

Feasibility and safety of research sigmoid colon biopsy in a cohort of Thai men who have sex with men with acute HIV-1

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Abstract

Background: The gut-associated lymphoid tissue (GALT) is a major reservoir of HIV-1 established early in acute HIV-1 infection (AHI). Sampling tissue from GALT can provide information about viral reservoirs and immune responses but may be complicated during AHI for reasons such as high viral replication, CD4 T cell depletion and immune activation. Risk of adverse events (AEs) associated with research sigmoid colon biopsies was assessed in participants with AHI in Bangkok, Thailand.

Methods: Between 2009 and 2016, 170 biopsies collected from the sigmoid colon were performed during AHI and at follow-up visits (median 24 weeks post AHI diagnosis). Adverse event incidence was evaluated, as well as the associations of procedure timing, repetition and clinical parameters with AE risk. Negative binomial regression models were used to calculate incidence rate ratios and 95% confidence intervals.

Results: Among 103 participants (median age of 27 years, 97.1% male, 96.1% men who have sex with men), 87 sigmoidoscopies were completed during AHI and 83 at a follow-up visit. Approximately 30 biopsies were obtained per procedure for assessment of colonic viral load and HIV-1 reservoir, immunohistochemistry or phenotypic assays. All 11 AEs were grade 1 (6.5%) and included abdominal discomfort ($n=5$, 2.9%), mild rectal bleeding ($n=5$, 2.9%) and difficulty passing stool ($n=1$, 0.6%). Biopsy-related AE risk was not significantly associated with age, HIV-1 RNA, CD4 T cell count, or number and time of biopsy.

Conclusions: Complications of sigmoidoscopy with biopsy in participants with AHI were infrequent and mild. Longitudinal sampling of the sigmoid colon to evaluate the gut-associated HIV-1 reservoir can be safely performed as part of research studies.

Keywords: research risk, colon biopsy, acute HIV-1 infection

Introduction

In treated HIV-1 infection, gut-associated lymphoid tissue harbours a pool of latently infected CD4 T cells despite antiretroviral therapy (ART) [1–4] and is a barrier to HIV-1 cure. HIV-1 infects lymphoid tissue during acute HIV-1 infection (AHI) with rapid gut CD4 T cell depletion, inflammation and immune activation [5,6]. The gastrointestinal mucosa contains a large proportion of the body's lymphocytes and is an important tissue compartment for monitoring changes in CD4 T cell populations [7,8].

Flexible sigmoidoscopy has been used to collect colon mucosal biopsies for research and is well tolerated in people without HIV-1 [9–13]. Complications are rare and include bleeding, abdominal pain, diarrhoea, flatulence, bloating, colitis, diverticulitis and infection [9–13]. Dark coloured stool can be expected for up to several days post procedure [9]. Minimal bowel preparation is required for sigmoid biopsy, allowing sampling in acute infection prior to ART initiation if immediate initiation is desired [14]. Few

studies have reported the safety and tolerability of this procedure in the context of HIV-1 infection, and none have been done during AHI or in an international setting. It is unknown whether high viral replication or immune activation in AHI could affect procedure-associated risks [8]. In this article we examined the safety and tolerability of one or multiple sigmoid colon biopsies in a cohort of predominantly young Thai men who have sex with men (MSM) enrolled during AHI. Our findings may help researchers, participants and ethical review committees in their considerations for including sigmoidoscopy and research biopsies in studies of populations.

Methods

Study population

Individuals were prospectively screened at the Thai Red Cross Anonymous Clinic and offered enrolment in the RV254/SEARCH010 cohort (ClinicalTrials.gov identification number NCT00796146) after AHI was confirmed by fourth generation HIV antigen/antibody immunoassay, pooled nucleic acid testing and sequential less sensitive HIV antibody immunoassay (IA), as previously described [15,16]. Participants in the cohort were offered ART initiation during AHI, and follow-up assessments,

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including peripheral CD4 T cell count and plasma HIV RNA viral load, were at days 0, 3, 7; weeks 2, 4, 8, 12; and every 12 weeks thereafter [17].

The study protocol and consent forms were approved by the institutional review boards of Chulalongkorn University in Thailand and the Walter Reed Army Institute of Research in the US. The analysis included all participants in RV254/SEARCH010 who underwent at least one sigmoidoscopy with colon biopsy between May 2009 and May 2016. All participants provided informed consent prior to any study procedures.

Sigmoidoscopy with colon biopsy

Sigmoid colon biopsy was optional and offered at enrolment and after 24, 96 and 240 weeks on study. Biopsies were performed via flexible sigmoidoscopy by qualified gastroenterologists. Pre-procedure bowel preparation included diet modification and saline enemas the day prior to the procedure. Bowel preparation quality was determined for the left side colon according to the Boston Bowel Preparation Scale [18]. During the procedure, participants were offered meperidine 25mg intravenously and/or midazolam 2.5mg intravenously to relieve discomfort and anxiety. If any polyps were found during the sigmoidoscopy, the gastroenterologist would perform a polypectomy. Approximately 30 biopsies were obtained from gut tissue in the sigmoid area using Radial Jaw 3 biopsy forceps (Boston Scientific, Natick, MA). Two of the thirty biopsy pieces were sent to the pathology laboratory to assess for malignancies. The participant was referred to the gastroenterologist or a private medical practitioner if there were any abnormal findings. Participants were advised to refrain from anal intercourse for 7 days after the procedure.

Biopsy pieces were either cryopreserved or embedded in paraffin for subsequent assessment of colonic viral load, colonic HIV-1 reservoir or immunohistochemistry. A subset of mucosal mononuclear cells was isolated after collection to assess the phenotype of different cell subsets as well as HIV-specific T cell responses in the mucosa by multiparameter flow cytometry.

Adverse event monitoring and grading

Participants were monitored in the procedural suite until fully awake and were urged to contact the study nurses as soon as possible if abnormal symptoms developed after the procedure. At every study visit, physicians questioned participants for any pre-existing or current adverse events (AEs), including biopsy-related AEs that were deemed likely to be related to the procedure. Each AE was graded according to the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events [19].

Statistical methods

Unadjusted negative binomial regression models were used to calculate incidence rate ratios (IRRs) and 95% confidence intervals (CIs) to determine whether AE risk was associated with procedures at AHI, repetition of procedures, age, peripheral CD4 T cell count and plasma HIV RNA viral load. Significance was determined by P -value <0.05 , and P -values 0.05 to <0.10 were considered suggestive of a trend. All analyses were performed using Stata Statistical Software: Release 15 (StataCorp LLC, College Station, TX).

Results

Clinical characteristics of research participants

A total of 170 biopsies were performed (87 at baseline during AHI and 83 at a follow-up visit) in 103 consenting participants

between May 2009 and May 2016. Most study participants were men who have sex with men (MSM) ($n=99$, 96.1%). Sixty-three participants completed one procedure, 16 completed two, 21 completed three and 3 completed four procedures. Clinical characteristics of research participants at either AHI or follow-up are summarised in Table 1. All except one were on ART during the follow-up biopsy, and median HIV RNA was undetectable for participants completing a biopsy at least 24 weeks post AHI diagnosis.

Incidence of sigmoid colon biopsy-related adverse events

After 170 sigmoid colonoscopies with biopsies, 11 AEs occurred in seven participants who were deemed related to the procedure by the investigators, for an overall AE rate of 6.5%. All AEs were grade 1, and the predominant AEs were abdominal discomfort ($n=5$, 2.9%) and rectal bleeding ($n=5$, 2.9%). There was one case of difficulty passing stool (0.6%). Rectal bleeding included bloody or dark stool and did not require any intervention. Three participants had multiple AEs either at AHI or at follow-up biopsies. All AEs resolved spontaneously within a median of one (interquartile range 1–3) day, except in one case in which the abdominal discomfort was prolonged for 3 months and occurred concomitantly with untreated urethral gonorrhoeal infection. The gonorrhoeal infection was eventually treated with a single dose of 400mg of cefixime and 1000mg of azithromycin at 3 months after the colon biopsy, which coincided with the resolution of abdominal discomfort.

Factors associated with biopsy-related adverse events

Undergoing sigmoidoscopy with biopsies during AHI compared with follow-up and undergoing more than one sigmoidoscopy with biopsies were not significantly associated with AEs. CD4 T cell count and HIV RNA at the time of biopsies were also not associated with AE risk. The frequency of AEs in participants aged between 18 and 25 years (8 AEs in 67 procedures) compared with participants aged >26 years (3 AEs in 103 procedures) suggested a trend of increased AE risk in younger participants [IRR 4.10 (95% CI 0.95–17.64), $P=0.06$] (Table 2).

Discussion

In this cohort of mostly Thai MSM with AHI, sigmoid colon biopsies were safe and well tolerated. The biopsy-related AEs were all grade 1, were largely expected and did not interfere with daily functioning or require medical intervention. All symptoms resolved without sequelae.

Rectal bleeding or dark coloured stool can be expected for up to 2 days after colon biopsy procedures performed during routine clinical care [8]. All AEs were transient except for one case of mild abdominal discomfort that resolved after treatment for urethral gonorrhoeal infection. Two large studies have identified significant but rare sigmoidoscopy-related complications in HIV-uninfected populations: 24 hospitalisations out of 109,534 flexible sigmoidoscopies between 1994 and 1996 in northern California and two perforations out of 49,501 sigmoidoscopies between 1987 and 1996 at the Mayo Clinic Scottsdale [10,11]. A study of Peruvian MSM at high risk of HIV-1 reported mild AEs, and their total complication rate of 7.7% (3/39 procedures) included one case of mild flatulence, one of mild bloating and one moderate adverse drug reaction [12]. Our complication rate of 6.5%, of which all AEs were grade 1, supports the safety of the procedure in our cohort.

In a study conducted at the University of Pittsburgh, multiple flexible sigmoidoscopies in HIV-uninfected, healthy participants

Table 1. Characteristics of participants who completed sigmoid colon biopsy

| Characteristics | Biopsy at baseline, week 0 (N=87) | Biopsy at follow-up, week 24–240 (N=83) |
|---|--------------------------------------|--|
| Age (years), median (IQR) | 27 (23–32) | 28 (25–34) |
| 18–25 | 39 (44.8) | 28 (33.7) |
| 26–30 | 24 (27.6) | 27 (32.5) |
| 31–40 | 17 (19.5) | 14 (16.9) |
| >41 | 7 (8.1) | 14 (16.9) |
| Sex, n (%) | | |
| Female | 3 (3.5) | 7 (8.4) |
| Male | 84 (96.5) | 76 (91.6) |
| Weight (kg), median (IQR) | 62 (57–70) | 62 (56–70) |
| Days since history of HIV exposure, median (IQR) | 18 (13–23) | – |
| Antiretroviral therapy | 0 (0) | 82 (98.8) |
| Duration on ART (weeks), median (IQR) | – | 24 (24–96) |
| Fiebig stage* | | |
| I | 12 (13.8) | – |
| II | 23 (26.4) | – |
| III | 37 (42.5) | – |
| IV | 7 (8.1) | – |
| V | 7 (8.1) | – |
| VI | 1 (1.1) | – |
| CD4 T cells (cells/mm ³), median (IQR) | 381 (288–538) | 616 (488–758) |
| <350 | 37 (42.5) | 3 (3.6) |
| 350–500 | 24 (27.6) | 19 (22.9) |
| >500 | 26 (29.9) | 61 (73.5) |
| HIV RNA (log ₁₀ copies/mL), median (IQR) | 5.8 (5.1–6.9) | UND (UND–UND) |

* Fiebig staging: F1, NAT+, p24 antigen–, HIV IgM–; FII, NAT+, p24 antigen+, HIV IgM–; FIII, HIV IgM+, Western blot–; FIV, HIV IgM+, Western blot indeterminate; FV, HIV IgM+, Western blot+ (without p31 band); FVI, HIV IgM+, Western blot+ (with p31 band). ART: antiretroviral therapy; IQR: interquartile range; N: total number of participants; UND: undetectable.

Table 2. Colon biopsy-related adverse events and association factors

| Procedure timepoint | AE association with | | | |
|-------------------------|-------------------------------------|-----------------|-------------------|---------|
| | Number of AE/number of participants | Rate (95% CI) | IRR (95% CI) | P-value |
| Baseline (week 0) | 8/87 | 9.2 (4.6–18.4) | 2.54 (0.57–11.27) | 0.22 |
| Follow-up (week 24–240) | 3/83 | 3.6 (1.2–11.2) | Ref. | |
| Completed procedures, n | Number of AE/number of procedures | Rate (95% CI) | IRR (95% CI) | P-value |
| 1 | 4/63 | 6.3 (2.4–16.9) | Ref. | |
| 2–4 | 7/107 | 6.5 (3.1–13.7) | 1.03 (0.30–3.52) | 0.96 |
| Age (years) | Number of AE/number of procedures | Rate (95% CI) | IRR (95% CI) | P-value |
| 18–25 | 8/67 | 11.9 (6.0–23.9) | 4.10 (0.95–17.64) | 0.06 |
| >26 | 3/103 | 2.9 (0.9–9.0) | Ref. | |

Univariate negative binomial regression model was used to compare: 1) AE rate between participants completing baseline biopsy at week 0 of enrolment and those completing a procedure at follow-up visits during weeks 24 to 240 after enrolment; 2) AE rate for first biopsy, second repeated biopsy third or fourth; and 3) AE rate between participants ages 18 and 25 and those above age 26. CD4 T cell count and HIV RNA (not shown) were not significantly associated with AE risk. AE: adverse event; CI: confidence interval; IRR: incidence rate ratios.

were considered safe and unrelated to AE risk. The average number of procedures per participant in the study was 3.6 with an AE rate of 1.6% per procedure. Of 1004 procedures, 14 grade 1 AEs and 2 grade 2 AEs were reported. Four of the AEs included

rectal bleeding that did not require transfusion. Our overall complication rate of 6.5% and the rectal bleeding rate of 2.9% are low, although higher than the rates in the Pittsburgh study. The difference may be due to frequent AE documentation as per the

RV254/SEARCH010 protocol and the number of biopsy pieces collected, which was approximately 30 pieces compared with the range of 7 to 21 in the Pittsburgh study [13].

Although not statistically significant, complication risk tended to be greater in younger participants. We cannot conclude why this may be so, although it is possible that young participants in the MSM cohort may have had higher rates of sexual activity and receptive anal intercourse that could be linked to susceptibility to complications in the colon. In this study, sexual activity was not recorded at each biopsy procedure. These analyses are limited by a relatively small sample size, especially of participants who underwent more than one procedure. The study relied heavily on self-reporting; thus AE documentation may not be complete if a participant decided not to inform the study team of a complication. Furthermore, the population of mainly young Thai MSM may limit generalisation of the findings to other populations.

We conclude that sigmoid colon biopsies are safe and well tolerated by participants with AHL in an international research setting. All reported AEs were infrequent, mild and self-limited. This information can be reassuring for participants, researchers and institutional review boards in designing and evaluating future studies that incorporate sigmoid biopsy.

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Conflicts of interest

MC interned at Royalty Pharma. TC has received a speaker fee from Gilead Sciences. SS co-directs a clinical research study that receives donated study medications from ViiV Healthcare. JA has received honorarium for participating in advisory meetings for ViiV Healthcare, Merck, Gilead, AbbVie and Roche. The remaining authors have no competing interests to declare.

Disclaimer

The views expressed are those of the authors and should not be construed to represent the positions of the participating institutions, the US Army, the Department of Defense or the Department of Health and Human Services. The investigators have adhered to the policies for protection of human subjects as prescribed in AR-70. Clinical trial number: NCT00796146.

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