Inguinal Lymph Node and Anorectal Mucosal Biopsies for Human Immunodeficiency Virus Research Protocols in an Emerging Nation: Patient Outcomes and Lessons Learned

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Abstract

Background: Lymph nodes and gut-associated lymphatic tissue are important reservoirs of the human immunodeficiency virus (HIV). Little is known about these reservoirs in different geographic populations. We report the surgical outcomes of excisional lymph node and anorectal mucosal biopsies performed internationally and describe the lessons learned.

Methods: Patients were recruited through the Joint Clinical Research Center (JCRC) in Kampala, Uganda, where procedures were performed. Studies were approved by the Institutional Review Boards of the JCRC and the University of Minnesota. Instruments and supplies were shipped to Uganda and prepared onsite. Drugs and skin preparations were purchased locally. Lymph nodes were removed through 1–3 cm incisions with ligatures on lymphovascular pedicles. Incisions were closed with subcuticular sutures and epidermal tape. Two to four pieces of anorectal mucosa were obtained through anoscopes using biopsy forceps.

Results: One hundred thirty-eight lymph node biopsies and 98 anorectal mucosal biopsies were performed on 71 patients. Forty-one patients were HIV-positive. Many patients had multiple procedures. Two minor complications resulted: One hematoma and one lymphocele. Despite the cost of travel and lodging, cost per biopsy was lower in Uganda compared with the United States.

Conclusion: Invasive clinical research can be performed with minimal morbidity in emerging nations with outcomes similar to those found in the United States, but with lower cost.

THE HUMAN IMMUNODEFICIENCY VIRUS (HIV) and acquired immune deficiency syndrome (AIDS) is endemic throughout the world. Although the virus can be controlled with antiretroviral drugs (ARVs) rendering blood viral counts undetectable, these therapies do not eradicate the virus as demonstrated by the recrudescence of viremia after treatment cessation and in the low-level viremia, which is detectable using highly sensitive assays in patients who are taking ARVs [1–5]. Understanding of the anatomic sites and cell types in which HIV persists (i.e., viral reservoirs) is a crucial step toward the development of novel treatments capable of producing a functional cure for HIV. Both peripheral lymph nodes and gastrointestinal-associated lymphatic tissue (GALT) are important viral reservoirs [6–8]. However, little is known about differences in these lymphatic reservoirs in geographically distinct populations. There is reason to be-

lieve that characteristics of the lymphatic reservoir are different in patients in Africa (where the largest burden exists of HIV/AIDS) compared with patients in the United States given underlying differences in lymphatic architecture, presumably because of differences in anti-genetic exposures [T.W. Schacker, unpublished data, 2012]. To study this appropriately, we obtained and processed lymphatic tissues in Africa.

We described previously our method for excisional biopsy of inguinal lymph nodes under local anesthesia and reported the safety of this procedure in HIV-infected subjects in the United States [9]. We report here the surgical outcomes of similar excisional lymph node and anorectal mucosal biopsies performed in the Republic of Uganda, East Africa, from several different research protocols. Additionally, we describe the challenges faced and lessons learned while

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performing clinical research involving invasive procedures in an emerging nation. Reports of the immunologic and viral studies performed on tissues are beyond the scope of this article and have been submitted for publication elsewhere.

Patients and Methods

All patients were recruited and managed through the Joint Clinical Research Centre (JCRC) in Kampala, Uganda, an HIV/AIDS care and research institution with a long history of conducting HIV clinical research with international collaborators. All biopsy procedures were performed in a wing of the JCRC's inpatient hospital in Kampala. The Institutional Review Boards (IRBs) of both the JCRC and the University of Minnesota approved all studies.

Surgical instruments were purchased from Northstar Surgical (Edina, MN) and on eBay. Surgical instruments were prepared locally and sterilized onsite with a steam autoclave. Other required supplies (surgical gowns, disposable anoscopes, gloves, drapes, etc.) were ordered from Cardinal Health (Dublin, OH) several months prior to planned procedures and shipped to Kampala.

Excisional lymph node biopsy

The surgical technique was modified from the original description because of available supplies and local institutional and cultural demands. Inguinal lymph node biopsies were performed with patients in the supine position. Cephalixin, 500 mg orally was given pre-operatively. The skin was prepared with iodine-based or chlorhexidine solutions. Lidocaine was used for local anesthesia but concentrations varied from 1%–2% depending on local availability. Skin incisions varied from 1–3 cm depending on patient size. All nodes were removed completely. Silk or Vicryl[®] ligatures (Ethicon, Somerville, NJ) were placed on vascular pedicles as needed. Incisions were closed with subcuticular Vicryl sutures with Steri-Strips[™] and tincture of benzoin reinforcement (3M, St. Paul, MN). Post-operatively, patients were given paracetamol for pain and continued cephalixin for 3 d according to local medical customs.

Rectal mucosal biopsies

Anorectal mucosal biopsies were obtained using Sani-Scope[®] Disposable Anoscopes (Cooper Surgical, Trumbull, CT) with water-soluble lubricant. Biopsies in the first 12 subjects were performed using Radial Jaw 4[®] Standard Capacity Biopsy forceps (Boston Scientific, Natick, MA) and the remaining were performed using Yeoman rectal biopsy

forceps (3.5 mm). Mucosal biopsies were obtained from the anorectal junction or proximal anal canal, cephalad to the pectinate or dentate line. Patients were given a saline enema (133 mL; CB Fleet Co, Lynchburg, VA), which was evacuated prior to the procedure. Two to four pieces of mucosa were obtained from each subject until a total of 4 mm² of tissue was obtained. Bleeding was controlled with pressure, initially with gauze followed by the collapsing anal canal after removal of the anoscope. We initially used petroleum gauze to pack the anal canal but found that it was not necessary. Subjects were told to expect some blood with bowel movements and to inform the study coordinators if it persisted for more than 2 d or if they passed large clots with bowel movements. Because of the lack of sensory nerves above the pectinate line, no analgesics were necessary.

Results

Between November 2009 and February 2012, our group made four trips to Uganda for a total of 44 d in-country. During that time we performed surgery on a total of 27 d. One surgeon traveled on the first two trips and both surgeon investigators were present for the majority of the operating days on the final two trips. The total surgeon-operating-days were 40 (Table 1).

During these four trips, we performed a total of 236 procedures (138 lymph node biopsies and 98 rectal biopsies) in 71 subjects. Mean age of subjects at study entry was 32 years (range, 18–43). In contrast to our experience in the United States where the majority of study subjects have been male, 62% of participants in these studies were female. All were natives of East Africa and were residing in Uganda. Thirty subjects were HIV-uninfected and 41 were HIV-infected with a mean CD4⁺ cell count of 387 cells/mm³ (range, 59–1,352) at study entry (Table 2). Seventeen subjects were not taking ARVs at time of first biopsy and had a mean HIV-1 viral load of 128,862 copies per milliliter blood (range, 1,040–1,643,055).

Over this 39-mo period, many subjects underwent repeat biopsy procedures, separated by as few as 7 d and as long as 3 y. Among HIV-infected subjects, eight underwent three lymph node and anorectal biopsies and five underwent two lymph node and anorectal biopsies. Of the 30 HIV-uninfected subjects, 11 had a total of three lymph node biopsies and nine had two. Anorectal biopsies were performed in 21 of these subjects on one occasion only. To assist the local inpatient needs, we also performed five other elective procedures on non-study patients (outcomes not included), including two diagnostic lymph node biopsies, one excisional biopsy of a skin lesion, one central line insertion, and one excision of 2 cm subcutaneous mass.

TABLE 1. TIME SPENT IN UGANDA, DAYS OPERATING, AND PER SURGEON WITH NUMBER AND TYPE OF BIOPSY PERFORMED

Surgeon	Dates	Days in Uganda	Surgical days	Surgeon days	Biopsies performed (n)	
					Lymph node	Rectum
1	11/4/09 – 11/12/09	8	5	5	7	8
2	1/27/10– 2/5/10	9	7	7	20	20
Both	1/29/11– 2/11/11	13	7	12	41	40
Both	1/20/11– 2/3/12	14	8	16	70	30
Total		44	27	40	138	98

TABLE 2. TYPE OF BIOPSY PERFORMED, DEMOGRAPHICS, HUMAN IMMUNODEFICIENCY VIRUS INFECTION STATUS OF THE PATIENTS, AND COMPLICATIONS

	<i>HIV positive (41)</i>	<i>HIV negative (30)</i>	<i>All (n = 71)</i>
Total # lymph node biopsies	77	61	138
Total # anorectal biopsies	77	21	98
Mean age (range)	34 (20–43)	29 (18–40)	32 (18–43)
Male (%)	34%	43%	38%
Mean CD4 ⁺ cell count (range)	387 (59–1,352)	N/A	N/A
Complications ^a	Lymphocele (1)	Hematoma (1)	2

^aThere were no complications resulting from anorectal biopsies.
HIV = human immunodeficiency virus; NA = not applicable.

Local study coordinators performed all post-operative follow-up. One patient was reported to have prolonged post-operative pain and swelling. Follow-up one year later revealed an unrelated inguinal hernia as the cause. There were no surgical site infections and only one lymphocele (which occurred in a HIV-infected subject). During our final visit, 20 HIV-uninfected subjects underwent two lymph node biopsies separated by 7–9 days. Only one small hematoma was noted from the previous week's procedures (Table 2). No complications related to the anorectal biopsies were reported.

The average total cost for each lymph node biopsy, including medications, surgical supplies, and subject travel and compensation was \$107 in Uganda and \$684 in the United States. The cost of anorectal biopsies in Uganda was \$55 compared with \$205 in the United States. These differences would have been far greater had we not been able to use a procedure room in the United States free of charge. Interestingly, even when factoring in costs of supply shipment and travel and lodging for a team of four U.S. clinical researchers, cost per biopsy procedure (with both lymph node and anorectal sampling) in Uganda was \$619, still lower than the cost of a biopsy procedure in the United States.

Discussion

With attention to logistics and support of a collaborating local institution, invasive clinical research can be performed with minimal morbidity and patient risk in emerging nations. Patient outcomes are similar to those in patients undergoing excisional lymph node biopsies in the United States. The cost per procedure is lower in Uganda, even when factoring in all travel and shipping costs.

Our low infection rate is consistent with series reported previously [9] and consistent with the infection rate after sentinel lymph node biopsy [10]. Similarly, the lack of complications found with the anorectal mucosal biopsies is consistent with more invasive procedures performed in the same anatomic region. In a large cohort of males undergoing transrectal prostate biopsies, only 3.5% developed fever and 0.5% were treated for prostatitis or urosepsis [11].

There are many aspects that led to the success of this research project. First, the principal investigator made several trips to Uganda and the JCRC to establish institutional relationships, identify collaborative partners, coordinate and plan protocols, investigate the physical resources present, and determine equipment needs. Second, all necessary supplies

were purchased and shipped to Uganda well in advance of surgical trips. We minimized cost by purchasing surgical instruments on eBay and shipping disposable supplies directly from the manufacturer. After the first visit, instruments and remaining supplies were stored for subsequent visits in a locked cabinet kept in the hospital. Finally, we had the support of a skilled local team consisting of a study nurse, project coordinator, and a nurse's aide. Although none had previous experience in protocols involving invasive procedures, all had experience managing patients in standard clinical research studies.

Flexibility and ingenuity were helpful in overcoming unexpected challenges. Surgical drapes were fashioned from sterile gowns, sterile wrap, and even from fabric purchased locally, which was cut to size, hemmed by local help, and then sterilized. We learned where to purchase lidocaine and iodine-based surgical scrubs locally because these could not be sent from the United States because of shipping restrictions. Given inconsistency in the quality of these supplies (the lidocaine was occasionally ineffective), we discovered the importance of keeping a well-stocked supply cabinet during all procedure days. Most importantly, we learned to remain flexible regarding procedure start times because patient delays were common and most often related to the heavy local traffic and occasionally long travel distances.

Over time, as we became familiar with the hospital and local staff, our capacity increased and we increased the number of procedures performed during each visit. We found that having two surgeons was helpful, particularly in the occasional procedure in which a second pair of experienced hands facilitated success in difficult biopsies. We accomplished more with two surgeons than could have been done with one surgeon alone in twice the time. As the number of planned procedures increased, travel plans were arranged to ensure that we had a full day prior to procedures to set up instrument trays and prepare the procedural areas.

We were also able to provide expertise and resources that otherwise would be cost prohibitive to some patients or difficult to obtain locally. Surgeons performed needed diagnostic biopsies and central intravenous access to local patients hospitalized at the JCRC. Much of our remaining equipment and supplies were given to the hospital at the end of the visits.

There is clearly a risk of being coercive when considering the amount to reimburse subjects for their time, effort, and travel in study participation. This is particularly true when relative monetary values vary greatly between a researcher's

home country and that of the study subject, especially if one location is financially disadvantaged compared with the other. To avoid crossing the line of coercion, we utilized our JCRC partners and IRB to determine an appropriate remuneration. The JCRC also provided the crucial role of recruiting subjects and obtaining consent for the study, which can be challenging considering the potential for many languages and dialects spoken by study subjects.

Finally, to obtain local IRB approval and to satisfy the expectations of participants, protocols were adapted to conform to local clinical practices where the potential negative impact was believed to be negligible. For example, pre-operative and post-operative antibiotic administration would not be considered necessary for a clean procedure such as an excisional lymph node biopsy. However, antibiotics are used routinely for such procedures in much of Africa because of the perceived risk of infection. To remain consistent with local practices, we prescribed oral antibiotics before and after the procedure. We acknowledge this as a possible compromise necessary to facilitate the research objective.

Conclusions

Research involving invasive procedures can be performed safely with substantial cost savings in countries with fewer health care resources than the United States. Establishing relationships and understanding local personnel and equipment capabilities is a crucial step before embarking on any research project. Unanticipated problems can be expected and flexibility is a necessity.

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Author Disclosure Statement

No competing financial interests exist.

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