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Safety of Excisional Inguinal Lymph Node Biopsies Performed for Research Purposes in HIV-1-Infected Women and Men

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Abstract

Background: Most HIV-1 replication occurs in secondary lymphoid tissues, and evaluating these tissues is crucial to investigations of pathogenesis. Inguinal lymph nodes (LN) are obtained frequently for these studies as they are readily detectable in most individuals and provide abundant numbers of cells. Knowledge of the outcomes of inguinal LN excision for research purposes is important to inform accurately study participants and researchers of the potential risks.

Methods: Data on surgical complications were collected in real time in HIV-1-infected subjects who underwent excisional inguinal LN biopsies for research purposes from February 1997 through June 2011. Data were analyzed retrospectively to determine the frequency of surgical complications using the Fisher exact test and nonparametric testing.

Results: Eighty-seven research subjects underwent a total of 95 LN excisions. Thirty-six percent of subjects were female, 53% were white, 26% were black, 16% Hispanic, and 2% Native American. Median age was 36 y (22–52). The median CD4+ T cell count was 478 cell/mm^3 (range, 57–1117) and the median plasma HIV-1 RNA concentration was 4.1 $\log_{10}$copies/mL (range, 1.7–5.9). Minor complications including seroma, transient lymphedema, hematoma, and allergic reaction to surgical tape, occurred in 10% of procedures. Complications that required medical attention occurred in an additional 10% of procedures, and included cellulitis (5%), superficial incisional surgical site infection (3%), and seroma requiring aspiration (1%). Subjects with complications had a lower BMI (25; range, 16–38; $n = 12$) than others (28; range, 19–57; $n = 40$; $p = 0.05$) and tended to have higher platelets, (median, 259×10^9/L; range, 196–332; vs. 233×10^9/L; range, 44–633; $p = 0.07$). No other clinical or laboratory characteristics were associated with complications ($p \geq 0.3$).

Conclusions: Lymph node excision for research purposes is generally safe in a diverse group of chronically HIV-1-infected women and men, but can result in complications in a minority of subjects. No predictors of complications were identified.

The majority of HIV-1 replication occurs in secondary lymphoid tissues, including lymph nodes, spleen, and mucosal associated lymphoid tissues such as those in the lung and the gastrointestinal tract. Lymph nodes are of particular importance because they harbor the largest proportion of CD4+ T cells [1,2], and phylogenetic analyses of virus in plasma and secondary lymphoid tissues of humans and macaques suggest that LNs are a major source of plasma viremia [3,4]. As a consequence, researchers frequently seek to obtain LNs from HIV-1-infected individuals in order to understand better HIV-1 immunopathogenesis. Strategies to obtain LN specimens include autopsy, fine needle aspirate, and excisional biopsy. A limitation of autopsy specimens is that cell viability may be suboptimal. Fine needle aspirations provide limited numbers of cells, and furthermore do not allow for architectural analyses. Inguinal LNs obtained by excisional biopsy are often sought as they can be removed readily during an outpatient procedure, they provide large numbers of viable cells, and allow for in situ tissue analyses.

Complication rates following sentinel inguinal lymph node biopsy (SLNB) for staging of melanoma and penile cancer have been reported from multiple studies. Infection is one of

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the most common complications, with reported rates ranging from 3% to 23% of cases [5,6] following SLNB for melanoma. Seroma is also a common complication. A recent study [5] reported a rate of seroma of 9.7%, more than one-half of which required aspiration (5.7%) (n = 139). In a single-site study evaluating outcomes following SLNB for penile cancer, rate of surgical site infection and abscess was 4% and seroma was 2%, which was similar to a multi-center study that demonstrated an infection rate of 3% and a seroma rate of 3.1% [7]. It is notable that rates of complications are significantly lower in retrospective studies, with overall complication rates reported from < 1% to 2% [8,9]. Nonetheless, comparing the risks of elective inguinal LN excision to the risks for excisions performed for clinical indications has some limitations because clinical procedures may involve multiple LN excisions or more extensive dissection [5–10]. Only one group to date has reported on outcomes following excisional inguinal LN biopsies for research purposes. This group reported an overall complication rate of 5% following 137 LN biopsies for research purposes in 44 HIV-1-infected subjects [11]. Specific complications reported included one infection, one lymphocele, two hematomas, one dorsal vein thrombosis, and two suture extrusions [11]. As researchers have recognized increasingly the importance of understanding HIV-1 immunopathogenesis in secondary lymphoid tissues, more excisional biopsies will be performed. Knowledge of the risks of excisional LN biopsy for research purposes in HIV-1-infected individuals is crucial in order to provide accurate information on the risk of these procedures. The purpose of this study was to characterize complications of inguinal LN biopsies in HIV-1-infected individuals undergoing the procedure as part of a research study.

Patients and Methods

Study subjects

HIV-1-infected men and women were recruited to donate inguinal LNs from February 1997 until June 2011 for multiple different studies of HIV-1 immunopathogenesis in secondary lymphoid tissues [12–16]. Initially, recruitment was not limited by CD4 + T cell count. Nevertheless, as inguinal LNs in approximately one-half the subjects with low CD4 + T cell counts were found to contain few cells or to be absent—conditions that are well described in the pathologic literature [17,18] to occur in AIDS—recruitment was limited to subjects with CD4 + T cells > 200 cells/mm^3 after July 1997. Subjects were selected based on willingness to undergo inguinal LN excision and absence of medical problems that would compromise safety in the judgment of the primary investigator. Individuals suspected to have pathologic processes involving their LNs besides HIV-1 were excluded from participation. Written informed consent was obtained from all study participants and the study was approved by the Colorado Multiple Institutional Review Board.

Peripheral blood was collected on the same day as LN excision. Peripheral blood CD4 + and CD8 + T cell counts were determined by flow cytometry, plasma HIV-1 RNA concentration was measured using Roche Amplicor HIV-1 Monitor™ (1997–2002), Roche COBAS® Amplicor HIV-1 test (2002–2008), and Roche COBAS® Ampliprep/Taqman 96 HIV-1 test (2008–current) (Roche Diagnostics Corporation, Indianapolis, IN). Complete blood counts were performed at the University of Colorado Hospital laboratory.

Procedure

Inguinal LNs were palpated and the side/site selected based on patient and surgeon preference. Two surgeons performed the majority of procedures in a clinic-based procedure room. Following a surgical site infection in a patient in 1997, which required admission for IV antibiotics, all subsequent subjects received one dose of cefazolin IV or vancomycin IV prior to the procedure, depending on allergies and history of disease with methicillin-resistant *Staphylococcus aureus*.

Some patients were administered mild sedation at their request. The inguinal region was shaved and prepared with Hibiclens® (Mölnlycke Health Care US, LLC, Norcross, Georgia). Using sterile technique, one percent lidocaine with epinephrine was infiltrated into the skin and subcutaneous tissues. A 2–3 cm incision was made inferior and parallel to the inguinal crease and sharp dissection was used to identify inguinal LNs along the sapheno-femoral junction. Electrocautery was used intermittently to provide hemostasis. Afferent and efferent lymphatics were ligated. The subcutaneous tissues were re-approximated in layers with absorbable suture. A sterile dressing was applied and left in place for 48 h prior to changing and bathing. Subjects were prescribed narcotics and non-steroidal anti-inflammatory drugs (NSAIDs) for pain relief. An ice bag was applied to the area at the patient's discretion.

Following the procedure, subjects were called daily or every other day for 2 wks to monitor for complications. In addition, all subjects were provided with the pager number or cell phone number of the investigators, to facilitate contact after-hours in the event of an emergency. If major problems were identified, arrangements were made for either a physician investigator or primary care doctor to evaluate the patient. All events were recorded by a physician who did not perform nodal surgery in real time and evaluated retrospectively as a part of this study. Surgical site infection was determined according to U.S. Centers for Disease Control and Prevention (CDC) guidelines [19]. Additional events, not defined by CDC or American College of Surgeons National Surgical Quality Improvement Program, were also collected.

Statistical methods

All statistical analyses assumed a two-sided significance level of 0.05 and were performed using GraphPad Prism version 6.00 for Windows (GraphPad Software, La Jolla, California). Fisher exact test was used for comparisons of categorical outcomes. Continuous outcomes were compared using non-parametric tests (Mann–Whitney U, Wilcoxon signed rank).

Results

Subject characteristics

A total of 87 subjects underwent 95 excisional LN biopsies for research purposes. Six subjects had two excisional biopsies and one underwent three procedures. Demographic and clinical characteristics of these subjects are shown in Table 1. The median age was 36 y and more than one-third of subjects

Table 1. Patient Demographics

Characteristic	*LN Excision (n = 87 subjects)*
No. (%) of men	56 (64%)
Age median (range)	36 (22–52)
Race/Ethnicity, No. (%)	
White	48 (55%)
Black	23 (26%)
Hispanic	14 (16%)
Native American	2 (2%)
Risk Factors, No. (%)	
Male-to-male sexual contact (MSM)	40 (46%)
MSM and history of IVDU	9 (10%)
Heterosexual contact[1]	24 (28%)
History of IVDU	10 (12%)
Needle Stick	2 (2%)
Blood Transfusion	2 (2%)
BMI median (range), n = 51	26 (16–57)
Comorbidities, No., (%)[2]	
0	70 (81%)
1	16 (19%)
AIDS[3]	7 (8%)
History of psychiatric diagnosis	22 (25%)
HCV Co-infected	14 (16%)
HBV Co-infected	2 (2%)
Antiretroviral therapy (ART) No., (%)	
Prior history ART	11 (13%)
On ART for at least one of LN excisions	11 (13%)

[1]Heterosexual contact and IVDU in one female.
[2]Chronic obstructive lung disease, reactive airway disease, diabetes mellitus, hypertension
[3]Based on CD4+ T cell count < 200 cells/mm^3
AIDS = acquired immunodeficiency syndrome; BMI = body mass index; LN = lymph node; IVDU = intravenous drug use.

were women. The majority of subjects were white (55%) and 26% were black. Overall, almost half of the subjects described male-to-male sexual contact (MSM) and a one-quarter reported heterosexual contact as their primary risk factor for HIV-1 acquisition. When HIV-1 risk behaviors were analyzed by gender, the majority (88%) of men described MSM and more than one-half (56%) of women described heterosexual contact as their primary risk factor. Approximately one-fifth of subjects had a non-infectious general medical problem, such as chronic obstructive lung disease, reactive airway disease, diabetes mellitus, or hypertension, and 25% of subjects described a past history of psychiatric illness. Only a small proportion of this cohort had a history of acquired immunodeficiency syndrome (AIDS) or antiretroviral therapy secondary to recruitment criteria. The median CD4+ T cell count was 478 cells/mm^3 (range, 57–1117; n = 95) and the median plasma viral load was 4.1 $\log_{10}$copies/mL (range, 1.7–5.9; n = 93).

Complications associated with elective inguinal lymph node biopsy

Overall, complications occurred after 20% of the excisional biopsies (Table 2). None of the subjects who underwent multiple LN biopsies had a complication at more than one time point, therefore each complication represents one subject. Nine (10%) procedures were associated with a complication requiring a specific intervention and nine (10%) were more minor complications, which did not require intervention. An isolated seroma complicated seven procedures, and one required aspiration to facilitate resolution. In general, seromas lasted one to two weeks, but in one subject it persisted for three months before resolution. Lymphedema, hematoma, and reaction to adhesive tape were infrequently identified and resolved without intervention. Eight procedures were complicated by infection. Three infections met criteria for superficial incisional surgical site infection (SSI) according to the CDC definition. One subject with SSI required bedside incision and drainage and culture showed Group A *Streptococcus*. Another subject with SSI had an associated culture-negative seroma. SSI resolved in these two subjects after intravenous followed by oral antibiotics. The other SSI spontaneously drained and resolved with oral antibiotics alone. Five subjects were categorized as having a clinical concern for potential cellulitis that resolved following oral antibiotics. One of the subjects with cellulitis also had an associated seroma that resolved without aspiration. Nerve damage, allergies to medications, or long-term symptoms from groin incision were not identified as complications in this cohort.

Relationship of demographic and clinical characteristics to complications of inguinal lymph node biopsy

The frequency of complications did not differ significantly between women (23%) and men (20%; p = 0.8). In addition, there was no significant difference in median age between individuals experiencing a complication (36 y; range 24–51) and those who did not (37 y; range 22–52; p = 0.9, n = 95). Among those with complications, 61% were white, 17% were black and 22% were Hispanic, which was not significantly different than the ethnic/racial makeup of the cohort (p = 0.6). Body mass index was available in a subset of subjects. Subjects with a complication had a slightly lower BMI (median, 25; range 16–38; n = 12) than those who did not have a complication (median, 28; range 19–57; n = 40; p = 0.05). Having a comorbid condition, AIDS, hepatitis C virus (HCV) co-infection, psychiatric diagnosis, or history of IVDU was not associated with having a complication (p ≥ 0.2). In addition,

Table 2. Complications Associated with Elective Inguinal Lymph Node (LN) Biopsy

	Number (%) of LN excisions (n = 95)
Major Complications	
Superficial incisional surgical site infection	3 (3%)
Cellulitis	5 (5%)
Aspirated seroma	1 (1%)
Total	9 (10%)
Minor Complications[1]	
Seroma	6 (6%)
Lymphedema	1 (1%)
Hematoma	1 (1%)
Reaction to adhesive tape	1 (1%)
Total	9 (10%)

[1]Events defined as not requiring specific medical intervention.

there was no association with HIV-1 risk factor, or which surgeon performed the procedure and having a complication ($p \geq 0.8$). The lack of association of clinical characteristics and complication rate did not change when limiting analysis to subjects classified as having a clinically significant complication. There were no significant differences in white blood cell count, CD4 + T cell count, CD8 + T cell count, and HIV-1 RNA concentration between subjects with and without complications ($p \geq 0.3$). There was a trend toward higher median platelet count in subjects with a complication (259×10^9/L; range, 196–332; n = 17) compared with those without (233×10^9/L; range, 44–633; n = 74, p = 0.07).

Discussion

This is the largest study and only the second one to date describing the safety of inguinal LN excision for HIV-1 research purposes. Importantly, this study included a substantial proportion of women as well as non-white participants. This study used meticulous and standardized monitoring of participants following the biopsy procedure to identify events and found that 10% of subjects had a complication that required a medical intervention (incision and drainage, antibiotics, aspiration of seroma), and an additional 10% of subjects experienced minor complications that spontaneously resolved. The most common complication was infection. In this selected population, we did not identify significant clinical or laboratory characteristics associated with likelihood of complication from inguinal LN biopsy. Taken together, these findings suggest that LN excision in HIV-1-infected individuals for research purposes is generally safe, although complications do occur in a minority of individuals.

A complication rate of 10% events requiring medical intervention is in the expected range based on the literature. Specifically, compared to studies evaluating complications from inguinal sentinel LN biopsy for malignant disease, the rate described here was higher than some [5,6], but lower than others [7,10]. Further, infection and seroma were the most common events reported in these procedures performed for clinical indications [5–7,10], which is also consistent with our findings. Notably, the rate of complications requiring medical intervention in this study is higher than one previously reported study describing LN excision in HIV-1 infected subjects for research purposes, which reported five events (3.6%) requiring intervention [11]. One potential reason for the difference in complication rates between the two studies is sampling differences. The present study included more women and more non-white individuals than the previous study, although neither of these factors was associated with complications. In addition, surveillance bias may have led to over-reporting of infection in our study. Regular phone contact with participants following research procedures prompted participants to evaluate their wounds in detail and may have led to identification of sub-clinical symptoms being classified as a complication. In addition, identification of complications relied on assessment from non-surgeon physician investigators who took an aggressive approach, empirically treating for infection when an incision looked suspicious rather than just monitoring for infection. For example, a sub-clinical finding such as post-operative incision erythema may have been considered possible cellulitis and treated with oral antibiotics. Importantly, this close monitoring may have averted the need for surgical intervention because all but one of the infections described herein resolved with medical intervention alone. Finally, the method of hair removal could have contributed to infection rate as shaving is known to be associated with higher infection rates than clipping [20,21]. Lymphedema, hematoma, and reactions to tape were detected at a very low frequency in this study. The low rate for these more minor events is consistent with other studies [6,7,10,11], although one study evaluating sentinel LN biopsy for melanoma reported a higher rate of hematoma and lymphedema [5]. The present study suggests that the complication rate of inguinal LN biopsy for research purposes could be higher than reported previously [11] and that close monitoring could identify more events.

We did not find any predictors of wound complications from inguinal LN biopsy. It was noted that subjects who experienced a complication had a lower BMI and higher platelet count. Nonetheless, these values were within the normal range; therefore, the clinical significance of these findings is unclear. The lack of association of complication rate with typical predictors of surgical complications is likely due to recruitment of subjects who had minimal comorbid conditions, and who were generally not obese or underweight, and were less than 60 y old.

This study of surgical complications has a few limitations. Some of the common factors associated with surgical complications such as smoking, recent weight loss, and serum albumin concentrations [22] were not recorded. Nonetheless, in general, the subjects were relatively healthy therefore recent weight changes or abnormalities in albumin were unlikely. No mechanism for collecting long term complications was set up as a part of the study, although no problems were relayed to investigators from subjects' primary providers to date. Finally, published guidelines for collecting surgical complications are limited to complications that are generally associated with higher morbidity [19]. The identification and quantification of less morbid events, such as cellulitis, is challenging because it is often based on clinical judgment of the investigators, which may limit comparability between studies.

This retrospective review of clinical outcomes of a diverse group of HIV-1 infected subjects who underwent inguinal LN excision for research purposes provides important new information regarding the risk of this procedure. Overall, 90% of study subjects experienced no or minor complications. In addition, a portion of the 10% of individuals who experienced a morbid event may have been the result of surveillance bias. Nevertheless, the meticulous follow up and empiric treatment for early symptoms and wound findings likely contributed to the overall safety of the study in which only one bedside incision and drainage was required to resolve infection. Importantly, this study establishes further that the performance of excisional inguinal LN biopsy in HIV-1-infected individuals for research purposes is generally safe. This information is important because it provides a reference for future investigators designing studies and local institutional review boards.

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No competing financial interests exist.

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