commentaries

Molecular Diagnostics for AIDS Lymphoma Diagnosis in South Africa and the Potential for Other Low- and Middle-Income Countries

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In 2015, the HIV prevalence in adults 15 to 49 years of age in South Africa was 19.2%, which represented an estimated 7 million HIV-infected individuals.[1] In 2004, the government of South Africa started its public health antiretroviral therapy (ART) program[2] and during the past 12 years, has initiated ART for more than 3 million people.[3] Coincident with this, the overall prevalence of HIV-associated lymphomas has increased during the past decade.[4,5] There are many possible reasons for this increase despite increased ART coverage, such as late initiation of ART therapy, incomplete ART coverage, and perhaps a long enough lifespan for some patients to develop lymphoma.

HIV infection is associated with an increased risk of lymphomas.[6-9] The most common subtypes diagnosed in people living with HIV (PLWH) are diffuse large B-cell lymphoma, Burkitt's lymphoma, and Hodgkin lymphoma (HL).[10] In South Africa, plasmablastic and non-Hodgkin lymphoma (NHL) with intermediate features between diffuse large B-cell lymphoma and Burkitt's lymphoma are being increasingly recognized in PLWH.[4] Clinical signs and symptoms of aggressive lymphoma often are indistinguishable from common opportunistic infections, the most notable of which is *Mycobacterium tuberculosis* infection (ie, tuberculosis [TB]). According to the WHO Global Tuberculosis Report in 2016, South Africa has the highest burden of HIV and TB coinfection—473 occurrences per 100,000 people annually and an estimated 10,000 occurrences of multidrug-resistant (MDR) TB each year.[11] In addition, TB is the leading cause of death recorded on death certificates in South Africa[12] and autopsy studies suggest it is the leading infection found postmortem in HIV-infected adults receiving ART,[13] in those who die in the hospital,[14] and in people who die at home without an apparent cause of death.[15]

TB in PLWH often is difficult to confirm by traditional microscopy, because roughly half of HIV-infected patients present with smear-negative disease[16]; those who present with fever, night sweats, weight loss, and lymphadenopathy often are started empirically on TB treatment.[17] For those patients who do not respond to initial therapy, MDR TB is always a consideration. Empiric treatment of TB can result in major delays in diagnosis and initiation of appropriate treatment. Indeed, a retrospective review conducted in KwaZulu-Natal, South Africa, documented that 18 of 21 patients with lymphoma had been treated empirically for TB for a median of 5 months before the diagnosis of lymphoma was established and they were referred for lymphoma treatment.[18] In addition, a study from Uganda showed that 30.6% (56 of 183) of patients with HIV-associated lymphoma had received an average of 3.5 months of empiric TB treatment before the lymphoma diagnosis,[19] which suggests that this problem of possible delayed diagnoses is not isolated to South Africa.

Simply increasing the number of biopsies is not practical given the limited resources available. After biopsy specimens are prepared, an anatomic pathologist is required for interpretation. Minimal training for a pathologist requires 4 years beyond medical school. South African pathologists are already hard pressed to interpret the biopsies that are currently obtained and would struggle to accommodate a large increase in biopsies. Also, the numbers of pathologists in South Africa far exceed those in many low-and middle-income countries (LMICs).[20]

These limitations notwithstanding, increased capacity to diagnose lymphoma may be lifesaving. Studies from developed countries suggest overall long-term survival rates of at least 50% for systemic HIV-associated lymphoma[21-25] by using standard chemotherapy paradigms in combination with ART. Although limited data exist about the outcomes of HIV-associated lymphoma in South

Africa, initial reports suggest that long-term survival can be achieved with combined ART and chemotherapy in approximately half of the patients. Poor performance status, CNS involvement, compromised hepatic or renal function, and bulky disease all adversely affect outcomes and are more common in patients with delayed diagnoses. A mechanism to better prioritize patients for timely diagnostic biopsy likely would pay dividends in lives saved. Rapid advances in molecular diagnostics might facilitate such prioritization.

Antibody diversity is generated by recombination of immunoglobulin DNA loci in B cells. The recombination of various variable, diversity, and joining segments on the heavy-chain locus and the variable and joining segments on the two light-chain loci (κ and λ) in bone marrow create unique immunoglobulin molecules that can leave the bone marrow and travel to peripheral lymphoid organs (spleen, lymph nodes, and mucosa-associated lymphoid tissue). In these peripheral lymphoid organs, additional modification of the immunoglobulin molecule occurs through a process known as somatic hypermutation. Taken together, the various modifications that immunoglobulin molecules undergo increase the diversity of the immunoglobulin repertoire and create B cells that have their own unique signatures.

Lymphoid neoplasms are a clonal expansion of a particular B-cell population. This B-cell population can be detected and characterized by the unique signature of the immunoglobulin molecule present. Southern blot was an early technique used to detect clonal immunoglobulin gene rearrangements as a marker of lymphoma.[26-28] As rearrangement of the immunoglobulin gene segments alters the position of the restriction-endonuclease sites, a clonal population can be detected by the presence of a distinct band on SB that differs from that of germline sequences.[26-28] Subsequently, much more sensitive approaches that involve polymerase chain reaction technology with standardized primer sets have been developed.[29-31]

In parallel to progress in the technical approach to detecting clonal immunoglobulin rearrangements, appreciation has grown that tumor DNA often is present in patients' plasma.[32] Investigation of circulating tumor DNA (ctDNA; a technique often termed liquid biopsy) is actively being studied in many malignancies. Several groups have demonstrated the applicability to lymphoma diagnostics and monitoring, including in patients with AIDS lymphoma.[33-35]

More recently, next-generation sequencing (NGS) has emerged as a precise and efficient way to detect clonal immunoglobulin gene rearrangements in ctDNA; the level of detection is one tumor cell per million leukocytes.[36] Several studies that use NGS approaches (LymphoSight; Adaptive Technologies, Seattle, WA) have demonstrated an ability to detect clonal immunoglobulin gene rearrangements in the ctDNA of HIV-negative patients with NHL[37-41] and HL.[42] Wagner-Johnston et al[4] reported a sensitivity of 50% (in 24 of 48 samples) for detection of clonal immunoglobulin DNA by NGS in pretreatment samples taken from PLWH with either NHL or HL.

More research is needed to better understand the sensitivity and specificity of NGS of clonal immunoglobulin gene rearrangements in ctDNA from patients with aggressive B-cell lymphomas, including PLWH. Although inadequate alone for lymphoma diagnosis, NGS of ctDNA in blood samples could prioritize biopsies in those whose clinical presentation suggests a high risk of lymphoma. Such a two-stage diagnostic strategy could allow the surgical and histopathologic resources available in low-resource settings to be leveraged so as to maximize the diagnosis of lymphoma–especially when lymphoma diagnoses may be missed or delayed because of the high suspicion of TB or other infection.

Although NGS is not currently available in resource-limited settings for incorporation into the diagnostic algorithm for lymphoma, precedence exists for the introduction of sophisticated diagnostic approaches that use DNA technology in South Africa. The GeneXpert platform developed by Cepheid (Sunnyvale, CA) first emerged for the detection of *Bacillus anthracis* by the US Postal Service.[43] The system produced rapid results (within 30 to 40 minutes) and because each individual cartridge contains the polymerase chain reaction primers for the target nucleic acid sequence of interest, the technology has been adapted for various infectious and oncologic diagnostic purposes. In South Africa, GeneXpert has replaced microscopy as the first-line diagnostic test for TB and was endorsed by WHO in 2010. GeneXpert machines in South Africa can be loaded by technicians who require only 2 weeks of training beyond basic schooling. Of particular interest, GeneXpert allows for the diagnosis of rifampin-resistant TB.[44]

As alluded to previously, HIV-associated TB can be particularly challenging to diagnose in a timely fashion with smear microscopy and culture. A

meta-analysis of seven studies conducted in HIV-positive patients who lived in LMICs evaluated the sensitivity of sputum microscopy compared with that of the GeneXpert TB assay with culture positivity serving as the gold standard. The results showed a median sensitivity with smear microscopy of 52.8% (range, 22.2% to 68.9%) and a median sensitivity with GeneXpert TB of 84.0% (range, 58.3% to 91.7%).[45] Although the majority of these studies were conducted in symptomatic patients, at least one study evaluated active-case finding in a group of ART-naïve, HIV-infected patients establishing care at an ART clinic in Cape Town, South Africa.[46]

In a relatively short amount of time, the GeneXpert platform was successful in garnering WHO approval, accelerating the diagnosis and treatment of TB in symptomatic patients[47] and contributing to the diagnosis of at-risk individuals in the form of active-case finding[46] and to the successful negotiation of decreased costs for LMICs.[48] With the appropriate international collaboration, we believe that NGS could be similarly successful for lymphoma diagnoses. In addition to using NGS of ctDNA for the detection of clonal Ig gene rearrangements to help prioritize PLWH for lymphoma diagnosis, several studies have evaluated this approach for early detection of relapsed or refractory disease.[38,39] Although more research is needed to better understand the kinetics of ctDNA during various lymphoma therapies, the idea of monitoring treatment response in blood would be desirable in a resource-limited setting, where radiologic evaluation of treatment response with positron emission tomography/computed tomography and traditional computed tomography is limited.

NGS requires a relatively modest investment in research to validate the approach. Thus, the NGS analysis of a plasma specimen or lymph node aspirate might facilitate efficient prioritization of patients for biopsy to establish a diagnosis of lymphoma and accelerate the initiation of curative therapy.

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REFERENCES

1. UNAIDS: South Africa HIV and AIDS estimates (2015). http://www.unaids.org/en/regionscountries/countries/southafrica/
2. Klausner JD, Serenata C, O'Bra H, et al: Scale-up and continuation of antiretroviral therapy in South African treatment programs, 2005-2009. J Acquir Immune Defic Syndr 56:292-295, 2011
3. WHO: 17 million people with access to antiretroviral therapy. http://www.who.int/hiv/mediacentre/news/global-aids-update-2016-news/en/
4. Abayomi EA, Somers A, Grewal R, et al: Impact of the HIV epidemic and anti-retroviral treatment policy on lymphoma incidence and subtypes seen in the Western Cape of South

Africa, 2002-2009: Preliminary findings of the Tygerberg Lymphoma Study Group. Transfus Apheresis Sci 44:161-166, 2011

5. Patel M, Philip V, Omar T, et al: The impact of human immunodeficiency virus infection (HIV) on lymphoma in South Africa. J Cancer Ther 06:527-535, 2015
6. Patel P, Hanson DL, Sullivan PS, et al: Incidence of types of cancer among HIV-infected persons compared with the general population in the United States, 1992-2003. Ann Intern Med 148: 728-736, 2008
7. Engels EA, Biggar RJ, Hall HI, et al: Cancer risk in people infected with human immunodeficiency virus in the United States. Int J Cancer 123:187-194, 2008
8. Grulich AE, van Leeuwen MT, Falster MO, et al: Incidence of cancers in people with HIV/AIDS compared with immunosuppressed transplant recipients: A meta-analysis. Lancet 370:59-67, 2007
9. Silverberg MJ, Lau B, Achenbach CJ, et al: Cumulative incidence of cancer among persons with HIV in North America: A cohort study. Ann Intern Med 163:507-518, 2015
10. Gibson TM, Morton LM, Shiels MS, et al: Risk of non-Hodgkin lymphoma subtypes in HIV-infected people during the HAART era: A population-based study. AIDS 28:2313-2318, 2014
11. WHO: Global tuberculosis report: South Africa tuberculosis profile. https://extranet.who.int/sree/Reports?op=Replet&name=%2FWHO_HQ_Reports%2FG2%2FPROD%2FEXT%2FTBCountryProfile&ISO2=ZA&LAN=EN&outtype=pdf
12. Statistics South Africa: Mortality and causes of death in South Africa: Findings from death notification, 2015. http://www.statssa.gov.za/?page_id=1854&PPN=P0309.3&SCH=6987
13. Wong EB, Omar T, Setlhako GJ, et al: Causes of death on antiretroviral therapy: A post-mortem study from South Africa. PLoS One 7(10), 2012:e47542
14. Cohen T, Murray M, Wallengren K, et al: The prevalence and drug sensitivity of tuberculosis among patients dying in hospital in KwaZulu-Natal, South Africa: A postmortem study. PLoS Med 7(6), 2010:e1000296
15. Omar T, Variava E, Moroe E, et al: Undiagnosed TB in adults dying at home from natural causes in a high TB burden setting: A post-mortem study. Int J Tuberc Lung Dis 19:1320-1325, 2015
16. Lawn SD, Mwaba P, Bates M, et al: Advances in tuberculosis diagnostics: the Xpert MTB/RIF assay and future prospects for a point-of-care test. Lancet Infect Dis 13:349-361, 2013
17. Theron G, Peter J, Dowdy D, et al: Do high rates of empirical treatment undermine the potential effect of new diagnostic tests for tuberculosis in high-burden settings? Lancet Infect Dis 14: 527-532, 2014
18. Puvaneswaran B, Shoba B: Misdiagnosis of tuberculosis in patients with lymphoma. S Afr Med J 103:32-33, 2012
19. Buyego P, Nakiyingi L, Ddungu H, et al: Possible misdiagnosis of HIV associated lymphoma as tuberculosis among patients attending Uganda Cancer Institute. AIDS Res Ther 14:13, 2017
20. Adesina A, Chumba D, Nelson AM, et al: Improvement of pathology in sub-Saharan Africa. Lancet Oncol 14:e152-e157, 2013
21. Boué F, Gabarre J, Gisselbrecht C, et al: Phase II trial of CHOP plus rituximab in patients with HIV-associated non-Hodgkin's lymphoma. J Clin Oncol 24:4123-4128, 2006
22. Dunleavy K, Little RF, Pittaluga S, et al: The role of tumor histogenesis, FDG-PET, and short-course EPOCH with dose-dense rituximab (SC-EPOCH-RR) in HIV-associated diffuse large B-cell lymphoma. Blood 115:3017-3024, 2010
23. Sparano JA, Lee JY, Kaplan LD, et al: Rituximab plus concurrent infusional EPOCH chemotherapy is highly effective in HIV-associated B-cell non-Hodgkin lymphoma. Blood 115:3008-3016, 2010
24. Montoto S, Wilson J, Shaw K, et al: Excellent immunological recovery following CODOX-M/IVAC, an effective intensive chemotherapy for HIV-associated Burkitt's lymphoma. AIDS 24:851-856, 2010

25. Hentrich M, Berger M, Wyen C, et al: Stage-adapted treatment of HIV-associated Hodgkin lymphoma: Results of a prospective multicenter study. J Clin Oncol 30:4117-4123, 2012
26. Arnold A, Cossman J, Bakhshi A, et al: Immunoglobulin-gene rearrangements as unique clonal markers in human lymphoid neoplasms. N Engl J Med 309:1593-1599, 1983
27. Cossman J, Uppenkamp M, Sundeen J, et al: Molecular genetics and the diagnosis of lymphoma. Arch Pathol Lab Med 112:117-127, 1988
28. Cleary ML, Chao J, Warnke R, et al: Immunoglobulin gene rearrangement as a diagnostic criterion of B-cell lymphoma. Proc Natl Acad Sci USA 81:593-597, 1984
29. Sioutos N, Bagg A, Michaud GY, et al: Polymerase chain reaction versus Southern blot hybridization: Detection of immunoglobulin heavy-chain gene rearrangements. Diagn Mol Pathol 4:8-13, 1995
30. Reed TJ, Reid A, Wallberg K, et al: Determination of B-cell clonality in paraffin-embedded lymph nodes using the polymerase chain reaction. Diagn Mol Pathol 2:42-49, 1993
31. van Dongen JJM, Langerak AW, Brüggemann M, et al: Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene recombinations in suspect lymphoproliferations: Report of the BIOMED-2 concerted action BMH4-CT98-3936. Leukemia 17:2257-2317, 2003
32. Stroun M, Anker P, Maurice P, et al: Neoplastic characteristics of the DNA found in the plasma of cancer patients. Oncology 46:318-322, 1989
33. Frickhofen N, Müller E, Sandherr M, et al: Rearranged Ig heavy chain DNA is detectable in cell-free blood samples of patients with B-cell neoplasia. Blood 90:4953-4960, 1997
34. Tohda S, Murakami N, Nara N: Polymerase chain reaction detection of rearranged immunoglobulin heavy chain DNA in plasma samples is useful in the diagnosis of B-cell lymphoma. Int J Hematol 72:74-78, 2000
35. Wagner-Johnston ND, Gellert L, Gocke CD, et al: Clonal immunoglobulin DNA in the plasma of patients with AIDS lymphoma. Blood 117:4860-4862, 2011
36. Faham M, Zheng J, Moorhead M, et al: Deep-sequencing approach for minimal residual disease detection in acute lymphoblastic leukemia. Blood 120:5173-5180, 2012
37. Armand P, Oki Y, Neuberg DS, et al: Detection of circulating tumour DNA in patients with aggressive B-cell non-Hodgkin lymphoma. Br J Haematol 163:123-126, 2013
38. Roschewski M, Dunleavy K, Pittaluga S, et al: Circulating tumour DNA and CT monitoring in patients with untreated diffuse large B-cell lymphoma: A correlative biomarker study. Lancet Oncol 16:541-549, 2015
39. Kurtz DM, Green MR, Bratman SV, et al: Noninvasive monitoring of diffuse large B-cell lymphoma by immunoglobulin high-throughput sequencing. Blood 125:3679-3687, 2015
40. Ladetto M, Brüggemann M, Monitillo L, et al: Next-generation sequencing and real-time quantitative PCR for minimal residual disease detection in B-cell disorders. Leukemia 28:1299-1307, 2014
41. Sarkozy C, Huet S, Carlton VEH, et al: The prognostic value of clonal heterogeneity and quantitative assessment of plasma circulating clonal IG-VDJ sequences at diagnosis in patients with follicular lymphoma. Oncotarget 8:8765-8774, 2017
42. Oki Y, Neelapu SS, Fanale M, et al: Detection of classical Hodgkin lymphoma specific sequence in peripheral blood using a next-generation sequencing approach. Br J Haematol 169:689-693, 2015
43. Ulrich MP, Christensen DR, Coyne SR, et al: Evaluation of the Cepheid GeneXpert system for detecting *Bacillus anthracis*. J Appl Microbiol 100:1011-1016, 2006
44. WHO: WHO endorses new rapid tuberculosis test. http://www.who.int/tb/features_archive/new_rapid_test/en/

45. Lawn SD, Kerkhoff AD, Vogt M, et al: HIV-associated tuberculosis: Relationship between disease severity and the sensitivity of new sputum-based and urine-based diagnostic assays. BMC Med 11: 231, 2013

46. Lawn SD, Brooks SV, Kranzer K, et al: Screening for HIV-associated tuberculosis and rifampicin resistance before antiretroviral therapy using the Xpert MTB/RIF assay: A prospective study. PLoS Med 8(7), 2011:e1001067

47. Boehme CC, Nicol MP, Nabeta P, et al: Feasibility, diagnostic accuracy, and effectiveness of decentralised use of the Xpert MTB/RIF test for diagnosis of tuberculosis and multidrug resistance: A multicentre implementation study. Lancet 377:1495-1505, 2011

48. FIND: Negotiated product pricing. http://www.finddx.org/find-negotiated-product-pricing/