SHORT REPORT

Antiretroviral therapy suppresses rectal HIV-RNA shedding despite inflammation in MSM with rectal *C. trachomatis* and *N. gonorrhoeae* infections—a cross-sectional, single-center study

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ABSTRACT

Objectives Rectal infections with *Chlamydia trachomatis* and/or *Neisseria gonorrhoeae* (CT/NG) are common in men who have sex with men (MSM) and are linked to HIV transmission. However, rectal CT/NG infections are often asymptomatic and it is not known how they contribute to HIV transmission. We assessed clinical and cytological signs of inflammation as well as rectal HIV-RNA in HIV-infected MSM with and without CT/NG infection.

Methods 112 HIV-positive MSM with or without rectal symptoms and with or without antiretroviral therapy who underwent high-resolution anoscopy (HRA) at the proctological outpatient centre of the University Hospital Essen, Germany, between November 2013 and February 2014 were included in this cross-sectional study. During the examination, rectal swabs for the assessment of CT/NG, HIV-RNA and inflammatory cells (granulocytes, lymphocytes, histiocytes) were collected. 110 patients were assessed according to the study protocol, and no imputation of missing data was performed.

Results Rectal infections with CT or NG were detected in 17 participants, and 4 participants were coinfected. Only symptomatic CT/NG infections (8/17) showed signs of inflammation in HRA. Symptomatic CT/NG infections were also associated with the detection of lymphocytes and histiocytes in rectal cytology (both P<0.001). In contrast, asymptomatic CT/NG infections neither resulted in clinical nor cytological signs of inflammation. Rectal HIV-RNA was undetectable in all participants with rectal CT/NG infections who received combined antiretroviral therapy (ART) when plasma HIV-RNA was below the limit of detection (n=13). Besides rectal CT/NG infections, syphilis (n=4) and HPV-associated lesions (n=37) were frequently detected, and proctological symptoms were associated with simultaneous infection with ≥2 STDs.

Conclusions Only symptomatic but not asymptomatic rectal infections with CT and/or NG were associated with clinical and cytological signs of inflammation. Rectal HIV shedding was not promoted by CT/NG infections in patients receiving ART with suppressed plasma HIV-RNA.

Trial registration number UTN: U1111-1150-4804. German Clinical Trials Register (DRKS): DRKS00005468.

INTRODUCTION

The prevalence of sexually transmitted diseases (STDs) is rising among men who have sex with men (MSM).[1] Rectal bacterial infections with *Chlamydia trachomatis* and/or *Neisseria gonorrhoeae* (CT/NG) are common.[2 3] Some authors regard the well-documented association of rectal CT/NG infections with newly diagnosed HIV infections primarily as a marker for risk-taking sexual behaviour,[4] while others suggest a causal link to HIV transmission.[5 6]

Inflammatory processes of (often asymptomatic) rectal CT/NG infections that might promote HIV transmission are not well characterised. CT/NG-induced rectal HIV shedding in patients receiving combined antiretroviral therapy (ART) has not been demonstrated,[7 8] and rectal CT infections were not associated with mucosal damage or elevated levels of proinflammatory cytokines.[9] However, these reports nearly exclusively cover asymptomatic rectal CT/NG infections. Additionally, clinical and cytological data are missing.

Here we assess clinical and cytological signs of inflammation in HIV-positive MSM with and without rectal CT/NG infections using high-resolution anoscopy (HRA) and anal cytology. Additionally, proctological symptoms and rectal HIV-RNA levels (as marker for rectal HIV shedding) are evaluated.

METHODS

Study population

The study centre offers HRA for the management of HPV-related conditions and acute proctological problems, and most patients are HIV-positive MSM. We estimated the prevalence of rectal CT/NG among HIV-positive MSM visiting our STD-focused proctological outpatient clinic to be around 15%, slightly higher than reported for (HIV-negative and HIV-positive) MSM in Germany.[2] With the goal to detect ≥15 infections, we aimed to include ≥100 HIV-positive MSM in this exploratory cross-sectional study. The primary aim was to assess the impact of CT/NG on rectal inflammation and rectal HIV-RNA shedding. Between November 2013 and February 2014, every HIV-positive MSM with a scheduled appointment for HRA or who presented with acute proctological problems was asked to participate.

Study visit

Before HRA, proctological symptoms were assessed and a rectal cytology sample was acquired using a tissue brush (Cytobrush Plus GT, Coopersurgical). Collected cells were immediately transferred into PreservCyt solution (Hologic). For HRA, a video colposcope (Leisegang, 3 MV) with a disposable anoscope coated with 2% lidocaine hydrochloride gel was used. After visualising the linea dentata, samples for HIV-RNA quantification and CT/NG detection were collected from the rectal mucosa using a polyurethane coated swab (Σ-Swab, MWE) and the Xpert CT/NG Specimen Collection Kit (Cepheid), respectively (see below). The distal rectum and anal canal were inspected to identify signs of inflammation and HPV-associated lesions (condyloma acuminata and anal intraepithelial neoplasia) at 15× magnification. Finally, plasma HIV-RNA, $CD4^+$ lymphocyte count, syphilis serology and the current status of ART were assessed.

Cytology, rectal HIV-RNA quantification, CT/NG assessment and descriptive statistics

In cytology samples, density of granulocytes, lymphocytes and histiocytes was assessed as follows: isolated cells: (+); <20% of all cells per visual field: +; 20%–50% of all cells per visual field: ++; >50% of all cells per visual field: +++. Additionally, epithelial dysplasia was assessed. For semiquantitative rectal HIV-RNA assessment, Σ-Swabs were fully saturated with 60–80 µL rectal exudate and placed into 1.5 mL ice-cold normal human plasma (NHP). HIV-1 RNA was recovered by centrifugation through a QIAShredder (20 000× g, 3 min), resuspended in ice-cold NHP and stored at −80°C. HIV-1 RNA was quantified with the Abbott m2000 System according to the instructions of the manufacturer with a lower limit of detection of 40 copies/mL. The theoretical lower limit of detection was approximately 65 copies/swab. CT and NG were detected according to the instructions of the manufacturer (Xpert CT/NG System, Cepheid).

Cytology samples, CT/NG samples and rectal HIV-RNA samples did not allow repeated analyses. Failed analyses were handled as missing data. For descriptive statistics, no data were imputed for missing values. Fisher's exact test was used to compare granulocytes, lymphocytes and histiocytes in cytology samples of participants with CT/NG infection (symptomatic and asymptomatic) and without CT/NG infections (GraphPad Prism V.7).

RESULTS

Study population and prevalence of STD

One hundred and twelve HIV-positive MSM were included in the study. One participant withdrew his consent after the study visit, one participant could not be examined due to diarrhoea, and the remaining 110 participants were analysed according to the study protocol. Mean age, mean time since HIV diagnosis, Centers for Disease Control and Prevention (CDC) stage of HIV infection, mean $CD4^+$ cell count and the proportion of participants receiving antiretroviral therapy (ART) are summarised in table 1. In 88% of the MSM receiving ART, plasma HIV-RNAs were <40 copies/mL.

Seventeen CT/NG infections were detected (16.5%), and four participants were coinfected with CT and NG. Additional STDs were common: the frequencies of rectal HPV-associated lesions, abnormal epithelia in anal cytology and early-stage syphilis are listed in table 1.

Table 1 Demographic data, HIV-related data and STD prevalence

Demographics, HIV infection	Mean (SD) or percentage (n/total)	Missing data (n)
Mean age (years)	41.9 (±10.5)	–
Mean duration since HIV diagnosis (years)	9.2 (±8.2)	–
CDC stage HIV infection		
A	54.5% (60/110)	–
B	23.6% (26/110)	–
C	21.8% (24/110)	–
1	6.4% (7/110)	–
2	59.1% (65/110)	–
3	34.5% (38/110)	–
Mean $CD4^+$ cell count (cells/µL)	540 (±236)	2*
Prescribed ART	90.9% (100/110)	–
With HIV plasma viral load <40 copies/mL	88.0% (88/100)	–
Rectal shedding of HIV-RNA	13.5% (14/104)	6†
When HIV plasma viral load was <40 copies/mL	2.4% (2/83)	5†
When HIV plasma viral load was ≥40 copies/mL	57.1% (12/21)	1†
STD prevalence	**Percentage (n/total)**	**Missing data (n)**
Detection CT and/or NG	16.5 (17/103)	7‡
CT	70.6 (12/17)	
NG	52.9 (9/17)	
CT/NG coinfections	23.5 (4/17)	
Current syphilis	3.7 (4/109)	1§
Abnormal epithelia in anal cytology¶	26.6 (29/109)	1**
Detection of HPV-associated lesions††	33.6 (36/107)	3‡‡
Condyloma/AIN I	83.3 (30/36)	
AIN II/AIN III	16.7 (6/36)	

*CD4 count missing.
†Rectal HIV-RNA amplification failed.
‡Nucleic acid amplification test for CT/NG failed.
§Serology missing.
¶Atypical cells of undetermined significance, low-grade intraepithelial lesion or high-grade intraepithelial lesion.
**Insufficient quality of sample.
††HPV-associated lesions were either of typical clinical appearance on (in?) high-resolution anoscopy or confirmed by biopsy.
‡‡Participants with suspected AIN refused biopsy.
AIN, anal intraepithelial neoplasia; ART, antiretroviral therapy; CDC, Centers of Disease Control and Prevention.

Proctological symptoms and HRA results

Of the participants without rectal CT/NG infection, 20% and 15% presented with proctological symptoms and signs of rectal inflammation, respectively. The most common symptom was bleeding (n=10), followed by itching (n=6), and the most common sign of inflammation was serous exudate (n=11). In contrast, eight participants (47%) with CT/NG infection indicated proctological symptoms, most commonly pain (n=7), followed by bleeding and discharge (both n=3). Correspondingly, seven out of eight symptomatic CT/NG infections resulted in ≥1 of the following clinical signs of inflammation: pussy/serous exudate (n=6), bloody exudate (n=4) and erythema of rectal mucosa (n=4). No asymptomatic rectal CT/NG infections resulted in clinical signs of inflammation.

Simultaneous infections with ≥2 STDs were common. Seventy-five per cent of symptomatic but only 11% of asymptomatic rectal infections were associated with simultaneous detection

Table 2 Comparison of inflammatory cells in rectal cytology samples of HIV-infected MSM with asymptomatic, symptomatic and without rectal CT/NG infection

	No CT/NG infection (n=85*)		Asymptomatic CT/NG infection (n=9*)			Symptomatic CT/NG infection (n=8*)		
	Cytologies pos. n/total (%)	Median density†	Cytologies pos. n/total (%)	Median density†	Relative risk‡ (95% CI, P)	Cytologies pos. n/total (%)	Median density†	Relative risk‡ (95% CI, P)
Granulocytes	39/85 (45.9)	(+)	3/9 (33.3)	(+)	0.812 (0.559 to 1.565, P=0.727)	7/8 (87.5)	++	4.329 (1.120 to 24.310, P=0.030)
Lymphocytes	7/85 (8.2)	(+)	1/9 (11.1)	(+)	1.032 (0.900 to 1.629, P=0.568)	5/8 (62.5)	+	2.447 (1.314 to 6.717, P<0.001)
Histiocytes	5/85 (5.9)	(+)	1/9 (11.1)	(+)	1.059 (0.931 to 1.669, P=0.463)	5/8 (62.5)	+	2.519 (1.355 to 6.909, P<0.001)

*Total n=102: seven data sets excluded due to failed CT/NG nucleic acid amplification tests and one data set excluded due to insufficient quality of cytology sample (compare with table 1).
†Median cell density of cytology samples positive for the respective cell type (from (+) to +++; see the Methods section); samples negative for the respective cell type were not included in the analysis.
‡Relative risk for the detection of the respective cell type compared with cytology samples from participants without CT/NG infection (Fisher's exact test).
CT/NG, *Chlamydia trachomatis* and/or *Neisseria gonorrhoeae*; MSM, men who have sex with men.

of ≥2 STDs: CT/NG coinfections (3 symptomatic vs 1 asymptomatic), concomitant early syphilis (3 vs 0) and concomitant HPV-associated lesions (4 vs 1).

Cytological signs of rectal inflammation associated with CT/NG infections

Without CT/NG infection, isolated granulocytes were found in nearly half of the cytology samples, and isolated lymphocytes and histiocytes were rare. Asymptomatic CT/NG infections were not associated with the detection of inflammatory cells (table 2).

Symptomatic CT/NG infections on the other hand frequently resulted in the detection of granulocytes (P=0.030), lymphocytes (P<0.001) and histiocytes (P<0.001). When inflammatory cells were detected, their density was also increased (table 2).

Rectal shedding of HIV-RNA

While rectal HIV-RNA was only detectable in 2.4% of MSM with plasma HIV-RNA <40 copies/mL, rectal HIV-RNA was detected frequently (57.1%) in MSM with plasma HIV-RNA levels ≥40 copies/mL (table 1). Plasma HIV-RNA concentration correlated with rectal HIV-RNA concentration (data not shown). Rectal CT/NG infections (with or without symptoms) on the other hand were not associated with rectal shedding of HIV-RNA: 0 out of 13 MSM with CT/NG infection and plasma HIV-RNA <40 copies/mL (five reporting symptoms) had detectable concentrations of rectal HIV-RNA.

DISCUSSION

In this study on HIV-positive MSM, patients with rectal CT/NG infections more often presented with proctological symptoms than has been reported,[1 2] most probably because symptomatic patients proactively seek help at a proctology consultancy. Therefore, we were able to compare symptomatic with asymptomatic rectal CT/NG infections. Only symptomatic infections resulted in inflammation. In line with potentially promoting HIV transmission, histiocytes and lymphocytes were detectable in rectal cytology samples of patients with symptomatic CT/NG infection. However, rectal HIV-RNA shedding was effectively suppressed by ART even in the presence of rectal inflammation. Asymptomatic rectal CT/NG infection did neither lead to rectal inflammation nor enhance rectal HIV shedding. While symptomatic infections might lead to the transmission of HIV-infected cells, we did not find any evidence for increased infectiousness of patients with asymptomatic rectal CT/NG infections.

In contrast to previously published data,[10] proctological symptoms were frequently associated with CT/NG coinfections or other additional STDs. Thus, the high rate of symptomatic/inflammatory rectal CT/NG infections may partly be driven by infection with more than one STD.

Detection of inflammation in cytology depends on leaky mucosa allowing the exudation of immune cells. Cytology cannot rule out minor inflammatory responses possibly connected to asymptomatic CT/NG infections. However, for HIV transmission, a mucosal barrier breach is a key factor. Thus, cytology is a sufficient method to detect relevant inflammatory reactions for HIV transmission.

Additionally, we could neither assess rectal shedding of HIV-infected cells nor minimal HIV-RNA shedding (with or without CT/NG infection). However, suppression of rectal HIV shedding despite (mostly asymptomatic) rectal bacterial infections has previously been reported.[7 8] And the absence of (potentially HIV-infected) histiocytes and lymphocytes in the rectal exudate of asymptomatic, CT/NG-infected patients confirms their very low level of HIV infectiousness.[11]

The small sample size and the exploratory nature of this study are the most important limitations. In particular, the number of CT/NG-infected MSM who did not receive ART was small (n=4), and we were therefore unable to assess the impact of inflammation on rectal HIV shedding in patients with viraemia. Additionally, we assessed a population of HIV-positive MSM with a high rate of proctological symptoms and multiple STDs. Transferability of our clinical findings to a broader population is therefore limited.

To understand potential differences of the biological role of rectal CT/NG infections for HIV susceptibility, inflammatory responses in HIV-negative patients need to be studied. For HIV infectiousness, rectal infections with CT and NG primarily seem to be markers for risk-taking sexual behaviour; ART is an effective measure to mitigate the risk of HIV transmission.

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Contributors JS designed the study, performed HRA, collected samples, contributed to the establishment of the method for detection of rectal HIV-RNA, contributed to the interpretation of the data, and prepared and revised the

manuscript. JV contributed to the establishment of the method for detection of rectal HIV-RNA and performed HIV-RNA detection. EW prepared the data set. JW established and performed inflammatory cell analysis in rectal cytology. EHvH performed CT/NG testing. DS gave important intellectual input to study design and to the manuscript. SE designed the study, performed HRA, collected samples, contributed to the interpretation of the data and revised the manuscript critically for important intellectual content. All authors have read and commented on the manuscript, and all authors saw and approved the final version of the manuscript. All authors agreed to be accountable for all aspects of the work.

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Patient consent Obtained.

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