot study, we set out to investigate whether obtaining high-quantity and high-quality cfDNA is feasible and suitable for sophisticated sequencing analyses in PLWH in Soweto, SA.

METHODS

Study Participants and Ethics

As part of an ongoing Institutional Review Board–approved, cross-sectional study, PLWH with and without lymphoma were recruited through the Perinatal HIV Research Unit located on the campus of Chris Hani Baragwanath Academic Hospital in Soweto, SA. To be eligible for this study, all participants were required to be 18 years or older. Participants with lymphoma were required to have a documented HIV diagnosis and a newly diagnosed aggressive B-cell lymphoma. Participants with TB without lymphoma were identified and recruited at outpatient clinics in Soweto. Potential participants with documented HIV infection and a positive Xpert MTB/RIF Ultra (Cepheid, Sunnyvale, CA) result within the preceding week were contacted by study personnel. After enrollment, monthly follow-up calls were conducted with patients with TB to assess response to TB treatment and to document signs and symptoms suggestive of lymphoma or interval diagnosis of lymphoma. This study was approved by the Johns Hopkins School of Medicine Institutional Review Board (#00107027) and the University of the Witwatersrand Human Research Ethics Committee (#161608).

Specimen Collection

Between October 2018 and October 2019, 10 PLWH with newly diagnosed aggressive B-cell lymphoma (four diffuse large B-cell lymphoma, one plasmablastic lymphoma, one Burkitt lymphoma, and four classical HL) and eight PLWH with newly diagnosed TB were consented and enrolled. Study participants provided whole blood samples and consent for record review. Blood specimens were collected in four 10 mL cell-stabilizing Streck BCT tubes (Streck, Omaha, NE) and transferred by courier at ambient temperatures (6°C-37°C) to a central laboratory in Johannesburg for same-day processing (Fig 1).

Specimen Processing

Blood was centrifuged twice following the manufacturer's protocols. The initial spin was performed at 2,000*g* for 10 minutes at 4°C. Plasma was then separated and centrifuged a second time at 4,000*g* for 20 minutes at 4°C. Plasma that is free of cell debris obtained after the second spin was then frozen at –80°C until subsequent transport. Specimens were shipped to the United States on dry ice. Once received, specimens were stored at –80°C until further processing.

Plasma DNA Isolation

Approximately 10-15 mL of frozen plasma, per sample, was thawed at room temperature. Following this, plasma was

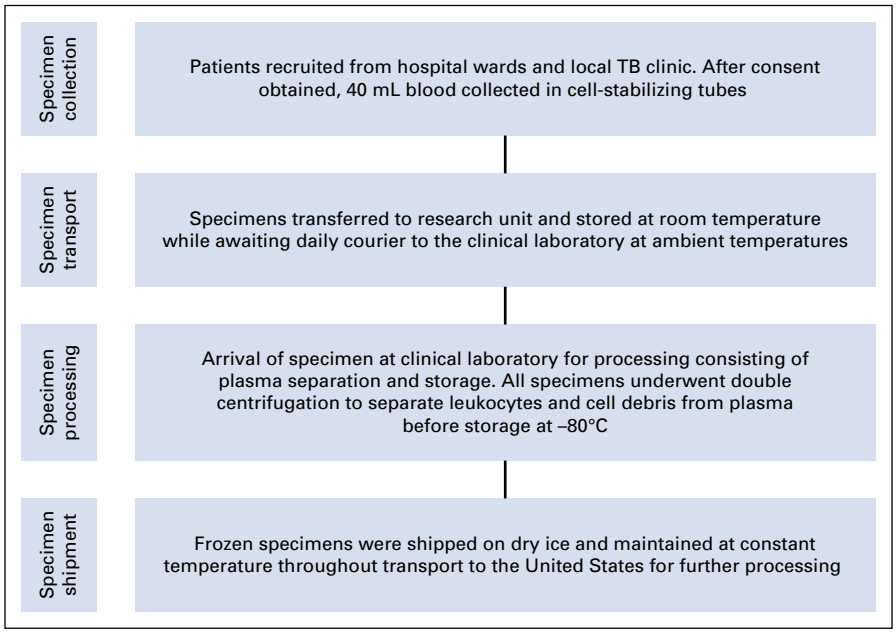

FIG 1. Study design. Sample collection occurred in Soweto/Johannesburg, South Africa. Patients referred from the Hematology Department with newly diagnosed HIV–associated lymphoma were approached at Chris Hani Baragwanath Academic Hospital. Patients with newly diagnosed TB were recruited from the surrounding TB clinics in the Soweto area. After appropriate consent was obtained, 40 mL of blood was collected in cell-stabilizing tubes. Specimens were then transferred to the Perinatal HIV Research Unit located on the hospital campus awaiting daily courier transport to the local clinical laboratory in Johannesburg. After initial processing, specimens were shipped to United States for further processing. TB, tuberculosis.

centrifuged at 4,500*g* for 10 minutes at 4°C. cfDNA extraction was performed using the NucleoSnap DNA Plasma Isolation Kit (Cat#74030; Macherey-Nagel, Bethlehem, PA) following a modified vendor protocol to proportionally adjust for the increased plasma volume being processed. cfDNA was ultimately eluted from spin columns with 80 μL of a 1:1 dilution of Macherey-Nagel Elution buffer in DNAse-free water. Total DNA quantity was assessed by Qubit dsDNA High Sensitivity Assay (Thermo Fisher, Waltham, MA) following vendor specifications. cfDNA quantity and quality were also assessed by the Agilent High Sensitivity TapeStation assay (Agilent Technologies, Santa Clara, CA) according to vendor protocols and analyzed using the TapeStation analysis software (version 3.2). cfDNA was then stored at –20°C until further analysis.

Immunoglobulin Heavy Chain Library Preparation

cfDNA libraries were prepared in duplicate using the LymphoTrack *IGH*-MiSeq (frameworks 1, 2, and 3) panel (Cat#71210129, Invivoscribe, San Diego, CA). Vendor protocols were followed with minor adjustments. To achieve the DNA input requirements within the volume constraints for the assay, samples with lower DNA concentrations (< 10 ng/μL) were vacuum centrifuged to dryness before the addition of master mix. This was then reconstituted to the recommended input volume and combined with master mix. A total of 40 ng or 50 ng of input DNA per framework was used for library preparations in duplicate for a total of up to 240-300 ng of input DNA per sample. Polymerase chain reaction amplification was carried out for 31 cycles according to vendor protocol.

Immunoglobulin Heavy Chain Sequencing

Final amplified libraries up to 100 pM were prepared. All libraries were first pooled according to framework and then combined into a single sequencing pool comprising 40 μL of each of the framework pools. This final pool was denatured for 5 minutes at room temperature using 15 μL of 1N NaOH. Denaturing was stopped by the addition of 15 μL of chilled 1N HCl, 450 μL of chilled HT1 buffer, and 50 μL of denatured 20 pM PhiX control (Cat# FC-110-3001, Illumina, San Diego, CA), resulting in a final sequencing library concentration of 20 pM This final library was sequenced on the Illumina MiSeq using v2 500 cycle kits (Cat# MS-102-2003, Illumina).

Data and Statistical Analyses

Raw sequencing data were processed, aligned, and analyzed using the LymphoTrack analysis software (version 2.4.3). Additional analyses were performed using Microsoft Excel (2016) and GraphPad Prism (Version 8.4.3, GraphPad Software, San Diego, CA). Statistical analyses were performed using GraphPad Prism. Continuous variables were compared using unpaired two-tailed *t* tests, if parametric, or Mann-Whitney tests, if nonparametric. Anderson-Darling and D'Agostino-Pearson tests were used to test for normality.[25-27]

RESULTS

High-Quality and High-Quantity cfDNA Was Obtained

Plasma DNA was isolated from nine patients with lymphoma and eight patients with TB. Plasma from one patient with lymphoma was found to be hemolyzed and was not analyzed. The abundance of cfDNA molecules was evaluated by high-sensitivity electrophoresis, and

representative electropherograms are shown in Figure 2. Fragments measuring within 90-400 bp with characteristic profiles were designated as cfDNA, and fragments larger than 500 bp were designated as genomic DNA (gDNA). There was typically no detectable DNA between 400 and 500 bp. Dominant cfDNA peaks of shorter fragment lengths were readily detected in all samples and likely represent mononucleosomal molecules.[28] Eighty-eight percent of samples (15 of 17) also demonstrated prominent secondary peaks of larger fragment lengths likely representing dinucleosome molecules. One lymphoma sample showed a measurable tertiary peak (data not shown) likely representing trinucleosomal cfDNA molecules.

Quantification of cfDNA showed a trend toward higher concentration of cfDNA (Fig 3A) in patients with lymphoma when compared with patients with TB (median 65.10 ng/mL plasma, 95% CI, 29.40 to 111.3 ng/mL *v* median 26.75 ng/mL plasma, 95% CI, 10.80 to 107.8 ng/mL; $P = .0745$). The concentration of either mono- or dinucleosomal molecules was similarly higher in patients with lymphoma than patients with TB (58.90 ng/mL *v* 18.90 ng/mL, $P = .0580$; 9.350 ng/mL *v* 3.80 ng/mL, $P = .3357$), but the differences were not statistically significant. However, cfDNA was more abundant in patients with lymphoma (Fig 3B) since cfDNA accounted for a median 91% (95% CI, 89 to 96) of all isolated DNA in patients with lymphoma versus 86% (95% CI, 74 to 93) in patients with TB ($P = .0039$). The contribution from either mono- or dinucleosomal molecules to total cfDNA was similar in both groups (median 86% *v* 85% and median 14% *v* 15%, respectively).

With respect to the fragment length of cfDNA molecules, both mono- and dinucleosomal molecules were measured at similar lengths in patients with lymphoma and patients with TB. The median dominant cfDNA molecules were 130 bp (95% CI, 119.0 to 136.0) in length in patients with lymphoma and 139.5 bp (95% CI, 119.0 to 184.0) in patients with TB. The secondary cfDNA molecules had a median measurement of 236.0 bp (95% CI, 205.0 to 287.0) in patients with lymphoma, as compared with 252.0 bp (95% CI, 222.0 to 335.0) in patients with TB. A single lymphoma sample showed a distinct tertiary cfDNA molecule that measured to be 374.0 bp, which comprised 3% of all cfDNA in that sample.

Immunoglobulin Heavy Chain Sequencing Showed Variable Amplification Robustness Correlating With Amplicon Size

The immunoglobulin heavy chain (*IGH*) locus was sequenced using primers that amplify multiple framework regions of the *IGH* variable gene (*IGHV*) (Fig 4) to evaluate for the presence of clg gene rearrangements. Sequencing across three framework regions not only increases sensitivity but also requires the presence of longer fragments of B-cell DNA when primers target framework 1 (FR1) or FR2 regions of the *IGHV* gene. A nonclonal pattern appears as a

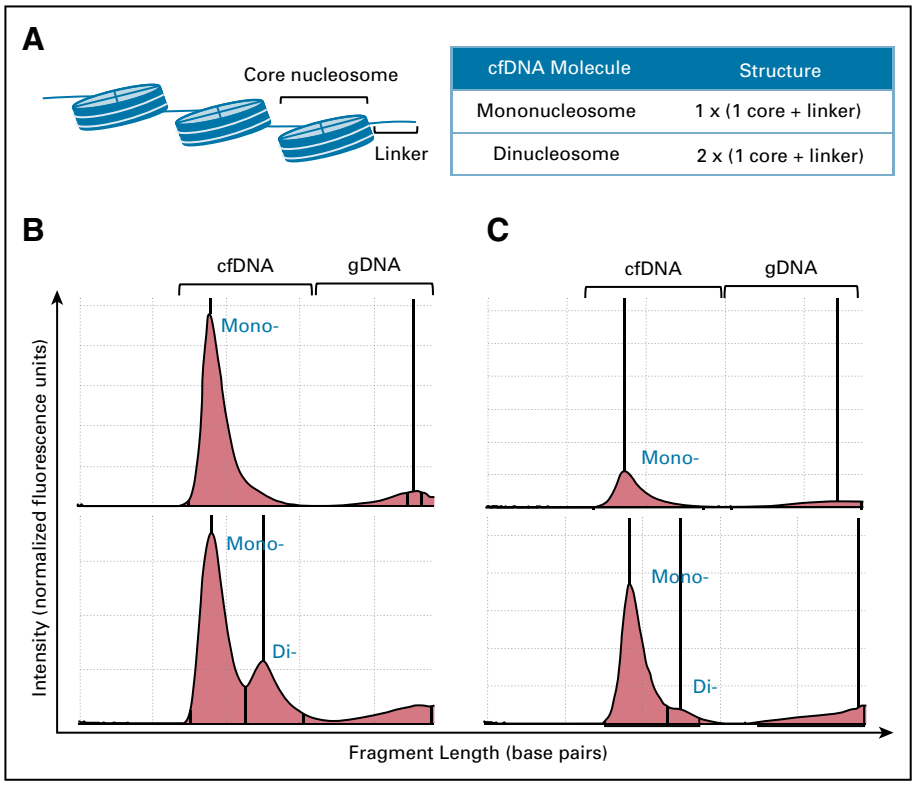

FIG 2. Types of cfDNA molecules found in plasma. (A) Illustration of cfDNA molecules as either mononucleosomal or dinucleosomal structures. Representative high sensitivity electropherograms of plasma DNA from (B) two patients with lymphoma and (C) two patients with tuberculosis without lymphoma demonstrating cfDNA with predominantly mononucleosomal molecules in the top panels and cfDNA with mono- and dinucleosomal molecules in the bottom panels. cfDNA, cell-free DNA; gDNA, genomic DNA.

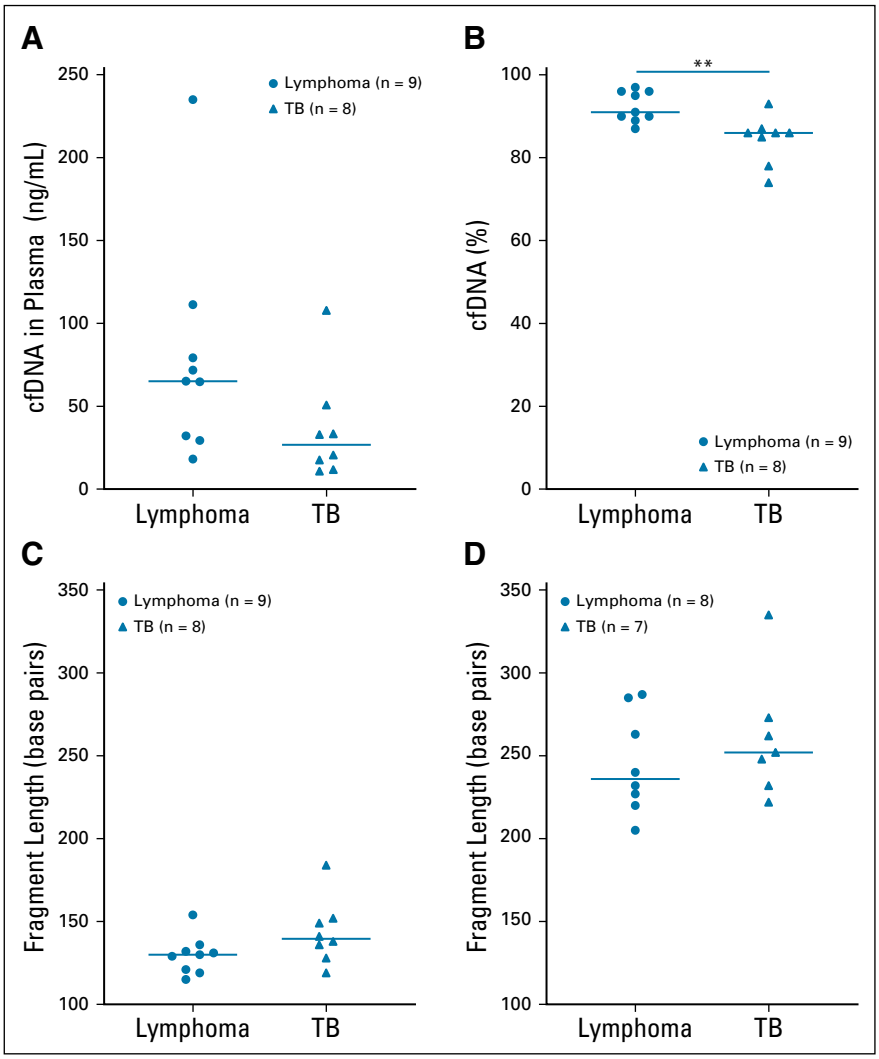

FIG 3. Quantity and quality of plasma cfDNA in patients with lymphoma and TB. (A) Concentration of cfDNA in circulation, as quantified as amount of cfDNA in each milliliter of plasma (median 65.10 ng/mL *v* 26.75 ng/mL). (B) Percent cfDNA as a proportion of all plasma DNA (median 91% *v* 86%, $P = .0039$). Fragment lengths of (C) the dominant mononucleosomal cfDNA molecules (median 130 bp *v* 139 bp) and (D) the secondary dinucleosomal cfDNA molecules (median 236 bp *v* 252 bp). Bars in each graph represent median values. None of the comparisons in A, C, or D reached statistical significance. cfDNA, cell-free DNA; TB, tuberculosis.

polyclonal distribution of unique VDJ sequences (Fig 4B) when separated by amplicon length, whereas a clonal sequencing pattern is represented as a single, or two, dominant VDJ sequence(s) with little-to-no polyclonal sequences (Fig 4C).

To assess amplification robustness of different fragment lengths of B-cell DNA, we compared the frequency distributions of the V_HFR1-J, V_HFR2-J, and V_HFR3-J amplicons based on amplicon length (see Appendix Fig A1 for normality test of the frequency distributions). The profiles obtained from plasma cfDNA in patients either with lymphoma or without (with TB) were compared against the expected profiles obtained from an internal control sample (Fig 5). The internal control is a contrived B-cell gDNA mixture with a variety of polyclonal sequences and no clonal sequence. In gDNA, intact DNA length is not a limiting factor for amplification efficiency; however, length may be a limiting factor in cfDNA. Since cfDNA is generally short, sequences amplified using FR3 primer sets (smallest amplicon) showed the strongest amplification signal in both patients with lymphoma and TB, whereas FR2 and FR1 primer sets (larger amplicons) showed reduced amplification, although there appears to be robust amplification up to 300 bp. Although the amplification profiles obtained from cfDNA from patients with lymphoma and TB did not differ from one another for any of the primer sets, both groups differed significantly from the internal control gDNA for the largest (FR1: lymphoma *v* gDNA $P = .030$; TB *v* gDNA $P = .034$) and smallest (FR3: lymphoma *v* gDNA $P = .006$; TB *v* gDNA $P = .007$) amplicons. This suggests that there are fewer available B-cell DNA fragments of the appropriate size in plasma at either extreme (< 100 bp and > 250 bp). In contrast, the amplification profiles obtained from the intermediate-size amplicon (FR2) showed no difference among the three groups, suggesting adequate abundance of B-cell DNA molecules of approximately 200-250 bp in plasma cfDNA irrespective of diagnosis. Although the amplification profiles from cfDNA did not differ based on diagnosis, the median amplicon length differed significantly for the largest FR1 amplicons. The median amplicon size was 272 bp, compared with

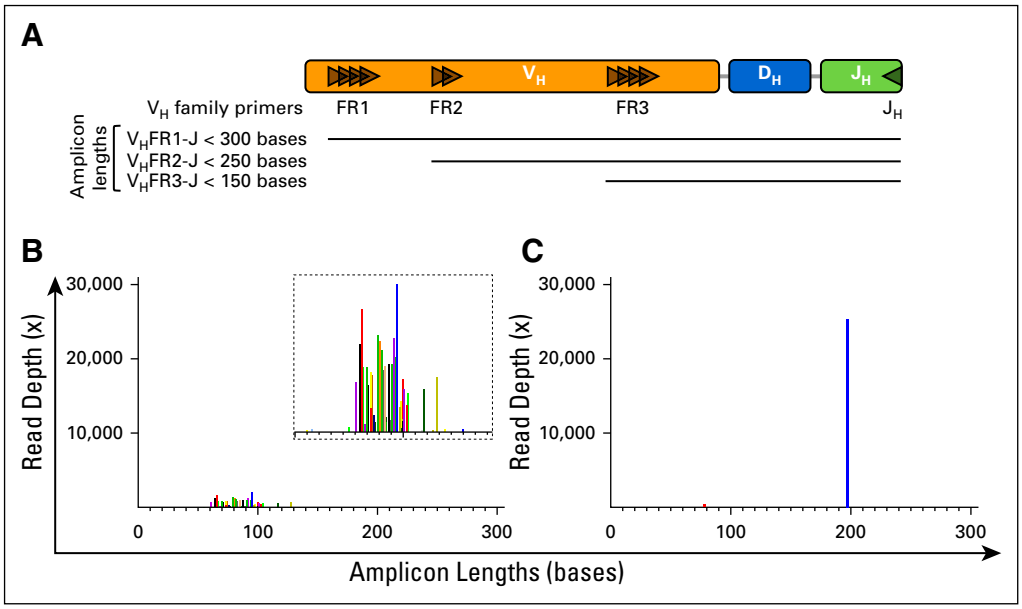

FIG 4. Immunoglobulin heavy chain (IGH) sequencing of plasma cell–free DNA. (A) Schematic of the primer sets used to amplify and sequence variable, diversity, and joining (VDJ) rearrangements from the *IGH* gene. V_H gene family primers target each framework (FR1, FR2, and FR3) separately and amplify across the D_H gene to a common set of J_H primers. The expected amplicon lengths are shown. Representative histograms of (B) a nonclonal or polyclonal pattern and (C) a clonal pattern of VDJ sequences. Unique VDJ sequences are color coded with the most dominant sequence in blue. The sequences are separated by amplicon length (*x*-axis) with the corresponding abundance represented as read depth (*y*-axis). (B) A nonclonal or polyclonal pattern of VDJ sequences amplified and sequenced using the V_HFR3-J primers. The inset shows an enlarged view at 1:10 scale. (C) A clonal pattern of VDJ sequences with a single dominant sequence, and little appreciable polyclonal sequences, amplified and sequenced using the V_HFR2-J primers. V_H, D_H, and J_H, variable, diversity, and joining of the immunoglobulin heavy chain gene; V_HFR1-J, immunoglobulin heavy chain variable region framework 1 amplified across the J gene; V_HFR2-J, immunoglobulin heavy chain variable region framework 2 amplified across the J gene; V_HFR3-J, immunoglobulin heavy chain variable region framework 3 amplified across the J gene.

275 bp in internal control gDNA, in patients with lymphoma, and only 219 bp in patients with TB ($P < .0001$).

IGH Sequencing Positively Identifies Clonal Sequences in Patients With Lymphoma

Using the above technique, clg sequences were detected in cfDNA in five of nine (56%) patients with lymphoma but were not detected in any of the patients with TB (Table 1). Amplification and sequencing using multiple framework regions increased sensitivity, as two of five samples only demonstrated clonality in a single framework, and two of five samples only showed clonality in the longer amplicons (FR1 and/or FR2), but not FR3.

DISCUSSION

clg DNA is routinely used on tissue biopsies to aid in the diagnosis of lymphoma and is increasingly being assessed in plasma cfDNA as a disease monitoring marker, particularly for mantle cell lymphoma.[29] However, the analysis of clg DNA in cfDNA has not previously been used to facilitate the diagnosis of lymphoma. Traditionally, definitive diagnosis requires a biopsy of suspicious lesions identified by physical examination and/or imaging during the healthcare practitioner interval. However, in SSA, this interval is often prolonged in PLWH. We focused on HIV patients with newly diagnosed lymphoma and a population of PLWH with newly diagnosed TB because the two disease processes are easily confounded and confused with regard to clinical signs and symptoms. Our findings suggest that with appropriate specimen tubes, it is straightforward to collect and transport blood specimens to a central lab and isolate high-quality cfDNA.

Although clg DNA is present in plasma of patients with untreated lymphoma, DNA quantity and quality have been a long-standing barrier to cfDNA investigations. Poor-quality DNA can yield false-negative results, and contamination with cellular DNA resulting from lysis of white blood cells ex vivo can mask a clonal pattern. In this small series of patients, we identified dominant mononucleosomal cfDNA molecules in all patient samples and prominent dinucleosomal molecules in the majority of patient samples (lymphoma and TB) reflecting the high quality of cfDNA collected. Despite the fact that similar amounts, and proportions, of larger dinucleosomal cfDNA molecules were found in both cohorts and the amplification

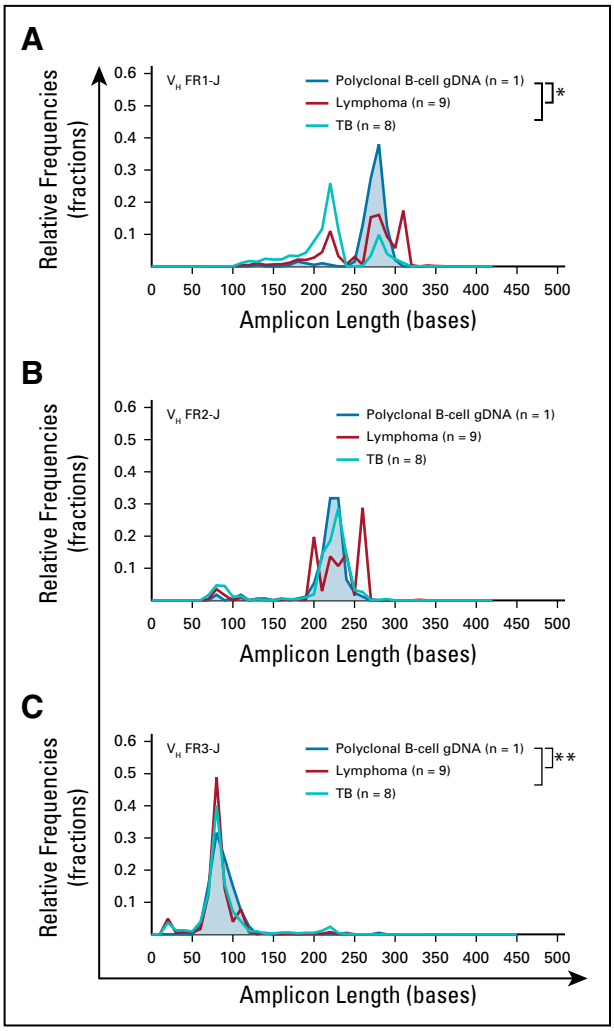

FIG 5. Amplicon lengths of variable, diversity, and joining sequences in plasma cell–free DNA. The frequency distribution of amplicon lengths amplified using the different primer sets in patients with lymphoma and TB was compared against the expected distribution obtained from a polyclonal B-cell DNA control sample. (A) V_HFR1-J amplicons. (B) V_HFR2-J amplicons. (C) V_HFR3-J amplicons. The distribution of amplicon lengths obtained from plasma DNA differed significantly from the expected distribution for both the largest (V_HFR1-J) and smallest (V_HFR3-J) amplicons irrespective of diagnosis, whereas the intermediate-size amplicons (V_HFR2-J) showed no difference among the three groups. Statistical significance was evaluated using two-tailed unpaired Mann-Whitney test, as these distributions were not parametric. gDNA, genomic DNA; TB, tuberculosis; V_HFR1-J, immunoglobulin heavy chain variable region framework 1 amplified across the J gene; V_HFR2-J, immunoglobulin heavy chain variable region framework 2 amplified across the J gene; V_HFR3-J, immunoglobulin heavy chain variable region framework 3 amplified across the J gene. *P = .01-.05, **P = .001-.01.

profiles obtained from both cohorts were similar, sequencing data showed that the largest B-cell DNA molecules (> 250 bp) were more abundant in patients with lymphoma. In the absence of systemic lymphoma to continuously release tumor DNA in circulation, larger B-cell cfDNA molecules may not be readily available in individuals without lymphoma. This conjecture is supported by the fact that only a minute fraction of cfDNA represents B-cell DNA in healthy individuals.[30,31] Although these larger B-cell DNA molecules were likely derived from dinucleosomal cfDNA molecules, the true source of this DNA is unknown. Since circulating tumor cells are rarely found in aggressive B-cell lymphoma, or HL,[32] this DNA may simply represent gDNA from dying lymphoma cells.

With this high-quality cfDNA, we were able to detect clg in most (56%) lymphoma plasma specimens, and none of the specimens taken from patients with TB. The primer sets targeting frameworks 1 and 3 were able to identify clg sequences in three samples, whereas primer sets targeting framework 2 were able to identify clg sequences in four samples. Although these numbers remain small, the sensitivity afforded by targeting all three primer sets is superior to the sensitivity of single individual primer sets, which is a finding that has been consistently demonstrated when evaluating tumor tissue biopsies.[33] With an approximate sensitivity of 50%, our results are similar to previous reports of clonal Ig detection in plasma when the tumor-specific sequence is unknown, and clone detection is performed in an uninformed manner.[20,34-36] This somewhat lower detection rate may also be related to somatic hypermutation abrogating primer binding sites,[33] which is a phenomenon that affects germinal center and post–germinal center–derived lymphomas. Finally, lower clg detection may be due to lower abundance of circulating tumor DNA[18] in some of the studied samples. Future studies will include the analysis of other lymphoma-specific markers, such as light chain gene rearrangements and characteristic tumor mutations, to improve diagnostic sensitivity.

In the current study, we note that the next-generation sequencing (NGS) was performed in Maryland and not in SA. This fact not only highlights the robustness of our collaboration and our ability to preserve specimen integrity but also highlights the work that is still needed to be done to build the infrastructure in SA to be able to sequence these specimens in country. To that end, we note a collaboration with a key stakeholder on the ground in Johannesburg, SA, the iLEAD initiative. This Gates-funded, African laboratory initiative has provided resources to increase NGS capacity in SA. Future work will focus on building in-country capacity to sequence specimens locally using paired specimens. Establishing initial feasibility of specimen collection and processing in SA is an important first step in achieving this goal.

The small sample size in this study limits our ability to assess the clinical utility of the NGS technique. Future investigations will include more patients and an expanded menu of molecular markers to assess the diagnostic utility of these assays. Markers to be studied include clg, somatic mutation panels, and Epstein-Barr virus DNA analysis to

TABLE 1. Immunoglobulin Heavy Chain Gene Sequencing Results

Study ID	Diagnosis	V_HFR1-J	V_HFR2-J	V_HFR3-J	Combined Result
P1448-059	NHL	Nonclonal	Nonclonal	Nonclonal	Nonclonal
P1448-062	NHL	Clonal (biallelic)	Clonal (biallelic)	Clonal (biallelic)	Clonal
P1448-064	NHL	Nonclonal	Clonal (monoallelic)	Nonclonal	Clonal
P1448-055	CHL	Nonclonal	Nonclonal	Clonal (monoallelic)	Clonal
P1448-066	NHL	Nonclonal	Nonclonal	Nonclonal	Nonclonal
P1448-067	NHL	Clonal (monoallelic)	Clonal (monoallelic)	Clonal (biallelic)	Clonal
P1448-069	NHL	Nonclonal	Nonclonal	Nonclonal	Nonclonal
P1448-070	CHL	Nonclonal	Nonclonal	Nonclonal	Nonclonal
P1448-072	CHL	Clonal (monoallelic)	Clonal (monoallelic)	Nonclonal	Clonal
P1448-083	TB control	Nonclonal	Nonclonal	Nonclonal	Nonclonal
P1448-084	TB control	Nonclonal	Nonclonal	Nonclonal	Nonclonal
P1448-090	TB control	Nonclonal	Nonclonal	Nonclonal	Nonclonal
P1448-091	TB control	Nonclonal	Nonclonal	Nonclonal	Nonclonal
P1448-092	TB control	Nonclonal	Nonclonal	Nonclonal	Nonclonal
P1448-094	TB control	Nonclonal	Nonclonal	Nonclonal	Nonclonal
P1448-086	TB control	Nonclonal	Nonclonal	Nonclonal	Nonclonal
P1448-093	TB control	Nonclonal	Nonclonal	Nonclonal	Nonclonal

Abbreviations: CHL, classical Hodgkin lymphoma; NHL, non-Hodgkin lymphoma; TB, tuberculosis; V_HFR1-J, immunoglobulin heavy chain variable region framework 1 amplified across the J gene; V_HFR2-J, immunoglobulin heavy chain variable region framework 2 amplified across the J gene; V_HFR3-J, immunoglobulin heavy chain variable region framework 3 amplified across the J gene.

distinguish virion DNA from tumor DNA.[37] Which tests will prove most sensitive, specific, and economically feasible and what the most useful algorithm for applying these investigations are yet to be determined. The feasibility results described herein suggest that these approaches have promise. As the value of plasma-based approaches is better defined, we look forward to the possibility that plasma DNA diagnostics may play a role in facilitating more rapid lymphoma diagnosis in the future, particularly in PLWH in resource-limited settings.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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APPENDIX

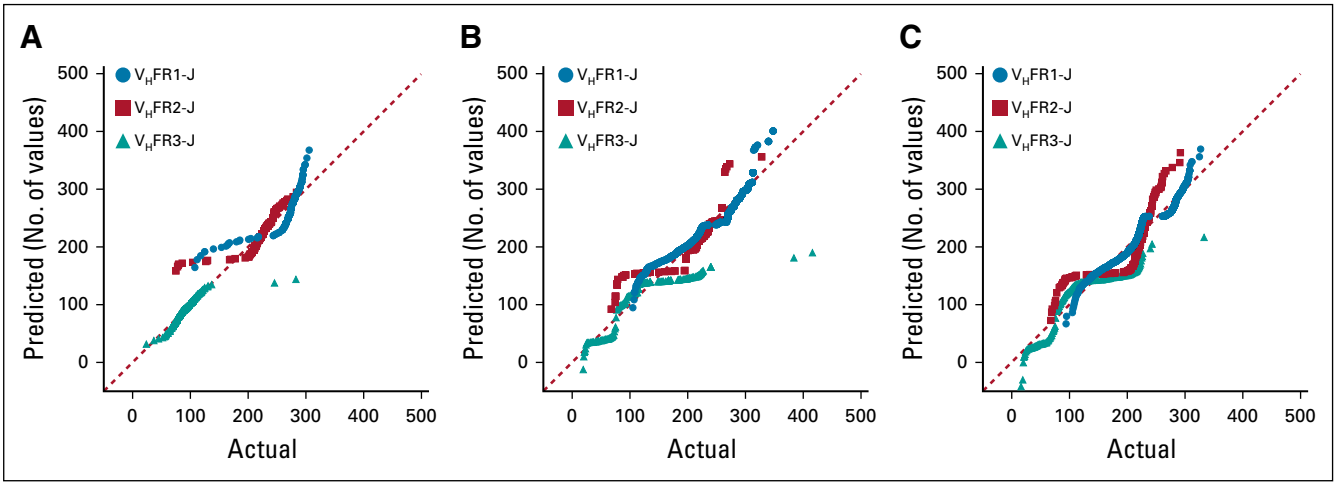

FIG A1. Normal quantile-quantile (QQ) plot of the frequency distributions of V_HFR1-J, V_HFR2-J, and V_HFR3-J amplicon lengths. Normal QQ plots of amplicon lengths obtained from (A) a B-cell DNA (+) control, (B) cell-free DNA (cfDNA) from patients with lymphoma, and (C) cfDNA from patients with tuberculosis. V_HFR1-J, immunoglobulin heavy chain variable region framework 1 amplified across the J gene; V_HFR2-J, immunoglobulin heavy chain variable region framework 2 amplified across the J gene; V_HFR3-J, immunoglobulin heavy chain variable region framework 3 amplified across the J gene.