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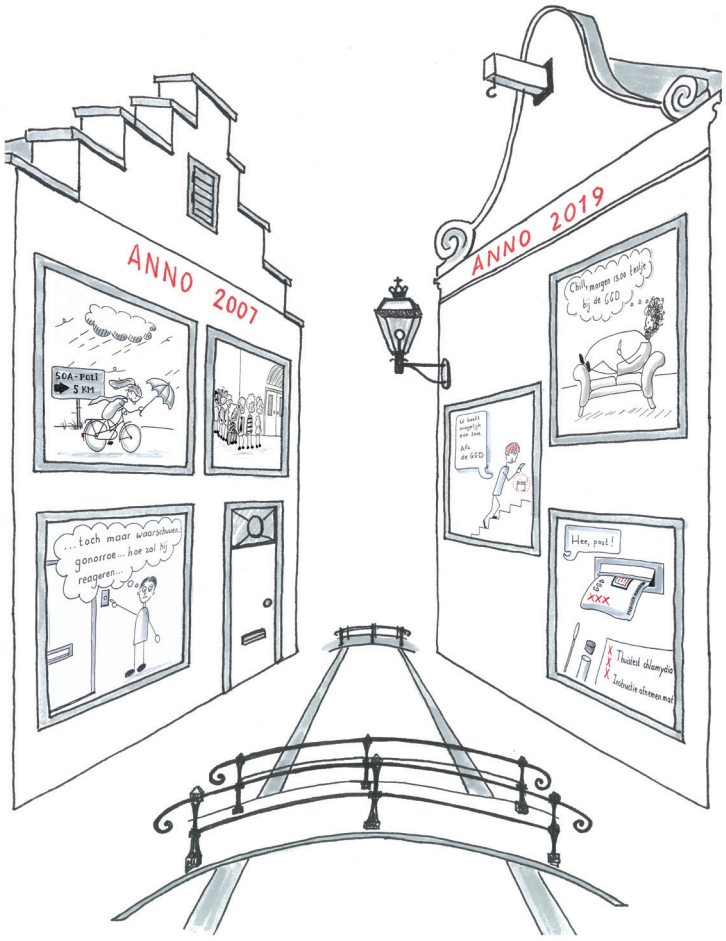

Martijn van Rooijen

STRATEGIES FOR CLINICAL AND PUBLIC HEALTH MANAGEMENT OF SEXUALLY TRANSMITTED INFECTIONS

Martijn S. van Rooijen

Strategies for clinical and public health managment of sexually transmitted infections
Martinus Sebastiaan van Rooijen

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STRATEGIES FOR CLINICAL AND PUBLIC HEALTH MANAGEMENT OF SEXUALLY TRANSMITTED INFECTIONS

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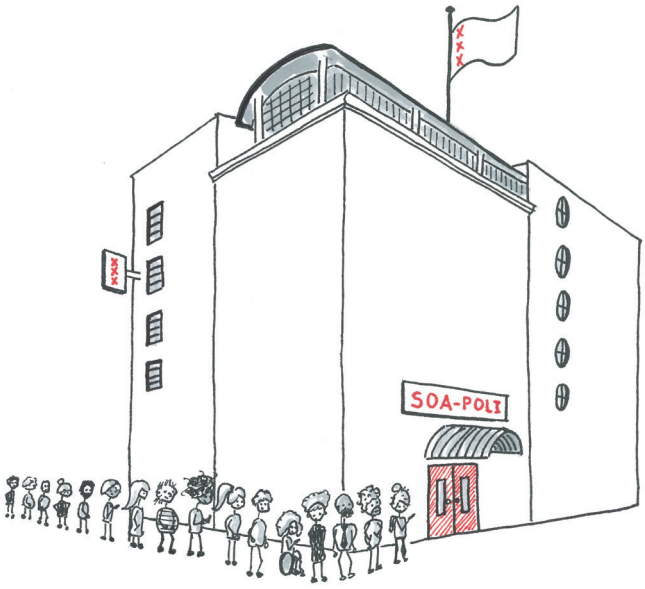
TABLE OF CONTENTS

CHAPTER 1



General introduction

Sexually transmitted infections (STI) encompass a group of infections that can be spread or acquired through sexual contact.[1] Each infection has the potential to pose significant immediate and long-term harm, affecting health and well-being. An important tool in the control of STI is early diagnosis and treatment of those infected.

In my position as a data manager at the Amsterdam STI clinic, I have primarily been working at the data processing and analysis site of the collected patient data. During processing of surveillance data and monitoring of our clinical process, a variety of relevant clinical and public health questions were raised. The setting at the Public Health Service with experienced researchers resulted in many of these questions being addressed, leading to research and subsequently findings published in peer-reviewed papers. In this thesis a selection of the published papers is presented.

(1.1) STI CARE IN THE NETHERLANDS

In the Netherlands, several organisations are active to promote sexual education, implement sexual prevention campaigns, and perform STI testing. Since 2012, sexuality has been adopted in the official national curriculum guidelines for secondary education.[2]

STI care in the Netherlands is primarily provided by general practitioners (GPs) and specialized STI centres.[3] STI clinics are subsidised by the government and provide low threshold, free of charge STI/HIV testing and care, targeted at high-risk groups.[4] High-risk groups are those notified of STI exposure, those reporting STI-related symptoms, sex workers, men who have sex with men (MSM), first or second generation immigrants from STI/HIV endemic areas, those with a sex partner from an STI/HIV endemic area, those aged below 25 years, and victims of sexual violence.[4]

In accordance with the subsidy requirements, prior to 2012 all clients had to be tested for *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, syphilis, and – using the opt-out strategy – HIV.[4] From 2012 up to and including 2014, attendees below the age of 25 years were tested for chlamydia only and from 2015 onwards for chlamydia and gonorrhoea.[4]

Since 2008, a compulsory deductible at the expense of the patient applies for the first consumed health care.[5] As a result, costs of GP prescribed STI laboratory tests

and treatment needs to be paid out-of-pocket in cases where little to no healthcare is consumed. In contrast, care delivered by STI clinics is free of charge, and the deductible does not apply here. As a result of this disbalance, more clients apply for a consultation at the STI clinic to get access to STI testing while avoiding having to pay the deductible. In 2016 the demand for STI testing and the STI positivity rate at STI clinics were historically high.[4]

(1.2) STI OUTPATIENT CLINIC AMSTERDAM

The STI outpatient clinic of the Public Health Service of Amsterdam annually performs around 44,000 free of charge and anonymous STI consultations (STI clinic data 2016, unpublished). The number of consultations among MSM is high: 15,812 consultations in 2016. This is about 40% of all nationwide performed consultations at STI clinics in MSM (STI clinic data 2016, unpublished).[4] Clients visit our clinic on their own initiative and they can book an appointment online or by telephone. When all test results are available, the client is informed with an SMS or email to log-in to a website to read their diagnosis. In case of an STI, patients can directly book a treatment appointment at this webpage.

STI clinics need to evaluate and assess their management policies and get acquainted with new technological developments through scientific research. Also, surveillance tasks need to be fulfilled to keep track of changing risk behaviour patterns and STI prevalences. Surveillance and research might lead to adjustment or alteration of current public health policies or the adoption of new interventions. In the following four chapters of this thesis, various key components of STI management are addressed: evaluation of diagnostic tests, clinical management, public health surveillance, and public health interventions.

(2) EVALUATION OF DIAGNOSTIC TESTS

The key characteristics of a diagnostic test designed to distinguish infected from uninfected individuals are sensitivity (i.e. the probability that a truly infected individual will test positive), and specificity (i.e. the probability that a truly uninfected individual will test negative).[6] Sensitivity and specificity are usually determined against a reference standard test, sometimes referred to as a 'gold standard' test.[6] If the diagnostic accuracy is known, a cost-effectiveness analysis can be performed to evaluate the health-economic impact as well as other possible societal consequences.[7]

(2.1) GENITAL HERPES SIMPLEX VIRUS TYPE-SPECIFIC SEROLOGY

Genital herpes is caused by herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2).[8] Apart from recurrent discomfort and distress to the patient, genital herpes infections can cause meningitis, life-threatening infections in newborns (neonatal herpes) and contribute to the spread of HIV.[8]

Commercially available type-specific serologic assays for HSV-1 and HSV-2 might be useful in: (1) patients with recurrent genital symptoms or atypical symptoms with negative HSV cultures, (2) patients with a clinical diagnosis of genital herpes without laboratory confirmation, and (3) patients with a partner with genital herpes.[9] A non-commercially available Western blot for HSV-1 and HSV-2 is seen as the reference test but is primarily used in research settings.[9] Because higher rates of HSV-2 shedding occur in HIV/HSV-2 co-infected persons and treatment of co-infected persons with acyclovir has been shown to reduce both HIV and HSV-2 viral shedding, experts in the US endorse screening for HSV-2 in HIV-infected populations.[10]

Initially we aimed to assess whether there was a trend in the number of patients attending the STI clinic with a primary HSV-1 or HSV-2 episode. For this project we needed a test to distinguish primary from recurrent HSV episodes; we therefore acquainted ourselves with HSV type-discriminating antibody tests (glycoprotein G directed). During validation of these serologic tests, it appeared that in some samples from patients with a recurrent HSV episode, the HSV type-specific test was negative. This finding raised a new research question: how do three commercially available type-specific serological HSV-1 and HSV-2 tests perform in a unique selection of consecutive sera samples (chapter 2.1)? The Western blot reference test for herpes simplex virus type 1 and 2 was performed on samples with a discordant test result.

(2.2) GRAM-STAINED URETHRAL SMEAR POINT-OF-CARE TEST FOR CHLAMYDIA

Point-of-care tests - laboratory diagnostic testing performed at or near the site where clinical care is delivered[11] - are important in the management of STIs because they allow the clinician to provide immediate test results and treatment.[12] In case of point-of-care testing, the benefit might also concern the earlier interruption of transmission. In the absence of point-of-care testing and treatment, transmission can occur in the period

between sample collection and provision of treatment or even over a longer period if patients do not return for treatment.

At every initial consultation at the Amsterdam STI clinic, clients are instructed to abstain from sex or to practice safe sex until they have received definitive test results. However, in a study among MSM with an STI who returned for treatment (mean time between testing and treatment being 13 days), 34.7% of the participants reported having had sexual contacts in this period.[13] Of those who had had sex, 22.9% reported unprotected anal intercourse with a steady or casual partner.

Clients of the Amsterdam STI clinic can retrieve their test results online (after logging-in with a personal code) or by telephone. Patients with an STI who do not show up for treatment are contacted by STI clinic nurses. Despite all these efforts, 4.0% of the clients with a confirmed STI does not obtain their test result (STI clinic data 2016, unpublished).

Urethral Gram-stained smears - microscopic examination of a urethral exudate for the presence of polymorphonuclear leucocytes - could be used as a point-of-care test to distinguish patients with urethritis from those without urethritis.[14] Chapter 2.2 examines the benefit of performing point-of-care urethral Gram-stained smears to diagnose chlamydia in all high-risk men compared to symptomatic men only. In this analysis we considered costs, sensitivity and specificity, loss to follow-up, and number of patients who received immediate treatment.

(3) CLINICAL MANAGEMENT

The availability of laboratory techniques for the identification of STIs raises the question who should be offered testing. The decision to start testing depends among other things on the prevalence of the disease, short and long term benefit of testing to the patient, the effect on the ongoing transmission, and the availability of financial resources. In this chapter on clinical management the focus lies on gaining knowledge about the prevalence of pathogens at specific anatomical locations in a subset of clients. Prior to these studies, STI clinic clients were not routinely tested at the specific anatomical locations.

(3.1) PHARYNGEAL CHLAMYDIA

From 1989, the STI clinic started to routinely test all female clients for urogenital chlamydia using culture; men were only tested if they had microscopy proven urethritis. From 1991, the enzyme-linked immunosorbent assay (ELISA) on chlamydia was introduced for both men and women and in 1995, the first nucleic acid amplification test (NAAT) for chlamydia was implemented at the STI clinic. The Centers for Disease Control and Prevention (CDC) started in 2014 to recommend NAATs for the detection of *C. trachomatis* and *N. gonorrhoeae* infections as screening or diagnostic tests.[15]

Since 2008, pharyngeal gonorrhoea is tested with the transcription-mediated amplification (TMA) combination test (Hologic, Marlborough, MA, USA): a NAAT. Although only intended to test for *N. gonorrhoeae*, *C. trachomatis* results became available also due to the multiplex nature of the test. In some cases the sample was positive for chlamydia. Next, a study was started to estimate the prevalence of pharyngeal chlamydia, and among those who tested positive, clearance of chlamydial RNA was studied (chapter 3.1).

(3.2) URETHRAL LYMPHOGRANULOMA VENEREUM

Lymphogranuloma venereum (LGV) is a condition caused by invasive serovars of *C. trachomatis* (L1, L2, or L3).[16] Today in developed countries, LGV is predominantly associated with rectal infections, and the classical finding of inguinal lymphadenopathy is uncommon.[16] Differentiation of chlamydia infections caused by LGV versus non-LGV strains is of importance because of therapeutic implications: whereas non-LGV infections are treated with a 7-day regime, LGV infections require a regimen of doxycycline for 21 days.[17]

At our clinic, MSM with anorectal chlamydia are routinely tested for LGV. However, in MSM with urethral chlamydia LGV testing is not performed. To establish the positivity rate for urethral LGV and to find any evidence for the possibility of LGV transmission from the urethra to the rectum, a selection of MSM with urethral chlamydia was tested for urethral LGV (Chapter 3.2).

(3.3) MRSA AMONG MEN WHO HAVE SEX WITH MEN

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a bacterium with potentially dangerous implications in immunocompromised and hospitalised patients.[18] MRSA was thought to be exclusively associated with hospitals, but it also circulates in the general population and is then referred to as community-associated MRSA (CA-MRSA).[18] Since the mid-1990s, there has been an increase in the number of MRSA infections in populations, which was unrelated to health care system exposure.[19] CA-MRSA strains appear to have rapidly disseminated among the general population in most areas of the United States and affect patients with and without exposure to the health care environment.[19] CA-MRSA has become an increasingly important cause of skin and soft tissue infections.[20] In the United States and Canada CA-MRSA infections have been shown to be more prevalent in certain populations, including MSM.[18] Within MSM populations, CA-MRSA has been linked to methamphetamine use, sex with multiple partners, use of Internet for sexual contacts, and history of STI.[18] Chapter 3.3 describes the prevalence of MRSA and associated risk factors in MSM at the Amsterdam STI clinic with and without clinical signs of a skin or soft tissue infection.

(4) PUBLIC HEALTH SURVEILLANCE

Public health surveillance is the ongoing systematic collection, analysis, interpretation and dissemination of health data.[21] Public health surveillance enables the collection of data required for the implementation of evidence based public health management and interventions programs.[21] In the Netherlands, all data from the STI clinics are collected centrally for surveillance purposes.[22] Large-scale surveillance at STI clinics is important to detect changes in the prevalence of STI.

(4.1) HEPATITIS C SCREENING AMONG HIV POSITIVE MSM AND MSM OPTING-OUT OF HIV TESTING

Since 2000 there has been recognition in the postindustrialized world that there has been a dramatic rise in the incidence of hepatitis C virus (HCV) in HIV-positive MSM.[23] Retrospectively, since 1995, an increase in the prevalence of HCV has been observed in HIV-positive MSM at the Amsterdam STI clinic.[24] Whereas it remains controversial whether sexual transmission of HCV occurs in the general (heterosexual) population, it has now been accepted that permucosal - sexual risk behaviour and mucosally

administered drugs - rather than parenteral risk, is a key factor in HCV transmission in HIV-positive MSM.[23] Co-infection with HIV and HCV is associated with accelerated liver fibrosis and a shorter time to progression to cirrhosis and hepatic decompensation when compared with HCV mono-infected patients.[25]

The national HIV treatment guideline recommends specialised HIV clinics to screen for HCV antibodies at first consultation after HIV diagnosis and to screen for alanine transaminase (ALT) at every consultation.[5] In addition, MSM at risk should be tested for HCV antibodies annually.[5] Many HIV positive MSM who visit the Amsterdam STI clinic report high risk behaviour and may be at risk of contracting HCV. In 2007, the STI clinic started HCV antibody screening in HIV positive MSM. As a result, MSM visiting the STI clinic for STI testing and visiting the HIV clinic for their HIV treatment, will be screened for HCV at both sites. In chapter 4.1 the added value of HCV screening in HIV positive MSM at an STI clinic is discussed. In addition, MSM who opted-out of HIV testing (these men are possibly at higher risk of HIV) were also offered HCV antibody screening.

(4.2) STI IN MSM REQUESTING FOR POST-EXPOSURE PROPHYLAXIS FOR HIV

Post-exposure prophylaxis (PEP) is the use of short-term antiretroviral therapy (ART) to reduce the risk of acquisition of HIV infection following exposure.[26] Since 2010, clients who experienced a sex accident in the preceding 72 hours may request PEP at the Amsterdam STI clinic. Next to initiating PEP, clients are screened for STI. Testing for *C. trachomatis* and *N. gonorrhoeae* during this PEP consultation might be too early as an infection has not yet been established. To preclude missing any infections, clients were invited to return to the STI clinic after 2 weeks for repeat *C. trachomatis* and *N. gonorrhoeae* testing. In chapter 4.2, the prevalence of *C. trachomatis* and *N. gonorrhoeae* during initial and repeat visit are reported and interpreted.

(4.3) STI IN VICTIMS OF A SEXUAL ASSAULT

Sexual assault refers to a broad spectrum of non-consensual sexual activity, including sexual contact (such as unwanted kissing, fondling, or touching) with or without penetrative sex.[27] Sexual assault may include the use of physical force, psychological coercion, or non-consent due to younger or older age, disability, or incapacitation with drugs or alcohol.[27] Rape is a legal term that includes non-consensual penetration of the mouth, anus, or vagina.[27] During a sexual assault transmission of STI may occur.

At the Amsterdam STI clinic, sexual assault victims receive a priority appointment. During consultation at the STI clinic, victims are screened for STI and counselled by a professional.

The number of studies about STI in victims of a sexual assault is limited and they report variable STI prevalences.[28] Some studies showed that the follow-up rate in victims of a sexual assault is low [28], and for this reason some clinics offer presumptive antibiotic treatment for STIs at the first visit. The Amsterdam STI clinic however does not provide presumptive therapy. Therefore, if an STI is diagnosed in a victim he/she needs to return to the STI clinic for treatment. In chapter 4.3, the number of sexual assault victims requesting STI care, the STI infection rate and the treatment uptake are evaluated.

(5) PUBLIC HEALTH INTERVENTIONS

Public health challenges in the field of STI are ongoing epidemics and increasing difficulties to reach those at risk. In recent years, online methods became available for STI care providers. Next to one-way publishing information on STI related topics, the Internet can also be used to interact with patients. The Amsterdam STI clinic started in 2010 with online triage and appointment opportunities. Yet, new (online) media offer opportunities to implement innovative interventions that could address today's challenges in public health. These interventions should be evaluated to study their effectiveness and utility in the target population.

(5.1) CHLAMYDIA TESTING AT HOME

Since August 2010, the Amsterdam STI clinic prioritizes high risk clients applying for an appointment. Clients with the highest priority - those reporting STI related symptoms, those notified of STI exposure, victims of a sexual offence and clients requesting post-exposure prophylaxis for HIV - need to be seen within one working day. The second priority group - clients aged below 20 years, MSM, commercial sex workers, and first or second generation immigrants from STI/HIV endemic areas - ideally should be seen within 10 working days. If appointment slots are still available the third priority group – youngsters between 20 and 25 years with no other indication – should be seen within 10 working days.[29]

As a result of high demand, the third priority group, youngsters, were ill served, and were referred to their GP. A policy change dropped mandatory syphilis and HIV blood tests in this group from 2012 onwards; only testing on *C. trachomatis* was required.[22] This change made it possible to offer this group home collection of samples for STI testing using less invasive specimen collection.[15] To explore the preference of STI clinic clients, the STI clinic started in September 2012 to offer chlamydia specimen collection at home to youngsters at low risk of STI. In home-testers who tested chlamydia positive, follow-up (treatment, partner notification) was provided at the STI clinic. In chapter 5.1 the preference of home testing, testing outcomes and follow-up are described.

(5.2) ONLINE PARTNER NOTIFICATION TOOL

Partner notification is the process whereby the sexual partner(s) of a patient diagnosed with an STI are identified and informed of their exposure to an STI.[30] These notified persons are invited to attend the clinic for testing, counselling and, where necessary, treatment.[30] The goal is to identify and treat undiagnosed infections, thus preventing late sequelae and interrupt ongoing transmission.[31] Partner notification can be in the form of patient referral (the index patient notifies partners), provider referral (the care provider notifies partners) or contract referral.[32 33] With contract referral, the patient and the provider make an agreement that the patients notifies partners on their own by a specific date, and if the patient has not done so by the agreed date, the provider contacts the partner.[32 33] Partner notification services are routinely offered by the STI clinic of Amsterdam.

Despite the efforts of Dutch STI clinics, it appears that a considerable proportion of identifiable sex partners – especially those of heterosexual male index patients – were not notified.[34] To lower the threshold to notify sex partners, an online partner notification tool called Suggestatest.nl was developed by the STI clinics of Rotterdam and Amsterdam. The use of this tool is evaluated in chapter 5.2 and the acceptability and usability rating of those who used this application is discussed in chapter 5.3.

OUTLINE OF THIS THESIS AND USED DATA SOURCES

For this thesis several data sources – ranging from routinely collected at the STI clinic to data specifically collected at several STI clinics – have been used. In the table below, per study a summary of the used data is provided.

Data sources used in this thesis

Chapter	Research question	Study design	Data source	Study population (including size[1]) and location	Pathogen of interest / outcome	Period of data collection
Chapter 2: Evaluation of diagnostic tests: implications for clinical practice						
2.1	What is the diagnostic accuracy of three commercially available type-specific (glycoprotein G (gG) directed) serological HSV-1 and HSV-2 assays among a selection of consecutive sera samples?	Longitudinal	EPR, retrospective serological test results	Male and female clients of the AMS STI clinic: n=17 patients with a recurrent HSV-1 and n=33 with a recurrent HSV-2 episode	HSV 1 & 2 serology	2000 - 2012
2.2	What is the benefit of performing urethral Gram-stained smears to diagnose chlamydia in all high-risk men compared to symptomatic men only?	Cross-sectional	EPR, consultation costs	High risk male clients of the AMS STI clinic: n=7185 in 2008-2009 and n=18,852 in 2010-2011	Urogenital chlamydia, urethral gram-stained smear, consultation costs, loss to follow-up	2008 - 2011
Chapter 3: Clinical management: implications for testing protocols						
3.1	What is the prevalence of pharyngeal chlamydia, and among those who tested positive, the clearance of chlamydial RNA?	Cross-sectional, cohort	EPR, additional questionnaire, pharyngeal swab	MSM (n=13,111) and high-risk women (n=6915) at the AMS STI clinic	Positivity and clearance of pharyngeal chlamydial RNA	January 2011 - July 2012
3.2	What is the positivity rate for urethral LGV among MSM with urethral chlamydia who are potentially at risk for urethral LGV?	Cross-sectional	EPR, retrospective LGV typing urethral chlamydia samples	Urethral chlamydia positive MSM: (1) with anorectal LGV (n=33) (2) with an anorectal LGV positive partner at the AMS STI clinic (n=59)	Urethral LGV	January 2008 - August 2012

Chapter	Research question	Study design	Data source	Study population (including size[1]) and location	Pathogen of interest / outcome	Period of data collection
3.3	What is the prevalence of MRSA and associated risk factors in MSM at the Amsterdam STI clinic with and without clinical signs of a skin or soft tissue infection?	Cross-sectional	EPR, additional questionnaire, *S. aureus* and MRSA cultures	MSM with (n=74) and without (n=137) clinical signs of a skin or soft tissue infection at the AMS STI clinic	*Staphylococcus aureus* and MRSA	October 2008 - April 2010
Chapter 4: Public health surveillance						
4.1	What is the added value of HCV screening at an STI clinic among HIV positive MSM and among MSM opting-out of HIV testing?	Cross-sectional and longitudinal	EPR, HIV centres	HIV positive MSM (n=5297) and MSM unaware of their HIV status (n=824) tested for HCV at the AMS STI clinic	(date of) HCV diagnosis	November 2007 - January 2011
4.2	What is the prevalence of *C. trachomatis* and *N. gonorrhoeae* 2 weeks after an STI consultation following a recent (<72 hours) sex accident?	Cross-sectional and longitudinal	EPR	MSM with an indication for HIV PEP at the AMS STI clinic: n=473	*C. trachomatis* and *N. gonorrhoeae* infections at baseline and follow-up	April 2010 - December 2012
4.3	What is the number of sexual assault victims requesting STI care, the STI infection rate and the treatment uptake?	Cross-sectional	EPR	Female (n=1066) and male (n=135) victims of a sexual assault at the AMS STI clinic	STI positivity and treatment uptake	January 2005 - September 2016
Chapter 5: Public health interventions						
5.1	Do low-risk youngsters prefer home testing for chlamydia? What are the testing outcomes and their follow-up for treatment?	Cross-sectional	EPR, appointment database	Young (<25 year) low-risk clients of the AMS STI clinic (n=1804) applying for an appointment	Specimen collection preference, chlamydia positivity, no-show/return, treatment uptake	September 2012 - July 2013

Chapter	Research question	Study design	Data source	Study population (including size[1]) and location	Pathogen of interest / outcome	Period of data collection
5.2	How often do STI positive patients use the online tool to notify their partner(s) and which method do they use?	Cross-sectional	EPR, Suggestatest.nl database	Clients of the AMS and Rotterdam STI clinic diagnosed with an STI (n=988) and partners notified through Suggestatest.nl (n=505)	Use of Suggestatest.nl	March to July - 2012
5.3	How do users (both index patients and their notified partner(s)) rate the acceptability and usability of this application?	Cross-sectional	Suggestatest.nl database, additional questionnaire	Users of Suggestatest.nl who filled-in a questionnaire: patients (n=112) and notified partners (n=163)	Acceptability and usability of online partner notification tool	March 2012 - June 2013

Abbreviations:
AMS: Amsterdam; EPR: electronic patient record; HCV: hepatitis C virus; HIV: human immunodeficiency virus; HSV: herpes simplex virus; LGV: lymphogranuloma venereum; MRSA: methicillin-resistant Staphylococcus aureus; MSM: men who have sex with men; PEP: post-exposure prophylaxis; STI: sexually transmitted infection.

[1]Study sizes mentioned concerns the total number of consultations (not unique individuals), except in chapters 2.1, 3.2, 3.3, 5.3.

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CHAPTER 2

Evaluation of diagnostic tests

CHAPTER 2.1

False-negative type-specific glycoprotein G antibody responses in STI clinic patients with recurrent HSV-1 or HSV-2 DNA positive genital herpes, the Netherlands.

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ABSTRACT

Objectives

Herpes simplex virus (HSV) type-discriminating antibody tests (glycoprotein G (gG) directed) are used to identify naïve persons and differentiate acute infections from recurrences. We studied test characteristics of three commercially available antibody tests in patients with recurrent (established by viral PCR tests) herpes simplex virus type 1 (HSV-1) or herpes simplex virus type 2 (HSV-2) genital herpes episodes.

Methods

Serum samples (at minimum 3 months after t=0) were examined for the presence of gG-1-specific or gG-2-specific antibodies using the HerpeSelect 1 and 2 Immunoblot IgG, the HerpeSelect 1 and 2 enzyme linked immunoassays IgG and the LIAISON HSV-1 and HSV-2 IgG indirect chemiluminescence immunoassays.

Results

The immunoblot was HSV-1 positive in 70.6% (95% CI 44.0% to 89.7%), the LIAISON in 88.2% (95% CI 63.5% to 98.5%) and the ELISA in 82.4% (95% CI 56.6% to 96.2%) of the 17 patients with a recurrent HSV-1 episode. From 33 patients with a recurrent HSV-2 episode, the immunoblot was HSV-2 positive in 84.8% (95% CI 68.1% to 94.9%), the LIAISON in 69.7% (95% CI 51.3% to 84.4%) and the ELISA in 84.8% (95% CI 68.1% to 94.9%). Among 15/17 (88.2%; 95% CI 63.5% to 98.5%) patients with HSV-1 and 30/33 (90.1%; 95% CI 75.7% to 98.1%) patients with HSV-2, HSV-1 or HSV-2 antibodies, respectively, were detected in at least one of the three antibody tests.

Conclusions

Commercial type-specific gG HSV-1 or HSV-2 antibody assays were false negative in 12–30% of patients with recurrent HSV-1 or HSV-2 DNA positive genital lesions. The clinical and epidemiological use of type-specific HSV serology can be hampered by false-negative results, especially if based on a single test.

INTRODUCTION

Genital herpes is the major cause of genito-ulcerative disease affecting a considerable number of individuals worldwide.[1] Genital herpes infections can cause meningitis, life-threatening infections in newborns (neonatal herpes) and contribute to the spread of HIV.[1] Genital herpes is caused by herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2). Antibodies against the structural glycoprotein G (gG-1 in HSV-1 and gG-2 in HSV-2) are used for type-discriminating serology.[2] At present, there are several US Food and Drug Administration (FDA)-approved commercial kits available with high sensitivity and specificity to establish type-specific immunoglobulin antibodies (IgG) to gG-1 and gG-2.[2] These serological tests are indicated for testing sexually active adults or expectant mothers to diagnose past HSV-1 and/or HSV-2 infections and can be used in sero-epidemiological studies as a proxy marker to identify high-risk sexual behaviour.[3-8]

Type-specific herpes simplex virus (HSV) antibodies can take from 2 weeks to 3 months to develop,[1] but it has been shown that seroconversion for HSV-gG can take longer.[9] Additionally, the loss of antibodies (seroreversion) has been described.[10-12] To gain insight into the clinical reliability of type-specific HSV immunoglobulin antibody tests, we aimed to examine antibodies to gG-1 and gG-2 in sequential serum samples from patients with recurrent HSV-1 or HSV-2 DNA positive genital lesions.

MATERIALS AND METHODS

Study population and setting

The sexually transmittable infections (STI) outpatient clinic of the Public Health Service in Amsterdam annually performs approximately 40,000, new, free-of-charge and anonymous STI consultations.[13] In patients with anogenital ulcerative disease, ulcer swabs were obtained to investigate with PCR the presence of DNA from HSV-1 and HSV-2 (targets two conserved regions; gB and IE2 genes).[14] Serum samples taken at the time of each consultation were stored at –20°C for research purposes. Patient characteristics, clinical findings, diagnoses and subsequent treatment were routinely recorded in an electronic patient file. For this study, we used anonymized routinely collected data; therefore, ethical clearance was not sought.

Patient and sample selection

Between 2000 and 2012, patients with a first episode (t=0), and at least one return visit with recurrent HSV-1 or HSV-2 DNA positive genital lesions more than 3 months after the first episode, were selected. Serum samples were obtained from all recurrent patients with HSV-1, and (due to financial constraints) from the 35 most recent patients with HSV-2.

Serological testing procedure

All sequential serum samples were examined for the presence of gG-1-specific or gG-2-specific antibodies using three gG-specific commercial tests. The HerpeSelect 1 and 2 Immunoblot IgG (Focus Diagnostics, San Juan Capistrano, California, USA) was performed on de Auto-Lipa 48 from Fujirebio (Chuo-ku, Tokyo, Japan). The HerpeSelect 1 and 2 enzyme linked immunoassays IgG (ELISA, Focus Diagnostics, San Juan Capistrano, California, USA) were performed on the Dynex DSX (Dynex Laboratories, Chantilly, Virginia, USA). The LIAISON HSV-1 and HSV-2 type-specific IgG indirect chemiluminescence immunoassays (CLIA) (Diasorin, Stillwater, USA) were performed on the LIAISON analyser. The above mentioned tests were executed according to the product inserts and were all performed blinded by one lab technician and for the interpretation of the immunoblot by an additional lab technician. In addition, all negative or equivocal sequential serum samples in at least one of the three assays were tested for HSV-1 and HSV-2 antibodies at the University of Washington by Western blot (WB).[2 15] The above tests were executed at different moments; therefore, samples were thawed and re-frozen.

Statistical analysis

Allowing 3 months of antibody maturation, the selected sequential sera were assumed to contain at least type-specific IgG antibodies to the HSV type found with PCR at t=0. If the antibody test was negative or equivocal, this result was denoted false negative. Additionally, we focused on indicators—pregnancy, anti-herpes therapy ((val)acyclovir) and HIV status—for false-negative test results. Groups were compared with the Fisher's exact test for categorical variables. Exact 95% CIs around sensitivities were calculated.

RESULTS

Genital HSV-1 and HSV-2 diagnosis

Between January 2000 and December 2011, 19 (1.5%) patients returned with recurrent genital HSV-1 DNA positive lesions and 66 (2.9%) with recurrent genital HSV-2 DNA positive lesions. From the latter group, the 35 (53.0%) most recent patients were selected. Two patients with HSV-1 and two patients with HSV-2 were excluded because their serum samples were not available.

Patient characteristics

The majority of both patients with HSV-1 (n=10) and patients with HSV-2 (n=18) were men who have sex with men (respectively, 58.9% and 54.5%), and, respectively, 4 (23.5%) and 17 (51.5%) patients were HIV-positive (table 1).

Serological test results

HSV-1

At the first recurrent HSV-1 episode, the immunoblot was HSV-1 positive in 12/17 (70.6%; 95% CI 44.0% to 89.7%), the LIAISON in 15/17 (88.2%; 95% CI 63.5% to 98.5%) and the ELISA in 14/17 (82.4%; 95% CI 56.6% to 96.2%) patients (table 2).

For samples that were negative or equivocal in the LIAISON – from the 3 used tests the most sensitive assay – no gG-1 antibodies were detected in either the immunoblot or the ELISA. None of the three women with discrepant test results were pregnant. The proportion of patients with seronegative or equivocal results at the recurrent HSV-1 episode who had received anti-herpes therapy at t=0 (3/5, 60.0%) or had an HIV-positive status (1/5, 20.0%) did not differ significantly from patients with seropositive results at the recurrent HSV-1 episode (5/12, 41.7%, p=0.62 and 3/12, 25.0%, p=1.00, respectively) (see supplementary tables 1 and 2a). From the five patients with seronegative or equivocal results at the recurrent HSV-1 episode, three proved HSV-1 positive in the WB analysis, for one patient not enough serum was available for WB, and for one patient the WB was consistently HSV-1 negative (but HSV-2 positive). However, after repeated discrepancy testing this sample was both HSV-1 and HSV-2 positive with HSV-1 on the border of a positive reaction.

HSV-2

At the first recurrent HSV-2 episode, the immunoblot was HSV-2 positive in 28/33 (84.8%; 95% CI 68.1% to 94.9%), the LIAISON in 23/33 (69.7%; 95% CI 51.3% to 84.4%) and the ELISA in 28/33 (84.8%; 95% CI 68.1% to 94.9%) patients (table 2). Combining the results of the ELISA and the immunoblot, the sensitivity raised from 84.8% to 90.9% (95% CI 75.7% to 98.1%).

Among the serum panel with seronegative or equivocal results –all men–the proportion of patients who had received antiherpes therapy at t=0 (3/11, 27.3%) or with an HIV-positive status (5/11 45.5%) was not significantly different from the panel with concordant results (6/22, 27.3%, p=1.0 and 12/22, 54.5%, p=0.62, respectively) (see supplementary tables 1 and 2b). From the 11 patients with seronegative or equivocal results at the first recurrent HSV-2 episode, two proved HSV-2 positive in the WB analysis, for three patients WB was performed in serum from another recurrent genital HSV-2 episode (all HSV-2 positive) and for four patients not enough serum was available. Samples from the remaining two patients with an inter-recurrent window of 4 and 8 months were consistently WB HSV-2 negative (but HSV-1 positive) at first and after discrepancy testing.

Out of four patients with negative or equivocal HSV-2 serology at the first recurrent genital HSV-2 episode, three patients had one and one patient had three additional recurrent episodes with HSV-2 DNA positive lesions (see supplementary table 2b, including type-specific HSV antibody test results showing cases with seroconversion and seroreversion).

Table 1. Main characteristics of patients with recurrent HSV-1 or HSV-2 DNA positive genital lesions, STI clinic, Public Health Service of Amsterdam, the Netherlands, 2000-2011[1]

	HSV-1 n=17	**HSV-2 n=33**
Variable	**n (%)**	**n (%)**
Demographics		
Median age (IQR) in years	34 (IQR 27-40)	39 (IQR 32-48)
Ethnicity		
Dutch	14 (82.4)	18 (54.5)
Surinamese/Dutch Antillean	1 (5.9)	9 (27.3)
South-American (other)	1 (5.9)	4 (12.1)
Other	1 (5.9)	2 (6.0)

	HSV-1 n=17	HSV-2 n=33
Variable	**n (%)**	**n (%)**
Sexual preference		
Heterosexual male	2 (11.8)	10 (30.3)
MSM	10 (58.9)	18 (54.5)
Female	5 (29.4)	5 (15.2)
New STI diagnosis and HIV status at current visit		
HIV status[2]		
Unknown	3 (17.6)	0 (0)
Negative	10 (58.8)	16 (48.5)
Positive	4 (23.5)	17 (51.5)
Early syphilis	1 (5.9)	1 (3.0)
Chlamydia[3]	4 (23.5)	4 (12.1)
Gonorrhoea[4]	1 (5.9)	2 (6.1)
HSV recurrent episodes		
1	16 (94.1)	28 (84.8)
2	1 (5.9)	3 (9.1)
3	0 (0)	1 (3.0)
4	0 (0)	1 (3.0)
Time to first recurrence (months)		
≥3-5	0	9
6-11	3	7
12–23	6	3
24–35	1	5
36–47	3	3
48–59	3	2
≥60	1	4

Abbreviations:
HSV, herpes simplex virus; HSV-1, herpes simplex virus type 1; HSV-2, herpes simplex virus type 2; IQR: interquartile range; MSM: men who have sex with men; STI, sexually transmittable infections.

[1]Patient characteristics are from the consultation with the first recurrent genital HSV-1 or HSV-2 episode with a minimal window of 3 months from the first episode.
[2]Only patients who were not already known to be HIV- positive were offered an HIV test. HIV status was considered unknown when no history of HIV was reported and no HIV test was performed at current visit; negative when the HIV serology test was negative at current visit; and positive when reporting a history of HIV or when the HIV serology test was positive at current visit.
[3]Being diagnosed with urogenital and/or anorectal chlamydia.
[4]Being diagnosed with urogenital and/or anorectal and/or oropharyngeal gonorrhoea.

Table 2. Type-specific HSV IgG outcomes for HerpeSelect 1/2 immunoblot, HerpeSelect-1 and HerpeSelect-2 ELISA and LIAISON HSV-1 and HSV-2 CLIA in sera from patients with recurrent HSV-1 or HSV-2 DNA positive genital lesions, STI clinic, Public Health Service of Amsterdam, the Netherlands, 2000-2011[1]

		ELISA HerpeSelect HSV-1 HSV1		
		Positive n=14	**Negative n=2**	**Equivocal n=1**
Variable		**n**	**n**	**n**
HerpeSelect 1/2 immunoblot[2]	**LIAISON HSV-1 CLIA**			
Positive	Positive	12	0	0
Negative	Positive	0	0	1
	Negative	0	1	0
	Equivocal	0	1	0
Equivocal	Positive	2	0	0
		ELISA HerpeSelect HSV-2 HSV2		
		Positive n=28	**Negative n=4**	**Equivocal n=1**
Variable		**n**	**n**	**n**
HerpeSelect 1/2 immunoblot[3]	**LIAISON HSV-2 CLIA**			
Positive	Positive	22	1	0
	Negative	4	0	1
Negative	Positive	0	0	0
	Negative	2	3	0

Abbreviations:
CLIA, chemiluminescence immunoassays; HSV, herpes simplex virus; HSV-1, herpes simplex virus type 1; HSV-2, herpes simplex virus type 2; STI, sexually transmittable infections.

[1]If patients had more than one recurrent genital HSV episode, in this table serum from first recurrent episode is reported.
[2]Immunoblot result notated as negative if HSV-1 was not detected.
[3]Immunoblot result notated as negative if HSV-2 was not detected.

DISCUSSION

In respectively, 29.4% (5/17) and in 33.3% (11/33) of the patients with a recurrent episode of genital HSV-1 or HSV-2 DNA positive lesions, type-specific antibodies to the causative HSV-type were not detected in at least one of the three used gG directed serological tests.

A strength of this study is the unique data on recurrent genital HSV-1 episodes that occur less often than for HSV-2.[1] Moreover, all recurrent HSV-1 and HSV-2 episodes, including antiviral treatment, and epidemiological data are well documented.

Unfortunately, for some patients the serum volume left was insufficient or the WB analyses were performed on sera from another episode. The patient selection in our study might have influenced the sensitivity, and - due to the relative small numbers - the CIs around the calculated sensitivities are wide. The quality of the used sera might have decreased over time. Although no association of HIV status with false-negative HSV type-specific serology has been shown, no data were available about the actual immune status of the patients.

Earlier, 89% sensitivity was shown for the HerpeSelect-1 ELISA, 96% for the HerpeSelect-2 ELISA, 99–100% for the HerpeSelect-1 immunoblot and 97–100% for the HerpeSelect-2 immunoblot.[16] Comparable with our study, a lower sensitivity (82.6%) of the HerpeSelect-2 ELISA was shown.[17] In a previous Swedish study, 106 WB positive patients with a recurrent genital culture-proven infection were all HerpeSelect-2 ELISA positive.[18] Here, we found two WB HSV-2 positive patients with HSV-2 DNA positive recurrent genital lesions and HerpeSelect-2 ELISA negative results. In a comparison between the HSV-1 and HSV-2 LIAISON CLIA and the HerpeSelect ELISA, the overall agreement was 99.6% for HSV-1 and 100% for HSV-2.[19] In our study, the LIAISON was more often positive in patients with recurrent HSV-1 (88.2%) than in patients with recurrent HSV-2 (69.7%).

In two patients with HSV-2, the WB result was HSV-1 positive only. Based on WB results from newly infected patients with HSV, it can take up to 6 months to seroconvert to HSV-gG.[9] In our study, out of eight patients with an inter-recurrent HSV-2 window between 3 and 6 months, six patients were discordant in at least one of the three serological tests.

In two patients with more than one documented recurrent HSV-2 episode, gG-2 serology was positive at the first recurrent episode but reconverted to negative at subsequent recurrent episode in all three commercial tests (see supplementary data). This phenomenon of seroreversion for gG-2 antibodies was previously described.[10-12]

Next to seroreversion and a seroconversion interval of up to 6 months, a mutated gG-2 gene could explain the false-negative type-specific serological results.[20 21] Possibly, the ethnic background of our study population has influenced the type-specific serological results as previously geographic variation in the performance of the Focus HerpeSelect ELISA test has been shown.[22]

False-negative HSV type-specific serological test results can have far-fetching consequences for pregnant women (erroneous indication for caesarean delivery), for HSV serodiscordant sexual couples (unnecessary prophylactic measures) and for sero epidemiological studies (biased proxy for sexual risk behaviour).[8] Development of a test algorithm for commercially available type-specific tests should be considered to confirm negative HSV serological results more accurately. Further research is needed to develop more sensitive type-specific antibody tests.

In conclusion, type-specific antibodies to HSV-1 or HSV-2 were frequently (29.4% and 33.3%, respectively) not detected with gG directed commercial assays in sera from patients with recurrent, viral DNA positive genital HSV-1 or HSV-2 lesions. The clinical and epidemiological use of type-specific HSV serology can be hampered by false-negative results, especially if based on one test only.

Key messages

- Glycoprotein G directed type-specific human herpes simplex virus type 1 (HSV-1) and herpes simplex virus type 2 (HSV-2) antibody tests are used to identify naïve persons for partner and maternity counselling purposes.
- Here, we show that three serological herpes simplex virus (HSV) tests can be false negative in 12–30% of patients with recurrent genital HSV-1 and HSV-2 lesions.
- When used for clinical management and counselling purposes in a comparable population, the lower sensitivity of type-specific serological HSV tests should be taken into consideration.

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Contributors

MSvR, WR, DK and HJCdV designed the study protocol. MSvR was responsible for the data collection at the STI clinic. DK and GH were responsible for the type-specific serology at the laboratory. MSvR performed the statistical analyses. MSvR, WR and HJCdV drafted the paper, all authors commented on draft versions and all approved the final version.

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Competing interests

None declared.

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SUPPLEMENTARY TABLES

Supplementary table 1. Patient characteristics and type-specific HSV IgG outcomes (HerpeSelect 1/2 immunoblot, HerpeSelect-1 and HerpeSelect-2 ELISA, LIAISON HSV-1 and HSV-2 CLIA, and Western blot) in sera (first recurrent HSV-1 or HSV-2 episode) from patients with consecutive HSV-1 or HSV-2 DNA positive genital lesions with discordant results in type-specific glycoprotein G directed serologic tests, STI clinic, Public Health Service of Amsterdam, the Netherlands, 2000-20111

Discordant HSV-1									
Age	**Sexual orientation**	**HIV-status[2]**	**Antiviral drug at first episode**	**Pregnant**	**Time between episodes (months)**	**Immunoblot 1/2**	**ELISA HerpeSelect-1**	**LIAISON HSV-1 CLIA**	**Western blot**
50-59	MSM	+	no	n/a	52	-	-	±	missing
20-29	MSW	-	yes	n/a	65	±	+	+	HSV-1
20-29	WSM	unk	no	no	13	±	+	+	HSV-1
20-29	WSM	-	yes	no	8	-	±	+	HSV-1
20-29	WSM	-	yes	no	37	HSV-2	-	-	HSV-1&2[3]
Discordant HSV-2									
Age	**Sexual orientation**	**HIV-status[2]**	**Antiviral drug at first episode**	**Pregnant**	**Time between episodes (months)**	**Immunoblot 1/2**	**ELISA HerpeSelect-2**	**LIAISON HSV-2 CLIA**	**Western blot**
40-49	MSM	+	yes	n/a	5	HSV-1&2	-	+	Missing
30-39	MSM	+	no	n/a	4	HSV-2	+	-	HSV-1&2[4]
40-49	MSW	-	no	n/a	96	HSV-1	+	-	HSV-1&2
30-39	MSM	-	no	n/a	4	-	+	-	HSV-1&2[5]
20-29	MSW	-	no	n/a	8	HSV-1	-	-	HSV-1[6]
30-39	MSM	+	no	n/a	76	HSV-1&2	+	-	Missing
30-39	MSM	+	no	n/a	6	HSV-1&2	±	-	HSV-1&2[7]
40-49	MSM	+	yes	n/a	8	HSV-1&2	+	-	Missing
40-49	MSW	-	yes	n/a	4	HSV-1	-	-	HSV-1[6]
30-39	MSM	-	no	n/a	3	-	-	-	HSV-2
20-29	MSW	-	no	n/a	4	HSV-1&2	+	-	Missing

Abbreviations:
MSM: men who have sex with men; MSW: men who have sex with women only; n/a; not applicable; neg: negative; pos: positive; unk: unknown; WSM: women who have sex with men; -: negative; +: positive; ±: equivocal.

[1]If patients were having more than one recurrent genital HSV episode, in this table serum from the first recurrent episode is reported.
[2]Only patients who were not already known to be HIV-positive were offered an HIV test. HIV status was considered unknown when no history of HIV was reported and no HIV test was performed at current visit; negative when the HIV serology test was negative at current visit; and positive when reporting a history of HIV or when the HIV serology test was positive at current visit.
[3]At first, the WB analysis for this sample was HSV-2 positive only; during repeated discrepancy testing, this sample was both HSV-1 and HSV-2 positive in the WB with HSV-1 on the border of a positive reaction.
[4]Western blot result from serum sample taken at a consultation 20 months after the initial genital herpes episode.
[5]Western blot result from serum sample taken at a consultation 34 months after the initial genital herpes episode.
[6]Consistently WB HSV-2 negative (but HSV-1 positive) at first and after repeated testing.
[7]Western blot result from serum sample taken at a consultation 3 months after the initial genital herpes episode.

Supplementary table 2a. Patient characteristics, STI diagnosis and type-specific glycoprotein G directed serologic test outcomes in sera from patients with recurrent HSV-1 DNA positive genital lesions, STI clinic, Public Health Service of Amsterdam, 2000-2011

Rows in grayscale are consecutive serum samples with negative or equivocal HSV-1 results in at least 1 of the 3 glycoprotein G directed serological tests.

pt	visit	age	sex[1]	ethn[2]	HIV[3]	pregnant[4]	med[5]	time[6]	syphilis	CT	NG	IB IgG	Liaison gG-1	Liaison gG-2	ELISA gG-1	ELISA gG-2	WB IgG[7]
1	1	40-49	MSM	NL	unk	n/a	no		-	-	-	HSV-1			+	-	
	2	50-59	MSM	NL	pos	n/a	no	15.9	-	-	-	HSV-1	+	-	+		
	3	50-59	MSM	NL	pos	n/a	no	26.9	-	-	-	HSV-1	+	-	+		
2	1	50-59	MSM	NL	unk	n/a	no		-	-	-	-			-	-	
	2	50-59	MSM	NL	pos	n/a	no	52.0	+	-	-	-	±	-	-		n.d.
3	1	50-59	MSW	NL	neg	n/a	yes		-	-	-	±			+	-	
	2	50-59	MSM	NL	neg	n/a	no	16.3	-	-	-	HSV-1	+	-	+		
4	1	30-39	MSM	NL	neg	n/a	no		-	-	-	-			+	-	
	2	40-49	MSM	NL	neg	n/a	no	24.9	-	-	-	HSV-1	+	-	+		
5	1	30-39	MSM	SA	unk	n/a	no		+	-	-	HSV-1&2			+	+	
	2	30-39	MSM	SA	neg	n/a	yes	14.8	-	-	-	HSV-1&2	+	+	+		
6#	1	30-39	MSM	NL	unk	n/a	no		-	-	+						
	2	30-39	MSM	NL	unk	n/a	no	22.6	-	+	+	HSV-1&2	+	+	+		
7	1	30-39	MSM	NL	unk	n/a	yes		-	-	-	HSV-1&2			+	+	
	2	30-39	MSM	NL	neg	n/a	no	44.3	-	+	-	HSV-1&2	+	+	+		
8#	1	30-39	MSM	other	pos	n/a	no		-	-	-						
	2	40-49	MSM	other	pos	n/a	yes	52.3	-	-	-	HSV-1	+	-	+		
9#	1	20-29	MSM	NL	unk	n/a	yes		-	-	-						
	2	30-39	MSM	NL	unk	n/a	no	51.3	-	-	-	HSV-1	+	-	+		
10	1	20-29	MSW	NL	unk	n/a	yes		-	-	-	-			-	-	
	2	20-29	MSW	NL	neg	n/a	no	64.9	-	-	-	±	+	-	+		HSV-1
11	1	20-29	WSM	Sur	unk	no	no		-	-	-	Neg			-	-	
	2	20-29	WSM	Sur	unk	no	no	12.6	-	-	-	±	+	-	+		HSV-1
12	1	20-29	WSM	NL	unk	no	yes		-	+	-	-			+	+	
	2	20-29	WSM	NL	neg	no	yes	11.6	-	+	-	HSV-1	+	-	+		

Supplementary table 2a. Continued

pt	visit	age	sex[1]	ethn[2]	HIV[3]	pregnant[4]	med[5]	time[6]	syphilis	CT	NG	IB IgG	Liaison gG-1	Liaison gG-2	ELISA gG-1	ELISA gG-2	WB IgG[7]
13	1	20-29	WSM	NL	neg	no	no		-	-	-	-			+	-	
	2	20-29	WSM	NL	neg	no	no	13.6	-	-	-	HSV-1	+	-	+		
14	1	30-39	MSM	NL	pos	n/a	yes		-	-	-	HSV-1&2			+		
	2	30-39	MSM	NL	pos	n/a	no	6.1	-	+	-	HSV-1&2	+	+	+		
15	1	30-39	MSW	NL	neg	n/a	no		-	-	-	-			-	-	
	2	30-39	MSW	NL	neg	n/a	yes	41.1	-	-	-	HSV-1	+	-	+		
16	1	20-29	WSM	NL	neg	no	yes		-	-	-	-			-	-	
	2	20-29	WSM	NL	neg	no	yes	8.3	-	-	-	-	+		±		HSV-1
17	1	20-29	WSM	NL	neg	no	yes		-	-	-	HSV-2			-	+	
	2	20-29	WSM	NL	neg	no	no	37.1	-	-	-	HSV-2	-	+	-		HSV-2[8]

Abbreviations:
CT: *Chlamydia trachomatis*; IB: immunoblot; MSM: men who have sex with men; MSW: men who have sex with women only; n/a; not applicable; n.d. not done; neg: negative; NG: *Neisseria gonorrhoeae*; NL: Dutch; pos: positive; pt: patient; SA: South American; Sur: Surinamese; unk: unknown; WB: Western blot; WSM: women who have sex with men; -: negative; +: positive; ±: equivocal.

[1]sex: sexual preference in the previous 6 months
[2]ethn: ethnicity
[3]HIV: HIV-serostatus; only patients who were not already known to be HIV-positive were offered an HIV test. HIV status was considered unknown when no history of HIV was reported and no HIV test was performed at current visit; negative when the HIV serology test was negative at current visit; and positive when reporting a history of HIV or when the HIV serology test was positive at current visit.
[4]pregnant: pregnant at time of visit
[5]med: medication given at time of genital herpes episode, either Valaciclovir 500 mg, twice daily for 5 days or Aciclovir 200 mg, five times daily for 5 days
[6]time: time between initial and recurrent genital HSV-1 episode in months
[7]one serum sample from a patient with first recurrent HSV-1 episode with negative or equivocal HSV-1 results in at least 1 of the 3 glycoprotein G directed serological tests, was not determined with the Western blot because not enough serum was available
[#]serum from initial genital HSV-1 episode was not available
[8]at first, the WB analysis for this sample was HSV-2 positive only; during repeated discrepancy testing, this sample was both HSV-1 and HSV-2 positive in the WB with HSV-1 on the border of a positive reaction.

Supplementary table 2b. Patient characteristics, STI diagnosis and type-specific glycoprotein G directed serologic test outcomes in sera from patients with recurrent HSV-2 DNA positive genital lesions, STI clinic, Public Health Service of Amsterdam, 2000-2011

Rows in grayscale are consecutive serum samples with negative or equivocal HSV-2 results in at least 1 of the 3 glycoprotein G directed serological tests.

pt	visit	age	sex[1]	ethn[2]	HIV[3]	pregnant[4]	med[5]	time[6]	syphilis	CT	NG	IB IgG	ELISA gG-1	ELISA gG-2	Liaison gG-1	Liaison gG-2	WB IgG[7]
1	1	40-49	MSM	NL	pos	n/a	no		-	-	+	HSV-2	-	+	-	+	
	2	40-49	MSM	NL	pos	n/a	no	11.8	-	-	-	HSV-2		+	-	+	
2	1	30-39	MSW	NL	neg	n/a	yes		-	-	-	HSV-1&2	+	+	+	+	
	2	30-39	MSW	NL	neg	n/a	no	52.0	-	-	-	HSV-1&2		+	+	+	
3	1	30-39	MSW	SA	unk	n/a	no		-	-	-	HSV-1&2	+	+	+	+	
	2	30-39	MSW	SA	neg	n/a	no	35.2	-	-	-	HSV-2		+	+	+	
4	1	40-49	MSM	NL	unk	n/a	yes		-	-	-	HSV-1	+	-	+	-	
	2	40-49	MSM	NL	pos	n/a	no	5.2	+	-	-	HSV-1&2	-	-	+	+	n.d.
	3	40-49	MSM	NL	pos	n/a	no	57.1	-	-	-	HSV-1&2		+	+	+	
5	1	40-49	MSM	NL	pos	n/a	yes		-	-	-	HSV-1&2	+	+	+	+	
	2	40-49	MSM	NL	pos	n/a	no	14.7	-	-	-	HSV-1&2		+	+	+	
6	1	30-39	MSM	SA	pos	n/a	yes		-	-	+	HSV-2	-	+	-	+	
	2	30-39	MSM	SA	pos	n/a	no	46.6	-	-	+	HSV-2		+	-	+	
7	1	40-49	MSM	NL	unk	n/a	no		-	-	-	HSV-1&2	+	+	+	+	
	2	50-59	MSM	NL	pos	n/a	yes	67.9	-	-	-	HSV-2		+	+	+	
8	1	40-49	MSM	NL	unk	n/a	no		-	-	-	HSV-1&2	-	+	+	+	
	2	40-49	MSM	NL	neg	n/a	no	58.3	-	-	-	HSV-1&2		+	+	+	
9	1	30-39	MSM	NL	unk	n/a	no		-	-	+	±	±	-	+	-	
	2	30-39	MSM	NL	pos	n/a	yes	4.0	-	-	-	HSV-2	-	+	+	-	n.d.
	3	30-39	MSM	NL	pos	n/a	yes	15.6	-	+	+	HSV-1&2	±	+	+	±	n.d.
	4	30-39	MSM	NL	pos	n/a	yes	20.3	-	-	-	HSV-2	-	+	-	+	HSV-1&2
	5	30-39	MSM	NL	pos	n/a	no	39.4	-	-	-	HSV-2		+	-	+	
10	1	30-39	MSW	AT	unk	n/a	no		-	-	-	HSV-1	+	+	+	-	
	2	40-49	MSW	AT	neg	n/a	no	96.2	-	-	-	HSV-1		+	+	-	HSV-1&2

Supplementary table 2b. Continued

pt	visit	age	sex[1]	ethn[2]	HIV[3]	pregnant[4]	med[5]	time[6]	syphilis	CT	NG	IB IgG	ELISA gG-1	ELISA gG-2	Liaison gG-1	Liaison gG-2	WB IgG[7]
11	1	30-39	MSM	NL	unk	n/a	no		-	+	-	±	-	-	-	-	
	2	30-39	MSM	NL	neg	n/a	no	3.6	-	-	-	-	+	+	-	-	n.d.
	3¥	40-49	MSM	NL	neg	n/a	yes	33.8	-	-	-	-		-	+	-	HSV-1&2
12#	1	50-59	MSM	NL	pos	n/a	no		-	-	-						
	2	60-69	MSM	NL	pos	n/a	no	23.8	-	-	-	HSV-2		+	-	+	
13	1	40-49	MSW	SUR	unk	n/a	no		-	-	-	HSV-1&2	+	+	+	+	
	2	50-59	MSW	SUR	neg	n/a	yes	96.1	-	-	-	HSV-1&2		+	+	+	
14	1	50-59	MSM	NL	pos	n/a	no		-	+	-	HSV-1&2	+	+	+	+	
	2	50-59	MSM	NL	pos	n/a	no	31.8	-	-	-	HSV-1&2	+	+	+	+	
15	1	20-29	MSW	SUR	neg	n/a	no		-	+	-	HSV-1	+	-	+	-	
	2	20-29	MSW	SUR	neg	n/a	no	8.4	-	+	-	HSV-1	+	-	+	-	HSV-1[8]
16	1	20-29	MSM	SA	neg	n/a	no		-	-	-	HSV-2	+	+	+	±	
	2	30-39	MSM	SA	pos	n/a	no	75.8	-	-	-	HSV-1&2		+	+	-	n.d.
17	1	40-49	MSM	SUR	unk	n/a	no		-	-	-	HSV-2	-	-	-	+	
	2	40-49	MSM	SUR	pos	n/a	no	38.3	-	+	-	HSV-2		+	-	+	
18	1	30-39	MSM	NL	pos	n/a	no		-	+	-	HSV-1	+	-	+	-	
	2	30-39	MSM	NL	pos	n/a	yes	2.9	-	-	-						HSV-1&2
	3	30-39	MSM	NL	pos	n/a	no	6.0	-	-	-	HSV-1&2	+	±	+	-	n.d.
	4¥	40-49	MSM	NL	pos	n/a	no	33.8	-	+	-	HSV-1		-	+	-	n.d.
19	1	20-29	WSM	EE	unk	no	no		-	-	+	HSV-1&2	+	+	+	±	
	2	20-29	WSM	EE	neg	no	yes	11.7	-	-	-	HSV-1&2	+	+	+	+	
	3	20-29	WSM	EE	neg	no	no	31.2	-	-	-	HSV-1&2		+	+	+	
20	1	20-29	WSM	SA	neg	no	no		-	-	-	HSV-1&2	+	+	+	+	
	2	20-29	WSM	SA	neg	no	no	6.3	-	-	-	HSV-1&2		+	+	+	
21	1	40-49	MSM	NL	pos	n/a	yes		-	-	-	HSV-1	+	-	+	-	

Supplementary table 2b. Continued

pt	visit	age	sex[1]	ethn[2]	HIV[3]	pregnant[4]	med[5]	time[6]	syphilis	CT	NG	IB IgG	ELISA gG-1	ELISA gG-2	Liaison gG-1	Liaison gG-2	WB IgG[7]
	2	40-49	MSM	NL	pos	n/a	no	8.1	-	-	-	HSV-1&2		+	+	-	n.d.
22	1	40-49	MSW	NL	neg	n/a	yes		-	-	-	±	+	-	+	-	
	2	40-49	MSW	NL	neg	n/a	no	4.1	-	-	-	HSV-1		-	+	-	HSV-1[8]
23[#]	1	30-39	MSM	AT	pos	n/a	no		-	+	+						
	2	40-49	MSM	AT	pos	n/a	no	25.3	-	-	-	HSV-1&2		+	+	+	
24	1	40-49	MSM	NL	pos	n/a	yes		-	+	-	HSV-2	-	+	-	+	
	2	50-59	MSM	NL	pos	n/a	yes	36.6	-	+	-	HSV-2		+	-	+	
25	1	30-39	MSM	NL	pos	n/a	no		-	-	-	HSV-1&2	+	+	+	+	
	2	30-39	MSM	NL	pos	n/a	no	7.9	-	-	-	HSV-1&2		+	+	+	
26	1	30-39	WSM	NL	neg	no	yes		-	-	-	-	-	-	-	-	
	2	30-39	WSM	NL	neg	no	no	25.6	-	-	-	HSV-2		+	-	+	
27	1	20-29	MSM	NL	neg	n/a	no		-	-	-	-	-	-	-	-	
	2	30-39	MSM	NL	neg	n/a	no	3.3	-	-	-	-		-	-	-	HSV-2
28	1	20-29	WSM	SUR	pos	unk	no		-	-	-	HSV-1&2	+	+	+	+	
	2	20-29	WSM	SUR	pos	yes	yes	6.0	-	-	-	HSV-1&2		+	+	+	
	3	20-29	WSM	SUR	pos	no	yes	17.1	-	-	-	HSV-1&2		+	+	+	
29	1	50-59	MSW	SUR	neg	n/a	no		-	-	-	HSV-1	+	-	+	-	
	2	50-59	MSW	SUR	neg	n/a	yes	28.1	-	-	-	HSV-1&2		+	+	+	
30	1	20-29	MSW	SUR	neg	n/a	no		-	+	-	HSV-1	+	-	+	-	
	2	20-29	MSW	SUR	neg	n/a	no	3.7	-	-	-	HSV-1&2		+	+	-	n.d.
31	1	<20	WSM	NL	neg	no	yes		-	-	-	±	+	-	-	-	
	2	<20	WSM	NL	neg	no	yes	4.2	-	+	+	HSV-2		+	-	+	
32	1	40-49	MSW	other	neg	n/a	no		-	-	-	HSV-2	-	+	-	+	
	2	40-49	MSW	other	neg	n/a	no	15.7	-	-	-	HSV-2		+	-	+	
33	1	20-29	MSW	SUR	neg	n/a	no		-	-	-	HSV-1&2	+	+	+	-	
	2	20-29	MSW	SUR	pos	n/a	no	4.8	-	-	-	HSV-1&2		+	+	+	

Abbreviations:
AT: Dutch Antillean; CT: *Chlamydia trachomatis*; EE: Eastern Europe; IB: immunoblot; LA: Latin American; MSM: men who have sex with men; MSW: men who have sex with women only; n/a; not applicable; n.d. not done; neg: negative; NG: *Neisseria gonorrhoeae*; NL: Dutch; pos: positive; pt: patient; SA: South American; Sur: Surinamese; unk: unknown; WB: Western blot; WSM: women who have sex with men; -: negative; +: positive; ±: equivocal.

[1]sex: sexual preference in the previous 6 months
[2]ethn: ethnicity
[3]HIV: HIV-serostatus; only patients who were not already known to be HIV-positive were offered an HIV test. HIV status was considered unknown when no history of HIV was reported and no HIV test was performed at current visit; negative when the HIV serology test was negative at current visit; and positive when reporting a history of HIV or when the HIV serology test was positive at current visit.
[4]pregnant: pregnant at time of visit
[5]med: medication given at time of genital herpes episode, either Valaciclovir 500 mg, twice daily for 5 days or Aciclovir 200 mg, five times daily for 5 days
[6]time: time between initial and recurrent genital HSV-2 episode in months
[7]seven serum samples from patients with first recurrent HSV-2 episode with negative or equivocal HSV-2 results in at least 1 of the 3 glycoprotein G directed serological tests, were not determined with the Western blot because not enough serum was available.
[¥]possible seroreversion for gG-2 antibodies
[#]serum from initial genital HSV-2 episode was not available
[8]consistently WB HSV-2 negative (but HSV-1 positive) at first and after repeated testing.

CHAPTER 2.2

Point-of-care management of urogenital *Chlamydia trachomatis* via Gram-stained smear analysis in male high-risk patients. Diagnostic accuracy and cost-effectiveness before and after changing the screening indication at the STI Clinic in Amsterdam.

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ABSTRACT

Objectives

To measure the effect of changing the point-of-care (POC) testing algorithm of urogenital chlamydia for all male high-risk patients to those with only symptoms with respect to: diagnostic accuracy, loss to follow-up, correctly managed consultations and costs.

Methods

Retrospective comparison of the diagnostic accuracy and cost-effectiveness of Gram-stained urethral smear analysis for the POC management of urogenital *Chlamydia trachomatis* infections. Between 2008 and 2009 Gram-stained urethral smear analysis was offered to all men irrespective of symptoms; between 2010 and 2011 only to those with symptoms. The Aptima CT assay was the reference diagnostic test.

Results

The number of examined Gram-stained smears in the two periods was respectively 7185 (2008–2009 period) and 18,852 (2010–2011 period). The sensitivity of the Gram stain analysis was respectively 83.8% (95% CI 81.2% to 86.1%) and 91.0% (95% CI 89.5% to 92.3%) ($p<0.001$). The specificity was respectively 74.1% (95% CI 73.0% to 75.2%) and 53.1% (95% CI 51.8% to 54.4%) ($p<0.001$). The positive predictive value was low in both periods, respectively 31.7% (95% CI 29.8% to 33.6%) and 35.6% (95% CI 34.1% to 37.1%) ($p=0.002$), whereas the negative predictive value was high, respectively 97.0% (95% CI 96.4% to 97.4%) and 95.4% (95% CI 94.6% to 96.1%) ($p=0.002$). The loss to follow-up rate between 2008–2009 and 2010–2011 was, respectively, 1.8% (95% CI 1.0% to 2.9%) vs 2.3% (95% CI 1.7% to 3.0%) ($p=0.36$). There was a small difference in overtreatment, 68.0% (95% CI 66.0% to 69.8%) vs 64.1% (95% CI 62.6% to 65.5%) ($p=0.001$). The cost per correctly managed consultation was 14.3% lower in the 2010–2011 period (€94.31 vs €80.82). The percentage of delayed treated infections was significantly lower in the 2008–2009 period (10.5%) compared with the 2010–2011 period (22.8%) ($p<0.001$).

Conclusions

With a high sensitivity in male high-risk patients, the Gram-stained urethral smear is a useful POC test to detect urogenital *C. trachomatis*. When offered only to men with urogenital symptoms the specificity decreases but the cost per correctly managed consultation is reduced with 14.3% without a significant difference in loss to follow-up but with a significantly higher rate of delayed treatment.

INTRODUCTION

Chlamydia trachomatis (CT) urogenital infection is a common sexually transmitted infection (STI) causing life threatening conditions like ectopic pregnancy and a high burden of morbidity like infertility and pelvic inflammatory disease.[1] In 2005, according to WHO, there were approximately 98 million adults infected with CT and it was estimated that yearly 101 million new cases of CT occurred globally.[2]

Point-of-care (POC) tests are important in the management of STIs because they allow the clinician to provide immediate test results and treatment.[3] Recently, several companies have developed lipopolysaccharide based POC tests that provide rapid results for the detection of CT. However, low sensitivity (25–65%) precludes more widespread use in clinical settings.[4 5]

In most STI clinics male patients are treated promptly for CT if a non-gonococcal urethritis (NGU) is diagnosed. NGU in men is characterised by discharge and urethral symptoms such as dysuria or urethral itching, but may be asymptomatic.[6] NGU is based on the microscopic analysis of a smear from the urethra or urine sediment. NGU can be indicative for CT infections but other causative organisms such as *Mycoplasma genitalum* (MG), *Ureaplasma urolyticum*, *Trichomonas vaginalis*, anaerobic colon flora or herpes simplex virus may be involved. In many settings NGU is diagnosed if more than five polymorph nucleated leucocytes (PMNL) per high power field (hpf) are seen in a urethral Gram-stained smear, in the absence of intracellular negative diplococci. In the Netherlands >10 PMNLs/hpf is used as a threshold to diagnose NGU. For female high-risk patients a Gram-stained smear of genital samples is not a suitable POC test for urogenital CT because of the low positive predictive value.[7]

Irrespective of symptomatology all high-risk male patients who visited the STI Outpatient Clinic of the Public Health Service of Amsterdam before February 2010 were offered a Gram-stained smear as a POC test for CT and *Neisseria gonorrhoeae* (NG). Since February 2010, only symptomatic high-risk patients were offered a Gram-stained smear, because of structural understaffing of the POC laboratory. Here we compared the Gram stain analysis in the POC management of urogenital CT in male high-risk patients in these two time periods. We evaluated the diagnostic accuracy, loss to follow up, percentage correctly managed consultations and the costs. The data set used in the current study was collected and described in an earlier study where we investigated the Gram stain analysis for POC management of urogenital NG.[8]

METHODS

Study setting

The STI Outpatient Clinic in Amsterdam, the Netherlands, is a nurse-led clinic that offers free STI screening and treatment. Annually approximately 38,000 screenings are performed.[9] During online enrolment patients are stratified into different risk groups. Patients are classified as high-risk if one of the following criteria is met: having STI-related symptoms, notified of an STI by a sexual partner, paid for sexual contact, men who had sex with men or uninsured patients from sub-Saharan Africa. More information about the study population can be found in the earlier published study about POC management of urogenital NG.[8]

Study design and selection of patients

We performed a retrospective analysis of data from the electronic patient file. Since all data were collected for routine purposes and anonymized before the analysis, ethical clearance was not sought. Only male high-risk patients were selected. The male low-risk patients were excluded from the study because this group was not offered Gram stain examination. Some patients visited the STI clinic more than once and were offered a new standard testing procedure each time. The number of patients used in our calculations refers to the number of consultations and not to the number of unique patients. Consultations with a missing or failing Gram stain and/or confirmation test result were excluded from the analysis. Consultations that took place on days that Gram stain examination was not available (because of laboratory understaffing) were also excluded. NGU diagnoses based on urine sediment were excluded from the diagnostic accuracy analysis. In the period between 1 January 2008 and 31 December 2009 (referred to as the 2008–2009 period) urethral Gram-stained smears for light microscopic examination were obtained from all male patients who were identified as high-risk irrespective of signs and/or symptoms. Between 12 February 2010 and 31 December 2011 (referred to as the 2010–2011 period), Gram stain analysis was performed only in male high-risk patients with urogenital signs or symptoms (discharge, painful and/or frequent urination).

Gram stain analysis and confirmation testing

If >10 PMNL/hpf were seen (in the absence of intracellular Gram-negative diplococci) in at least 3 different hpfs under a light microscope a presumptive diagnosis of NGU was made. In the case of a negative result, a first-void urine sample was examined under a light microscope after centrifugation at 1000 rpm for 3 min (urine sediment) and if more than 10 PMNL/hpf were seen in the urine also the presumptive diagnosis of NGU was made. Those diagnosed with NGU were given an instant oral dose of 1000 mg azithromycin plus post-test counselling and contact tracing. If Gram-negative diplococci were seen in the PMNLs, patients were presumptively treated for NG with ceftriaxone 500 mg intramuscularly and for CT with azithromycin 1000 mg orally. Patients with a Gram stain result of <10 PMNLs/hpf were not treated at the initial visit but were managed 1 week later when the definite results (serological, culture and nucleic acid amplification test) became available. Aptima CT assay (Genprobe, USA) was used as the standard reference test for urogenital CT. Samples for reference testing were obtained from a first void urine sample. More information about the management of high-risk patients is described in the supplementary data and also in an earlier published study.[8]

Confirmed and treated infections, prompt and delayed treatment, loss to follow-up and overtreatment

The percentage of confirmed (by standard test) and treated infections was calculated as all confirmed CT infections treated at our clinic, out of all confirmed CT infections. The percentage of promptly tested and correctly treated infections was calculated as all confirmed CT infections treated at the initial visit out of all confirmed CT infections. The proportion of delayed treated infections was calculated as all infections treated at the return visit out of all confirmed CT infections. The percentage of loss to follow-up was calculated as the proportion of confirmed CT infections that were not treated at our clinic within 12 weeks after the definite diagnosis was available (upon three attempts to inform the patient), out of all confirmed CT infections. The percentage of overtreatment was calculated as those who received treatment upon a false positive Gram stain (negative Aptima CT assay) out of all infections that had to be treated upon a positive Gram stain.

Costs per consultation and per correctly managed consultation

Costs of the consultations were estimated from a health services perspective. Costs included were direct staff time (salary plus benefits), clinic space, supplies, overhead and medication. Costs for the patient, like loss of productivity due to waiting hours,

were not accounted for. We calculated the cost per consultation by dividing the total costs by all consultations. To combine both the costs and the diagnostic accuracy in one outcome the cost per correctly managed consultation was calculated by dividing the total costs by the number of correctly managed consultations. Incorrect management of CT was defined as delayed treatment (treatment after the first visit), no treatment at all (loss to follow-up) or overtreatment (a positive Gram stain result without confirmed CT). The remaining consultations fell into the group of correctly managed consultations, that is, treated presumptively at the clinic upon a confirmed (by standard test) CT infection or those not treated with a negative standard test result. More information about the costs calculations can be found in the supplementary data and table S1.

Urethritis caused by NG

Gram-stained smear analysis was also used for the detection of urogenital NG. In case a presumptive NG diagnosis was made, patients were treated with ceftriaxone plus azithromycin to cover NG and CT. During the 2010–2011 period, Gram-stained smear analysis was offered only to symptomatic patients. Since in men urogenital NG is more frequently symptomatic then urogenital CT, we calculated the prevalence of NG in both periods to exclude possible bias caused by the presumptive treatment of NG infections.

Analysis

Statistical analyses were done using Stata/SE V.12.1 for Windows and IBM SPSS Statistics V.21. Differences in proportions between the two study periods with 95% CIs were tested for the equality of proportions using large-sample statistics (binomial approximation) in the two study periods. Data of the consultation costs were analysed in Excel.

RESULTS

In the 2008–2009 period 30,079 consultations were performed in men, of which 20,492 (68.1%) were considered high-risk (figure 1). After exclusion, the final analysis set consisted of 7185 (23.9%) high-risk consultations.

In the 2010–2011 period 30,460 consultations were performed in men, of which 20,349 (66.8%) were considered high-risk. After exclusion the final analyses set consisted of 18,852 (61.9%) high-risk consultations.

Although the percentage of consultations included in the analysis is much higher in the 2010–2011 period as compared with the 2008–2009 period (mainly because of exclusion of the consultations in the 2008–2009 period because of days with lack of laboratory staff), the general characteristics like sex, age, nationality and various risk factors in those included and excluded are comparable (see supplementary table S2).

Diagnostic accuracy

The sensitivity of the Gram stain for CT was 83.8% (95% CI 81.2% to 86.1%) in the 2008–2009 period and 91.0% (95% CI 89.5% to 92.3%) in the 2010–2011 period (p<0.001). The specificity of the Gram stain for CT was 74.1% (95% CI 73.0% to 75.2%) in the 2008–2009 period and 53.1% (95% CI 51.8% to 54.4%) in the 2010–2011 period (p<0.001). The positive predictive value was low in both periods; 31.7% (95% CI 29.8% to 33.6%) in the 2008–2009 period and 35.6% (95% CI 34.1% to 37.1%) in the 2010–2011 period. In both periods there was a comparable high negative predictive value of 97.0% (95% CI 96.4% to 97.4%) and 95.4% (95% CI 94.6% to 96.1%), respectively. Detailed information about diagnostic accuracy can be found in the supplementary table S3.

Confirmed and treated infections, prompt and delayed treatment, loss to follow-up and overtreatment

The proportion of confirmed CT infections treated at the clinic was comparably high in both periods: 98.2% vs 97.7% (p=0.26) (table 1). In the 2008–2009 period the percentage of promptly treated infections based on Gram stain POC management was significantly higher compared with the 2010–2011 period, respectively, 87.7% and 74.9% (p<0.001). Consequently the percentage of delayed treated infections was significantly lower in the 2008–2009 period (10.5%) compared with the 2010–2011 period (22.8%) (p<0.001).

There was no statistically significant difference in the loss to follow-up percentage between both periods: 1.8% (95% CI 1.0% to 2.9%) vs 2.3% (95% CI 1.7% to 3.0%) (p=0.36), but a small statistically significant difference in the percentage overtreated, 68.0% (95% CI 66.0% to 69.8%) vs 64.1% (95% CI 62.6% to 65.5%) (p=0.001) (table 1).

Costs per consultation and per correctly managed consultation

The average cost per consultation was estimated to be €71.60 in the 2008–2009 period versus €67.20 in the 2010–2011 period, a savings of €4.40 or 6.2% per consultation. On the basis of 75.9% correctly managed CT infections in the first period and 83.2% correctly managed CT infections in the second period, the cost per correctly managed

consultation was €94.31 in the first period compared with €80.82 in the second period, a savings of €13.49 or 14.3% per correctly managed consultation (table 2).

Urethritis caused by NG

In the 2008–2009 period 10.7% (95% CI 8.6% to 13.2%) (81/775) patients with a true positive Gram stain for CT had a co-infection with NG compared with 13.8% (95% CI 12.0% to 15.6%) (201/1461) of the patients with a true positive Gram for CT in the 2010–2011 period (p=0.043).

In the 2008–2009 period 14.1% (95% CI 12.4% to 15.9%) (229/1627) of the patients with a false positive Gram stain for CT turned out to have an NG infection compared with 20.6% (95% CI 19.1% to 22.2%) (546/2645) of the patients with a false positive Gram stain for CT in the 2010–2011 period (p<0.001).

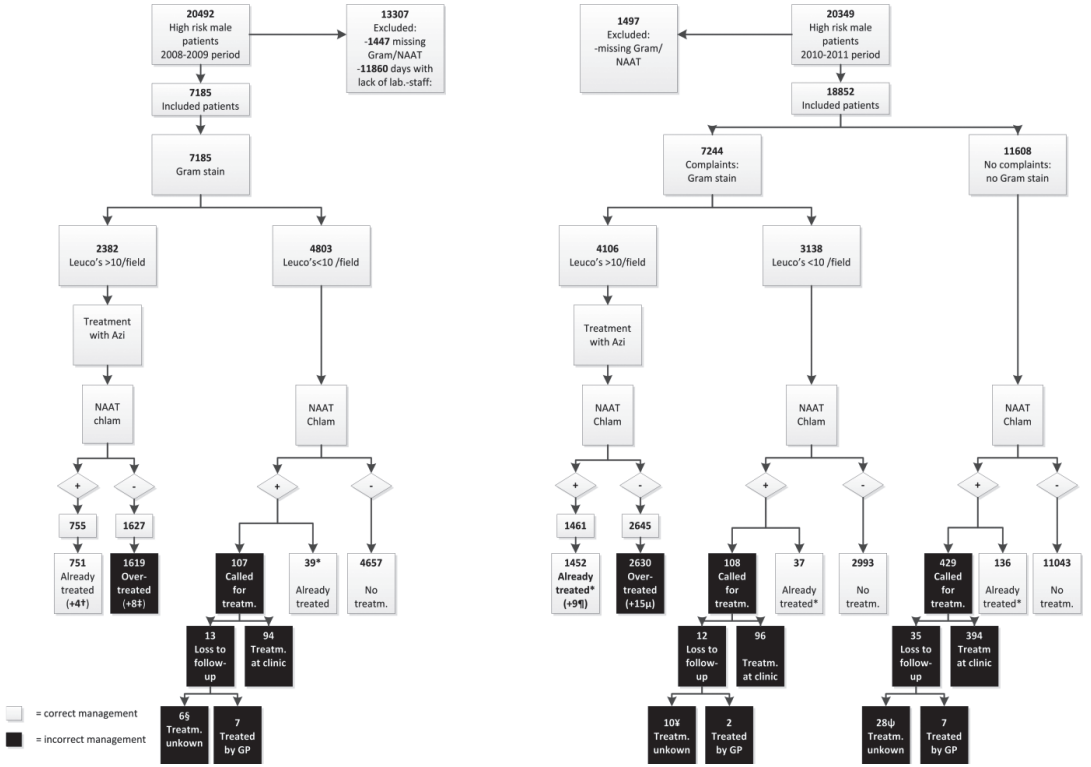

Figure 1. Flow chart of the management of urogenital *Chlamydia trachomatis* among high-risk male patients, STI outpatient clinic, Amsterdam, the Netherlands, 2008-2011.

Abbreviations: STI, sexually transmitted infection.

*Already treated for other reason (positive urine sediment, urogenital gonorrhoea, anorectal chlamydia/gonorrhoea, epididymitis, partner with chlamydia), †Four patients were not treated at the first visit (one patient was treated at the second visit, three patients were loss to follow-up), ‡Eight patients were not treated presumptively for unknown reason, §Four patients did read their results on internet, two patients didn't, ¶Nine patients were not treated at the first visit (six patients were treated during the second visit and three patients were loss to follow-up), µFifteen patients were not treated presumptively at the first visit for unknown reason, ¥Five patients did read their results on internet, five patients didn't.ψFourteen patients did read their results on internet, 14 patients didn't.

Table 1. Confirmed* and treated urogenital *Chlamydia trachomatis* (CT) infections, promptly treated and delayed treated infections, loss to follow-up and overtreated, in high-risk male patients, STI Outpatient Clinic, Amsterdam, the Netherlands, 2008–2011

	Confirmed CT treated†	Promptly treated‡	Delayed treated§	Loss to follow-up¶	Overtreatment**
Overall					
2008-2009	885††/901 (98.2%)	790‡‡/901 (87.7%)	95§§/901 (10.5%)	16¶¶/901 (1.8%)	1619***/2382 (68.0%)
2010-2011	2121†††/2171 (97.7%)	1625‡‡‡/2171 (74.9%)	496§§§/2171 (22.8%)	50¶¶¶/2171 (2.3%)	2630****/4106 (64.1%)

Abbreviations:
STI, sexually transmitted infection.

*Confirmed with Aptima CT assay (Genprobe, USA).
†Proportion (%) at the clinic treated CT infections, out of all Aptima CT assay confirmed infections.
‡Proportion (%) promptly (at initial visit) treated CT infections, out of all Aptima CT assay confirmed infections.
§Proportion (%) delayed (at return visit) treated CT infections, out of all Aptima CT assay confirmed infections.
¶Proportion (%) of untreated CT infections (not treated at the STI outpatient clinic) out of all Aptima CT assay confirmed infections, 12 weeks after the confirmed diagnosis became available.
**Proportion (%) overtreated patients was those who received treatment upon a false positive Gram stain (negative Aptima CT assay) out of all infections that had to be treated upon a positive Gram stain.
††The cases with a confirmed CT that were treated at the clinic were calculated as follows (see left flow chart and legend of figure 1): 751+39 (those already treated)+1 (not according to point-of-care (POC) protocol treated at the second visit)+94 (treated at the second visit)=885.
‡‡The cases with a confirmed CT that were treated promptly (at initial visit) were calculated as follows (see left flow chart and legend of figure 1): 751+39 (in the 'already treated' boxes)=790.
§§The cases with a confirmed CT with delayed treatment (at the second visit) were calculated as follows (see left flow chart and legend of figure 1): 94 (treated at second visit)+1 (not according to POC protocol treated at the second visit)=95.
¶¶The loss to follow-up cases were calculated as follows (see left flow chart and legend of figure 1): 13 (in the 'loss to follow-up box')+3 (exceptions in the 'already treated box')=16.
***The cases that were overtreated based on a false positive Gram stain (see left flow chart and legend of figure 1): 1619 (in the 'overtreated box').
†††The cases with a confirmed CT that were treated at the clinic were calculated as follows (see right flow chart and legend of figure 1): 1452+37+136 (those already treated)+96+394 (those treated at the second visit) +6 (not according to POC protocol treated at the second visit)=2121.
‡‡‡The cases with a confirmed CT that were treated promptly (at initial visit) were calculated as follows (see right flow chart and legend of figure 1): 1452+37+136 (in the 'already treated boxes')=1625.
§§§The cases with a confirmed CT with delayed treatment (at the second visit) were calculated as follows (see right flow chart and legend of figure 1): 96+394 (treated at second visit)+6 (not according to POC protocol treated at the second visit)=496.
¶¶¶The cases of loss to follow-up were calculated as follows (see right flow chart and legend of figure 1): 12+35 (in the 'loss to follow-up boxes')+3 (exceptions in the 'already treated box')=50.
****The cases that were overtreated based on a false positive Gram stain (see right flow chart and legend of figure 1): 2630 (in the 'overtreated box').

Table 2. Cost per consultation and per correctly managed consultation of urogenital *Chlamydia trachomatis* (CT), among high-risk male patients, STI Outpatient Clinic, Amsterdam, the Netherlands, 2008–2011

Study period	2008-2009	2010-2011
Total cost (TC)	€ 514479.17	€ 1266876.18
Total number of consultations (N)	7185	18852
Total number of correctly managed consultations (%) (NCM)	5455 (75.9%)	15676 (83.2%)
Mean cost per consultation (TC/N)	€ 71.60	€ 67.20
TC of incorrect management*	€30957.46 (n=1730)	€ 50578.89 (n=3176)
Due to delayed treatment	€ 75.27 (n=95)	€ 393.00 (n=496)
Due to loss to follow up; no treatment	€ 12.68 (n=16)	€ 39.62 (n=50)
Due to overtreatment	€ 30869.51 (n=1619)	€ 50146.27(n=2630)
Mean cost per correctly managed consultation	€ 94.31	€ 80.82

Abbreviations:
STI, sexually transmitted infection.

*Incorrect management of CT is defined as delayed treatment (treatment after the first visit), no treatment at all (loss to follow-up after a positive nucleic acid amplification test (NAAT)) or overtreatment (based on a positive Gram stain result without confirmed CT). The remaining consultations fall into the group of correct management, that is, treated presumptively at the clinic upon a confirmed (by standard test) CT infection or those not treated with a negative standard test result. More information about the costs calculations can be found in the online supplementary data.

DISCUSSION

POC management is a highly valued public health principle to prevent sequelae, loss to follow-up consultations and ongoing transmission.[3] Faced with budget cuts we had to economise our POC laboratory around 2010. Whereas before 2010 all high-risk patients were offered a Gram stain smear testing to diagnose urogenital CT promptly, only those with urogenital symptoms received POC management from 2010 onwards. This measure resulted in a cost reduction of 14.3% per correctly managed consultation of urogenital CT.

In this study we found a relatively high sensitivity of Gram stain analysis for urogenital CT in both periods (83.8% in the 2008–2009 period and 91.0% in the 2010–2011 period) compared with the sensitivity reported by other studies, ranging from 23% to 71%.[10 11] The relative high sensitivities in our study could be due to a selective study population that consisted of high-risk patients of which the majority were men who have sex with men (respectively 56.5% and 64.8% of the study populations) compared with more

general populations in other studies. Also different analysis techniques and thresholds used for a prompt NGU diagnosis (5 PMNLs/hpf in other studies vs 10 PMNLs/hpf in the present study) make a comparison on outcomes difficult.[12] According to national guidelines the outpatient clinic in Amsterdam uses more than 10 PMNLs/hpf as a threshold for NGU whereas most international guidelines advise to use more than 5 PMNLs/hpf as a threshold. The higher threshold in our study could have resulted in a higher specificity but lower sensitivity.

The higher sensitivity of the Gram stain to detect CT in the 2010–2011 period (91.0%) compared with the 2008–2009 period (83.8%) could be explained by fewer false negative outcomes when urethral smear analysis was performed solely in symptomatic men. Probably symptomatic CT infections are more likely to cause Gram stain positive smear results as opposed to asymptomatic CT infections.

On the other hand, the specificity was relatively low in both periods (74.1% and 53.1%). This can be partly explained by infection with NG which may have caused symptomatic urethritis in many patients. The lower specificity in the 2010–2011 period (53.1%) compared with the 2008–2009 period (74.1%) is remarkable. A possible explanation is that CT infections are mostly asymptomatic in contrast with infections with urogenital NG. That also explains the higher percentage of NG infections found among the patients with a false positive Gram stain for CT in the 2010–2011 period when the Gram-stained smear was only offered to symptomatic patients (20.6% vs 14.1%, $p<0.001$).

Moreover, other micro-organisms we did not test for routinely could have caused positive smear results and so could be responsible for the high amount of false positivity in both periods. NGU is reported to be caused by CT in 15–40% of cases, MG in 15–25%, *T. vaginalis* in 5–15% and less commonly, herpes simplex virus and adenovirus in 2–4%. *Ureaplasma urealyticum* has been associated in some but not all studies.[7 13 14] However in 20–50% of NGU cases the aetiology remains unknown.[15] Although not excluded, most MG infections are symptomatic; the organism is found in only 5–6% of asymptomatic men.[16 17] Likewise, urogenital infections caused by herpes simplex virus and adenovirus are to cause symptoms more frequently than on average in bacterial related NGU.[5] These non-detected pathogens can further explain the low specificity found in the study.

The cost per correctly managed consultation was 14.3% lower (a difference of €13.49 per consultation) in the 2010–2011 period compared with the 2008–2009 period. This difference can be partially explained by the higher sensitivity in the 2010–2011 period resulting in a higher percentage of correctly managed consultations. Moreover there was less overtreatment in the 2010–2011 period. Also the relative decrease in the amount of Gram stains analyses performed in the 2010– 2011 period contributed in the reduction of the cost per correctly managed consultation. Yet the percentage of delayed treatment doubled. In a future mathematical modelling study we want to quantify ongoing transmission due to delayed treatment.

The strength of our study is the analysis of a large data set of comparable study populations over both periods. The outcomes of this study are in line with the outcomes of an earlier published study in which we compared the cost-effectiveness of the Gram stain in detecting urogenital NG in both periods.[8] In that study we also reported a lower cost of urethral Gram stain analysis when offered solely to symptomatic high-risk patients as opposed to all patients irrespective of symptomatology.

A limitation of both our studies is that we focused only on CT and NG as possible causative agents of urethritis. We could not evaluate urethral infections by other micro-organisms known to cause urethritis. It is still debateable if microorganisms like MG should be routinely screened for, or only in case of symptoms. More prospective studies are needed to give additional insight in the pathology and treatment of MG and other causative micro-organisms of NGU.[16 17]

To summarise, Gram stain smear analysis as a POC test for urogenital CT in symptomatic high-risk men only is more cost-effective compared with the analysis of all men, irrespective of symptoms. Screening symptomatic men only, saved 14.3% per correctly managed consultation, resulted in a higher sensitivity but a lower specificity, less overtreatment and a comparable loss to follow-up. Since there is no accurate and affordable pathogen-specific POC test for CT available, Gram stain smear analysis remains the preferred test for the prompt management of urogenital CT in high-risk men.

Key messages

‣ Microscopic analysis of Gram-stained urethral smears is the most reliable point-of-care (POC) test to date for the presumptive management of urogenital chlamydia in men.
‣ The Gram-stained smear POC system is accurate for the presumptive management of urogenital chlamydia in high-risk men.
‣ When offered only to high-risk patients with urogenital symptoms, the cost per correctly managed consultation is reduced by 14.3% with less overtreatment and comparable loss to follow-up but a higher rate of delayed treatment.

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Contributors

HJCdV, MSvR and MS were the authors responsible for the design of the study. MSvR and MB collected and interpreted the data of the diagnostic outcomes. SA did the statistical analysis of the diagnostic outcomes. KV collected and analysed the data of the costs. MB drafted the paper. HJCdV, MS and WRF supervised the overall study. All authors reviewed and approved the final article.

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Competing interests

None.

Ethics approval

All data used in this study was collected as part of routine management and anonymized before the analysis. Therefore ethical approval was not considered necessary.

Provenance and peer review

Not commissioned; externally peer reviewed.

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SUPPLEMENTARY DATA

Supplementary table S1. Costing parameters in the point-of-care management of urogenital *Chlamydia trachomatis*, STI outpatient clinic, Amsterdam, the Netherlands, 2008-2011

Activity	Description	Cost (EUR)
Consultation	Initial patient visit; nurse takes patient history and conducts physical exam and NAAT swab	12.11
Reference test (Nucleic Acid Amplification Test (NAAT*))	NAAT test by on-site laboratory	48.53
Gram test	Gram swab and prep for analysis by nurse; reading by lab technician; doctor diagnosis	3.77
Treatment of patient with either positive Gram stain result or positive NAAT test	Azithromycin 1000 mg prescribed by doctor and administered by nurse; contact tracing and counselling by nurse	19.07
Gram negative result	Nurse informs patient test is gram negative; end of visit	0.81
Patient follow up in case of a false Gram negative result	Nurse attempts to contact patient up to three times for treatment	2.42 per attempt

*Aptima CT assay (Genprobe, USA)

Supplementary table S2. Characteristics of high-risk visitors, STI outpatient clinic, Amsterdam, the Netherlands, 2008-2011

	2008-2009		2010-2011	
	Excluded from analyses	**Included in analyses**	**Excluded from analyses**	**Included in analyses**
	13307*	**7185**	**1497**	**18852**
Confirmed CT by reference test [n (%)]:				
Yes	1385 (10.4)	901 (12.5)	58 (5.3)	2171 (11.5)
No	11858 (89.1)	6284 (87.5)	1360 (90.8)	16681 (88.5)
Missing	64 (0.5)	0	79 (5.3)	0
Age in years (median; SD)	35.0 (10.8)	34.0 (11.0)	35.0 (11.0)	34.0 (11.5)
Nationality [n (%)]:				
Dutch	9091 (68.3)	4831 (67.2)	1022 (68.3)	12965 (68.8)
Surinamese	797 (6.0)	530 (7.4)	97 (6.5)	1187 (6.3)
South-American	420 (3.2)	250 (3.5)	41 (2.7)	631 (3.3)
Eastern European	355 (2.7)	154 (2.1)	32 (2.1)	483 (2.6)
Sub-Saharan African	371 (2.8)	195 (2.7)	36 (2.4)	403 (2.1)
North African	276 (2.1)	168 (2.3)	28 (1.9)	358 (1.9)

Supplementary table S2. Continued

	2008-2009		2010-2011	
	Excluded from analyses	**Included in analyses**	**Excluded from analyses**	**Included in analyses**
	13307*	**7185**	**1497**	**18852**
Etnicity				
Asian	280 (2.1)	152 (2.1)	32 (2.1)	381 (2.0)
Antillean	179 (1.3)	113 (1.6)	24 (1.6)	292 (1.5)
Turkish	169 (1.3)	100 (1.4)	24 (1.6)	195 (1.0)
Other	1369 (10.3)	692 (9.6)	161 (10.8)	1957 (10.4)
Sex worker [n (%)]:				
Yes	145 (1.1)	68 (0.9)	7 (0.5)	140 (0.7)
No	13162 (98.9)	7117 (99.1)	1490 (99.5)	18712 (99.3)
Has paid for sex in past 6 months [n (%)]:				
Yes	467 (3.5)	215 (3.0)	51 (3.4)	401 (2.1)
No	12840 (96.5)	6970 (97.0)	1446 (96.6)	18451 (97.9)
Alerted by partner [n (%)]:				
Yes	2474 (18.6)	1355 (18.9)	312 (20.8)	3980 (21.1)
No	10833 (81.4)	8530 (81.1)	1185 (79.2)	14872 (78.9)
Complaints [n (%)]:				
Yes	7494 (56.3)	4332 (60.3)	1209 (80.8)	9237 (49.0)
No	5813 (43.7)	2853 (39.7)	288 (19.2)	9615 (51.0)
Man who had sex with men in the past 6 months [n (%)]:				
Yes	7954 (59.7)	4060 (56.5)	742 (49.6)	12216 (64.8)
No	5353 (40.2)	3125 (43.5)	755 (50.4)	6636 (35.2)

*In the 2008-2009 period relatively more high-risk patients were excluded because of the exclusion of days when Gram stain examination was not available because of lack of laboratory staff

Supplementary table S3. Positive and negative results of the Gram stain versus the standard reference test (Nucleic Acid Amplification Test (NAAT) *) for urogenital *Chlamydia trachomatis* in men, STI outpatient clinic, Amsterdam, the Netherlands, 2008-2011

	2008-2009				**2010-2011**		
	NAAT*		Total		NAAT*		Total
Gram	+	-		Gram	+	-	
+	755	1627	2382	+	1461	2645	4106
-	146	4657	4803	-	145	4657	4802
	901	6284	7185		1606	7302	8908

*Aptima CT assay (Genprobe, USA)

PATIENT MANAGEMENT

Gram stains from urethral smears in men were interpreted using high power field (hpf) light microscopic examination by an experienced laboratory technician on the POC laboratory. All permanent employed technicians (n=5) were trained at an intermediate to higher level of education for medical laboratory technology and had between 3 and 15 years of experience working in the POC laboratory of the STI clinic.

If more than 10 polymorph nucleated leucocytes (PMNL)/per high powerful field (hpf) were seen (in the absence of intracellular Gram negative diplococci) in at least three different fields under the light microscope a presumptive diagnosis of non-gonococcal urethritis (NGU) was made. In the case of a negative result, the cell pellet of a first-void urine sample (after centrifugation at 1000 rpm for 3 minutes) was examined under the light microscope and if more than 10 PMNL/hpf were seen in the urine, a presumptive diagnose of NGU was also made. Those diagnosed with NGU were given an oral dose of 1000 mg Azithromycin the same day, plus post-test counselling and contact tracing. If Gram negative diplococci were seen in the PMNL's, patients were presumptively treated for both gonorrhoea (NG) with Cefriaxone 500 mg intramuscularly and urogenital chlamydia (CT) with Azithromycin 1000 mg orally.

A presumptive treatment with Azithromycin was also given to partners of index patients with a documented CT or NGU diagnosis, either in the electronic patient database or via an official notification slip. Also presumptive treatment for CT was given to patients with a presumptive diagnose of epididymitis, non-gonoccal proctitis (NGP) or anorectal NG infection.

The Aptima CT assay (Genprobe, USA) was used as the gold standard reference test for urogenital CT. Samples for reference testing were obtained from the first void urine sample.

The microbiological technicians who interpreted the NAAT were blind to the Gram stain results. All microbiological technicians (n=17) from the Public Health Laboratory finished a higher laboratory education and worked between 2 and 17 years with NAAT's.

Within a week the definite diagnosis was based on the reference test result. In the case CT was confirmed but the patient had not received Azithromycin or Doxycycline

upon the initial visit he was summoned to the clinic for additional treatment and contact tracing. Those who did not show up were notified in 3 additional attempts via telephone, email, or surface mail within 12 weeks after the diagnosis was made. If all attempts failed, the patient was considered lost to follow up. Patients who had received Azithromycin or Doxycycline upon the initial visit did not have to return to the clinic, unless other infections were diagnosed which required additional care.

Costs per consultation and per correctly managed consultation
Costs were estimated from a health services perspective. Time spent on procedures by nurses and doctors were based on expert opinion. Two different doctors and two different nurses who worked at least for one year at the STI clinic made an estimation of the activities related to the process of diagnosing and follow-up of CT.

Time spent by laboratory technicians processing samples for Gram stain analysis was measured with a stopwatch. The financial department of the Public Health Service provided data of salaries, costs for materials, equipment and the overhead. All costs were reported in Euro (€), 2012 values, and exclusive of value added tax (VAT). Costs for the patient, like loss of productivity due to waiting hours, were not accounted for.

The cost of human resources was calculated by multiplying the time needed for each activity (in minutes) by the cost of one minute of working time of that staff type. Based on the above, the cost of the first visit was estimated to be 12.11 euro and the cost of treatment was estimated to be 19.07 euro. The cost of a Gram test was estimated to be 3.77 euro and the cost of a NAAT to be 48.53 euro. The costs spent on contacting patients (by phone or mail) who did not return for treatment was estimated to be 2.42 euro per attempt. The costs of each segment of a consultation were estimated and then multiplied by the actual number of patients receiving that part of the consultation in each of the two time periods, and then summed to give the total cost for each period. The total cost was divided by the number of consultations and number of correctly managed consultations to give the mean cost per consultation and the mean cost per correctly managed consultation. We calculated the cost per consultation and the cost per correctly managed consultation. Incorrect management of chlamydia is defined as delayed treatment (treatment after the first visit), no treatment at all (loss to follow-up after a positive NAAT), or over-treatment (based on a false-positive Gram stain result). The remaining consultations fall into the group of correct management.

2.2

CHAPTER 3

Clinical management of STI

CHAPTER 3.1

Spontaneous pharyngeal *Chlamydia trachomatis* RNA clearance. A cross-sectional study followed by a cohort study of untreated STI clinic patients in Amsterdam, the Netherlands.

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ABSTRACT

Objectives

Pharyngeal *Chlamydia trachomatis* (chlamydia) might contribute to ongoing chlamydia transmission, yet data on spontaneous clearance duration are rare. We examined the prevalence, spontaneous clearance, chlamydial DNA concentration and genotypes of pharyngeal chlamydia among sexually transmitted infection (STI) clinic patients.

Methods

Female patients at high risk for an STI who reported active oral sex and male patients who have sex with men (MSM) were screened for pharyngeal chlamydia RNA using a nucleic acid amplification test. A repeat swab was obtained to evaluate spontaneous clearance in untreated patients with pharyngeal chlamydia. Quantitative chlamydia DNA load was determined by calculating the chlamydia/human cell ratio.

Results

Pharyngeal chlamydia was detected in 148/13,111 (1.1%) MSM and in 160/6915 (2.3%) women. 53% of MSM and 32% of women with pharyngeal chlamydia did not have a concurrent anogenital chlamydia infection. In 16/43 (37%) MSM and in 20/55 (36%) women, the repeat pharyngeal swab was negative (median follow-up 10 days, range 4–58 days). Patients with an initial chlamydial DNA concentration above the median were less likely to clear. Of 23 MSM with pharyngeal chlamydia who had sex with a lymphogranuloma venereum (LGV)-positive partner recently or in the past, two (8.7%) were LGV biovar positive.

Conclusions

The pharynx is a reservoir for chlamydia and LGV, and may play a role in ongoing transmission. Although delay in ribosomal RNA decline after resolution of the infection might have led to an underestimation of spontaneous clearance, in high-risk STI clinic patients, testing the pharynx for chlamydia should be considered.

INTRODUCTION

Urogenital infection with *Chlamydia trachomatis* (CT) is the most prevalent bacterial sexually transmitted infection (STI) in most industrialised countries, including the Netherlands.[1] Lymphogranuloma venereum (LGV), a serious ulcerative STI caused by *C. trachomatis* biovar L, is endemic among men who have sex with men (MSM).[2] Although treatment is straightforward, STI screening programmes have been unable to stop the ongoing epidemics of both *C. trachomatis* and LGV.[3]

Dutch STI guidelines recommend screening for pharyngeal *C. trachomatis* (PhCT) on indication only[4] and the United States Centers for Disease Control does not recommend testing for PhCT.[5] The British Association for Sexual Health and HIV recommends PhCT screening of MSM and commercial sex workers reporting sexual risk behaviour that may result in pharyngeal infection.[6]

The relevance of PhCT for the individual patient and for ongoing chlamydia transmission is unknown. Testing for PhCT in various STI clinic populations using a nucleic acid amplification test (NAAT) revealed a PhCT prevalence between 1% and 3%.[7 8] Although one study showed an association between upper respiratory tract symptoms and PhCT,[9] most patients with PhCT are asymptomatic.[8 10 11] Recently, acquisition of urethral chlamydia through fellatio has been reported in heterosexual males and MSM.[12 13] Depending on the duration of pharyngeal infection, PhCT might play an important role in the ongoing transmission of chlamydia.

In this study, we investigated pharyngeal *C. trachomatis* RNA prevalence, spontaneous clearance and bacterial DNA load in a large group of MSM and women visiting the STI clinic in Amsterdam, the Netherlands. We also performed LGV genotyping on PhCT samples from a subset of MSM to examine whether the pharynx is a potential reservoir for LGV.

METHODS

Study setting

The STI outpatient clinic at the Public Health Service of Amsterdam (GGD Amsterdam) annually performs around 36,000 free-of-charge and anonymous STI consultations. All patients are routinely tested for *C. trachomatis*, gonorrhoea, syphilis, hepatitis B[14] and also for HIV using an opt-out strategy.[15] Routinely collected data (e.g., age, country of birth, number and type of sexual contacts in the previous 6 months, history of HIV, symptoms, laboratory results, diagnoses and spontaneously reported pharyngeal symptoms) are registered in an electronic patient database.

In this article, four substudies are reported: (1) *C. trachomatis* prevalence among those routinely tested for PhCT, (2) spontaneous clearance of PhCT, (3) chlamydial DNA concentration in PhCT samples and (4) LGV typing in PhCT samples from MSM at increased risk for LGV. Inclusion of subjects was between January 2011 and July 2012.

Substudy 1: prevalence of and predictors for pharyngeal chlamydia

At our clinic, women are regarded as having a high-risk profile if they report STI-related symptoms, if they were notified by a sexual partner with an STI or if they are involved in commercial sex. High-risk women who report active fellatio in the previous 6 months (regardless of condoms use) and all MSM (irrespective of reported active fellatio) are routinely tested for pharyngeal *Neisseria gonorrhoeae* and *C. trachomatis*. This test is performed on a nurse-collected pharyngeal swab using the APTIMA *Combo* 2 assay (GEN-PROBE, San Diego, California, USA). For self-collected urine samples (MSM), nurse-collected cervical swabs (women) and nurse-collected rectal samples (from those reporting passive anal sex in the previous 6 months) *C. trachomatis* is tested using the APTIMA CT assay (GEN-PROBE). Approximately 1 week after the initial consultation, definitive results are available and patients who need treatment are invited to come to the clinic at the shortest notice for a follow-up visit and treatment; the date of treatment consultation is determined by the patient. In case of PhCT, a single dose of azithromycin 1000 mg is offered, unless azithromycin or doxycycline (100 mg twice daily for a minimum of 7 days) was already prescribed as presumptive treatment at the initial consultation.

Substudy 2: spontaneous clearance of pharyngeal chlamydia

PhCT-positive patients who returned for treatment, who were over 18 years of age and did not take antibiotics at their first visit were asked for written informed consent to participate in the substudy on spontaneous clearance of pharyngeal chlamydia. During the first 5 months, only patients with PhCT and without concurrent anogenital *C. trachomatis*, anogenital gonorrhoea and pharyngeal gonorrhoea infection were invited to participate. When the diagnosis became available, eligible visitors consenting to participate were asked to have pharyngeal swabs collected three more times (when the diagnosis was communicated, plus 1 and 2 weeks later), where after treatment was given. However, due to the high number of patients with a co-infection and the low proportion of patients who agreed to participate, we decided to change the inclusion criteria (approved by the ethical committee), and henceforth (remaining 13 months) all patients with PhCT were invited to participate. In the new design, upon inclusion, after the diagnosis became available, a nurse collected a single follow-up pharyngeal swab (APTIMA *Combo* 2 assay) and, using a questionnaire, interviewed the patient about oral sexual behaviour between the initial and follow-up visits. Participants were subsequently treated for PhCT as described above.

Substudy 3: quantification of chlamydial DNA concentration

In all chlamydia-positive pharyngeal samples from initial and follow-up consultations, quantitative chlamydia bacterial load determination was done, using a real-time PCR targeting the cryptic plasmid (expressed as inclusion-forming units (IFU)). In the same samples, the number of human leucocyte antigen (HLA) copies was determined using a quantified serial dilution. The chlamydia/human cell ratio (chlamydial DNA concentration) was calculated as described previously[16] and expressed as IFU/100 million HLA copies. As the majority of PhCT samples tested negative in the less sensitive real-time PCR, analysis on a linear scale had very limited power. As a solution, samples were categorised in three groups: (1) chlamydial DNA undetectable (*C. trachomatis*-positive in the APTIMA *Combo* 2 assay but negative in the real-time PCR), (2) chlamydial DNA concentration equal or below and (3) chlamydial DNA concentration above the median. Samples in which no HLA was detected were excluded from this analysis.

Substudy 4: testing for pharyngeal LGV

Pharyngeal samples from MSM with PhCT who also had (1) a concurrent diagnosis of anorectal or inguinal LGV or (2) a history of LGV, or (3) a history of sexual contact with a partner with LGV, were selected for pharyngeal *C. trachomatis* biovar L testing.

PhCT samples from initial consultation were tested with a *pmpH*-based in-house real-time PCR to discriminate between LGV and non-LGV genotypes, as described previously.[17 18] If both the LGV and non-LGV test results were negative in the *pmpH* test, the result was considered inconclusive.

Statistical analysis and data collection

All statistical analyses were performed using SPSS V.19 (SPSS, Chicago, Illinois, USA) and STATA Intercooled V.11.0 (STATA, College Station, Texas, USA). Sexual preference and commercial sex work refers to the period 6 months prior to the consultation. Ethnicity was defined based on criteria of Statistics Netherlands (CBS)[19] and was categorised in Dutch and non-Dutch. HIV status was based on the HIV test result at the initial consultation or on self-reported HIV-positive status. A concurrent diagnosis of STI was defined as being diagnosed with chlamydia at another anatomical location, gonorrhoea, infectious hepatitis B, HIV and/or infectious syphilis at the initial consultation. Anorectal chlamydia status (only tested when reporting receptive anal sex) was categorised in three groups (not tested, negative and positive).

$\chi 2$ Test or Fisher's exact test was used to compare categorical variables between groups; the Mann–Whitney U test was used to compare continuous variables between groups. p Values of <0.05 were considered statistically significant. Univariate and multivariable logistic regression analyses were conducted to identify independent determinants of PhCT. Multivariable model building was done using a backward step-wise procedure, including only those variables with a univariate p value of <0.10. Age and number of sexual partners in the previous 6 months were forced into and kept in the model. Other variables were kept in multivariable models if p<0.05. Due to small numbers, multivariable analyses of determinants for PhCT spontaneous clearance could not be performed.

RESULTS

Prevalence of and predictors for pharyngeal chlamydia

Between January 2011 and July 2012, 48,653 consultations were performed at the STI clinic, of which 13,339 with MSM and 8078 with high-risk women (38.5% of all female patients). At 13,111 and 6915 consultations, respectively, pharyngeal swabs were tested for chlamydia (figure 1).

The PhCT prevalence in MSM was 1.1% (148 diagnoses; 95% CI 0.9 to 1.3, table 1). Two MSM with PhCT did not return to the clinic to obtain their treatment. Pharyngeal symptoms were reported by 285 (2.2%) PhCT-negative MSM and by 2 (1.4%) PhCT-positive MSM (p=0.49). Concurrent anogenital chlamydia infections were found in 1280 (9.9%) PhCT-negative MSM and in 70 (47.3%) PhCT-positive MSM (p<0.001). In multivariable analyses, PhCT positivity was associated with being notified of any STI, concurrent anorectal chlamydia, concurrent urogenital chlamydia, more than 10 sexual partners in the previous 6 months, and an unknown HIV status (versus HIV-negative).

PhCT prevalence among women was 2.3% (160 diagnoses; 95% CI 2.0 to 2.7, table 2). Four women with PhCT did not return to the clinic to obtain their treatment. Pharyngeal symptoms were reported by 26 (0.4%) PhCT-negative women and 1 (0.6%) PhCT-positive woman (p=0.47). Concurrent anogenital chlamydia infections were found in 658 (9.7%) PhCT-negative and in 109 (68.1%) PhCT-positive women (p<0.001). In multivariable analyses, women reporting commercial sex work had a lower risk for PhCT, while those with pharyngeal gonorrhoea, those notified of an STI, those with a concurrent urogenital chlamydia and those with more than 10 sexual partners in the previous 6 months had a higher risk for PhCT.

Spontaneous clearance of pharyngeal chlamydia

Out of 148 MSM and 160 women with PhCT, 81 (54.7%) and 106 (66.3%) met the inclusion criteria, respectively (figure 1). Not showing up for treatment, not willing to participate and mistakenly not being invited resulted in 43 (53.1%) MSM and 55 (51.9%) women with PhCT participating in the follow-up study.

MSM who participated were significantly less often notified of an STI (16.3% vs 34.3%; p=0.028), had less often STI-related symptoms (14.0% vs 37.1%; p=0.005) and had less often an anogenital chlamydia infection (32.6% vs 53.3%; p=0.022). Women who participated were significantly older (median age 24 vs 22 years; p=0.005), received more often payment for sex in the previous 6 months (37.0% vs 17.0%; p=0.005), were less often notified of an STI (25.9% vs 49.1%; p=0.005) and had less often a concurrent urogenital chlamydia infection (55.6% vs 74.5%; p=0.015).

The median time between initial and follow-up consultation was 12 days for MSM (range, 7–58 days) and 9 days for women (range, 4–58 days). At follow-up, PhCT was cleared in 16/43 (37.2%) MSM and in 20/55 (36.4%) women (see supplementary table S1). Half

of MSM and 46.3% of women reported active fellatio without a condom since initial consultation, but this did not affect spontaneous clearance (p=1.0 in MSM and p=0.88 in women). Median follow-up time in MSM and women who cleared PhCT (13.0 (IQR 9–19) and 8.5 (IQR 8– 13.5)) did not significantly differ from those who did not clear (10.0 (IQR 8–14), p=0.15; and 9 (IQR 8–15), p=0.67, respectively). No significant determinants for PhCT spontaneous clearance were detected among MSM (see supplementary table S2), but among women, increasing age was associated with spontaneous clearance (per-year increase in age: OR 1.13; 95% CI 1.01 to 1.27); see supplementary table S3). Concurrent urogenital chlamydia infection was inversely associated with spontaneous clearance (OR 0.32; 95% CI 0.10 to 1.00).

Quantification of chlamydial DNA concentration

In 88 (91.7%) out of 96 participants of the clearance substudy with available samples, HLA could be detected in both (initial and follow-up) PhCT samples (figure 1). From the 88 initial samples, 46 (52.3%) had an undetectable, 23 (26.1%) a low and 19 (21.6%) a high chlamydial DNA concentration (table 3).

Among the 46 participants with an undetectable chlamydial DNA concentration, 26 (56.5%) had cleared the infection, compared with only 7 (30.4%) and 2 (10.5%) among those with low and high chlamydial DNA concentrations (p=0.001). No consistent pattern was observed in the chlamydial DNA concentration in follow-up samples compared with the initial samples (table 3).

Lymphogranuloma venereum

Totally, 23 MSM with PhCT at initial consultation had a current or past LGV diagnosis or reported sexual contact with a partner with LGV (figure 1). Out of 23 pharyngeal samples, two were LGV biovar positive (8.7%; these were not included in the bacterial clearance substudy), 7 (30.4%) negative and 14 (60.9%) inconclusive.

Table 1. Associations of baseline characteristics with pharyngeal chlamydia in 13,111 men who have sex with men (MSM), STI clinic, Public Health Service of Amsterdam, 2011-2012

	Pharyngeal chlamydia		Univariate analysis		Multivariable analysis	
	Negative n=12,963	Positive n=148				
Variable	**n (%)**	**n (%)**	**OR (95%CI)**	**p Value**	**aOR (95%CI)**	**p Value**
Demographics						
Median age (IQR) in years[a]	39 (31-47)	36 (29-46)		0.028		
Age in years						
<30	2,713 (20.9)	37 (25.0)	1	0.12	1	0.37
30-39	3,908 (30.1)	51 (34.5)	0.96 (0.63-1.47)		0.95 (0.61-1.49)	
40-49	3,964 (30.6)	43 (29.1)	0.80 (0.51-1.24)		0.81 (0.51-1.30)	
>=50	2,378 (18.3)	17 (11.5)	0.52 (0.29-0.93)		0.61 (0.33-1.11)	
Ethnicity						
Dutch	8,069 (62.2)	81 (54.7)	1	0.062		
Non-Dutch	4,894 (37.8)	67 (45.3)	1.36 (0.99-1.89)			
Sexual behaviour						
Getting paid for sex in previous 6 months	259 (2.0)	4 (2.7)	1.36 (0.50-3.71)	0.55		
Paying for sex in previous 6 months	106 (0.8)	1 (0.7)	0.83 (0.11-5.95)	0.85		
Median number of sexual partners in previous 6 months (IQR)[a]	5 (3-12)	8 (4-20)		0.003		
Number of sexual partners in previous 6 months						
0-4	5,413 (41.8)	47 (31.8)	1	0.008	1	0.023
5-10	4,200 (32.4)	47 (31.8)	1.29 (0.86-1.94)		1.19 (0.78-1.80)	
>10	3,350 (25.8)	54 (36.5)	1.86 (1.25-2.75)		1.74 (1.16-2.63)	

Table 1. Continued

	Pharyngeal chlamydia		Univariate analysis		Multivariable analysis	
	Negative n=12,963	Positive n=148				
Variable	**n (%)**	**n (%)**	**OR (95%CI)**	**p Value**	**aOR (95%CI)**	**p Value**
Active oral sex in previous 6 months				0.29		
Unknown	423 (3.3)	1 (0.7)				
No	247 (1.9)	2 (1.4)				
Yes, with condom	184 (1.4)	1 (0.7)				
Yes, without condom	12,109 (93.4)	144 (97.3)				
Reason for visit						
Was notified by sexual partner	1,967 (15.2)	43 (29.1)	2.29 (1.60-3.28)	<0.001	1.56 (1.07-2.27)	0.020
Had STI-related complaints	3,602 (27.8)	45 (30.4)	1.14 (0.80-1.62)	0.48		
Had pharyngeal complaints	285 (2.2)	2 (1.4)	0.61 (0.15-2.47)	0.49		
New STI diagnosis at current visit						
STI diagnosis[b]	2,667 (20.6)	84 (56.8)	5.07 (3.65-7.03)	<0.001		
Chlamydia (excl. pharyngeal CT)[c,d]	1,280/12,955 (9.9)	70/148 (47.3)	8.19 (5.90-11.36)	<0.001		
Anorectal chlamydia[c]						
Not tested	2,945 (22.7)	26 (17.6)	1.29 (0.81-2.03)		1.22 (0.76-1.95)	
Negative	9,168 (70.7)	63 (42.6)	1	<0.001	1	<0.001
Positive	850 (6.6)	59 (39.9)	10.10 (7.03-14.51)		8.14 (5.54-11.97)	
Urogenital chlamydia[d]	550/12,918 (4.3)	20/147 (13.6)	3.54 (2.19-5.72)	<0.001	1.98 (1.19-3.29)	0.008
Anorectal gonorrhoea[d]	569/12,891 (4.4)	18/148 (12.2)	3.00 (1.82-4.94)	<0.001		
Urogenital gonorrhoea[d]	357/12,926 (2.8)	5/148 (3.4)	1.23 (0.50-3.02)	0.65		
Pharyngeal gonorrhoea	709 (5.5)	15 (10.1)	1.95 (1.14-3.34)	0.015		
HIV status[e]						
Unknown	212 (1.6)	8 (5.4)	3.43 (1.64-7.14)		3.09 (1.44-6.61)	
Negative	8,533 (65.8)	94 (63.5)	1	0.001	1	0.002
Positive	4,218 (32.5)	46 (31.1)	0.99 (0.69-1.41)		0.76 (0.52-1.12)	
Early syphilis[d]	321/12,956 (2.5)	7/148 (4.7)	1.95 (0.91-4.21)	0.087		

Abbreviations:
aOR: adjusted odds ratio; CT: *Chlamydia trachomatis*; excl.: excluding; incl.: including; IQR: interquartile range; MSM: men who have sex with men; OR: odds ratio; STI: sexually transmitted infection; 95%CI: 95% confidence interval.

All variables with p<0.10 in univariate analysis were included in the multivariable model, except the two summarizing variables STI diagnosis and chlamydia (excl. pharyngeal CT). Patients who had missing values in STI testing were excluded from the multivariable model. The total number of records included in the multivariable model was 12,986.

[a]Mann-Whitney U Test for comparing not normally distributed continuous variables.
[b]STI diagnosis is defined as being diagnosed with *Chlamydia trachomatis* (excluding pharyngeal CT), gonorrhoea (any location), infectious hepatitis B, HIV, and/or early syphilis at time of current visit. This combined variable was not included in the multivariable analyses.
[c]Only MSM reporting passive anal sex were tested for anorectal chlamydia.
[d]In some cases no material was available or the laboratory test failed.
[e]Only MSM who were not already known to be HIV-positive were offered an HIV-test. HIV status was considered unknown when no history of HIV was reported and no HIV test was performed at current visit; negative when the HIV serology test was negative at current visit; and positive when reporting a history of HIV or when the HIV serology test was positive at current visit.

Table 2. Associations of baseline characteristics with pharyngeal chlamydia in 6,915 high-risk women, STI clinic, Public Health Service of Amsterdam, 2011-2012

	Pharyngeal chlamydia		Univariate analysis		Multivariable analysis	
	Negative n=6,755	Positive n=160				
Variable	**n (%)**	**n (%)**	**OR (95%CI)**	**p Value**	**aOR (95%CI)**	**p Value**
Demographics						
Median age (IQR) in years[a]	25 (22-30)	22 (20-26)		<0.001		
Age in years						
<20	453 (6.7)	22 (13.8)	1	<0.001	1	0.37
20-24	2,534 (37.5)	80 (50.0)	0.65 (0.40-1.05)		0.99 (0.59-1.67)	
25-29	1,884 (27.9)	39 (24.4)	0.43 (0.25-0.73)		0.87 (0.49-1.55)	
30-34	838 (12.4)	10 (6.3)	0.25 (0.12-0.52)		0.68 (0.30-1.52)	
>=35	1,046 (15.5)	9 (5.6)	0.18 (0.08-0.39)		0.50 (0.21-1.15)	
Ethnicity						
Dutch	2,821 (41.8)	68 (42.5)	1	0.85		
Non-Dutch	3,934 (58.2)	92 (57.5)	0.97 (0.71-1.33)			
Sexual behaviour						
Getting paid for sex in previous 6 months	2,354 (34.8)	38 (23.8)	0.58 (0.40-0.84)	0.004	0.25 (0.09-0.76)	0.014
Median number of sexual partners in previous 6 months (IQR)[a]	3 (2-200)	3 (1-15)		0.030		
Number of sexual partners in previous 6 months						
0-2	2,828 (41.9)	76 (47.5)	1	0.15	1	0.010
3-10	1,661 (24.6)	42 (26.3)	0.94 (0.64-1.38)		0.89 (0.59-1.34)	
>10	2,266 (33.5)	42 (26.3)	0.69 (0.47-1.01)		4.85 (1.64-14.34)	
Active oral sex in previous 6 months				0.023		
Unknown	29 (0.4)	0 (0)				
No	32 (0.5)	1 (0.6)				
Yes, with condom	633 (9.4)	5 (3.1)				
Yes, without condom	6,061 (89.7)	154 (96.3)				

Table 2. Continued

	Pharyngeal chlamydia		Univariate analysis		Multivariable analysis	
	Negative n=6,755	Positive n=160				
Variable	**n (%)**	**n (%)**	**OR (95%CI)**	**p Value**	**aOR (95%CI)**	**p Value**
Reason for visit						
Was notified by sexual partner	968 (14.3)	66 (41.3)	4.20 (3.04-5.79)	<0.001	1.75 (1.18-2.58)	0.005
Had STI-related complaints	4,270 (63.2)	97 (60.6)	0.90 (0.65-1.24)	0.50		
Had pharyngeal complaints	26 (0.4)	1 (0.6)	1.63 (0.22-12.07)	0.63		
New STI diagnosis at current visit						
STI diagnosis[b]	791 (11.7)	113 (70.6)	18.13 (12.80-25.68)	<0.001		
Chlamydia (excl. pharyngeal CT)[c,d]	658/6,751 (9.7)	109/160 (68.1)	19.79 (14.06-27.86)	<0.001		
Anorectal chlamydia[c]	135/1,622 (8.3)	19/34 (55.9)				
Not tested	5,133 (76.0)	126 (78.8)	2.43 (1.42-4.17)			
Negative	1,487 (22.0)	15 (9.4)	1	<0.001		
Positive	135 (2.0)	19 (11.9)	13.95 (6.93-28.08)			
Urogenital chlamydia[d]	625/6,751 (9.3)	108/160 (67.5)	20.36 (14.48-28.63)	<0.001	15.67 (10.78-22.77)	<0.001
Anorectal gonorrhoea[d]	52/6,699 (0.8)	5/159 (3.1)	4.15 (1.64-10.54)	0.003		
Urogenital gonorrhoea[d]	103/6,690 (1.5)	13/159 (8.2)	5.69 (3.13-10.37)	<0.001		
Pharyngeal gonorrhoea	101 (1.5)	12 (7.5)	5.34 (2.87-9.93)	<0.001	2.29 (1.15-4.58)	0.019
HIV status[e]				1.0		
Unknown	59 (0.9)	1 (0.6)				
Negative	6,680 (98.9)	159 (99.4)				
Positive	16 (0.2)	0 (0)				
Early syphilis[d]	4/6,747 (0.1)	0/160 (0)		1.0		

Abbreviations:
aOR: adjusted odds ratio; CT: *Chlamydia trachomatis*; excl.: excluding; incl.: including; IQR: interquartile range; OR: odds ratio; STI: sexually transmitted infection; 95%CI: 95% confidence interval.
All variables with p<0.10 in univariate analysis were included in the multivariable model, except the two summarizing variables STI diagnosis and chlamydia (excl. pharyngeal CT). Patients who had missing values in STI testing were excluded from the multivariable model. The total number of records included in the multivariable model was 6,781.
[a]Mann-Whitney U Test for comparing not normally distributed continuous variables.
[b]Big-five STI is defined as being diagnosed with *Chlamydia trachomatis* (excluding pharyngeal CT), gonorrhoea, infectious hepatitis B, HIV, and/or early syphilis at time of current visit. This combined variable was not included in the multivariable analyses.
[c]Only women reporting passive anal sex were tested for anorectal chlamydia.
[d]In some cases no material was available or the laboratory test failed.
[e]Only women who were not already known to be HIV-positive were offered an HIV-test. HIV status was considered unknown when no history of HIV was reported and no HIV test was performed at current visit; negative when the HIV serology test was negative at current visit; and positive when reporting a history of HIV or when the HIV serology test was positive at current visit.

Table 3. Chlamydial DNA concentration in initial and follow-up pharyngeal *C. trachomatis* positive samples and association with spontaneous clearance of pharyngeal chlamydia in 88 patients, STI clinic, Public Health Service of Amsterdam, 2011-2012

	Chlamydial DNA concentration[a] in initial pharyngeal chlamydia samples[b]			
	Undetectable IFU	Low	High	
	n=46	n=23	n=19	
	n (%)	n (%)	n (%)	P Value[c]
Pharyngeal chlamydia in follow-up sample				
Cleared (n=35)	26 (56.5%)	7 (30.4%)	2 (10.5%)	0.001
Not cleared (n=53)	20 (43.5%)	16 (69.6%)	17 (89.5%)	
Chlamydial DNA concentration[a] follow-up PhCT samples[b]				
Undetectable IFU	8 (17.4%)	3 (13.0%)	1 (5.3%)	-
Low	3 (6.5%)	7 (30.4%)	10 (52.6%)	
High	9 (19.6%)	6 (26.1%)	6 (31.6%)	

Abbreviations:
CT: *Chlamydia trachomatis*; IFU: inclusion forming units; IQR: interquartile range; PhCT: pharyngeal *Chlamydia trachomatis*; STI: sexually transmitted infection; 95%CI: 95% confidence interval.

[a]Chlamydial DNA concentration defined as; low: equal or lower than median (3.4 log IFU/100 million HLA copies); high: higher than median (3.4 log IFU/100 million HLA copies); undetectable IFU: IFU undetectable.
[b]This analysis included all patients who had a *C. trachomatis*-positive initial result, who also provided a pharyngeal sample at follow-up, and in whose samples (both initial and follow-up) HLA could be detected.
[c]p Value calculated with chi-squared test; chlamydial DNA concentration of initial PhCT samples in those who cleared PhCT compared with those who did not clear PhCT.

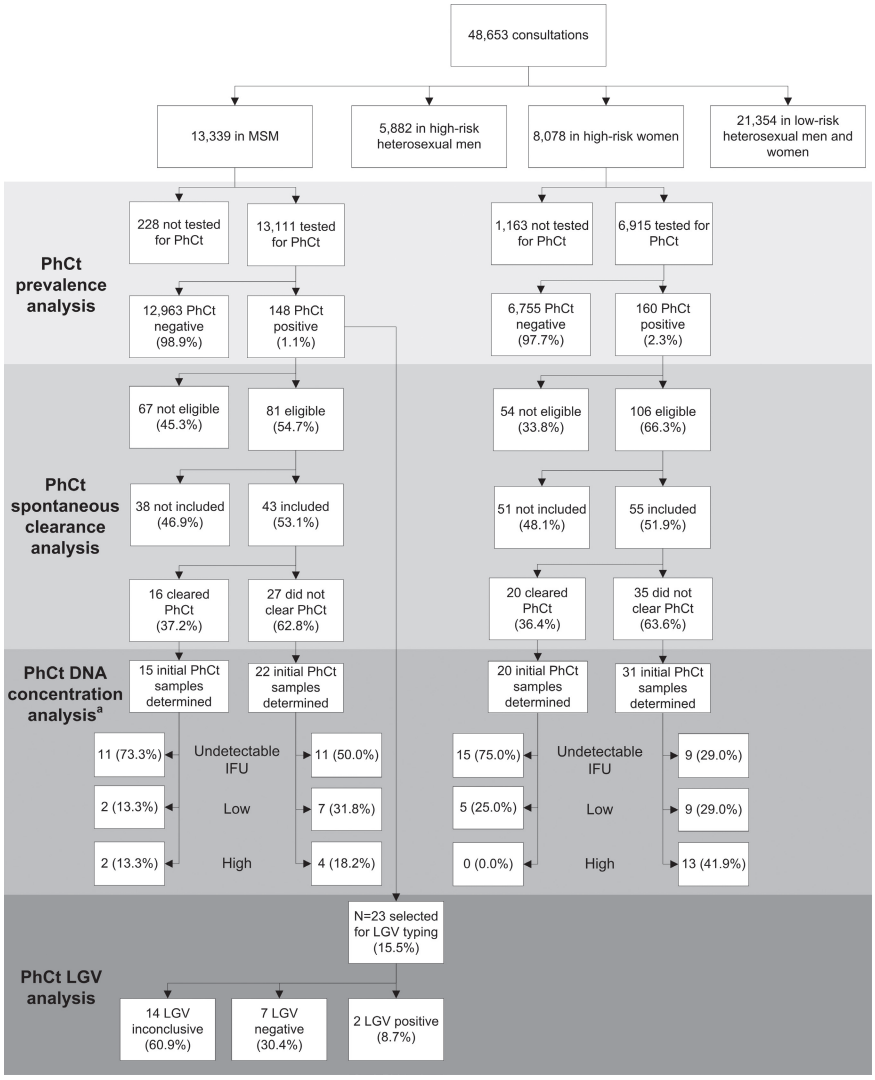

Figure 1. Flow chart of pharyngeal *Chlamydia trachomatis* (PhCT) sub-studies: (1) prevalence of PhCT; (2) spontaneous clearance of pharyngeal *C. trachomatis*; (3) analysis of *C. trachomatis* DNA concentration in pharyngeal infections; (4) prevalence of pharyngeal LGV, STI clinic, Public Health Service of Amsterdam, 2011–2012.

Abbreviations:
HLA, human leucocyte antigen; IFU, inclusion forming units; LGV, lymphogranuloma venereum; MSM, men who have sex with men; PhCT, pharyngeal *C. trachomatis*; STI, sexually transmitted infection.
[a]Chlamydial DNA concentration defined as; low: equal or lower than median (3.4 log IFU/100 million HLA copies); high: higher than median (3.4 log IFU/100 million HLA copies); undetectable IFU: IFU undetectable.

DISCUSSION

Principal findings

In comparison with urogenital and anorectal *C. trachomatis*, the prevalence of pharyngeal *C. trachomatis* is lower among MSM and high-risk women tested at this large STI clinic. Nonetheless, 52.7% and 31.9% of MSM and female patients with PhCT did not have a concurrent anogenital *C. trachomatis* infection; they would not have been treated if screening had only been performed for anogenital *C. trachomatis* infections. During follow-up, a minority (36.7%) of patients spontaneously cleared PhCT. Those with a higher chlamydial DNA concentration were less likely to spontaneously clear PhCT.

Strengths and weaknesses

A strength of this study is the high number of MSM and women screened for PhCT. To date, this study reports the largest number of MSM and women screened for PhCT at both initial and follow-up consultations. A limitation of this study was that less than half of the patients with PhCT had a follow-up consultation at which they were retested.

Whereas *C. trachomatis* DNA can be positive in the presence of remnants from non-viable organisms, the presence of *C. trachomatis* RNA implies that bacterial replication occurs and is, therefore, seen as an indicator of potential infectious bacterial viability.[20] Although *C. trachomatis* cultivation in follow-up samples would definitely prove bacterial persistence, the sensitivity of *C. trachomatis* cultivation is too low to be considered in clinical studies. Testing for chlamydial ribosomal RNA after azithromycin treatment in women with urogenital chlamydia showed that after 10 days 34% were still rRNA positive.[21] This delay in rRNA decline after resolution of the infection might have led to an underestimation of the proportion with spontaneous PhCT clearance in our study.[21 22] Also, it is unknown whether pharyngeal swabs are the preferred method to detect PhCT. Instead of pharyngeal swabs, the use of oral wash specimens (whereby a larger surface of the oral mucosa can be sampled) might be considered for future studies.[23]

The real-time PCR used for the quantification of *C. trachomatis* load is less sensitive than the APTIMA *Combo* 2 assay.[24 25] In this study, the majority of PhCT samples were negative in the real-time PCR implying a low level of chlamydial DNA in these samples.

Relation to other studies

In San Francisco, sentinel surveillance among MSM in 2010 showed a PhCT prevalence of 1.7%, slightly higher than the 1.1% found in our study.[7] In a Dutch study among female STI clinic patients reporting fellatio (January 2007–July 2008), PhCT prevalence was similar to that among high-risk women in our study (1.9% vs 2.3%).[8]

In another report from San Francisco in 2003, PhCT without concurrent anogenital *C. trachomatis* infections (solitary PhCT) was found in 30 out of 50 MSM (60.0%),[26] comparable with the 52.7% found in our study. In the Dutch study among women, the proportion of women with solitary PhCT was similar to that among high-risk women in our study (31% vs 31.9%).[8]

A relative small proportion of MSM and women spontaneously cleared PhCT during follow-up. In two earlier studies with a limited number of PhCT-positive participants, in 1 out of 2 (50%) cases with a median follow-up of 11 days[27] and in 6 out of 18 (33.3%) with a mean follow-up of 8 days (SD±6), PhCT was cleared during follow-up.[23]

Meaning of the study findings

This study shows that the pharynx is a potential reservoir for both chlamydia and LGV. Oral-to-genital transfer of *C. trachomatis* might be possible since PhCT RNA is detectable in a majority of patients for more than 1–2 weeks. Therefore, screening for PhCT may be relevant from a public health point of view. In this study, more than 93.0% of MSM and 85.6% of high-risk women reported fellatio in the previous 6 months, of whom, only 1.5% and 9.2% always used a condom during oral sex, respectively. The high frequency of unprotected orogenital contact in combination with the observed low spontaneous clearance might result in onward transmission of chlamydia.

Participants with an undetectable chlamydial DNA concentration at initial consultation more often cleared their PhCT, suggesting that the bacterial load present in the pharynx determines spontaneous clearance. Participants with PhCT but with an undetectable bacterial load at initial consultation more often cleared the infection, suggesting that the bacterial load influences risk of spontaneous clearance. Patients with PhCT and with an undetectable chlamydial DNA load may have less impact on the ongoing transmission, as in many of them *C. trachomatis* resolves within a short time. However, patients with a high chlamydial DNA load carry the bacteria for a longer period and likely transmit it via fellatio.

Strong predictors for PhCT in both women and MSM were concurrent urogenital chlamydia, being notified of an STI by a sexual partner, and high numbers of sexual partners. Among MSM, anorectal chlamydia was also significantly associated with PhCT. These predictors can be explained by increased risk for pharyngeal exposure to a chlamydia-positive partner. Unknown HIV status was a predictor for PhCT in MSM. These MSM actively declined being tested for HIV; they are known to exhibit higher sexual risk behaviour.[15] Female sex workers were less likely to have PhCT. This can be explained by more consistent and professional condom use. Women with pharyngeal gonorrhoea were at higher risk for PhCT. Possibly these women are at high risk for having sexual contact with a chlamydia-infected partner or pharyngeal gonorrhoea might increase the susceptibility for PhCT. Spontaneous reported pharyngeal symptoms were rare in patients with PhCT. Consequently, there are no symptom-based indications to test for PhCT, and testing only those patients who are at higher risk for PhCT will result in missed cases.

Unanswered questions and future research

To date, no large analysis has been performed to estimate the risk of oral-to-genital transfer of *C. trachomatis*. Our study focused on high-risk women and MSM. The observed prevalence and clearance may differ from those in other female and heterosexual male patients at the STI clinic. Since PhCT might contribute to the urogenital chlamydia epidemic, cost-effectiveness studies are needed to justify routine PhCT screening.

In conclusion, PhCT is found in 1.1% of MSM and 2.3% of high-risk women visiting the STI clinic in Amsterdam, the Netherlands. A minority of the PhCT patients cleared PhCT RNA within 1–2 weeks, and a lower *C. trachomatis* DNA concentration was associated with spontaneously cleared PhCT. PhCT might be a reservoir for the transmission of chlamydia and LGV.

Key messages

- After a median follow-up of 10 days, only 37% of patients with pharyngeal *Chlamydia trachomatis* had cleared their infection.
- Clearance was associated with a lower chlamydial DNA concentration.
- 32% (women) and 53% (men who have sex with men) of cases with pharyngeal chlamydia did not have concurrent anogenital chlamydia infections.

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Contributors

MSvR, MFSvdL and HJCdV designed the study protocol, supported by APvD and AGCLS. MSvR was responsible for implementation and data collection at the STI clinic. APvD and AGCLS were responsible for the chlamydia and LGV diagnostics at the laboratory. SAM was responsible for the chlamydial DNA load determination. MSvR and MFSvdL performed the statistical analyses. MSvR, MFSvdL and HJCdV drafted the paper, all authors commented on draft versions, and all approved the final version.

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Competing interests

None.

Ethics approval

This study was approved by the Medical Ethical Committee of the Academic Medical Centre, University of Amsterdam, Amsterdam, the Netherlands (ethics approval number MEC 10/216 # 11.17.416).

Provenance and peer review

Not commissioned; externally peer reviewed.

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SUPPLEMENTARY TABLES

Supplementary table S1. Spontaneous clearance of pharyngeal chlamydia 4 to 58 days after diagnosis in 43 men who have sex with men (MSM) and 55 high-risk women, STI clinic, Public Health Service of Amsterdam, 2011-2012

Clearance	Total n/N (%)	MSM n/N (%)	Women n/N(%)	p Value[a]
All participants	36/98 (36.7%)	16/43 (37.2%)	20/55 (36.4%)	0.93
those who reported active fellatio since diagnosis[b]	17/46 (37.0%)	8/21 (38.1%)	9/25 (36.0%)	0.88
those who did not report active fellatio since diagnosis[b]	19/50 (38.0%)	8/21 (38.1%)	11/29 (37.9%)	0.99
p Value[c]	0.92	1.0	0.88	

[a]p Value for pharyngeal chlamydia spontaneous clearance in MSM, versus female participants.
[b]At the follow-up visit, participants were asked if they had performed active fellatio (active oral-penile sex with a male partner) without a condom since the initial visit. Data missing from 1 MSM and 1 female participant; both failed to clear.
[c]p Value for comparison of proportion spontaneous cleared pharyngeal chlamydia between clients who reported fellatio and those who did not.

Supplementary table S2. Associations with spontaneous clearance of pharyngeal chlamydia in 43 men who have sex with men (MSM), STI clinic, Public Health Service of Amsterdam, 2011-2012

	No clearance n=27	Clearance n=16		Univariate analysis	
Variables	**n (%)**	**n (%)**	**p Value[a]**	**OR (95%CI)**	**p Value**
Median follow-up time in days (IQR)[b]	10.0 (8-14)	13.0 (9-19)	0.15	1.04 (0.98-1.11)	0.22
Demographics					
Median age (IQR) in years[b]	42 (29-46)	35 (31-49)	1.0	1.00 (0.94-1.07)	0.96
Age in years (tertiles)					
<33	9 (33.3)	6 (37.5)	0.93	1	0.93
33-43	8 (29.6)	5 (31.3)		0.94 (0.21-4.29)	
>43	10 (37.0)	5 (31.3)		0.75 (0.17-3.33)	
Ethnicity					
Dutch	14 (51.9)	8 (50.0)	0.91	1	0.91
Non-Dutch	13 (48.1)	8 (50.0)		1.08 (0.31-3.71)	
Sexual behaviour					
Median number of sexual partners in 6 months preceding initial visit (IQR)[b]	7 (5-15)	4 (1-10)	0.08		
Active fellatio without condom since initial visit[c]	13/26 (50.0)	8/16 (50.0)	1.0	1.0 (0.29-3.48)	1.0

Supplementary table S2. Continued

	No clearance n=27	Clearance n=16		Univariate analysis	
Variables	**n (%)**	**n (%)**	**p Value[a]**	**OR (95%CI)**	**p Value**
Reason for initial visit					
Was notified by sexual contact	4 (14.8)	3 (18.8)	0.74	1.33 (0.26-6.87)	0.74
Had STI related complaints	3 (11.1)	3 (18.8)	0.66	1.85 (0.33-10.49)	0.49
Had pharyngeal complaints	0 (0)	0 (0)	-	-	-
New STI diagnosis at initial visit					
STI diagnosis[d]	12 (44.4)	4 (25.0)	0.20	0.42 (0.11-1.63)	0.21
Anorectal chlamydia[e]	9/23 (39.1)	4/12 (33.3)	0.74	0.78 (0.18-3.36)	0.74
Urogenital chlamydia	3 (11.1)	0 (0)	0.28	-	-
Anorectal gonorrhoea	2 (7.4)	0 (0)	0.52	-	-
Pharyngeal gonorrhoea	1 (3.7)	1 (6.3)	1.0	1.73 (0.10-29.78)	0.71
HIV status[f]					
Unknown	1 (3.7)	2 (12.5)		2.83 (0.23-34.92)	
Negative	17 (63.0)	12 (75.0)	0.18	1	0.25
Positive	9 (33.3)	2 (12.5)		0.32 (0.06-1.73)	
Early syphilis	1 (3.7)	1 (6.3)	1.0	1.73 (0.10-29.78)	0.71

Abbreviations:
CT: *Chlamydia trachomatis*; excl.: excluding; incl.: including; IQR: interquartile range; MSM: men who have sex with men; OR: odds ratio; STI: sexually transmitted infection; 95%CI: 95% confidence interval.

[a]p Value calculated with chi-squared test or, in case of small numbers, with Fisher exact test.
[b]Mann-Whitney U Test for comparing not normally distributed continuous variables.
[c]Answer missing for one MSM without spontaneous clearance.
[d]STI diagnosis is defined as being diagnosed with *Chlamydia trachomatis* (excluding pharyngeal CT), gonorrhoea, infectious hepatitis B, HIV, and/or early syphilis at time of current visit.
[e]Only MSM reporting passive anal sex were tested for anorectal chlamydia.
[f]Only MSM who were not already known to be HIV-positive were offered an HIV-test. HIV status was considered unknown when no history of HIV was reported and no HIV test was performed at current visit; negative when the HIV serology test was negative at current visit; and positive when reporting a history of HIV or when the HIV serology test was positive at current visit.

Supplementary table S3. Associations with pharyngeal chlamydia spontaneous clearance in 55 high-risk women, STI clinic, Public Health Service of Amsterdam, 2011-2012

Variables	No clearance n=35 n (%)	Clearance n=20 n (%)	p Value[a]	Univariate analysis OR (95%CI)	p Value
Median follow-up time in days (IQR)[b]	9.0 (8-15)	8.5 (8-13.5)	0.67	0.99 (0.94-1.04)	0.78
Demographics					
Median age (IQR) in years[b]	24 (21-26)	25 (22-31)	0.038	1.13 (1.01-1.27)[c]	0.037
Age in years (tertiles)					
<23	15 (42.9)	5 (25.0)	0.28	1	0.29
23-25	11 (31.4)	6 (30.0)		1.64 (0.40-6.76)	
>25	9 (25.7)	9 (45.0)		3.00 (0.76-11.81)	
Ethnicity					
Dutch	14 (40.0)	7 (35.0)	0.71	1	0.71
Non-Dutch	21 (60.0)	13 (65.0)		1.24 (0.40-3.88)	
Sexual behaviour					
Getting paid for sex in 6 months preceding initial visit	10 (28.6)	10 (50.0)	0.11	2.50 (0.80-7.84)	0.12
Median number of sexual partners in 6 months preceding initial visit (IQR)[b]	2 (1-50)	78 (2-488)	0.077		
Active fellatio without condom since initial visit[d]	16/34 (47.1)	9/20 (45.0)	0.88	0.92 (0.30-2.79)	0.88
Reason for initial visit					
Was notified by sexual contact	12 (34.3)	2 (10.0)	0.047	0.21 (0.04-1.08)	0.061
Had STI related complaints	23 (65.7)	9 (45.0)	0.13	0.43 (0.14-1.31)	0.14
Had pharyngeal complaints	1 (2.9)	0 (0)	1.0	-	-
New STI diagnosis at initial visit					
STI diagnosis[e]	22 (62.9)	8 (40.0)	0.10	0.39 (0.13-1.22)	0.11

Supplementary table S3. Continued

Variables	No clearance n=35 n (%)	Clearance n=20 n (%)	p Value[a]	Univariate analysis OR (95%CI)	p Value
Anorectal chlamydia[f]	6/12 (50.0)	1/4 (25.0)	0.59	0.33 (0.03-4.19)	0.40
Urogenital chlamydia	22 (62.9)	7 (35.0)	0.047	0.32 (0.10-1.00)	0.050
Urogenital gonorrhoea	1 (2.9)	0 (0)	1.0	-	-
Pharyngeal gonorrhoea	1 (2.9)	0 (0)	1.0	-	-
HIV status (incl. test result of initial visit)[g]					
Negative	35 (100)	20 (100)			

Abbreviations:
CT: *Chlamydia trachomatis*; excl.: excluding; incl.: including; IQR: interquartile range; OR: odds ratio; STI: sexually transmitted infection; 95%CI: 95% confidence interval.

[a]p Value calculated with chi-squared test or, in case of small numbers, with Fisher exact test.
[b]Mann-Whitney U Test for comparing not normally distributed continuous variables.
[c]OR per year increase in age.
[d]Answer missing for one female without spontaneous clearance.
[e]STI diagnosis is defined as being diagnosed with *Chlamydia trachomatis* (excluding pharyngeal CT), gonorrhoea, infectious hepatitis B, HIV, and/or early syphilis at time of current visit.
[f]Only women reporting passive anal sex were tested for anorectal chlamydia.
[g]Only women who were not already known to be HIV-positive were offered an HIV-test. HIV status was considered unknown when no history of HIV was reported and no HIV test was performed at current visit; negative when the HIV serology test was negative at current visit; and positive when reporting a history of HIV or when the HIV serology test was positive at current visit.

CHAPTER 3.2

Urethral lymphogranuloma venereum infections in men with anorectal lymphogranuloma venereum and their partners: the missing link in the current epidemic?

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ABSTRACT

Urethral lymphogranuloma venereum (LGV) is not screened routinely. We found that in 341 men having sex with men with anorectal LGV, 7 (2.1%) had concurrent urethral LGV. Among 59 partners, 4 (6.8%) had urethral LGV infections. Urethral LGV is common, probably key in transmission, and missed in current routine LGV screening algorithms.

INTRODUCTION

Lymphogranuloma venereum (LGV) has re-established itself in Western society as an invasive sexually transmitted infection (STI) among men who have sex with men (MSM).[1] Lymphogranuloma venereum is caused by *Chlamydia trachomatis* (CT) biovar L. Prompt antibiotic treatment is effective and curative, although it is more extensive than anogenital biovar non-L CT infections. Broader awareness of LGV among clinicians is needed to enable appropriate investigation and management, prevent irreversible late sequelae, and accelerate the interruption of onward spread. Recently, we see a significant increase in the prevalence of LGV at the STI outpatient clinic in Amsterdam, which indicates that more extensive control measures are warranted.[2]

Most reported LGV cases among MSM involve anorectal infections.[2] Very few urogenital infections, also known as inguinal LGV, are described. Even fewer infections of the pharynx have been reported.[3] The overrepresentation of anorectal infections in the LGV epidemic is poorly understood but stresses that the mode of transmission of LGV needs additional clarification. It has been suggested that the transmission of rectal LGV may be related to sexual activities such as ''fisting'',[4] but this could not be confirmed later.[5] We postulated that anorectal LGV is transmitted via receptive anal intercourse and that there is a reservoir of missed genital LGV infections in MSM.

The primary aim of this study was to determine whether there is a reservoir of missed urethral LGV that might contribute to the ongoing LGV epidemic. We performed LGV typing on all CT-positive urethral samples in index patients with anorectal LGV infections and in visiting partners of indexes with anorectal LGV.

METHODS

Since 2005, all MSM patients reporting receptive anal intercourse in the past 6 months were tested on anorectal CT infections by Aptima CT system (GEN-PROBE, San Diego, CA) and tested further for LGV, as described before.[6 7] In case the *pmpH* test was inconclusive (mainly due to low CT load), biovar L could not be confirmed and the diagnosis was considered negative for LGV.

Patients who were diagnosed positive (index patients) could notify their partners who were registered at the STI clinic on a voluntary basis. The electronic patient

record of the index was linked to the record of the partner. This enabled immediate partner notification in case an infection was diagnosed in an index patient. Moreover, we distributed STI notification slips to infected index patients to distribute to their partners. The notification slip states the date plus the infection found, without personal identification. If the partner returned to the clinic, we could thus identify which infection he was exposed to. Lymphogranuloma venereum partner notification was implemented in case the partner had contact with the index within the past 60 days. For the study, we identified LGV contacts either on the electronic partner link or via the notification slip. Lymphogranuloma venereum contacts were screened routinely for other STIs and treated with a biovar non-L CT infection regimen (azithromycin 1000 mg orally single dose or doxycycline 100 mg orally twice a day for 7 days) in accordance with the Centers for Disease Control and Prevention 2010 sexually transmitted disease treatment guidelines.[8] The test and treat policy of the STI outpatient clinic in Amsterdam has been described in more detail in reference.[5]

We tested urethral biovar L infections retrospectively in urethral CT-positive consultations with an anorectal LGV infection and in urethral CT-positive sexual partners of consultations with an anorectal LGV infection on record between January 2008 and August 2012. All available CT-positive urine samples stored at -20-C, were retrieved, thawed, and tested with the biovar L specific polymerase chain reaction.[6 7] Patient data from LGV index patients and partners were retrieved from the electronic patient database. Because we used anonymous routine data, no ethical clearance for this study was needed.

RESULTS

During the study period, 27,504 MSM consultations reporting receptive anal intercourse in the past 6 months were screened at the STI clinic in Amsterdam. The prevalence of anorectal LGV in these men was 1.2% (n=341). Within this group, 33 (9.7%) urine samples were CT positive and 7 of these urine samples were biovar L positive (prevalence 2.1%). Nine (2.6%) urine samples were biovar non-L, 15 (4.4%) samples were inconclusive, and 2 (0.6%) samples were missing.

We located 59 sexual partners of MSM diagnosed as having an anorectal LGV on record. All partners were male, and none were diagnosed as having anorectal LGV. Among these partners, 10 (16.9%) urine samples were CT positive and 4 of these samples were

biovar L positive (prevalence 6.8%). One (1.7%) urine sample was biovar non-L, 3 (5.1%) were inconclusive, and 2 (3.4%) were missing.

All 11 patients with urethral LGV (based on a biovar L-positive urine sample) had sexual intercourse with exclusively men, and 9 were HIV-positive. Five of the 11 patients with urethral LGV did not report symptoms of urethritis, and only 1 of 11 had lymphadenopathy. Concurrent STIs including gonorrhoea (4/7), herpes simplex virus (1/7), and syphilis (1/7) were found among index patients with anorectal LGV. No co-infections besides urethral LGV were found in the partner group. In both groups, condom use was inconsistent, and multiple partners were reported in the preceding 6 months (between 2 and 10).

DISCUSSION

Various guidelines (British Association for Sexual Health and HIV [BASHH], World Health Organization/International Union against Sexually Transmitted Infections [IUSTI] and Centers for Disease Control and Prevention) recommend routine diagnostic methods for anorectal LGV in MSM, but none recommend routine screening of urethral LGV infections.[8-10] Here we show previously undiagnosed urethral LGV infections both in MSM with anorectal LGV and among partners of anorectal LGV index patients. Probably, urethral LGV is key in the transmission of LGV in MSM, but remains undetected to date. Many clinicians expect to find unilateral lymphadenopathy in an individual with genital LGV. Because only 1 of the 11 patients with urethral LGV had lymphadenopathy, the absence of this finding does not exclude an infection, and molecular testing is warranted.

Moreover, anorectal LGV infections are often missed because of unawareness of the disease,[11] lack of appropriate diagnostic tools,[12] or the asymptomatic nature of the infection in a considerable proportion of patients.[2] These missed anorectal infections might also contribute to the ongoing LGV epidemic among MSM.

Two above-mentioned guidelines recommend partner treatment of LGV index patients with azithromycin 1000 mg once or doxycycline 100 mg twice a day for 7 days, a regimen considered sufficient to eliminate biovar non-L CT infections. The new BASHH guideline, still under review, suggests extending the duration of partner treatment with doxycycline to 14 days.[9] We showed earlier that anorectal LGV can

persist under doxycycline treatment for 16 days, which stresses the importance of prolonged treatment for at least 21 days.[13]

Here we showed that partners of index patients with anorectal LGV (1) harbour asymptomatic urethral LGV infections, (2) had no other bacterial STI requiring antibiotic treatment, and (3) engaged in high-risk behaviour. Because under the current guidelines, they were treated insufficiently, it is feasible that their LGV infection was not eliminated, and further transmission was possible. We detected urethral LGV infections in 6.8% of the partners of patients with anorectal LGV. This is based on a small and incomplete number of partners necessitating further studies on the prevalence of urethral LGV. Until that time, it would be advisable to treat partners of patients with LGV with a 3-week course of doxycycline, considered effective to eliminate biovar L CT infections.

A limitation in our study is that we only included partners on record with a legitimate notification. Therefore, our results probably show an underestimation of urethral LGV among partners of LGV index cases. Of the CT-positive urethral samples, 48.4% and 37.5% of patients with anorectal LGV, respectively, and their partners were inconclusive for LGV determination. This is caused by a difference in sensitivity of the biovar-nonspecific commercial CT test and the LGV-specific in-house developed additional test. As a consequence, an underrepresentation of the true urethral LGV prevalence cannot be excluded.

Lymphogranuloma venereum infection is a serious concern for the MSM community in Western Europe and other industrialized countries. Awareness of, screening for, and prompt treatment of LGV are crucial for the individual patient and to prevent ongoing transmission. We see a significant increase in the prevalence of LGV in Amsterdam in the last year.[2] Urethral LGV might form a potential undetected reservoir. To further clarify this, we plan to estimate the prevalence of urethral LGV infections among the total MSM population visiting the outpatient clinic. Future findings might indicate the need for adjustment of LGV protocols, especially partner treatment and possibly routine screening for urethral LGV.

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3.2

CHAPTER 3.3

Low prevalence of methicillin-resistant *Staphylococcus aureus* among men who have sex with men attending an STI clinic in Amsterdam: a cross-sectional study

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ABSTRACT

Objective

Community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) is common among men who have sex with men (MSM) in the USA. It is unknown whether this is also the case in Amsterdam, the Netherlands.

Design

Cross-sectional study.

Setting

Sexually transmitted infection outpatient low-threshold clinic, Amsterdam, the Netherlands.

Participants

Between October 2008 and April 2010, a total of 211 men were included, in two groups: (1) 74 MSM with clinical signs of a skin or soft tissue infection (symptomatic group) and (2) 137 MSM without clinical signs of such infections (asymptomatic group).

Primary outcome measures

S. aureus and MRSA infection and/or colonisation. Swabs were collected from the anterior nasal cavity, throat, perineum, penile glans and, if present, from infected skin lesions. Culture for *S. aureus* was carried out on blood agar plates and for MRSA on selective chromagar plates after enrichment in broth. If MRSA was found, the spa-gene was sequenced.

Secondary outcome measures

Associated demographic characteristics, medical history, risk factors for colonisation with *S. aureus* and high-risk sexual behaviour were collected through a self-completed questionnaire.

Results

The prevalence of *S. aureus* colonisation in the nares was 37%, the pharynx 11%, the perianal region 12%, the glans penis 10% and in skin lesions 40%. In multivariable analysis adjusting for age, anogenital *S. aureus* colonisation was significantly associated with the symptomatic group (p=0.01) and marginally with HIV (p=0.06). MRSA was diagnosed in two cases: prevalence 0.9% (95% CI 0.1% to 3.4%). Neither had CA-MRSA strains.

Conclusions
CA-MRSA among MSM in Amsterdam is rare. Genital colonisation of *S. aureus* is not associated with high-risk sexual behaviour.

ARTICLE SUMMARY

Article focus

- Community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) is common among men who have sex with men (MSM) in the USA.
- It is unknown whether this is also the case in Amsterdam, the Netherlands.
- In a cross-sectional study at the sexually transmitted infection (STI) outpatient low-threshold clinic, Amsterdam, the Netherlands, we studied the prevalence of *S. aureus* and MRSA colonisation and infections among symptomatic and asymptomatic MSM.

Key messages

- Among MSM visiting the STI clinic in Amsterdam CA-MRSA is rare.
- Genital colonisation of *S. aureus* is not associated with high-risk sexual behaviour.

Strengths and limitations of this study

- The conclusions are based on systematically collected and aggregated data. The study was limited to the Amsterdam MSM population visiting the STI outpatient clinic.

INTRODUCTION

Staphylococcus aureus is a pathogen that can cause skin and soft tissue infections. Nasal carriage plays an important role in the epidemiology and pathogenesis of this infection.[1 2] Around 20% (range 12–30%) of individuals are persistent *S. aureus* nasal carriers, approximately 30% (range 16– 70%) are intermittent carriers and about 50% (range 16–69%) are non-carriers.[2 3] A causal relation between *S. aureus* nasal carriage and infection is supported by the fact that often the nasal *S. aureus* strain and the infecting strain are the same phage type or genotype.[4] Local antibiotic treatment of *S. aureus* from the nares results in the subsequent disappearance of *S. aureus* from other parts of the body.[1 2 4-6] Extranasal sites that typically harbour the organism include the skin, perineum and pharynx.[7 8]

Most strains of *S. aureus* are methicillin-susceptible *S. aureus* (MSSA), but some strains, called methicillin-resistant *S. aureus* (MRSA), are resistant to methicillin and all β-lactam antibiotics with the exception of the novel cephalosporin, ceftaroline, that can bind to penicillin-binding protein (PBP2a). MRSA has become a major nosocomial infection control problem (known as healthcare-associated MRSA, or HA-MRSA) in many parts of the world[9] with the exception of the Netherlands and of Scandinavian countries.[10 11] Later on, independent from healthcare institutions, MRSA emerged outside healthcare institutions, like the community-associated (CA-MRSA)[12 13] and livestock-associated (LA-MRSA) infections.[14 15] Resistance to methicillin and other β-lactams in *S. aureus* is based on an additional PBP which is coded by a mec gene such as mecA.[8] Homologues of mecA such as mecC were described recently.[14]

CA-MRSA is distinguished from HA-MRSA by clinical, laboratory and epidemiological characteristics.[16] A new CA-MRSA clone (USA300) has been described and was identified in several communities in the USA and Canada.[17-19] New clones of CA-MRSA have also been reported in Europe.[14 19] CA-MRSA often produces Panton-Valentine leukocidin (PVL), a toxin that causes polymorphonuclear leucocyte lysis and tissue necrosis.[17 19]

In men who have sex with men (MSM), outbreaks of CA-MRSA skin infections have been reported and the majority of the patients were HIV positive.[8 18 20-22] It has been suggested that CA-MRSA might be transmitted in this population from skin to skin during sexual contact.[20 21]

A cross-sectional study among 104 men conducted in Italy did not detect a single case of colonisation with CA-MRSA in HIV-positive MSM.[23] No other study in Europe has reported the prevalence of CA-MRSA colonisation in MSM. The purpose of this investigation was to evaluate the prevalence of, and sexual risk factors for *S. aureus* colonisation (MSSA and MRSA) and CA-MRSA infection among MSM visiting the sexually transmitted infection (STI) outpatient clinic in Amsterdam.

METHODS

Study population

Consecutive MSM attending the STI outpatient clinic in Amsterdam, the Netherlands, were invited to participate in this cross-sectional study. We aimed to include both symptomatic men (those who had clinical signs of a skin or soft tissue infection (pustules, abscesses, ulcerations, erythematous painful papules or plaques)) and asymptomatic men (those without aforementioned clinical signs). All participants were asked to complete a questionnaire concerning hospital admissions during the past year, factors known to be associated with *S. aureus* colonisation (e.g., sharing razor blades and tending animals), STI risk factors and sexual behaviour. STI risk factors were also obtained from the electronic files of the routine patient history.

Swabs were taken from the anterior nasal cavity, throat, perianal area and penile glans. From symptomatic men swabs from suspected infected skin lesions were also obtained. If MRSA was detected, the participant was referred to his general practitioner for an eradication therapy, and the sexual partners of the patient were invited for MRSA screening. The study was approved by the ethics committee of the Academic Medical Centre of the University of Amsterdam. All participants provided written informed consent.

Laboratory tests

Culture for *S. aureus* was carried out on blood agar plates and for MRSA on selective chromagar plates after enrichment in broth. *S. aureus* strains were confirmed by the SA442 (Martineau) nucleic acid amplification test (NAAT) and MRSA strains by the mecA NAAT.[24,25] If MRSA was found, the spa-gene was sequenced as described before.[26]

Statistical analysis

A sample size calculation was carried out prior to the study. The prevalence of MRSA carriage in the general population was estimated to be 0.03%.[24] We aimed to assess whether the MRSA prevalence among MSM patients of the STI clinic was at least 3%. To reject the null hypothesis (prevalence of 0.03% or less among the studied group) with a study power of 95% and a significance level of 0.05, a sample size of at least 113 asymptomatic men was needed; this was rounded up to 125. As men with signs suggestive of *S. aureus* skin or soft tissue infection were thought to be more likely to be infected with MRSA, we also aimed to include a group of 75 symptomatic men. Because the expected number of asymptomatic men was much larger than that of symptomatic patients, the inclusion period for the symptomatic participants was scheduled to be longer.

The electronic patient file with routine STI screening data, the questionnaire and the laboratory results were merged into one database. The $\chi 2$ test or Fisher's exact test were used to compare categorical variables between groups; the rank sum test was used to compare continuous variables between groups. Data analyses were performed with STATA software (STATA Intercooled, College Station, Texas, USA), V.11.0. p Values of less than 0.05 were considered statistically significant. Associations between possible risk factors and anogenital *S. aureus* colonisation were examined with multivariable logistic regressions, reporting adjusted OR with 95% CI. An initial model included all variables that were significant in bivariable analyses; variables were dropped one by one from the model based on the likelihood ratio test (if $p>0.05$); HIV was forced into the model.

RESULTS

In total 214 men were included, starting November 2008. The inclusion of asymptomatic men was completed in December 2008 and of symptomatic men in April 2010. From three participants laboratory results were not available and these were excluded, leaving 211 MSM for the analysis (137 asymptomatic and 74 symptomatic participants). The median age of men in the two groups was similar (38 and 41 years, respectively, $p=0.32$, see table 1). Men in the two groups were comparable regarding most non-sexual risk factors, sexual risk factors and STI diagnoses, but symptomatic men were more often found to be HIV positive (53% vs 31%; $p=0.002$) and were more often diagnosed with syphilis (12% vs 1%; $p<0.001$). Also, use of some drugs (cannabis,

cocaine and methamphetamine) was significantly more common in the symptomatic group.

The prevalence of *S. aureus* colonisation (including MSSA and MRSA) was 37% (78/211) in the nose, 11% (23/211) in the throat, 12% (26/210) in the perineum and 10% (22/211) on the glans penis. The prevalences were similar between symptomatic and asymptomatic men for nose and throat, but the symptomatic group had significantly higher penile and perineal colonisation prevalences (table 1). In the symptomatic group 40% (27/67) of men had an *S. aureus*-infected skin lesion. In one symptomatic and one asymptomatic case MRSA was detected, giving an overall prevalence of MRSA carriage of 0.9% (95% binomial exact CI (95% CI) 0.1% to 3.4%), and a prevalence of 0.7% (95% CI 0.02% to 4.0%) among asymptomatic MSM. Details of these two cases are described in the following. Neither known CA-MRSA nor PVL toxin-producing clones were detected.

S. aureus colonisation of the anogenital area (based on penile and perianal swabs) was found in 18% (38/211) of the participants. Participants with and without anogenital *S. aureus* colonisation were similar in respect to sexual risk behaviour, drug use, history or diagnosis of sexually transmitted diseases, antibiotic exposure, circumcision status and hygiene behaviour, but men with anogenital *S. aureus* colonisation were older (42 vs 38 years; p=0.05), and they were more often HIV positive (58% vs 34%, p=0.007; see table 2). In a multivariable logistic regression analysis, adjusting for age, HIV status (adjusted OR, aOR 2.2, 95% CI 0.96 to 4.9), belonging to the symptomatic group (aOR=2.7, 95% CI 1.2 to 5.9) and having had few sexual partners (aOR 3.1, 95% CI 1.2 to 8.3 for those with <5 sexual partners in the previous year compared to those with over 20 sexual partners) were independently and significantly associated with anogenital *S. aureus* colonisation.

We examined associations between nasal carriage of *S. aureus* and a long list of possible non-sexual risk factors, like admissions, operations, employment in healthcare sector, shaving habits, participation in team sports, sauna visits and other factors; none of these was associated with *S. aureus* colonisation of the nose.

Asymptomatic carrier of MRSA

An HIV-positive 40-year-old, asymptomatic, Dutch MSM was positive for MRSA at the perineum. The MRSA had spa-type t064 and was resistant to ciprofloxacin, gentamicin, oxacillin, penicillin and trimethoprim/sulfamethoxazole. Further genetic analysis revealed mecA+ and Martineau+; pUSA03 and PVL were both negative. Recently, the

patient was diagnosed with a hepatitis C infection. In the preceding year he had been hospitalised for an elective operation. He had travelled abroad frequently, including to the USA. He regularly used recreational drugs and visited a public gym and sauna. Furthermore, he engaged in high-risk sex, including unprotected anal intercourse, active and passive fisting, group sex, sex at sex parties and sex with partners met through the Internet. We attempted to trace six of his sexual partners of whom four were screened. None had MRSA and none had clinical signs of a skin infection.

Symptomatic carrier of MRSA

In an HIV-positive, 46-year-old Dutch MSM, MRSA was detected on swabs taken from a penile ulcer, the nasal cavity, the perineum and the glans penis. The MRSA had spa-type t002 and was resistant to ciprofloxacin, erythromycin, oxacillin and penicillin. Further genetic analysis revealed mecA+ and Martineau+; pUSA03 and PVL were both negative. In the preceding year the patient had neither been hospitalised, nor operated upon, nor had he travelled abroad. He did not use recreational drugs, and did not visit public gyms or saunas. He had not engaged in high-risk sex; he had had sex with one partner in the preceding year. This partner could not be traced for further screening.

Table 1. Demographic, behavioural and clinical characteristics, and *S. aureus* colonisation of five anatomical locations, of 211 MSM attending the STI clinic, Amsterdam, 2008-1010, according to symptomatology of skin or soft tissue infection.

Characteristic	Asymptomatic n=137		Symptomatic n=74		p Value
	n	%	n	%	
A. Demographics					
Median age in years (IQR)	38 (31-45)		41 (31-48)		0.32
Dutch ethnicity	111	81.0	54	73.0	0.18
B. Recreational drugs use					
None	21	15.3	7	9.5	0.23
Alcohol	92	67.2	47	63.5	0.60
Cannabis	34	24.8	33	44.6	0.003
XTC	40	29.2	27	36.5	0.28
GHB	36	26.3	25	33.8	0.25
Poppers (alkyl nitrites)	76	55.5	44	59.5	0.58
Cocaine	29	21.2	27	36.5	0.02
Ketamine	14	10.2	13	17.6	0.13
Amphetamine	8	5.8	7	9.5	0.33
Methamphetamine	3	2.2	6	8.1	0.04

Table 1. Continued

Characteristic	Asymptomatic n=137		Symptomatic n=74		p Value
	n	%	n	%	
C. Sexual history					
Number of sexual partners§					0.77
0	19	13.8	9	12.2	
1-4	30	21.9	14	18.9	
5-9	20	14.6	11	14.9	
10-19	28	20.4	15	20.3	
20-39	26	19.0	12	16.2	
40 or more	14	10.2	13	17.6	
Active anal sex with condom#	98	71.5	48	64.9	0.32
Passive anal sex with condom#	79	57.7	58	78.4	0.003
Active anal sex without condom#	68	49.6	32	43.2	0.38
Passive anal sex without condom#	56	40.9	37	50.0	0.20
Active fisting#	21	15.3	15	20.3	0.36
Passive fisting#	14	10.2	11	14.9	0.32
Had group sex§	60	43.8	38	51.4	0.29
Visited a sex club§	85	62.0	47	63.5	0.83
Visited a sex party§	31	22.6	17	23.0	0.95
Met sex partner through Internet§	75	54.7	45	60.8	0.40
Had sex abroad§	79	57.7	45	60.8	0.66
D. STI: history & diagnoses					
History of STI	34	24.8	24	32.4	0.24
HIV positive*	42	30.9	38	52.8	0.002
Syphilis diagnosis	1	0.7	9	12.2	<0.001
Gonorrhoea diagnosis	9	6.6	5	5.4	0.74
Chlamydia diagnosis	23	16.8	14	18.9	0.70
E. S. aureus colonisation					
Nares	50	36.5	28	37.8	0.85
Pharynx	13	9.5	10	13.5	0.37
Perineum	9	6.6	17	23.3	<0.001
Glans penis	10	7.3	12	6.2	0.043
Other locations†	--	--	27	40.3	--

Abbreviations:
GHB: γ-hydroxybutyric; HIV: human immunodeficiency virus; IQR: interquartile range; STI: sexually transmitted infection; XTC: MDMA; MSM: men who have sex with men.

§During the previous year
#In the past 6 months.
*HIV status missing for 3 patients who did not want to be tested
†Data available from 67/74 only.

Table 2. Demographic, behavioural and clinical characteristics of 211 MSM attending the STI clinic in Amsterdam, 2008–10, by *S. aureus* anogenital colonisation status

	***S. aureus* negative n=173**		***S. aureus* positive n=38**		**p Value**
	n	**%**	**n**	**%**	
A. Demographics					
Median age in years (IQR)	38 (30-45)		42 (35-49)		0.05
Dutch Ethnicity	138	79.8	27	71.1	0.24
B. Recreational drug use					
None	21	12.1	7	18.4	0.30
Alcohol	118	68.2	21	55.3	0.13
Cannabis	57	33.0	10	26.3	0.43
XTC	53	30.6	14	36.8	0.46
GHB	49	28.3	12	31.6	0.69
Poppers	96	55.5	24	63.2	0.39
Cocaine	44	25.4	12	31.6	0.44
Ketamine	23	13.3	4	10.5	0.64
Amphetamine	12	6.9	3	7.9	0.84
Methamphetamine	7	4.1	2	5.3	0.74
C. Sexual history					
Number of sexual partners[§]					0.08
0	18	10.4	10	26.3	
1-4	35	20.2	9	23.7	
5-9	29	16.8	2	5.3	
10-19	35	20.2	8	21.1	
20-39	33	19.1	5	13.2	
40 or more	23	13.3	4	10.5	
Active anal sex with condom[#]	121	69.9	25	65.8	0.62
Passive anal sex with condom[#]	110	63.6	27	71.1	0.38
Active anal sex without condom[#]	85	49.1	15	39.5	0.28
Passive anal sex without condom[#]	74	42.8	19	50.0	0.42
Active fisting[#]	29	16.8	7	18.4	0.81
Passive fisting[#]	22	12.7	3	7.9	0.41
Had group sex[§]	80	46.2	18	47.4	0.90
Visited a sex club[§]	109	63.0	23	60.5	0.78
Visited a sex party[§]	36	20.8	12	31.6	0.15
Met sex partner through Internet[§]	102	59.0	18	47.4	0.19
Had sex abroad[§]	105	60.7	19	50.0	0.23
D. STI: history & diagnoses					
History of STI	51	29.5	7	18.4	0.17
HIV positive[*]	59	34.3	21	58.3	0.007
Syphilis diagnosis	10	5.8	0	0	0.13
Gonorrhoea diagnosis	13	7.5	0	0	0.08
Chlamydia diagnosis	30	17.3	7	18.4	0.87

Table 2. Continued

	S. aureus negative n=173		*S. aureus* positive n=38		p Value
	n	%	n	%	
E. Other possible risk factors					
Regularly attends the gym§	109	63.0	20	52.6	0.24
Regularly plays in a team sport§	13	7.5	0	0	0.08
Regularly visits the sauna§	110	63.6	23	60.5	0.72
Regularly takes a public shower§	103	59.5	25	65.8	0.48
Regularly travels abroad§	149	86.1	32	84.2	0.76
Regularly shaves:					
Facial hair	165	95.4	38	100	0.18
Pubic hair	149	86.1	31	81.6	0.47
Body hair	110	63.6	20	52.6	0.21
Has pets	45	26.0	11	29.0	0.71
Works in health care	14	8.1	3	7.9	0.97
Was admitted to hospital§	9	5.2	4	10.5	0.22
Had surgery§	23	13.5	8	21.6	0.21
Had a blood transfusion§	6	3.5	1	2.6	0.79
Had enemas§	49	28.3	14	36.8	0.30
Frequent bites his fingernails	55	31.8	11	29.0	0.73
Has been circumcised	36	20.8	11	29.0	0.28

Abbreviations:
GHB: γ-hydroxybutyric; HIV: Human immunodeficiency virus; IQR: interquartile range; STI: sexually transmitted infection; XTC: MDMA; MSM: men who have sex with men

§During the previous year
#In the past 6 months
*HIV status missing for 3 patients who did not want to be tested

DISCUSSION

The prevalence of MRSA among MSM clients of the STI outpatient clinic in Amsterdam was 0.9% (95% CI 0.1% to 3.4%); among clients without skin lesions this was 0.7% (95% CI 0.02% to 4.0%). This is similar to the overall prevalence in Dutch hospitals (1.8%).[10] No CA-MRSA-associated clones were found in this population. These findings are in contrast to reports from the USA where considerable CA-MRSA prevalences were observed among MSM attending STI screening sites.[20 27] Although some MSM residing in Amsterdam frequently travel to the North American continent, this has not resulted in an extensive introduction of CA-MRSA within the Amsterdam population yet. The

low MRSA prevalence among the Dutch population in general and among the MSM STI clinic population may be attributed to the strict Dutch search-and-destroy policy and restrictive antibiotic prescription policy.[11] At present, there is no indication for routine screening of MSM STI clinic visitors for CA-MRSA in the Netherlands.

We observed similar prevalences of *S. aureus* nasal colonisation (37%) in MSM as observed in the general population.[2] Genital *S. aureus* colonisation was associated with HIV infection; although HIV infection may be considered a proxy for high-risk sexual behaviour, other markers for risk behaviour were not significantly associated with *S. aureus* colonisation. We attribute the more common colonisation among HIV-positive participants to their immunocompromised status. Unfortunately, we neither have data on virological, immunological or clinical parameters of HIV infection, nor about use of antiretroviral drugs. We did not observe an increased risk of *S. aureus* nasal carriage among HIV patients, as was reported earlier.[28]

In contrast to Southern European countries, the Netherlands has a low rate of HA-MRSA, probably owing to the aforementioned search-and-destroy policy. The restricted use of β-lactam antibiotics outside of hospitals, in the community, might explain the low prevalence of CA-MRSA. Europe has not yet been confronted with CA-MRSA on a wide scale[23] contrary to Canada and the USA where high prevalences are found.[9 12 13 20 21 23] Yet, in contrast to low prevalences of HA-MRSA and CA-MRSA in the Netherlands, screening of Dutch workers with livestock has revealed that 39% of slaughterhouse pigs and >20% of pig farmers are asymptomatic carriers of LA-MRSA belonging to sequence-type (ST) 398.[15] The two MRSA strains isolated in our study were both unrelated to this LA-MRSA epidemic.

In conclusion, CA-MRSA among MSM visiting the STI outpatient clinic in Amsterdam is rare. Although genital *S. aureus* colonisation was more common among HIV-positive MSM, we found no association between high-risk sexual behaviour and genital colonisation with *S. aureus*. In the Netherlands there is no indication for MRSA screening of MSM attending STI clinics.

Contributors

IKCWJ analysed the data and wrote the first manuscript draft. MSvR collected the data and analysed the data, MFSvdL supervised the epidemiological analysis and wrote the article, AJdN performed the molecular strain typing and wrote the article, AvD

performed the cultivation experiments, designed the study and wrote the article, HJCdV conceptualised and supervised the study and approved the final manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors certify that they have no affiliation with or financial involvement in any organisation or entity with a direct financial interest in the subject matter or materials discussed in the manuscript (e.g., employment, consultancies, stock ownership or honoraria). This work was presented in an oral presentation titled 'Community acquired MRSA and MSSA among MSM' during the 17th meeting International Society for STD Research (ISSTDR) in Quebec, Canada, 10 July 2011.

Ethics approval

The study was approved by the ethical board of the Academic Medical Centre of the University of Amsterdam.

Provenance and peer review

Not commissioned; externally peer reviewed.

Data sharing statement

Technical appendix, statistical code and dataset available from the HJCdV at Dryad repository, who will provide a permanent, citable and open access home for the dataset. Extra data can be accessed via the Dryad data repository at http://datadryad.org/ with the doi:10.5061/dryad.569j0.

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3.3

CHAPTER 4

Surveillance of STI

CHAPTER 4.1

Earlier detection of hepatitis C virus infection through routine hepatitis C virus antibody screening of human immunodeficiency virus-positive men who have sex with men attending a sexually transmitted infection outpatient clinic: a longitudinal study.

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ABSTRACT

Background

In 2007, routine hepatitis C virus (HCV) antibody testing was introduced for men who have sex with men (MSM) with a human immunodeficiency virus (HIV)-positive or unknown status attending a Dutch sexually transmitted infection (STI) outpatient clinic. We evaluated whether this screening resulted in additional and earlier HCV diagnoses among MSM who also attend HIV clinics.

Methods

At first STI consultation, HIV-positive MSM and MSM opting-out of HIV testing (HIV-status-unknown) were tested for HCV antibodies (anti-HCV). During follow-up consultations, only previously HCV negative men were tested. Retrospectively, STI clinic and HIV clinic HCV diagnosis dates were compared.

Results

One hundred twelve (6.4%) of 1742 (95% confidence interval [CI], 5.3–7.6%) HIV-positive and 3 (0.7%) of 446 (95% CI, 0.2–2.0%) HIV-status-unknown MSM tested anti–HCV-positive at first consultation. During follow-up consultations, 32 HIV-positive (incidence HCV-positive: 2.35/100 person years (PY) (95% CI, 1.66–3.33)) and 0 (1-sided, 97.5% CI, 0.0–3.76) HIV-status-unknown MSM became anti–HCV-positive. Four (11.8%) of 34 HIV-positive MSM notified by their sexual partner of HCV tested anti–HCV-positive.

Of 163 HIV-positive MSM with HCV antibodies, 78 reported a history of HCV. HCV diagnosis data at the HIV clinic was requested for the remaining 85 MSM and available for 54 MSM. Of these 54 MSM, 28 (51.9%) had their first HCV diagnosis at the STI clinic, of whom 7 concurrently with HIV. At their next scheduled HIV clinic consultation, 3 HCV cases probably would have been missed.

Conclusions

The introduction of routine anti-HCV testing at the STI outpatient clinic resulted in additional and earlier HCV detection among HIV-positive MSM. Testing should be continued among HIV-positive MSM, at least for those not (yet) under the care of an HIV clinic and those notified of HCV by their sexual partner.

INTRODUCTION

Hepatitis C virus (HCV) infection is primarily a blood-borne infection, and sexual transmission among heterosexuals is considered inefficient.[1] However, since 2000, outbreaks of sexually transmitted HCV infections have been reported among HIV-positive men who have sex with men (MSM) and phylogenetic analysis has confirmed the presence of sexual transmission networks.[2-5]

The HCV-HIV co-infection has been associated with a more rapid progression of both HCV- and HIV-related disease.[6] Early HCV diagnosis and treatment of acute infection might reduce further spread as demonstrated by modelling studies.[7]

Hepatitis C virus testing is not included in the standard test package of sexually transmitted infection (STI) clinics in the Netherlands. A biannual cross-sectional survey among STI clinic attendees in Amsterdam showed in 2007 an alarming HCV prevalence among HIV-positive MSM.[8] In response to this finding, the STI clinic in Amsterdam started routine HCV antibody testing among HIV-positive MSM and MSM who opted-out of HIV testing (HIV-status-unknown MSM). In addition, HIV clinics intensified HCV testing among HIV-positive MSM during routine clinic visits, which usually take place at 6-month intervals.[2]

We hypothesized that as HIV-positive MSM usually visit the STI clinic due to high sexual risk behaviour, routine HCV testing would result in additional and earlier HCV detection. First, we measured the HCV antibody prevalence and incidence among HIV-positive and HIV-status-unknown MSM. Second, we evaluated whether and the extent to which the moment of HCV diagnosis at the STI clinic preceded HCV diagnosis at HIV clinics.

MATERIALS AND METHODS

STI clinic procedures

Annually, the STI clinic of the Public Health Service of Amsterdam (an outpatient clinic) performs around 40,000 free and, if desired, anonymous STI consultations (i.e., all visits pertaining to one STI screening episode), of which 10,000 among men who have sex with men (MSM). Patients receive a unique personal identification code that is used during follow-up consultations. All patients are routinely tested for *Chlamydia trachomatis*, gonorrhoea, syphilis, hepatitis B,[9] and HIV using the opt-out strategy.[10]

Most newly diagnosed MSM with HIV are referred to 6 HIV clinics in Amsterdam. Routinely collected demographic and risk behaviour data, laboratory results, diagnosis, and, if applicable, HIV clinic are registered in an electronic patient database.

Since November 2007, all HIV-positive and HIV-status unknown MSM have been offered HCV antibody (anti-HCV) testing using an opt-in strategy: at first consultation, the MSM who agreed were tested to obtain their HCV antibody status. During follow-up consultations at the STI clinic, only those formerly anti–HCV-negative or indeterminate at the STI consultation were tested for anti-HCV. If anti-HCV-positive, patients were categorized into those with a new or previously diagnosed HCV infection, based on HCV history. Self-reported HCV status was recorded for all patients from April 2008 onward.

Hepatitis C virus antibodies were tested by means of a third-generation commercial microparticle EIA system (AxSYM HCV version 3.0; Abbott). Positive and indeterminate AxSYM results were confirmed with an immunoblot (Chiron RIBA HCV 3.0 SIA; Ortho-Clinical Diagnostics). MSM with a negative AxSYM result, and MSM with an indeterminate/positive AxSYM result with a confirmed negative immunoblot were defined anti–HCV-negative, those with an indeterminate immunoblot anti– HCV-indeterminate, and those with a positive immunoblot, anti-HCV-positive.

For further evaluation and/or HCV treatment, newly diagnosed MSM were referred to their general practitioner or HIV clinic. If the anti-HCV test result was indeterminate, patients were referred for additional testing. In the present study, we included all eligible MSM who visited the STI clinic from November 2007 until December 2010.

HCV diagnosis data collection at HIV clinics

To evaluate our HCV testing policy, HIV clinics in the Amsterdam area were contacted by a clinician to obtain retrospective information for: (1) men who tested anti–HCV-positive at first consultation and did not report a positive HCV status (prevalent HCV infection); (2) men who changed from first consultation anti–HCV-negative to indeterminate during follow-up, from anti– HCV-negative to positive, or from anti–HCV-indeterminate to positive (incident HCV infection). Date of HCV diagnosis and available laboratory results (HCV RNA, HCV antibodies, alanine transaminase [ALT]) were collected from patient files at the HIV clinics.

Statistical analysis

We describe anti-HCV testing acceptance, prevalence, and incidence among HIV-positive and HIV-status-unknown MSM. Baseline characteristics of HIV-positive and HIV-status-unknown MSM tested (first consultation with anti-HCV test result during the study period) and those never tested (first consultation during the study period) for anti-HCV at the STI clinic were compared. Characteristics of unique MSM according to anti-HCV status at first consultation with HCV test result (negative, indeterminate, and positive) were compared separately for HIV-positive and HIV-status-unknown MSM. The following covariates were evaluated: age, country of birth, commercial sex work, injecting drug use (IDU), presence of STI-related complaints, being notified of an STI, sexual preference, unprotected anal intercourse (UAI) (no or infrequent use of condoms during anal sex), and STI diagnosis. Injecting drug use, sexual preference, commercial sex work, and UAI refer to the period 6 months before consultation. Sexually transmitted infection was defined as a diagnosis of infection with *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, hepatitis B virus, and/or *Treponema pallidum* at the time of consultation. Incidence of HCV was calculated per 100 PY including 95% or, in case of no events, 97.5% confidence intervals. Follow-up time was calculated as the time from first until last negative or indeterminate HCV antibody test date (in case of no seroconversion), or until HCV seroconversion. The date of HCV seroconversion was estimated as the midpoint between first positive test and preceding HCV test date.

Subsequently, we compared the HCV diagnosis date at the STI clinic with the HCV diagnosis date at the HIV clinic for the 2 groups described above. Patients diagnosed with HCV at an HIV clinic before the STI clinic implemented HCV testing (November 2007) were excluded from this analysis.

Using SPSS version 19 (SPSS Inc., Chicago, Ill), groups were compared with the $\chi 2$ test or Fisher exact test for categorical variables and the Mann-Whitney U test or Kruskal-Wallis rank sum test for continuous variables. Confidence intervals around proportions and incidences were calculated with STATA software (STATA Intercooled, College Station, Tex), version 11.0. *P* values of <0.05 were considered statistically significant.

RESULTS

Between November 2007 and January 2011, 23,197 STI consultations were performed among MSM. In total, 5297 (22.8%) of 23,197 consultations among 1793 unique HIV-

positive MSM and 824 (3.6%) of 23,197 consultations among 612 unique HIV-status-unknown MSM were eligible for HCV testing. During follow-up, 39 MSM shifted from the HIV-status-unknown to the HIV-positive group.

BASELINE CHARACTERISTICS OF MSM OFFERED HCV TESTING

HIV-positive MSM

For 51 (2.8%) of 1793 HIV-positive MSM, anti-HCV was not tested at any consultation. A total of 5032 consultations with anti-HCV testing were performed among 1742 unique HIV-positive MSM (table 1). The median age of these unique MSM at first HCV test was 41 years (interquartile range [IQR], 34–46 years), 64.1% were born in the Netherlands, 4.4% reported female sexual partner(s), 0.8% reported IDU, 61.2% reported UAI in the preceding 6 months, in 41.7% an STI was diagnosed, and 22.2% had a new HIV diagnosis. Compared with those who were never tested at the STI clinic for anti-HCV during the study period, those tested less often received payment for sex ($p<0.006$).

MSM with unknown HIV status

During the study period, of 612 unique MSM with unknown HIV status, 166 (27.1%) were never tested at the STI clinic for anti-HCV as these MSM either also opted-out of HCV testing or the test was accidentally not offered. Among 446 unique MSM 579 consultations with HCV testing were performed (table 1). The median age of these unique MSM at first HCV test was 41 years (IQR, 33-48 years), 72.2% were born in the Netherlands, 10.3% also reported female sexual partner(s), none reported IDU, 39.7% reported UAI in the preceding 6 months, and 26.2% were diagnosed with an STI. The characteristics of MSM who were never tested at the STI clinic for anti-HCV during the study period did not significantly differ from those tested.

HCV ANTIBODY RESULTS

Baseline

Anti-HCV prevalence at first HCV test at the STI clinic was 6.4% (112/1742; 95% CI, 5.3–7.6%) among HIV-positive MSM and 0.7% (3/446; 95% CI, 0.2–2.0%) among HIV-status-unknown MSM (table 1). Of 112 HIV-positive MSM who tested anti–HCV-positive, 34 (30.4%) did not report their HCV infection. The remaining 78 (69.6%) MSM reported a positive HCV status and were thus diagnosed preceding the first HCV test consultation at the STI clinic. All 3 anti–HCV-positive MSM with unknown HIV status did not report

a positive HCV status. Indeterminate anti-HCV results at first HCV test were found in 1.8% (31/1742; 95% CI, 1.2–2.4%) of HIV-positive MSM and in 0.2% (1/446; 95% CI, 0.0–1.3%) of HIV-status-unknown MSM. Fifteen of 31 HIV-positive MSM reported their HCV status: 11/15 HCV-positive and 4/15 HCV-negative. The HIV-status-unknown man with an indeterminate anti-HCV result reported that he had not been tested for HCV before.

In univariate analysis, HIV-positive MSM who tested anti–HCV-positive were significantly older and more frequently reported IDU in the previous 6 months than HIV-positive MSM who tested negative or indeterminate (table 1). HIV-positive MSM who tested indeterminate or positive more frequently reported UAI in the previous 6 months than HIV-positive MSM who tested anti–HCV-negative.

Follow-up

Of 1599 unique HIV-positive MSM who were initially tested anti–HCV-negative at first consultation, 856 (53.5%) had at least 1 follow-up consultation until January 2011. In 3259 follow-up consultations, the anti-HCV test was indeterminate in 19 unique MSM and positive in 29 unique MSM (figure 1). Of 13 HIV-positive MSM with follow-up consultations who initially tested anti–HCV-indeterminate at their first consultation, 3 became anti–HCV-positive during follow-up. In total, 51 of 869 unique HIV-positive MSM became anti–HCV-indeterminate or positive during follow-up at the STI clinic (19 anti–HCV-negative to indeterminate, 29 negative to positive and 3 indeterminate to positive, adding up to 32 HCV antibody seroconversions). The HCV incidence of HCV seroconversion is 2.35 per 100 PY (95% CI, 1.66-3.33). No HCV seroconversions were found in 133 follow-up consultations of 82 unique HIV status-unknown MSM (incidence: 0.0/100 PY; 1-sided, 97.5% CI, 0.0–3.76).

During the study period, 34 HIV-positive and no HIV status-unknown MSM visited the STI clinic because a sexual partner notified them of HCV. Four of these HCV-notified MSM (11.8%) tested anti–HCV-positive.

Site and moment of first HCV diagnosis in HIV-positive MSM

Hepatitis C virus diagnosis at the HIV clinic was requested for 34 HIV-positive MSM who tested anti–HCV-positive at first consultation and did not report a previous diagnosed HCV infection, and for 51 MSM whose HCV status changed to indeterminate or positive during follow-up (figure 2). Among these 85 HIV-positive MSM, 13 were already diagnosed with HCV at the HIV clinic before the STI clinic introduced HCV screening;

for 18 MSM, no HIV clinic data was available, leaving 54 MSM for comparison. Twenty-eight (51.9%) of 54 HIV-positive MSM with an HCV diagnosis who already were or entered into care at an HIV clinic after the STI clinic consultation, were first diagnosed at the STI clinic. These 28 MSM include 7 MSM concurrently newly diagnosed with HIV and HCV and consequently not yet in care at an HIV clinic at the time of STI consultation. From the remaining 21 patients, 18 (85.7%) would probably have been diagnosed during their next routine consultation at the HIV clinic as clinical data showed elevated ALT levels, a positive anti-HCV test and/or a positive HCV RNA test. This consultation occurred within 1 month for 8 MSM; within 1 to 3 months for 5; within 3 to 6 months for 3; and for 2 MSM, HCV infection was diagnosed at the HIV clinic later, but precise date is unknown. For these, 16 MSM already in HIV care for whom potential HCV diagnosis data was known, the STI clinic detected HCV at a median of 31 days (IQR, 12–49 days) before diagnosis at the HIV clinic. The remaining 3 MSM would not have been diagnosed at the first consultation at the HIV clinic after the HCV diagnosis at the STI clinic, because explicit HCV testing did not take place.

Table 1. Characteristics and HCV antibody results at first consultation of unique HIV-positive MSM and MSM who opted-out of HIV testing (HIV-status-unknown). STI clinic of the Public Health Service of Amsterdam, the Netherlands, November 2007 - December 2010.

		HCV antibody result			
HIV-positive MSM	**Negative**	**Indeterminate**	**Positive**	**Total**	
First consultation[1]	1,599 (91.8%)	31 (1.8%)	112 (6.4%)	1,742	
Characteristics[2]	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**p Value**[3,4]
Median age (years, IQR)	41 (34-46)	40 (32-46)	44 (38-49)	41 (34-46)	<0.001
Reported IDU in the previous 6 months	10 (0.6)	0 (0)	4 (3.6)	14 (0.8)	0.015
Born in the Netherlands	1,022 (63.9)	18 (58.1)	76 (67.9)	1,116 (64.1)	0.55
Reporting STI-related complaints	853 (53.3)	16 (51.6)	58 (51.8)	927 (53.2)	0.94
Notified of any STI by sexual partner[5]	405 (25.3)	5 (16.1)	31 (27.7)	441 (25.3)	0.42
Commercial sex work in the previous 6 months	20 (1.3)	0 (0)	3 (2.7)	23 (1.3)	0.36
Reported sex with both males and females in the previous 6 months	75 (4.7)	0 (0)	1 (0.9)	76 (4.4)	0.095
UAI reported in the previous 6 months	958 (59.9)	24 (77.4)	84 (75.0)	1,066 (61.2)	0.001
STI diagnosis (current consultation)[6]	666 (41.7)	12 (38.7)	49 (43.8)	727 (41.7)	0.86
MSM opting-out of HIV testing	**Negative**	**Indeterminate**[7]	**Positive**[7]	**Total**	
First consultation[1]	442 (99.1%)	1 (0.2%)	3 (0.7%)	446	
Characteristics[2]	**n (%)**			**n (%)**	
Median age (years, IQR)	41 (33-48)			41 (33-48)	
Reported IDU in the previous 6 months	0 (0)			0 (0)	
Born in the Netherlands	319 (72.2)			322 (72.2)	
Reporting STI-related complaints	271 (61.3)			274 (61.4)	
Notified of any STI by sexual partner[5]	87 (19.7)			88 (19.7)	
Commercial sex work in the previous 6 months	4 (0.9)			4 (0.9)	
Reported sex with both males and females in the previous 6 months	45 (10.2)			46 (10.3)	
UAI reported in the previous 6 months	176 (39.8)			177 (39.7)	
STI diagnosis (current consultation)[6]	115 (26.0)			117 (26.2)	

Abbreviations:
IDU=injecting drug use; IQR=interquartile range; MSM=men who have sex with men; STI=sexually transmitted infection; infection with chlamydia, gonorrhoea, infectious syphilis, and/or infectious hepatitis B; UAI=unprotected anal intercourse (in HIV-positive n=1 missing for HCV-antibody-indeterminate and n=8 for HCV-antibody-negative).

[1]Screened for HCV antibodies for the first time at the STI clinic, either during their first consultation ever to the clinic or at their first consultation following the start of HCV antibody screening at the STI clinic.
[2]Characteristics of unique MSM at first HCV antibody screening consultation.
[3]p Values for categorical variables calculated with chi-squared test or, in case of small numbers, with Fisher exact test.
[4]Kruskall-Wallis rank sum test for comparing not normally distributed continuous variables.
[5]Notified of an STI indicates notifications for all STI. In total, 34 HIV-positive and no HIV-status-unknown MSM were notified for HCV. Four (11.8%) HIV-positive MSM notified of HCV exposure tested HCV-antibody-positive.
[6]STI is defined as a diagnosis of infection with *Chlamydia trachomatis* (any location), *Neisseria gonorrhoeae* (any location), hepatitis B virus, and/or *Treponema pallidum* at time of current visit. Out of 1,742 HIV-positive MSM, 387 (22.2%) were newly diagnosed with HIV at their first HCV antibody screening consultation.
[7]Due to small numbers, no characteristics of MSM opting-out of HIV testing with an indeterminate or positive HCV antibody result are shown. In addition, no p values comparing the different groups based on HCV antibody result were calculated.

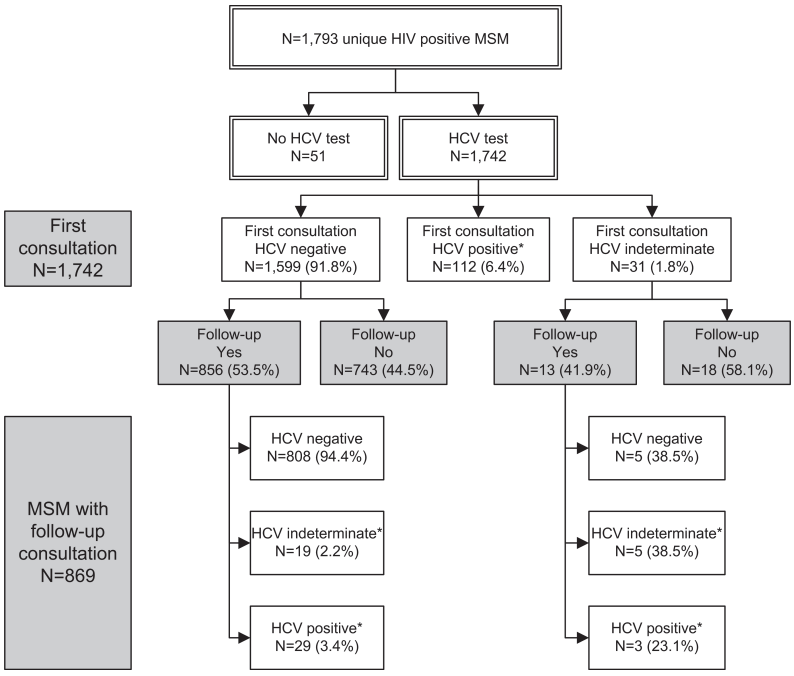

Figure 1. Flowchart for HCV antibody screening of 1793 HIV-positive MSM at the STI clinic of the Public Health Service of Amsterdam, the Netherlands, November 2007 until January 2011.
*These MSM were selected for the analysis on date of first HCV diagnosis (STI or HIV clinic).

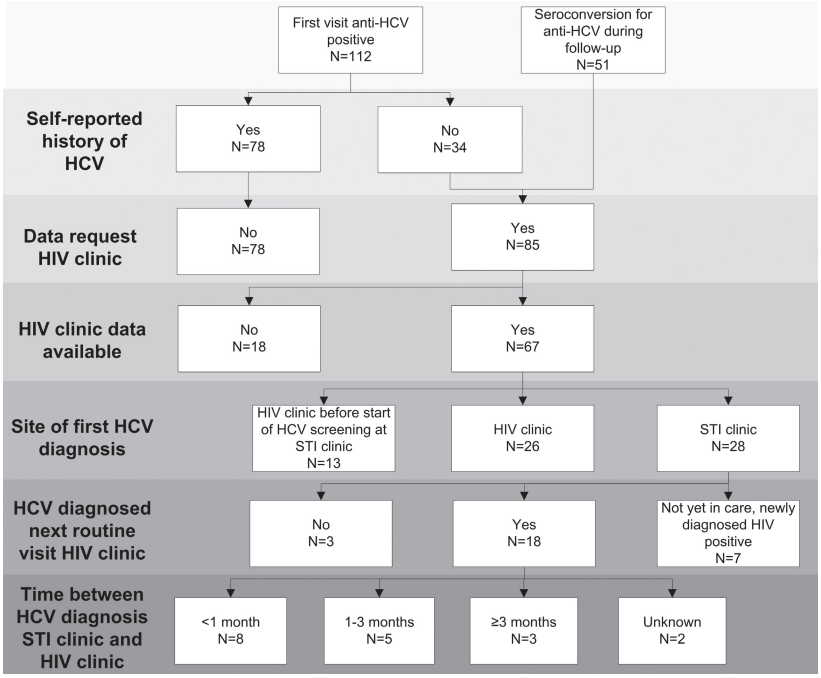

Figure 2. Flowchart for retrospective HIV clinic data of 163 HIV-positive MSM who tested HCV antibody positive at first screening consultation or indeterminate or positive during follow-up at the STI clinic of the Public Health Service of Amsterdam, the Netherlands, November 2007 until 2011.

DISCUSSION

We show that HCV antibodies are found at the first HCV test among HIV-positive MSM relatively often (6.4%). During follow-up, HCV incidence was 2.35 per 100 PY among HIV-positive men. For a considerable proportion of the HCV infections detected among HIV-positive MSM after implementation of routine HCV testing at our STI clinic, HCV diagnosis occurred earlier than at their HIV clinic. Importantly, although a relatively small number of HIV-positive MSM (n=34) was notified by their sexual partner of HCV exposure, a substantial proportion (11.8%) tested anti–HCV-positive.

Hepatitis C virus testing was also offered to HIV-status unknown MSM who declined HIV testing. In a previous study, it was shown that these men are at increased risk of testing HIV positive compared with MSM accepting HIV testing.[10] However, HCV prevalence (0.7%) among these HIV-status-unknown MSM seems comparable to the prevalence among the general population,[11] and no incident infection was found, suggesting that HCV testing is not indicated for this group. It should be noted that the

lower uptake (70.3%) of HCV testing among MSM who declined HIV testing indicates that this assumption should be made with caution. Offering HCV testing as opt-out option could increase HCV test uptake.

The proportion of HIV-positive MSM who tested anti–HCV positive at first consultation is lower than the HCV prevalence (15.3%) found in our STI clinic biannual surveys (2007–2010).[12] However, we screened only for anti-HCV, whereas the surveys also screened all HIV-positive MSM for HCV RNA. Additionally, 48.6% of HIV-positive MSM who opted-out of anti-HCV testing reported being HCV-positive.

The HCV prevalence found in this study among HIV-positive MSM attending the STI clinic in Amsterdam is higher than among non-IDU HIV-positive MSM attending an STI clinic in Italy (2008–2009; 2.46%).[13] The incidence in the present study is somewhat higher than other studies among HIV-positive MSM attending STI clinics in Australia (2002–2010; 0.9/100 PY), and the United Kingdom (2000–2006; 1.18/100 PY).[14 15] These differences might be explained by differences in sexual behaviour networks, observation period, and start of the HCV epidemic among MSM. In addition, the incidence - only calculated for MSM with follow-up consultations -might be overestimated due to high-risk MSM who (more often) reattended the STI clinic.

Our study demonstrates that indeterminate HCV antibody results should be considered as possible acute HCV infections. Several studies have shown a delay in development of HCV antibodies in HCV-naive HIV-positive individuals.[16 17] Although longer-lasting initial infections would be detected through the anti-HCV test policy at the STI clinic, alternative screening methods should be considered and implemented to detect acute initial infections and reinfections. Testing for HCV reinfections might also be useful at an STI clinic because the risk of reinfection among HIV-positive MSM who cleared their HCV infection is substantial at 8 to 15 per 100 PY.[18 19] Moreover, we showed that without routine HCV screening at the STI clinic, the HCV infection would have been missed for some patients at the HIV clinic.

The collected date of HCV diagnosis at HIV clinics reflects a routine consultation, usually taking place at 6-month intervals. However, after HCV was newly detected at the STI clinic, clients were actively referred to their HIV specialist with this new HCV diagnosis. Hence, date of HCV diagnosis at the HIV clinic might have been later without routine anti-HCV testing at the STI clinic. In our study, a relatively large proportion of patients

among whom the STI clinic detected HCV infection first were MSM newly diagnosed with HIV. A recent Dutch study demonstrates a delay of more than 4 weeks between the moment of HIV diagnosis at the STI clinic and entry into care at an HIV clinic for 30% of HIV-positive patients, of whom the majority are MSM.[20] Therefore, HCV screening at an STI clinic and direct referral remains important for newly diagnosed HIV patients.

A recent meta-analysis estimated that the annual HCV incidence rates in HIV-positive MSM ranged from a low of 0.42/100 person-years in 1991 (95% CI, 0.23–0.77) to 1.34/100 person-years in 2012 (95% CI, 0.76–2.36).[3] The raising and relative high incidence supports our opinion that from a public health perspective, HCV screening at an STI clinic might limit further spread in the community. However, research is needed to estimate the cost-effectiveness of HCV screening among HIV-positive MSM at STI clinics according to different test algorithms as well as risk assessment-based testing, and taking into consideration that HCV testing is also performed at HIV clinics. In 2010, the European AIDS Treatment Network recommended HCV antibody testing for newly diagnosed HIV individuals and that HIV-positive MSM at risk for contracting acute HCV should be screened with a liver function test (ALT) every 6 months and for HCV antibodies every 12 months.[2] However, risk behaviour is not clearly defined. In addition, the national clinical guideline does not dictate explicit HCV testing (HCV antibodies, antigen, or RNA) for HIV-infected MSM at every HIV clinic visit.[21] For those in care with an HIV specialist, more frequent explicit HCV screening at HIV clinics would probably make anti-HCV screening at the STI clinic redundant.

A limitation of this study was that although we referred MSM with an indeterminate anti-HCV status to their specialist, with anti-HCV screening only, we were unable to detect acute infections. In addition, for anti–HCV-positive MSM we do not know whether they had a chronic HCV infection. However, in the same period during biannual cross-sectional anonymous surveys at the STI outpatient clinic, 65% of the anti–HCV-positive HIV-infected MSM tested HCV RNA positive.[12] Also, as self-reported HCV status was not collected during the first 5 months of the study period, no reason for opting out can be provided for all MSM who declined HCV testing. Furthermore, additional HIV clinic data was missing for about one third of the HIV-positive MSM who tested anti–HCV-positive.

Strength of this study is the large number of HIV-positive and HIV-status-unknown MSM who were routinely screened for HCV during STI screening at the STI clinic. In addition,

this study highlights the added value of routine HCV screening among HIV-positive MSM at an STI clinic in addition to standard care at an HIV clinic.

In conclusion, routine HCV antibody testing at the STI clinic confirmed HCV is circulating among HIV-positive MSM, identified new HCV infections, and resulted in earlier HCV diagnosis for a considerable proportion of the HIV-positive MSM also in care at an HIV clinic. Among MSM opting out of HIV testing and accepting HCV testing, the HCV prevalence was low, and no incident cases were found. Therefore, STI clinics should consider including HCV testing among HIV-positive MSM, at least for those notified of HCV by their sexual partner, and those not (yet) under the care of an HIV clinic, like MSM newly diagnosed with HIV.

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Conflicts of Interest and Source of Funding
None declared.

Contributors
M.v.R., T.H., and M.P. designed the study protocol, supported by N.V. M.v.R. was responsible for data collection at the STI clinic and N.V. at the HIV treatment clinics. M.v.R. and M.P. completed the analyses. M.v.R., T.H., H.d.V., and M.P. drafted the article. All authors commented on draft versions, and all approved the final version.

Ethics approval
Because routinely collected anonymous data was used for this study, no ethical clearance was sought.

Previously presented
Preliminary result of the study have been presented as a poster at the NCHIV (27 November 2012, Amsterdam, the Netherlands abstract number 39) at the STI & AIDS World Congress (17 July 2013, Vienna, Austria abstract number P5.022).

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4.1

CHAPTER 4.2

Additional gonorrhoea and chlamydia infections found with rapid follow-up screening in men who have sex with men with an indication for HIV post-exposure prophylaxis.

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ABSTRACT

Sexually transmitted infection was found in 16.5% of the men who have sex with men with a postexposure prophylaxis indication. Chlamydia and gonorrhoea screening was repeated after 14 days. Among those who were initially sexually transmitted infection negative, 4.1% had chlamydia or gonorrhoea. In postexposure prophylaxis-indicated men who have sex with men, repeat chlamydia and gonorrhoea screening is advised to diagnose infections not apparent at baseline screening.

INTRODUCTION

HIV postexposure prophylaxis (PEP) is a 4-week course of antiretroviral treatment recommended to persons exposed to human body fluids possibly infected with HIV to prevent HIV acquisition.[1] It was first introduced to reduce the transmission risk after needle-stick accidents and other occupational exposures to HIV.[2] In 1997, it was introduced for use after sexual exposure.[3] Since 2010, the sexually transmitted infection (STI) outpatient clinic of the Public Health Service in Amsterdam, the Netherlands, offers PEP to HIV-negative patients who have had a considerable risk of recent HIV exposure through unsafe sex.

Individuals presenting for PEP after sexual exposure are at risk for concurrent STI. Therefore, a PEP request is an ideal opportunity for STI screening and safe sex promotion. Early incubating *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) infections acquired during the sexual exposure for which PEP is sought are possibly missed if STI screening is only performed at the consultation during which an indication for PEP initiation is established. Therefore, we aimed to determine if chlamydia and gonorrhoea screening should be repeated in men who have sex with men (MSM) 2 weeks after a PEP indication.

METHODS

If the client requests PEP or the history suggests that there has been considerable risk for HIV transmission in the previous 72 hours, the option to start PEP is discussed. A medical doctor decides whether PEP is indicated based on the criteria of the Dutch guidelines for sexual exposure,[4] which are in agreement with European guidelines[1] and the Centers for Disease Control and Prevention recommendations.[5]

At the STI clinic, testing for gonorrhoea, chlamydia, syphilis, hepatitis B, and HIV is offered to all MSM patients who visit the clinic, as described before.[6-9] Urine and rectal swabs are collected and screened for CT (Aptima CT single system; GEN-PROBE, San Diego, CA). Pharyngeal swabs are tested for CT and NG (Aptima Combo 2 system; GEN-PROBE). At the initial visit, a trained laboratory technician examines urethral and rectal Gram-stained smears for a presumptive gonorrhoea diagnosis (i.e., gram-negative diplococci in polymorphonuclear leukocytes) or a non-gonococcal infection (i.e., >10 polymorphonuclear leukocytes per light microscopic high-power field, no

gram-negative diplococci). Urethral and rectal cultures are used for the definitive NG diagnosis. HIV antibody rapid testing (Determine 1-2; Abbott Laboratories, Abbott Park, IL) is offered to all patients. Serum samples are also further tested by line immunoassay (Inno-Lia HIV I-II Score; Innogenetics, Ghent, Belgium); this result will follow after a few working days. Condylomata acuminata are diagnosed clinically.

Postexposure prophylaxis is not started if the HIV rapid test result is positive. In case the HIV antibody rapid test result is negative or inconclusive, but the result of the line immunoassay result, when it becomes available a few days later, is positive, continuation of PEP medication as treatment of HIV is reviewed with an infectious disease specialist. If the source person of the risk event is tested at the STI clinic and found to be HIV negative, PEP is considered not indicated, and if PEP was already started, it is therefore discontinued. Patients are free to decline PEP. If PEP is accepted, a visit is planned 2 weeks later to repeat screening for urethral, pharyngeal, and anal NG and CT infections.

We included all MSM visiting the STI outpatient clinic in Amsterdam with a PEP request after sexual exposure from April 2010 until December 2012. Men who have sex with men with multiple PEP requests during the inclusion period were included in the analysis multiple times, and thus, we report about ''presentations'' instead of individuals. An STI presentation could involve multiple sexually transmissible pathogens, or the same pathogen at more than 1 anatomical location. All statistical analyses were performed in SPSS version 19 (SPSS Inc., Chicago, IL). Ethical clearance was not sought because this was an analysis on routinely collected, anonymized data.

RESULTS

From April 2010 until December 2012, a total of 26,733 consultations were performed in MSM. We analysed 473 PEP requests from 438 unique individual MSM. In 78 (16.5%) of those 473 presentations, at least 1 STI was found (figure 1). In 334 (70.6%) presentations, PEP was indicated. The STI positivity among the 139 presentations in which PEP was not indicated was 16.5% (23/139): 11 attendees (7.9%) were HIV positive, and in 12 (8.6%) presentations, 16 STIs other than HIV were diagnosed. In the presentations in which PEP was indicated, 68 STIs were found in 55 (16.5%) of 334. Two attendees had a co-infection, 9 had the same STI at multiple anatomical places, and 1 had a triple infection (both NG and CT and NG at multiple anatomical places). The rectum was the most common anatomical place for an STI in 27 (49.1%) of 55 presentations. Only 6 (10.9%) of 55 presentations with a PEP indication and an STI were symptomatic at screening.

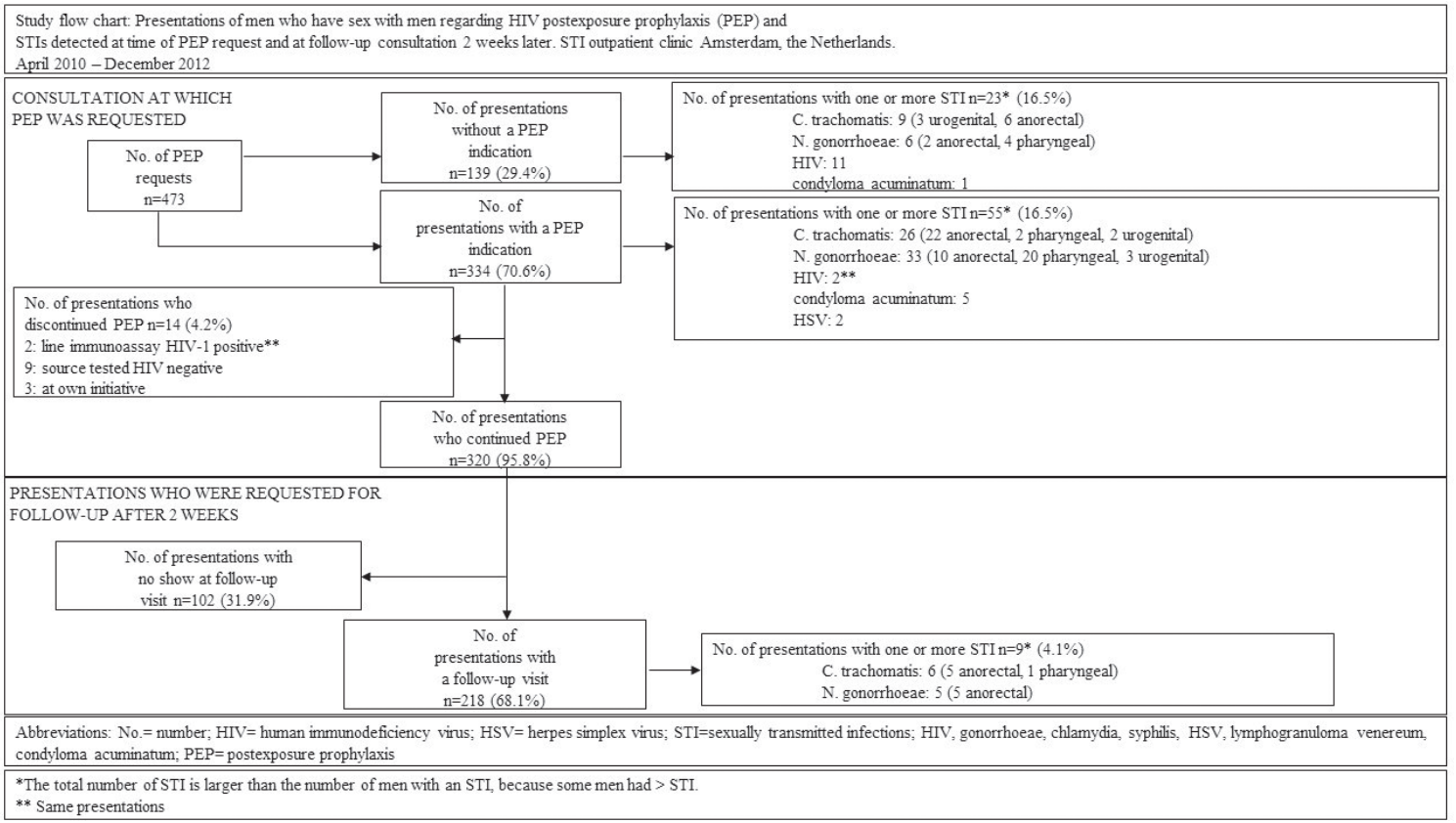

Figure 1. Study flowchart. Presentations of MSM regarding HIV PEP and STIs detected at the time of PEP request and at follow-up consultation 2 weeks later. STI outpatient clinic; Amsterdam, the Netherlands; April 2010 to December 2012.

In 14 presentations, PEP was discontinued: in 2, the confirmation of the line immunosorbent assay of the index proved positive; in 9, the partner involved in the sex accident proved HIV negative; in 3, the patient discontinued PEP at own initiative. The remaining 320 completed PEP. A follow-up visit after 2 weeks (range, 10-17 days) was performed in 218 (68.1%) of 320 presentations. In 9 (4.1%) of 218 presentations, at least 1 previously undiagnosed infection was found: 3 rectal CT, 3 rectal NG, 1 pharyngeal CT, and 2 rectal CT/NG co-infections. No urethral infections were detected at the 2-week visit. All of 9 presentations with a previously undiagnosed infection denied sexual contact since the last consultation.

DISCUSSION

This study demonstrates that screening for STI in MSM with a request for PEP after sexual HIV exposure is worthwhile. Of all 473 presentations requesting PEP, 16.5% had at least 1 STI. Of great importance are the 13 (2.7%) of 473 previously undiagnosed HIV infections. Moreover, reconfirming negative/inconclusive HIV rapid test results with HIV immunoassay in MSM is of importance because 2 additional HIV infections were thus diagnosed. We consider these by rapid test missed HIV primary infections. Because primary HIV infections are known to have very high viral loads, these are of great consequences for ongoing transmission.[10 11]

Repeated chlamydia and gonorrhoea screening 2 weeks after the PEP indication revealed 4.1% additional, possibly early incubating, CT and/or NG infections. Our observed prevalence of STI (16.5%) is higher compared with other reported studies in MSM receiving PEP.[12 13] Similar to earlier studies, in 31.9% consultations, the attendees in which PEP was indicated did not show for the follow-up visit.[12 14] Most MSM with an STI were asymptomatic; the same was reported recently by Jamani et al.,[14] who found that more than 90% of cases with chlamydia and gonorrhoea diagnoses were asymptomatic. This underlines the importance of providing STI screening to all individuals after high-risk exposures regardless of symptoms, unlike the recommendations in the UK PEP guidelines.[15] Offering PEP and prompt STI testing creates an opportunity to shorten the duration of infectiousness of an STI and facilitates discussion regarding risk reduction and safer sex.[16]

Strengths of this study are that our test policy did not change over time, and all PEP requesters were screened routinely, both at the initial visit and 14 days afterward.

Limitations of our study are the no-show ratio for a repeat screening of approximately 32% after 2 weeks and that we cannot exclude that attendees with a new CT or NG infection after 2 weeks did not have sexual intercourse within that period, although they all denied having had sex between the 2 visits. Our outcome differs from a similar report in which 28.1% of the presentations reported sexual activity between the baseline and the 2-week visit.[12] Sampling errors during the baseline visit could have produced false-negative results that were considered ''new'' infections found during the return visit. Because trained nurses supervised all sample collections and highly sensitive nucleic acid amplification tests were used, we consider the occurrence of sampling error highly unlikely.

Based on these data, we consider that STI and HIV screening should be offered to all MSM with a PEP request after a sexual HIV exposure regardless of symptoms. Immediate screening yields a high rate of especially gonorrhoea and chlamydia infections. Moreover, it seems advisable to repeat screening for gonorrhoea and chlamydia a few weeks later, to detect infections not apparent at the baseline screening. Whether these later diagnoses were early incubating or newly acquired infections remains unclear. The considerable proportion of patients that will not return for repeat screening a few weeks after the PEP request makes immediate screening indispensable.

Competing interests
None.

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None.

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CHAPTER 4.3

Sexually transmitted infection positivity rate and treatment uptake among female and male sexual assault victims attending the Amsterdam STI clinic between 2005 and 2016.

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ABSTRACT

Background

Victims could become infected with sexually transmitted infections (STIs) during a sexual assault. Several guidelines recommend presumptive antimicrobial therapy for sexual assault victims (SAVs).We assessed the STI positivity rate and treatment uptake of female and male SAVs at the Amsterdam STI clinic.

Methods

Sexual assault victims answered assault-related questions and were tested for bacterial STI (chlamydia, gonorrhoea, and syphilis), hepatitis B, and HIV during their initial visits. Sexual assault victim characteristics were compared with non-SAV clients. Backward multivariable logistic regression analysis was conducted to assess whether being an SAV was associated with a bacterial STI. The proportion of those returning for treatment was calculated.

Results

From January 2005 to September 2016, 1066 (0.6%) of 168,915 and 135 (0.07%) of 196,184 consultations involved female and male SAVs, respectively. Among female SAVs, the STI positivity rate was 11.2% versus 11.6% among non-SAVs (p=0.65). Among male SAVs, the STI positivity *r*ate was 12.6% versus 17.7% among non-SAVs (p=0.12). In multivariable analysis, female SAVs did not have increased odds for an STI (odds ratio 0.94; 95% confidence interval, 0.77–1.13), and male SAVs had significantly lower odds for an STI (odds ratio, 0.60; 95% confidence interval, 0.36–0.98). Of SAVs requiring treatment, 89.0% (female) and 92.0% (male) returned.

Conclusions

The STI positivity rate among female SAVs was comparable with female non-SAVs, but male SAVs had lower odds for having a bacterial STI than did male non-SAVs, when adjusting for confounders. The return rate of SAV for treatment was high and therefore does not support the recommendations for presumptive therapy.

INTRODUCTION

Approximately, 16.5% of women and 3.8% of men in the Netherlands have experienced vaginal or anal penetration, or oral sex without consent at least once in their lifetime.[1] Before the age of 16, 8.1% of women and 2.5% of men experience this kind of sexual assault.[1] Most earlier studies have shown a high positivity rate of sexually transmitted infections (STIs) at initial evaluation of sexual assault victims (SAVs).[2] However, Beck-Sagué and Solomon[3] argued that adolescents and adults frequently acquire STI through consensual sexual activity, whereas it is unclear whether victims are infected during an assault. Data on the STI positivity among female SAVs are scarce and even less is known about rates among male SAVs.[2]

The Centers for Disease Control and Prevention 2015 STD Treatment Guidelines recommend empirical presumptive antimicrobial therapy (before test results are available) targeting gonorrhoea, chlamydia, and trichomoniasis at the initial evaluation of SAVs, in view of their high STI positivity rates and low rate of return for follow- up visits.[2] In the Netherlands, there is no national guideline concerning STI testing and presumptive therapy for SAV.

The objective of this study was to assess the STI positivity rate and the follow-up rate in adolescent and adult female and male SAVs attending the STI clinic of Amsterdam, the Netherlands. In addition, we used data of both SAV and non-SAV clients to study whether being a victim of a sexual assault was associated with an STI diagnosis.

METHODS

Study population and procedures

The STI outpatient clinic of the Public Health Service of Amsterdam (GGD Amsterdam) annually performs around 45,000 free-of-charge and anonymous STI consultations. Before 2009, clients could walk in ("first-come, first-served" policy). Since 2009, clients had to apply for an appointment (online or by telephone): only high-risk clients and SAV received an appointment. Clients considered high-risk for STI included those reporting STI-related symptoms, those referred by a health care provider, those notified of an STI, men who have sex with men, commercial sex workers, clients who paid for sex (until 2015), clients younger than 25 years, clients reporting 3 or more sex partners (until 2015), clients of non–Western European and non–North American ethnicity, and/

or sexual partners of people of these ethnicities. All behaviour indicators refer to the 6 months before consultation. Demographics, detailed medical and sexual history, and test results were registered in an electronic patient database. Only in case of contact with an STI (proved with a notification card) or a positive Gram smear result, presumptive antibiotic treatment was given.

Since 2012, clients younger than 25 years without previously mentioned risk factors have only been offered chlamydia and gonorrhoea testing.[4] All other clients were tested for *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and syphilis. HIV testing was offered on indication before 2007. From 2007 onward, an opt-out strategy was adopted.

C. trachomatis was tested using nucleic acid amplification tests, and *N. gonorrhoeae* was tested using nucleic acid amplification tests or culture. Details on anatomical sites tested, laboratory tests used, and manufacturer details are presented in supplementary table 1.

STI clinic procedure in case of sexual assault

In this article, sexual assault refers to non-consensual penetration of the mouth, anus, or vagina, because these acts are associated with exposure to STI.[1,2] Sexual assault victims with a minimum age of 12 years were referred to the clinic by a health care provider (e.g., general practitioner or forensic physician) or by the police, or came on their own initiative. A professional asked all clients at the STI clinic whether their request for an STI test was related to a sexual assault. Sexual assault as reason for visit was only registered at the first STI consultation after an assault. If the clinic consultation took place within 72 hours after the assault, postexposure prophylaxis for HIV was considered according to a "risk of HIV exposure" assessment.[5] Unless already vaccinated, all SAVs were offered a hepatitis B vaccination. Sexual assault victims were routinely tested for chlamydia, gonorrhoea, syphilis, hepatitis B, and HIV. During consultation, questions were asked related to the sexual assault. From July 2013 onward, the time (≤7 days, >7 days) between the assault and STI consultation was registered.

Statistical analysis

The anonymized medical records from the electronic patient database were analysed in SPSS version 21.0 (IBM Corp, Armonk, NY). Sexual preference was determined by the sex of sexual partners in the preceding 6 months. Before 2011, ethnicity was self-reported. From 2011 onward, ethnicity was defined based on an algorithm combining

country of birth index, mother, and father.[6] Ethnicity was categorised into Dutch versus non-Dutch, consisting of 9 different groups (see table 1 for these groupings). Number of sex partners was categorised in quartiles. Age was categorised in 10-year age groups. A bacterial STI diagnosis was defined as being diagnosed as having *C. trachomatis*, *N. gonorrhoeae*, and/or infectious syphilis. HIV status - based on self-reported HIV-positive status and HIV test result at consultation - was categorised into known positive, newly diagnosed positive, negative, and unknown.

Whether someone was an SAV was registered per consultation, and all consultations, including those of clients with multiple consultations, were included in the analysis. For readability, this article will use the terms "SAV clients" and "non-SAV clients" instead of "consultations in which a sexual assault was (or was not) reported." Characteristics of SAV were compared with non-SAV for men and women separately. $\chi 2$ Test or Fisher exact test for categorical variables and the Mann-Whitney *U* test for continuous variables were used. For SAV with a bacterial STI having to return for treatment, the proportion lost to follow-up was assessed. Univariable and multivariable logistic regression analyses were performed to analyse whether being a victim of a sexual assault was an independent determinant for a bacterial STI diagnosis. To correct for repeated measurements (clients with multiple consultations), we used generalized estimating equations (STATA 13.0 software; STATA Intercooled, College Station, TX). Multivariable model building was done using a backward stepwise procedure, including only those variables with a univariable p value of less than 0.25.[7] All variables with a $p<0.05$ were kept in the final multivariable model. The variable of interest - being victim of a sexual assault - was forced into the model. The variables "physical symptoms" and "being notified" were excluded from the multivariable analysis, because they are consequences of a possible STI, and not risk factors or causes. To correct for possible differences introduced into the clinical population in 2009 by the transition from a walk-in to an appointment-based clinic, univariable and multivariable sub-analyses were performed for 2005–2008 and 2009–2016. p Values of less than 0.05 were considered statistically significant.

RESULTS

Between January 2005 and September 2016, 168,915 consultations were performed among female and 196,184 consultations among male clients. Of these, 1066 (0.63%) and 135 (0.07%) consultations involved female and male SAVs, respectively (figure 1).

Of 1066 consultations among female SAVs, 27 clients had 2, and 2 clients had 3 SAV consultations. Three male SAVs had 2, and 1 client had 3 SAV consultations.

Female SAVs

Compared with non-SAV female clients, SAVs were significantly older, less often Dutch, and more often of sub-Saharan African origin (table 1). Sexual assault victims less often lived in Amsterdam, reported more sexual partners, more frequently reported STI-related symptoms, were less often notified of STI exposure, and reported commercial sex work in the preceding 6 months less often. The proportion of SAV consultations diagnosed as having a bacterial STI (n=119; 11.2%) did not differ from non-SAVs (11.6%, p=0.65). However, SAVs did more frequently test hepatitis B surface antigen and anti-hepatitis B core-antigen positive (p=0.044 and p<0.001). In the univariable logistic regression analysis - except from being an SAV - all other determinants (age, ethnicity, residence, HIV status, number of sexual contacts, and commercial sex work) were significantly associated with having a bacterial STI (supplementary table 2). After adjusting for the previously mentioned variables in the multivariable logistic regression analysis, being an SAV was not associated with having a bacterial STI (odds ratio [OR], 0.94; 95% confidence interval [CI], 0.77–1.13). In the multivariable sub-analyses, SAVs did not have higher odds for diagnosis with a bacterial STI (2005–2008: OR, 1.16 [95% CI, 0.79–1.69]; 2009–2016: OR, 0.84 [95% CI, 0.67–1.05]).

Of 119 female SAVs with a bacterial STI, presumptive antibiotic treatment was given to 10. Of the remaining 109, 97 (89%) returned to the clinic for treatment and 12 (11.0%) did not return: 8 could not be reached, 2 were treated by their general practitioner, 1 was treated at her local health service, and in 1 case, the clinic sent a prescription to the client's pharmacy.

All the assailants of female SAVs were male, yet in 8 cases, a female was also involved (table 2). For 4.4% of female SAVs, condoms were used during the sexual assault. A minority of SAVs reported the assault to the police or underwent forensic examination. The interval between the assault and STI consultation was known for 385 (36.1%) of 1066 consultations; 33.5% of the assaults had occurred in the preceding 7 days, and the STI positivity rate in this group was similar to SAVs assaulted more than 7 days before consultation (10.1% vs 10.9%, p=0.80).

Male SAVs

Compared with non-SAV male clients, male SAVs less often lived in Amsterdam, were less often Dutch and Surinamese, and were more often from sub-Saharan African, North African, or Asian decent (table 3). In the 6 months preceding the STI clinic visit, 56% of the male SAVs and 39% of non-SAVs reported sexual contact with men only ($p<0.001$). Male SAVs reported a lower number of sexual partners, were more often paid for sex in the preceding 6 months, and were less often notified of STI exposure. The bacterial STI positivity rate was not significantly different between male SAVs (n=17; 12.6%) and non-SAVs (17.7%, $p=0.12$). However, significantly fewer male SAVs had a urogenital chlamydia and anal gonorrhoea diagnosis. In the univariable logistic regression analysis - except from being an SAV - all other variables (age, ethnicity, residence, HIV status, sex of sexual partner(s), number of sexual contacts, commercial sex work, and paying for sex) were significantly associated with having a bacterial STI (supplementary table 3). After adjusting for the previously mentioned variables in multivariable analysis, being a male SAV was associated with a lower risk of having a bacterial STI (OR, 0.60; 95% CI, 0.36–0.98). In the multivariable sub-analyses - although non-significant - male SAVs had lower odds of being diagnosed with having a bacterial STI (2005–2008: OR, 0.28 [95% CI, 0.07–1.11]; 2009–2016: OR, 0.68 [95% CI, 0.39–1.18]).

Of 17 male SAVs with a bacterial STI, presumptive antibiotic treatment was given to 3. Of the remaining 14, all but 1 (7.1%) returned to our clinic for treatment.

Most assailants were male, but in 11 cases, only female assailants were reported (table 2). Among 8.4% of the male SAVs, condoms were used during the sexual assault. A minority of SAVs reported the assault to the police or underwent forensic examination. The period between the assault and STI consultation was known for 49 (36.3%) of 135 consultations; 46.9% of the assaults occurred in the preceding 7 days, and the STI positivity rate in this group was not significantly different from SAVs assaulted more than 7 days before consultation (17.4% vs 23.1%, $p=0.73$).

Table 1. Demographics, sexual behaviour, and diagnosed STIs among 1066 clinic visits from female victims of a sexual assault and 165,742 clinic visits from female clients who were not a victim of sexual assault of the STI clinic in Amsterdam, the Netherlands; January 2005 to September 2016

	Victim of a sexual assault n=1066	**Not a victim of sexual assault n=165,742**	
Variable	**n (%)**	**n (%)**	**p Value**
Period			0.34
2005-2008	262 (24.6)	42,542 (25.7)	
2009-2012	354 (33.2)	51,643 (31.2)	
2013-2016	450 (42.2)	71,557 (43.2)	
Demographics			
Median age (IQR) in years[1]	24 (20-30)	24 (21-28)	0.003
Age in years[1]			<0.001
11- 25	575 (53.9)	97,050 (58.6)	
25 – 34	316 (29.6)	52,781 (31.8)	
35 – 44	117 (11.0)	10,570 (6.4)	
45 – 54	46 (4.3)	4234 (2.6)	
≥55	12 (1.1)	1104 (0.7)	
Ethnicity			
Dutch	641 (60.1)	113,056 (68.2)	<0.001[2]
Non-Dutch	425 (39.9)	52,686 (31.8)	
East European	40 (3.8)	9690 (5.8)	
Turkish	15 (1.4)	837 (0.5)	
North African	20 (1.9)	2223 (1.3)	
Sub-Saharan African	85 (8.0)	3503 (2.1)	
Antillean	15 (1.4)	2518 (1.5)	
Surinamese	100 (9.4)	13,513 (8.2)	
South American	34 (3.2)	4891 (3.0)	
Asian	34 (3.2)	4915 (3.0)	
Other	82 (7.7)	10,596 (6.4)	
Residence			<0.001
Amsterdam	759 (71.2)	126,140 (76.1)	
Province of North Holland	151 (14.2)	19,497 (11.8)	
Elsewhere in the Netherlands	72 (6.8)	11,536 (7.0)	
Other/unknown	84 (7.9)	8569 (5.2)	
Sexual behaviour in the preceding 6 months			
Median number of sexual partners (IQR)[3]	2 (2-4)	2 (1-4)	0.001
Commercial sex work[4]	52 (4.9)	14,996 (9.1)	<0.001
Reason for visit			
STI related symptoms[5]	358 (33.6)	40,075 (24.2)	<0.001
Notified of STI[6]	58 (5.4)	13,632 (8.2)	0.001
STI diagnoses[7]			
Bacterial STI[8]	119 (11.2)	19,239 (11.6)	0.65

Table 1. Continued

	Victim of a sexual assault n=1066	Not a victim of sexual assault n=165,742	
Variable	**n (%)**	**n (%)**	**p Value**
STI diagnoses[7]			
Chlamydia[9]	110 (10.3)	17,917 (10.8)	0.61
Anal	42/436 (9.6)	4572/39,230 (11.7)	0.23
Urogenital	96/1065 (9.0)	16,729/165,643 (10.1)	0.24
Pharyngeal[10]	11/495 (2.2)	772/30,670 (2.5)	0.68
Gonorrhoea[11]	19/1068 (1.8)	2175/158,030 (1.4)	0.26
Anal	9/876 (1.0)	755/74,503 (1.0)	0.97
Urogenital	15/1063 (1.4)	1680/157,763 (1.1)	0.27
Pharyngeal	5/745 (0.7)	594/55,704 (1.1)	0.30
Infectious syphilis	1 (0.1)	78/146,633 (0.1)	0.44
HIV status[12]			0.34
HIV negative	987 (99.7)	138,528 (99.8)	
HIV known positive	1 (0.1)	151 (0.1)	
HIV newly diagnosed[13]	2 (0.2)	143 (0.1)	
HIV not tested	76	26,920	
Hepatitis B[14]			
Hepatitis B infectious (HBsAg positive)	5/783 (0.6)	256/106,228 (0.2)	0.044
Hepatitis B immune (anti-HBc positive)	57/783 (7.3)	2378/106,228 (2.2)	<0.001

Abbreviations:
Anti-HBc: Antibodies to the hepatitis B core antigen, HBsAg: Hepatitis B surface antigen, IQR: Interquartile range, STI: Sexually transmitted infection.
[1]Missing for 3 non-victims. Youngest age victims: 13 years; non-victims: 11 years.
[2]p Value, Dutch versus non-Dutch.
[3]Missing before 2009 (263 victims and 42,748 non-victims).
[4]Missing for 4 victims and 57 non-victims.
[5]Missing for 19 non-victims.
[6]Missing for 1 victim and 30 non-victims.
[7]Not all were tested for each STI and at each anatomical location; behind the slash, the number of patients tested for the particular STI is mentioned.
[8]Bacterial STI is defined as being diagnosed as having *C. trachomatis*, gonorrhoea and/or infectious syphilis at the time of current visit.
[9]Anal, urogenital, and/or pharyngeal chlamydia positive.
[10]Tested from 2011.
[11]Anal, urogenital and/or pharyngeal gonorrhoea positive.
[12]Before 2007 HIV was only tested on indication. From January 2007 to December 2011, all clients were offered an HIV test. Since 2012, young low-risk women are not tested for HIV. p Value calculated for those women with a known HIV status.
[13]One female victim was newly diagnosed with HIV in the first consultation after the sexual assault; the other female victim was diagnosed during follow-up, 3 months after the sexual assault.
[14]Hepatitis B not tested in clients who were already vaccinated against hepatitis B or who were previously tested anti-HBc positive. Before April 2006 and from May 2014 onward, only high-risk clients were tested.

Table 2. Sexual assault history and contact with health care providers after the assault of 135 clinic visits from male and 1066 from female clients attending the STI clinic in Amsterdam, the Netherlands; January 2005 to September 2016

Variable	Men n=135[1] n (%)	Women n=1066[1] n (%)	p Value
Condom used during sexual assault			0.054
No	109 (91.6)	955 (95.6)	
Yes	10 (8.4)	44 (4.4)	
Sex of the perpetrator(s)			<0.001
Female	11 (9.2)	0 (0)	
Male	108 (90.0)	995 (99.2)	
Both male and female	1 (0.8)	8 (0.8)	
Reported the assault to the police			0.026
No	90 (75.0)	651 (64.8)	
Yes	30 (25.0)	354 (35.2)	
Examined by forensic doctor			0.021
No	105 (87.5)	787 (78.5)	
Yes	15 (12.5)	216 (21.5)	
Pregnancy test performed[2,3]			NA
No		541 (88.7)	
Yes		69 (11.3)	
Time between assault and STI clinic consultation[4]			0.080
<= 7 days	23/49 (46.9)	129/385 (33.5)	
> 7 days	26/49 (53.1)	256/385 (66.5)	

Abbreviations:
NA: not applicable

[1]History of the sexual assault and contact with health care providers missing for at maximum 16 men and 69 women
[2]Pregnancy test performed after the sexual assault but prior to attending the STI clinic
[3]Question about pregnancy test was not asked in the period July 2013 through September 2016
[4]Registered since July 2013 onwards. Missing for 86 male and 681 female sexual assault victims

Table 3. Demographics, sexual behaviour, and diagnosed STIs among 135 clinic visits from male victims of a sexual assault and 194,819 clinic visits from other male clients who were not a victim of sexual assault of the STI clinic in Amsterdam, the Netherlands; January 2005 to September 2016

	Victim of a sexual assault n=135	Not a victim of sexual assault n=194,819	
Variable	**n (%)**	**n (%)**	**p Value**
Period			0.19
2005 – 2008	32 (23.7)	55,755 (28.6)	
2009 – 2012	42 (31.1)	65,452 (33.6)	
2013 – 2016	61 (45.2)	73,612 (37.8)	
Demographics			
Median age (IQR)	28 (23-39)	30 (24-40)	0.199
Age in years[1]			0.45
13 – 25	42 (31.1)	51,320 (26.3)	
25 – 34	51 (37.8)	70,620 (36.2)	
35 – 44	20 (14.8)	40,691 (20.9)	
45 – 54	15 (11.1)	22,863 (11.7)	
≥ 55	7 (5.2)	9320 (4.8)	
Ethnicity			
Dutch	73 (54.1)	123,185 (63.2)	0.027[2]
Non-Dutch	62 (45.9)	71,634 (36.8)	
East European	3 (2.2)	4636 (2.4)	
Turkish	2 (1.5)	3053 (1.6)	
North African	7 (5.2)	5235 (2.7)	
Sub-Saharan African	11 (8.1)	5168 (2.7)	
Antillean	3 (2.2)	3918 (2.0)	
Surinamese	7 (5.2)	16371 (8.4)	
South American	3 (2.2)	6662 (3.4)	
Asian	7 (5.2)	7040 (3.6)	
Other	19 (14.1)	19,551 (10.0)	
Residence			0.002
Amsterdam	94 (69.6)	148,475 (76.2)	
Province of North Holland	15 (11.1)	20,878 (10.7)	
Elsewhere in the Netherlands	8 (5.9)	14,495 (7.4)	
Other/unknown	18 (13.3)	10,971 (5.6)	
Sexual behaviour in the preceding 6 months			
Sex of sexual partner(s)			<0.001
Female	29 (21.5)	111,633 (57.3)	
Male	75 (55.6)	75,230 (38.6)	
Both female and male	31 (23.0)	7956 (4.1)	

Table 3. Continued

	Victim of a sexual assault n=135	Not a victim of sexual assault n=194,819	
Variable	**n (%)**	**n (%)**	**p Value**
Median number of sexual partners (IQR)[3]	3 (2-6)	4 (2-8)	0.004
Commercial sex work[4]	6 (4.4)	2,472 (1.3)	0.008
Paying for sex[5]	3 (2.2)	11,458 (5.9)	0.70
Reason for clinic visit			
STI related symptoms[6]	38 (28.1)	66,146 (34.0)	0.15
Notified of STI[7]	11 (8.2)	31,968 (16.4)	0.010
STI diagnoses[8]			
Bacterial STI[9]	17 (12.6)	34,530 (17.7)	0.12
Chlamydia[10]	9 (6.7)	23,317 (12.0)	0.058
Anal	8/106 (7.5)	6592/66,418 (9.9)	0.41
Urogenital	3/134 (2.2)	17,326/194,541 (8.9)	0.007
Pharyngeal[11]	2/73 (2.7)	758/50,399 (1.5)	0.30
Gonorrhea[12]	6 (4.4)	11,834/193,361 (6.1)	0.42
Anal	2/117 (1.7)	5140/82,844 (6.2)	0.044
Urogenital	1 (0.7)	5369/193,137 (2.8)	0.19
Pharyngeal	5/110 (4.5)	4489/82,630 (5.4)	0.68
Infectious syphilis	4 (3.0)	3167/190,203 (1.7)	0.29
HIV status[13]			0.083
HIV negative	113 (92.6)	154,987 (87.7)	
HIV known positive	7 (5.7)	20,398 (11.5)	
HIV newly diagnosed[14]	2 (1.6)	1413 (0.8)	
HIV not tested	13	18,021	
Hepatitis B[15]			
Hepatitis B infectious (HBsAg positive)	1/96 (1.0)	739/125,294 (0.6)	0.43
Hepatitis B immune (anti-HBc positive)	8/96 (8.3)	7073/125,294 (5.6)	0.25

Abbreviations:
Anti-HBc: Antibodies to the hepatitis B core antigen, HBsAg: Hepatitis B surface antigen, IQR: Interquartile range, STI: Sexually transmitted infection

[1]Missing for 5 non-victims. Youngest age victims: 15 years; non-victims: 13 years.
[2]p Value, Dutch versus non-Dutch.
[3]Missing before 2009 (32 victims and 55,922 non-victims).
[4]Missing for 410 non-victims.
[5]Missing for 414 non-victims.
[6]Missing for 32 non-victims.
[7]Missing for 1 victim and 52 non-victims.

[8]Not all were tested for each STI and at each anatomical location; behind the slash, the number of clients tested for the particular STI is mentioned.
[9]Bacterial STI is defined as being diagnosed as having *C. trachomatis*, gonorrhoea and/or infectious syphilis at the time of current visit.
[10]Anal, urogenital, and/or pharyngeal chlamydia positive.
[11]Tested from 2011.
[12]Anal, urogenital, and/or pharyngeal gonorrhoea positive.
[13]Before 2007, HIV was only tested on indication. From January 2007 to December 2011, all clients were offered an HIV test. Since 2012, young low-risk heterosexual men are not tested for HIV. p Value calculated for those men with a known HIV status.
[14]Diagnosed in the first consultation after the sexual assault.
[15]Hepatitis B not tested in clients who were already vaccinated against hepatitis B or who were previously tested anti-HBc positive. Before April 2006 and from May 2014 onward, only high-risk clients were tested.

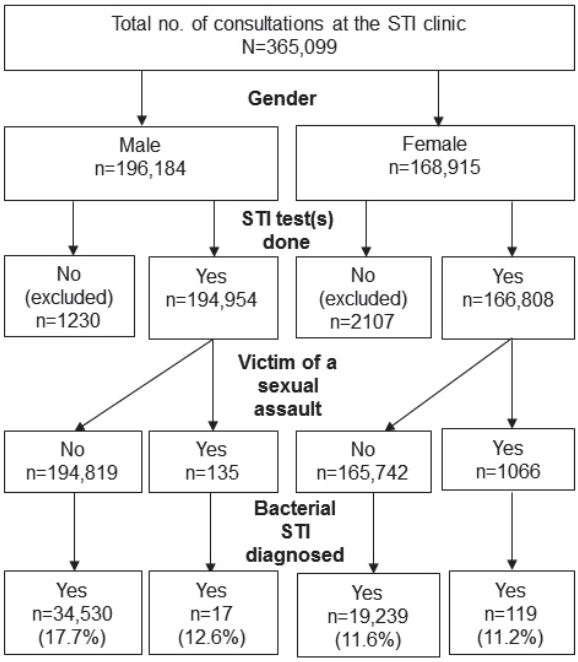

Figure 1. Flowchart of consultations at the STI clinic in Amsterdam, the Netherlands; January 2005 to September 2016.

DISCUSSION

This study is based on 12 years of clinical data and compares characteristics and STI positivity rates among SAVs with non-SAV STI clinic clients. Among female SAVs, the bacterial STI positivity rate was comparable with that among non-SAV female STI clinic clients. Although most sexual assaults reported among male SAVs could have resulted in a higher risk of STI acquisition (no condom use and male assailants), the relatively large group of male SAVs included in this study had a significantly lower risk of testing STI positive than did non-SAV male clients. Because we do not routinely offer presumptive treatment to SAV, we were able to investigate SAV follow-up. For both female and male SAVs, the return rate for treatment was very high (89% and 92%, respectively). Based on this finding, we believe that presumptive therapy is not necessary, at least not in our or similar settings.

Sexually transmitted infection screening among both preadolescent and adolescent clients in the United States showed an STI positivity rate (including herpes, human papillomavirus, and condylomata) of 19.6% among girls and 6.3% among boys.[8] Among 64 female and 1 male SAVs in an inner-city genitourinary medicine clinic in London, 2 cases (3.1%) with a bacterial STI (chlamydia) were detected.[9] In a Norwegian sexual assault centre, the chlamydia positivity rate (6.4%) among SAVs within 1 week after the assault was lower than that in a comparable clinical population (16% among 15- to 19-year and 12% among 20- to 24-year-olds).[10] Among 14.7% and 4.9% of SAVs examined at a French Department of Forensic Medicine, chlamydia and gonorrhoea were detected, respectively.[11] Our study mainly focused on bacterial STI, and comparable bacterial STI positivity rates among SAV and non-SAV clients were found. Among female clients, a sexual assault was not a risk factor, whereas male SAVs had a lower risk of a bacterial STI than did non-SAVs.

A study from the United Kingdom stratifying for recent consensual sexual intercourse showed an STI positivity of 25.6% among those who had and 4.3% among those who had not had intercourse 3 months before the assault.[12] The median number of sex partners in the present study (2 for female SAVs and 3 for male SAVs) also indicates that diagnosed STI could be unrelated to the assault. In addition to this possible effect of consensual sexual activity on contracting an STI, a longer period between the assault and the STI consultation might have influenced our results. The time between the assault and the STI clinic visit was only known for 36% of the consultations, and

approximately one third of the female and half of the male SAVs were assaulted in the 7 days preceding the clinic visit.

In a review, Seña et al.[2] observed that we do not know much about the STI positivity rate among male SAVs. In our study, male SAVs had a lower STI positivity rate (unadjusted; not significant). However, when adjusted for age, ethnicity, residence, HIV status, sex of sexual partner(s), number of sexual contacts, commercial sex work, and paying for sex, a significantly lower risk of bacterial STI was apparent. A British study showed that compared with female SAVs, male SAVs were more likely to access the routine walk-in genitourinary medicine clinic (compared with a specialised sexual assault clinic), perhaps because they are less likely to report a sexual assault to the police and therefore miss out on the forensic medical examiners referral pathway.[13] Our findings are in agreement with theirs: only one quarter reported the assault to the police compared with 35% of women, and 1 in 8 was examined by a forensic doctor compared with 22% of women. These male SAVs might have experienced a lower threshold to access the STI clinic compared with a specialised sexual assault clinic. Possibly, non-Dutch SAVs also experienced a lower threshold, because SAVs - compared with non-SAVs - were more often non-Dutch.

Both sub-Saharan African female and male victims reported a sexual assault more often. During the study period, a considerable group of asylum seekers from sub-Saharan regions affected by civil war and military unrest were living in the Netherlands. In these conflicts, rape was used as a weapon, which might explain the overrepresentation of this population in our study.[14] A US study among college students showed ethnic differences in the rate of reported sexual assaults.[15] African American and European American women reported similar rates and Asian American women reported lower rates of sexual assault.

In our study, the return rate for a follow-up visit was relatively high compared with the 48.8% among preadolescent and adolescent US SAVs.[8] This difference might be explained by the fact that SAVs in the United States study had to come for follow-up examinations and not for treatment. In other studies describing poor follow-up among recent SAVs, clients were treated presumptively, and a follow-up visit was indicated for repeat testing and examinations.[16-18] In our study, all clients received testing at the initial visit and only had to return to the clinic for treatment if indicated. Possibly, clients who were diagnosed as having an STI were more willing to return to the clinic than those

clients who had to return for additional testing. A different study population - SAV visiting a Dutch STI clinic - might also explain the difference in follow-up rates and treatment uptake. In addition, UK investigators in a study among female rape victims at a sexual assault clinic did not recommend presumptive treatment at initial evaluation.[12] Their arguments against presumptive treatment were a low incidence of STI among female SAVs, the lack of a simple antibiotic regime that can eradicate all bacterial STIs, and the hindrance of presumptive therapy in effective partner notification and treatment.

Compared with women seen at a Dutch sexual assault support centre, the SAVs in this study were relatively old (a mean of 24 years versus a median of 21 years), and this is probably due to the STI clinic policy of not providing services for children.[19] Compared with non-SAV STI clinic clients, SAVs were more often of non-Dutch origin and often reported STI-related symptoms. As a result, the included group of SAV might not be representative of other SAV study populations. In addition, some STI clinic clients may not have disclosed being assaulted.

For STI clinic policy, it is very important to assess whether SAVs are at risk for STI and should receive dedicated STI care. We found comparable to lower STI positivity rates among SAV clients versus non-SAV STI clinic clients; we also observed a high follow-up rate. These findings do not support the Centers for Disease Control and Prevention guideline to provide presumptive antimicrobial therapy targeted against gonorrhoea, chlamydia, and trichomoniasis at the initial evaluation.[2] This guideline is based on a high STI positivity rate in combination with low follow-up return rates among SAV clients.

Although this article shows that STIs are frequently identified among female and male SAV STI clinic clients, their rates do not exceed those found among non-SAV clients. Still, it is important that STI clinics offer SAV clients low-threshold, priority access. With specialised counselling and dedicated STI care, STI clinics can play an important role for this group. Although difficult to prove in practice, future research should focus on the fraction of STI attributable to sexual assaults. In line with antibiotic stewardship, STI clinics should consider treating only diagnosed bacterial STI with antibiotics among SAVs.

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Conflicts of Interest and Sources of Funding

None declared.

Contributors

M.v.R., L.v.K., M.S.v.d.L., and H.d.V designed the study protocol. M.v.R. was responsible for the data collection. M.v.R., L.v.K., and M.S.v.d.L. performed the statistical analyses, and M.v.R. and L.v.K. drafted the manuscript. All authors commented on draft versions, and all approved the final version.

Previously presented

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SUPPLEMENTARY TABLES

Supplementary table 1. Laboratory tests offered to STI clinic clients 2005-2016, Amsterdam, the Netherlands

STI (test)	Heterosexual men	MSM	Women
Chlamydia			
Urogenital (1,2)	▪ All	▪ All	▪ All
Anorectal (1,2,3)		▪ < May 2014: receptive anal sex ▪ >= May 2014: all MSM	▪ receptive anal sex ▪ >= May 2014: High risk women[a]
Oropharyngeal (2)		▪ >= January 2011: all MSM	▪ >= January 2011: High risk women[a] who reported receptive oral sex
Gonorrhoea			
Urogenital (1,2,4)	▪ <2012 all heterosexual men ▪ 2012-2014 at risk heterosexual men[b] ▪ >=2015 all heterosexual men	▪ All	▪ <2012 all women ▪ 2012-2014 at risk women[b] ▪ >=2015 all women
Anorectal (1,2,4)		▪ All	▪ Receptive anal sex[c] ▪ High risk women[a]
Oropharyngeal (1,2,4)		▪ All	▪ High risk women[a] who reported receptive oral sex
HIV (5,6,7)	▪ < 2007: on indication ▪ 2007-2011: all heterosexual men ▪ 2012-2016: at risk heterosexual men[b]	▪ < 2007: on indication ▪ 2007-2016: all MSM	▪ < 2007: on indication ▪ 2007-2011: all women ▪ 2012-2016: at risk women[b]
Syphilis (8,9)	▪ 2005-2011: all heterosexual men ▪ 2012-2016: at risk heterosexual men[b]	▪ 2005-2016: all MSM	▪ 2005-2011: all women ▪ 2012-2016: at risk women[b]
Hepatitis B[d] (10,11)	▪ April 2006 – May 2014: all heterosexual men (10,11) ▪ >= May 2014: commercial sex workers / non–Western-European and non-North-American ethnicity	▪ April 2006 – 2016: all MSM	▪ April 2006 – May 2014: all women ▪ >= May 2014: commercial sex workers / non–Western-European and non-North-American ethnicity

Abbreviations:
CT: *Chlamydia trachomatis*; NG: *Neisseria gonorrhoeae*; MSM: men who sex with men

[a]High risk women: women who were notified, reported symptoms, reported commercial sex work, reported receptive anal sex (until 2009), and/or Sub-Saharan African clients who were uninsured
[b]Reporting STI related symptoms, being notified of an STI, and/or a non–Western-European and non-North-American ethnicity or partners of people with these ethnicities. Until 2015, reporting 3 or more sex partners was also an indication to be classified as high-risk
[c]2012-2014: not to low risk women (women without any risk-factor from footnote b)
[d]clients were routinely screened for hepatitis B unless a client was known to be immune or having completed vaccination.

[1]< 2008 Cobas Amplicor (Roche, California, USA)
[2]>= 2008 Aptima CT and the CT/NG combo assays for the detection of rRNA (Hologic, Marlborough, MA, USA)
[3]>=2005, in MSM all anorectal mucosal, ulcer or bubo samples positive for CT were tested further with a *pmpH*-based in-house real-time PCR to discriminate between LGV and non-LGV genotypes
[4] In case of symptoms, being notified, sex work, or MSM, urogenital, anorectal (both until May 2014), and oropharyngeal NG (until 2008) were tested by culture (OXOID; CHOC, Wesel, Germany). Since May 2014 NG culture was only routinely performed in the case of a positive NAAT, to determine antimicrobial resistance.
[5]Rapid HIV test (Alere Determine HIV – 1/2 antibody test, Alere Inc., Waltham, MA, USA). Used in MSM, clients who reported STI related symptoms, clients who were notified, commercial sexworkers, until 2009 in women who reported receptive anal intercourse, until 2012 to uninsured Sub-Saharan African clients, and since 2012 to all Sub-Saharan African clients. Not used during outreach based consultations.
[6]Reactive or indeterminate samples were confirmed by HIV Ag/Ab combo test (until May 2013: AxSYM, Abbott Laboratories, Illinois, USA; from May 2013: LIAISON® XL Murex, Diasorin, Saluggia, Italy) and line immunoassay (Inno-Lia HIV I/II Score; Innogenetics, Ghent, Belgium). If these confirmations were indeterminate or negative, a Vidas P24-antigen test (Biomerieux, Marcy l'Etoile, France) was performed.
[7]Low-risk clients, outreach based consultations and since May 2014 MSM were screened with above (6) mentioned HIV Ag/Ab combo tests, and – if applicable – confirmatory tests.
[8]*Treponema pallidum* particle agglutination assay (until March 2013: Fujirebio, Tokyo, Japan) and from March 2013 with the Treponema Screen (LIAISON® XL, Diasorin, Saluggia, Italy).
[9]The Rapid Plasma Reagin (RPR) card test and the FTA-absorption test (Nosticon and Trepo-spot IF; Biomérieux, Marcy l'Etoile, France) were performed to diagnose, confirm, and classify the stage of syphilis infection.
[10]Anti-HBc test until June 2013: AxSYM; Abbott Laboratories; Illinois, USA; from June 2013: LIAISON® XL, Diasorin, Saluggia, Italy
[11]HBsAg (if Anti-HBc positive) until June 2013: AxSYM; Abbott Laboratories; Illinois, USA; from June 2013: LIAISON® XL, Diasorin, Saluggia, Italy.

Supplementary table 2. Univariable and multivariable logistic GEE analysis[1] of the associations of having a bacterial STI diagnosis[2] and being a victim of a sexual assault, demographics, sexual behaviour, and HIV status among 166,808 clinic visits from females attending the STI clinic in Amsterdam, the Netherlands, January 2005 - September 2016

	No bacterial STI	**Bacterial STI present**	**Univariable analysis**		**Multivariable analysis[3]**	
	n=147,450	**n=19,358**				
Variable	n (%)	n (%)	OR (95%CI)	p Value	aOR (95%CI)	p Value
Victim of sexual assault						
No	146,503 (88.4)	19,239 (11.6)	1	0.69	1	0.51
Yes	947 (88.8)	119 (11.2)	0.96 (0.80-1.16)		0.94 (0.77-1.13)	
Period[4]						
2005-2008	38,195 (89.2)	4609 (10.8)	1	<0.001		
2009-2012	45,957 (88.4)	6040 (11.6)	1.07 (1.03-1.12)			
2013-2016	63,298 (87.9)	8709 (12.1)	1.11 (1.06-1.15)			
Demographics						
Age in years[5]						
< 25	83,805 (85.8)	13,820 (14.2)	1	<0.001	1	<0.001
25 – 34	48,483 (91.3)	4614 (8.7)	0.59 (0.57-0.61)		0.53 (0.51-0.55)	
35 – 44	10,077 (94.3)	610 (5.7)	0.38 (0.35-0.41)		0.34 (0.31-0.37)	
45 – 54	4039 (94.4)	241 (5.6)	0.35 (0.31-0.41)		0.31 (0.27-0.36)	
≥ 55	1043 (93.5)	73 (6.5)	0.39 (0.28-0.53)		0.35 (0.25-0.48)	

Supplementary table 2. Continued

	No bacterial STI n=147,450	Bacterial STI present n=19,358	Univariable analysis		Multivariable analysis[3]	
Variable	n (%)	n (%)	OR (95%CI)	p Value	aOR (95%CI)	p Value
Ethnicity						
Dutch	101,124 (88.9)	12,573 (11.1)	1	<0.001	1	<0.001
East-European	8700 (89.4)	1030 (10.6)	1.02 (0.94-1.10)		1.13 (1.03-1.23)	
Turkish	729 (85.6)	123 (14.4)	1.36 (1.10-1.69)		1.39 (1.13-1.71)	
North-African	1924 (85.8)	319 (14.2)	1.34 (1.18-1.52)		1.39 (1.22-1.58)	
Sub-Saharan Africa	3223 (89.8)	365 (10.2)	0.90 (0.81-1.01)		0.96 (0.86-1.08)	
Antillean	2110 (83.3)	423 (16.7)	1.61 (1.44-1.81)		1.64 (1.46-1.84)	
Surinamese	11,307 (83.1)	2306 (16.9)	1.61 (1.53-1.70)		1.71 (1.62-1.81)	
South-American	4430 (89.9)	495 (10.1)	0.92 (0.83-1.02)		1.19 (1.07-1.32)	
Asian	4322 (87.3)	627 (12.7)	1.17 (1.07-1.28)		1.27 (1.16-1.39)	
Other/unknown	9581 (89.7)	1097 (10.3)	0.92 (0.86-0.99)		1.01 (0.94-1.08)	
Residence						
Amsterdam	112,569 (88.7)	14,330 (11.3)	1	<0.001	1	<0.001
Province of North-Holland	17,103 (87.0)	2545 (13.0)	1.15 (1.10-1.21)		1.09 (1.04-1.14)	
Elsewhere in the Netherlands	10,180 (87.7)	1428 (12.3)	1.11 (1.04-1.18)		1.05 (0.99-1.12)	
Other/unknown	7598 (87.8)	1055 (12.2)	1.10 (1.03-1.18)		1.09 (1.02-1.17)	
HIV status[6]						
HIV negative	122,525 (87.8)	16,990 (12.2)	1	<0.001	1	<0.001
HIV known positive	132 (86.8)	20 (13.2)	1.14 (0.68-1.91)		1.55 (0.90-2.69)	
HIV newly diagnosed	135 (93.1)	10 (6.9)	0.54 (0.29-1.01)		0.73 (0.39-1.39)	
HIV not tested	24,658 (91.3)	2338 (8.7)	0.67 (0.63-0.70)		0.57 (0.54-0.60)	

Supplementary table 2. Continued

	No bacterial STI n=147,450	Bacterial STI present n=19,358	Univariable analysis		Multivariable analysis[3]	
Variable	n (%)	n (%)	OR (95%CI)	p Value	aOR (95%CI)	p Value
Sexual behaviour in the preceding 6 months						
Number of sexual contacts						
0-1 sexual contacts	27,740 (88.3)	3684 (11.7)	1	<0.001	1	<0.001
2 sexual contacts	28,667 (87.6)	4052 (12.4)	1.08 (1.03-1.14)		1.09 (1.04-1.15)	
3-4 sexual contacts	29,809 (88.0)	4056 (12.0)	1.06 (1.01-1.12)		1.03 (0.98-1.09)	
>4 sexual contacts	22,844 (88.6)	2945 (11.4)	1.06 (1.00-1.12)		1.18 (1.11-1.26)	
No information[7]	38,390 (89.3)	4621 (10.7)	0.96 (0.91-1.00)		1.00 (0.96-1.05)	
Commercial sex work[8]						
No	133,744 (88.2)	17,955 (11.8)		<0.001	1	<0.001
Yes	13,652 (90.7)	1396 (9.3)	0.81 (0.76-0.87)		0.84 (0.77-0.91)	

Abbreviations:
95%CI: 95% confidence interval, aOR: adjusted odds ratio, GEE: generalized estimating equations, OR: odds ratio, STI: Sexually transmitted infection

[1]GEE analysis was performed to account for correlated data (repeated measures)
[2]Clients screened for bacterial STI (*Chlamydia trachomatis*, gonorrhoea, and/or infectious syphilis at time of current visit)
[3]N=166,744 consultations included. All variables in this table with a univariable p value < 0.25 were included in the multivariable model. The variable of interest, "being a victim of sexual assault", was forced in the multivariable model.
[4]Because of multicollinearity with "number of sexual partners", period was excluded from the multivariable model
[5]Missing for 3 consultations
[6]Before 2007 HIV was only tested on indication. From 2007 through 2011 all clients were offered an HIV test. Since 2012, young low-risk heterosexual women are not tested for HIV.
[7]Question about the number of sexual contacts in the preceding 6 months is part of the clinic protocol since 2009. Due to this information about number of sexual contacts is not available for 43,011 consultations.
[8]Missing for 61 consultations

Supplementary table 3. Univariable and multivariable logistic GEE analysis[1] of the associations of having a bacterial STI diagnosis[2] and being a victim of a sexual assault, demographics, sexual behaviour, and HIV status among 194,954 clinic visits from males attending the STI clinic in Amsterdam, the Netherlands, January 2005 - September 2016

	No bacterial STI n=160,407	**Bacterial STI present n=34,547**	**Univariable analysis**		**Multivariable analysis[3]**	
Variable	n (%)	n (%)	OR (95%CI)	p Value	aOR (95%CI)	p Value
Victim of sexual assault						
No	160,289 (82.3)	34,530 (17.7)	1	0.190	1	0.043
Yes	118 (87.4)	17 (12.6)	0.70 (0.41-1.19)		0.60 (0.36-0.98)	
Period[4]						
2005-2008	46,964 (84.2)	8823 (15.8)	1	<0.001		
2009-2012	53,832 (82.2)	11,662 (17.8)	1.07 (1.04-1.11)			
2013-2016	59,611 (80.9)	14,062 (19.1)	1.14 (1.10-1.18)			
Demographics						
Age in years[5]						
< 25	41,933 (81.6)	9429 (18.4)	1	<0.001	1	<0.001
25 – 34	58,465 (82.7)	12,206 (17.3)	0.89 (0.86-0.92)		0.80 (0.77-0.82)	
35 – 44	33,163 (81.5)	7548 (18.5)	0.88 (0.85-0.92)		0.63 (0.60-0.66)	
45 – 54	18,720 (81.8)	4158 (18.2)	0.84 (0.79-0.88)		0.51 (0.48-0.53)	
≥ 55	8122 (87.1)	1205 (12.9)	0.61 (0.56-0.66)		0.38 (0.35-0.42)	
Ethnicity						
Dutch	102,783 (83.4)	20,475 (16.6)	1	<0.001	1	<0.001
East-European	3756 (81.0)	883 (19.0)	1.25 (1.14-1.36)		1.11 (1.02-1.21)	
Turkish	2585 (84.6)	470 (15.4)	0.97 (0.86-1.09)		1.09 (0.97-1.22)	
North-African	4388 (83.7)	854 (16.3)	1.09 (1.00-1.18)		1.28 (1.17-1.39)	
Sub-Saharan Africa	4361 (84.2)	818 (15.8)	0.95 (0.87-1.04)		1.18 (1.08-1.29)	
Antillean	2946 (75.1)	975 (24.9)	1.68 (1.54-1.84)		1.69 (1.54-1.85)	

Supplementary table 3. Continued

Variable	No bacterial STI n=160,407 n (%)	Bacterial STI present n=34,547 n (%)	Univariable analysis OR (95%CI)	p Value	Multivariable analysis[3] aOR (95%CI)	p Value
Ethnicity						
Surinamese	12,683 (77.4)	3695 (22.6)	1.60 (1.52-1.68)		1.88 (1.79-1.97)	
South-American	5203 (78.1)	1462 (21.9)	1.43 (1.33-1.55)		1.12 (1.04-1.20)	
Asian	5698 (80.9)	1349 (19.1)	1.17 (1.08-1.26)		1.05 (0.98-1.13)	
Other/unknown	16,004 (81.8)	3566 (18.2)	1.13 (1.08-1.19)		1.00 (0.95-1.05)	
Residence						
Amsterdam	121,756 (82.0)	26813 (18.0)	1	<0.001	1	0.049
Province of North-Holland	17,521 (83.9)	3372 (16.1)	0.90 (0.86-0.94)		1.00 (0.95-1.04)	
Elsewhere in the Netherlands	11,831 (81.6)	2672 (18.4)	1.04 (0.99-1.10)		1.07 (1.02-1.13)	
Other/unknown	9299 (84.6)	1690 (15.4)	0.91 (0.86-0.96)		1.03 (0.98-1.09)	
HIV status[6]						
HIV negative	130,957 (84.4)	24,143 (15.6)	1	<0.001	1	<0.001
HIV known positive	13,669 (67.0)	6736 (33.0)	2.53 (2.42-2.64)		2.07 (1.97-2.17)	
HIV newly diagnosed	856 (60.5)	559 (39.5)	3.17 (2.83-3.54)		2.50 (2.24-2.81)	
HIV not tested	14,925 (82.8)	3109 (17.2)	1.14 (1.09-1.19)		1.18 (1.13-1.23)	
Sexual behaviour in the preceding 6 months						
Sex of sexual partner(s)						
Female	96,571 (86.5)	15,091 (13.5)	1	<0.001	1	<0.001
Male	57,062 (75.8)	18,243 (24.2)	2.00 (1.94-2.06)		1.95 (1.88-2.02)	
Both male and female	6774 (84.8)	1213 (15.2)	1.20 (1.12-1.28)		1.27 (1.18-1.36)	

Supplementary table 3. Continued

Variable	No bacterial STI n=160,407 n (%)	Bacterial STI present n=34,547 n (%)	Univariable analysis OR (95%CI)	p Value	Multivariable analysis[3] aOR (95%CI)	p Value
Number of sexual contacts						
0-2 sexual contacts	34,142 (85.3)	5865 (14.7)	1	<0.001	1	<0.001
3-4 sexual contacts	29,306 (83.1)	5977 (16.9)	1.19 (1.14-1.24)		1.15 (1.10-1.20)	
5-8 sexual contacts	25,373 (81.6)	5732 (18.4)	1.30 (1.25-1.36)		1.22 (1.17-1.28)	
>8 sexual contacts	24,477 (75.1)	8128 (24.9)	1.78 (1.70-1.86)		1.54 (1.47-1.61)	
No information[7]	47,109 (84.2)	8845 (15.8)	1.14 (1.10-1.19)		1.15 (1.11-1.19)	
Commercial sex work[8]						
No	158,083 (82.3)	33,983 (17.7)	1	0.001	1	<0.001
Yes	1985 (80.1)	493 (19.9)	1.23 (1.09-1.39)		0.80 (0.71-0.90)	
Paying for sex[9]						
No	149,528 (81.7)	33,551 (18.3)	1	<0.001	1	<0.001
Yes	10,540 (92.0)	921 (8.0)	0.47 (0.44-0.50)		0.56 (0.52-0.60)	

Abbreviations:
95%CI: 95% confidence interval, aOR: adjusted odds ratio, GEE: generalized estimating equations, OR: odds ratio, STI: Sexually transmitted infection

[1]GEE analysis was performed to account for correlated data (repeated measures)
[2]Clients screened for bacterial STI (*Chlamydia trachomatis*, gonorrhoea, and/or infectious syphilis at time of current visit)
[3]N=194,505 consultations included. All variables in this table with a univariable p value < 0.25 were included in the multivariable model. The variable of interest, "being a victim of sexual assault", was forced in the multivariable model.
[4]Because of multicollinearity with "number of sexual partners", period was excluded from the multivariable model
[5]Missing for 5 consultations
[6]Before 2007 HIV was only tested on indication. Between 2007 and 2012 all clients were offered an HIV test. Since 2012, HIV is not tested in young low-risk heterosexual men.
[7]Question about the number of sexual contacts in the preceding 6 months is part of the clinic protocol since 2009. Due to this information about number of sexual contacts is not available for 55,954 consultations.
[8]Missing for 410 consultations
[9]Missing for 414 consultations

CHAPTER 5

Public health interventions

CHAPTER 5.1

Young low-risk heterosexual clients prefer a chlamydia home collection test to a sexually transmitted infection clinic visit in Amsterdam, the Netherlands, a cross-sectional study.

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ABSTRACT

Background

Home-based self-collection of specimens for urogenital and anorectal chlamydia testing has been proven feasible and acceptable. We studied the efficiency of chlamydia home collection kits for young low-risk persons to optimise care at the Amsterdam sexually transmitted infection (STI) clinic.

Methods

Low-risk heterosexual persons under 25 years submitting an appointment request online were offered 3 different ways of chlamydia testing: (1) receiving a home collection kit, (2) coming to the clinic without, or (3) with sexual health counselling. The collection kit was sent to the client by surface mail and was used to self-collect a vaginal swab or urine sample (men). This sample was sent back to the laboratory for testing and the results could be retrieved online. Testing for gonorrhoea, syphilis, and human immunodeficiency virus was indicated after testing chlamydia-positive.

Results

Between September 2012 until July 2013, from 1804 online requests, 1451 (80%) opted for the home collection kit, 321 (18%) preferred an appointment at the clinic without, and 32 (2%) with sexual health counselling. Of the requested home collection kits, 88% were returned. Chlamydia was diagnosed in 6.0% of the clients receiving a home collection kit, and none of the chlamydia-positive clients tested positive for other STI.

Conclusions

Home collection is the preferred method for most young low-risk heterosexual clients who seek STI care. With a high compliance to collect and return the samples, home collection can be used as a tool to increase efficiency and dedicate STI clinic workers efforts to those at highest risk.

INTRODUCTION

Chlamydia trachomatis (CT) is the most commonly reported bacterial sexually transmitted infection (STI) in the Netherlands.[1] Because STI transmission is uniquely linked to human behaviour, its control depends on the identification of important risk groups and their risk behaviours associated with STI transmission.[2] To control chlamydia in the Netherlands, enhanced chlamydia screening was set up as a pilot project (Chlamydia Screenings Initiative) from 2008 to 2011 among the general population aged 16 to 29 years. This strategy, using home-collected samples, did not demonstrate statistical evidence of an impact on chlamydia positivity rates or estimated population prevalence and was therefore not implemented nationwide.[3]

A meta-analysis showed that, compared with swabs collected by clinicians, the sensitivity and specificity of self-collected urine, vaginal, and rectal swabs were high.[4] Most publications on STI tests with specimen collection at home are focused on actively invited participants.[5-7] These studies showed that the testing rate with home-based screening was greater than with clinic-based screening and home screeners rated testing as more convenient.[5] Home testing might overcome several barriers to get screened, including individual factors, such as privacy, embarrassment or discomfort, and access barriers, such as clinic inaccessibility and the lack of time or financial resources needed to attend appointments.[8]

At present, general practitioners and STI clinics in the Netherlands mainly provide chlamydia testing and treatment. In 2013, an estimation showed that about 40% of the chlamydia tests in Amsterdam were done by general practitioners, and 50% were provided by the STI clinic (van Rooijen et al., unpublished data). The STI clinics offer basic care targeted at high risk populations and are subsidised by the ministry of health.[9]

The STI clinic of the Public Health Service of Amsterdam (an outpatient clinic) performs around 40,000 free and, if desired, anonymous STI consultations. To select high-risk clients, the STI clinic works with prebooked appointments. These can be made either by telephone (40% of cases) or online (60%). Until 2012, the ministry of health ordered that all clients had to be screened for chlamydia, gonorrhoea, syphilis, and human immunodeficiency virus (HIV) using an opt-out strategy.[10] Since 2012, the testing policy is restricted to chlamydia only for low-risk clients (i.e., asymptomatic heterosexual youngsters <25).

To amend this testing policy adaption and to further increase the STI testing capacity of our STI clinic, the home collection method was introduced in September 2012. During the online request for an STI consultation, home collection is offered to young heterosexual clients with an indication to test for chlamydia only.

No literature is available about offering home collection to adolescents and young adults who want to get tested at an STI clinic. In this study, we assessed clients' preference for home collection kits or a face-to-face consultation at the clinic when seeking STI care. Diagnosis and treatment outcomes of home collectors and clinic clients were evaluated.

MATERIALS AND METHODS

Study setting

During the online appointment request, all clients were asked about the following indicators: commercial sex work, paying for sex, 3 or more sexual partners, male-to-male sex contacts, STI related complaints, STI notification, and non–Western-European or North-American ethnicity. All behaviour indicators were reported over the previous 6 months. Clients under the age of 25 years were assigned to a low-risk group if they did not report any risk factors as mentioned above. Low-risk clients were routinely tested for chlamydia, whereas high-risk clients were tested for chlamydia, gonorrhoea, syphilis, hepatitis B,[11] and also for HIV using an opt-out strategy.[10] After testing chlamydia-positive, low-risk patients had to come to the clinic and were additionally tested for gonorrhoea, syphilis, hepatitis B, and HIV.

Before September 2012, low-risk clients could only apply for a clinic visit which took about 15 minutes of consultation time for the nurse. From September 2012 onward, low-risk clients with a minimum age of 16 years who requested online an appointment at our clinic were offered 3 different ways of testing: (1) receive a home collection kit, (2) come to the clinic for specimen collection to test for chlamydia, or (3) come to the clinic for both a chlamydia test and sexual health counselling.

Home collection

In maximally 5 minutes, an administrative assistant prepared the self-composed home collection package (neutral envelope without any reference of the sending institution including sampling instructions and Aptima Collection Kit; Hologic, San Diego,

California, USA) and sent this to the client by mail. For men, a urine sample, and for women, a vaginal swab was collected. For women who reported anal sex contact in the previous 6 months, next to a vaginal swab, an anorectal swab was collected. Samples were returned to the laboratory by reply paid mail. If kits were not returned within 6 weeks (no reminders were sent), a new one had to be requested.

Clinic visit for chlamydia test (clinic testers)

Patients, who chose to visit the clinic for chlamydia testing only, were called back by an administrative employee, where after an appointment was booked. If no appointments were available for 10 working days onward, a standard rejection email informing the patient to retry at a later moment or to go to their general practitioner was sent without being called. Clients with an appointment were seen by a trained nurse or medical assistant who, after registration, a short medical history, and verbal sample instruction, provided collection material. Samples were self-collected at the toilet of the STI clinic. The specimens and sample instructions were the same as home collection.

Clinic visit for chlamydia test including sexual health counselling (clinic+ testers)

The routine described for clinic clients above also applied for clients who requested a clinic visit combined with sexual health counselling. Next to routine STI testing, clients were counselled in sexual health (e.g., contraception advice, (un)planned pregnancy or other sexuality issues) by a trained nurse or doctor.

Laboratory testing

Chlamydia was tested by means of the APTIMA CT assay (Hologic). Clients with an invalid test result (e.g., no or low volume of preservative fluid, no urine or swab) were called to come to the clinic to provide new samples.

Diagnosis and treatment

Approximately 1 week after receiving collected materials at the laboratory, definitive results were available. All clients could retrieve the test results online by using the provided personal login (included in sent collection kit or given during consultation) or by contacting the clinic by phone. Patients who needed treatment were invited to come to the clinic at the earliest for a follow-up visit and treatment. In case of urogenital chlamydia, a single dose of azithromycin 1000 mg was offered. In case of anorectal chlamydia in women who have used contraception (irrespective of condom use), doxycycline (100 mg twice daily 1 tablet for a minimum of 7 days) was offered. In

women with anorectal chlamydia at risk of being pregnant, a single dose of azithromycin 1000 mg including a test-of-cure visit after 3 weeks was offered. A nurse contacted chlamydia-positive patients who did not retrieve their results or treatment within 2 weeks (by email or phone). At the moment of treatment, partner notification was discussed. Partners could be notified by the case (with or without contact slip) or by a nurse. Partners notified with a contact slip or by a nurse were receiving presumptive treatment at our clinic.

Data collection

The following data were registered in an in-house developed appointment database: date of appointment request, preferred method of sample collection, date of sending the home collection kit, date of appointment at the clinic, age, postal code (home collectors only), country of birth, and number of sexual contacts in the previous 6 months. All clients with a home collection kit or a clinic visit at the STI clinic were also manually registered in our electronic patient file (EPF). Routinely collected data (e.g., postal codes, history of HIV, laboratory results, diagnoses, and treatment) were registered in this EPF. For this study, routinely collected records from the in-house developed database and EPF were linked and anonymized. Because all interventions were routine procedures, ethical approval was deemed unnecessary for this study.

Statistical analysis

All statistical analyses were performed using SPSS version 21 (SPSS Inc., Chicago, Ill) and STATA Intercooled 11.0 (STATA, College Station, Tex). Fisher exact test and $\chi 2$ test were used to compare categorical variables between groups; the Mann-Whitney *U* test was used to compare continuous variables between groups. p Values less than 0.05 were considered statistically significant.

Ethnicity was defined based on criteria of Statistics Netherlands (CBS) and was categorised in Dutch, European and other/unknown.[12] The HIV status was self-reported. A chlamydia diagnosis was defined as having a urogenital and/or anorectal chlamydia-positive nucleic acid amplification test. Time between sending and returning home collection specimen was calculated in days. Geographical distance (great circle distance) between clients' postal code and the STI clinic was calculated using the corresponding latitude and longitude coordinates obtained from Google Maps.[13 14]

In case of a duplicate request for an appointment (sent within 24 hours, preferring the same collection method, and from the same email address), only the first request was included. Clients, who first sent a request for a clinic appointment and, after receiving a rejection email, within 24 hours requested for a home collection kit, were excluded from the main analysis.

RESULTS

In the period from September 2012 until July 2013, we offered the option of home-based sample collection to 1878 clients with an online request for STI consultation (figure 1). We excluded 32 duplicates (multiple identical requests within 24 hours), and 42 requests from 20 clients who within 24 hours applied for both a home and clinic appointment.

Of 1804 unique requests, most opted for home collection (1451; 80.4%); 321 clients (17.8%) preferred to visit the clinic for chlamydia testing only, and 32 clients (1.8%) opted to visit the clinic for both chlamydia testing and sexual health counselling. In the study period, 97 clients had multiple requests for a home-based test; 93 clients requested twice and 4 requested 3 times.

In total, no linkage between appointment request and EPF data could be made for 132 (7.3%) consultation requests (14 (1.0%) home collectors, 108 (33.6%) clinic, and 10 (31.3%) clinic+ testers). The main reason of the missing linkage was that these requests did not result in an appointment because of invalid phone number (n=1), not answering the phone (n=40), or fully booked appointments (n=26). One patient whose home collection swab was lost during logistics at the laboratory, and 4 clients who showed up at their clinic appointment but were not tested for chlamydia as no risk for contracting STI was reported, were excluded from the following analysis.

Characteristics of the study population

Compared with clinic (67.9%) and clinic+ (59.1%) testers, home collectors were more often women (83.4%; p<0.001) (table 1). Home collectors had a higher median age of 22 years (interquartile range, 21–23 years) versus 21 years (20–22 years) in both clinic and clinic+ testers (p<0.001). Categorised in quartiles, clinic (36.8%) and clinic+ (36.4%) testers were more often from the youngest age category compared with home collectors (23.1%; p<0.001). In all 3 groups, more than 90% was Dutch (p=0.23). The

postal code was known for 97.0% of clients, and the proportion of clients who were living in Amsterdam was not statistically different between home collectors, clinic, and clinic+ testers (p=0.79). The median distance between clients' postal code and the STI clinic was 3.6 km (interquartile range, 2.0–7.5) and was not different between the 3 groups (p=0.51). When comparing home collectors versus all clinic testers, home collectors less often reported recent unprotected sex (46.1% vs 54.1%, p=0.024), and female home collectors reported more often anal sex (11.3% vs 5.7%, p=0.042).

Returned swabs and no show

Of clients opting for a home collection kit, 1262 (87.8%) returned their sample(s), most within 1 week (62.6%; figure 2). This return rate was not significantly different from the proportion that showed up at their appointment at the STI clinic (clinic: 86.1% and clinic+: 95.5%; p=0.45).

Chlamydia test results

The chlamydia positivity was 6.0% among home collectors (75/1258; 95% confidence interval [CI], 4.7–7.4), 5.6% among clinic testers (10/180; 95% CI, 2.7–10.0), and 4.8% among clinic+ testers (1/21; 95% CI, 0.1–23.8) (p=0.95; figure 1 and table 1). Although not significant, the overall urogenital chlamydia positivity in men (3.2%; 95% CI, 1.4–6.2) was lower than that in women (6.2%; 95% CI, 4.9–7.7, p=0.061). The urogenital nucleic acid amplification test was initially invalid in 6 (0.5%) home collectors, and in 3 (1.7%) clinic testers. Of those, 2 of 6 home collectors and all 3 clinic clients came to the clinic for repeated swab collection.

Among 115 female home collectors who returned samples and reported anal sex in the previous 6 months, 100 (87.0%) returned an anorectal swab of which one was invalid. Of 99 tested, 7 (7.1%; 95% CI, 2.9–14.0) had an anorectal chlamydia infection, of which 5 had a concurrent urogenital chlamydia infection (table 1). Of 7 clinic testers and 1 clinic+ tester who reported receptive anal sex, 1 clinic tester (14.3%; 95% CI, 0.4–57.9) was both urogenital and anorectal chlamydia-positive.

Obtaining results and receiving treatment

Of the chlamydia-negative clients, 5.3% of the home collectors, 1.8% of the clinic, and 0% of the clinic+ testers did not retrieve their diagnosis (p=0.084). All 86 chlamydia-positive patients obtained their results: 82 (95.3%) online and 4 (4.7%) by phone. Sixty-one (81.3%) of 75 chlamydia-positive home collectors were treated at our clinic;

6 (8.0%) were treated at their general practitioner or specialist; in 2 (2.7%) patients, a prescription was sent to their pharmacy; and for 6 (8.0%) patients, it is unknown whether they received treatment. All chlamydia-positive clinic testers and clinic+ testers were treated at our clinic. During chlamydia treatment, additional STI screening was performed in 53 (86.9%) home collectors, 10 (100%) clinic testers, and 1 (100%) clinic+ tester; none were positive for gonorrhoea, hepatitis B, syphilis, or HIV.

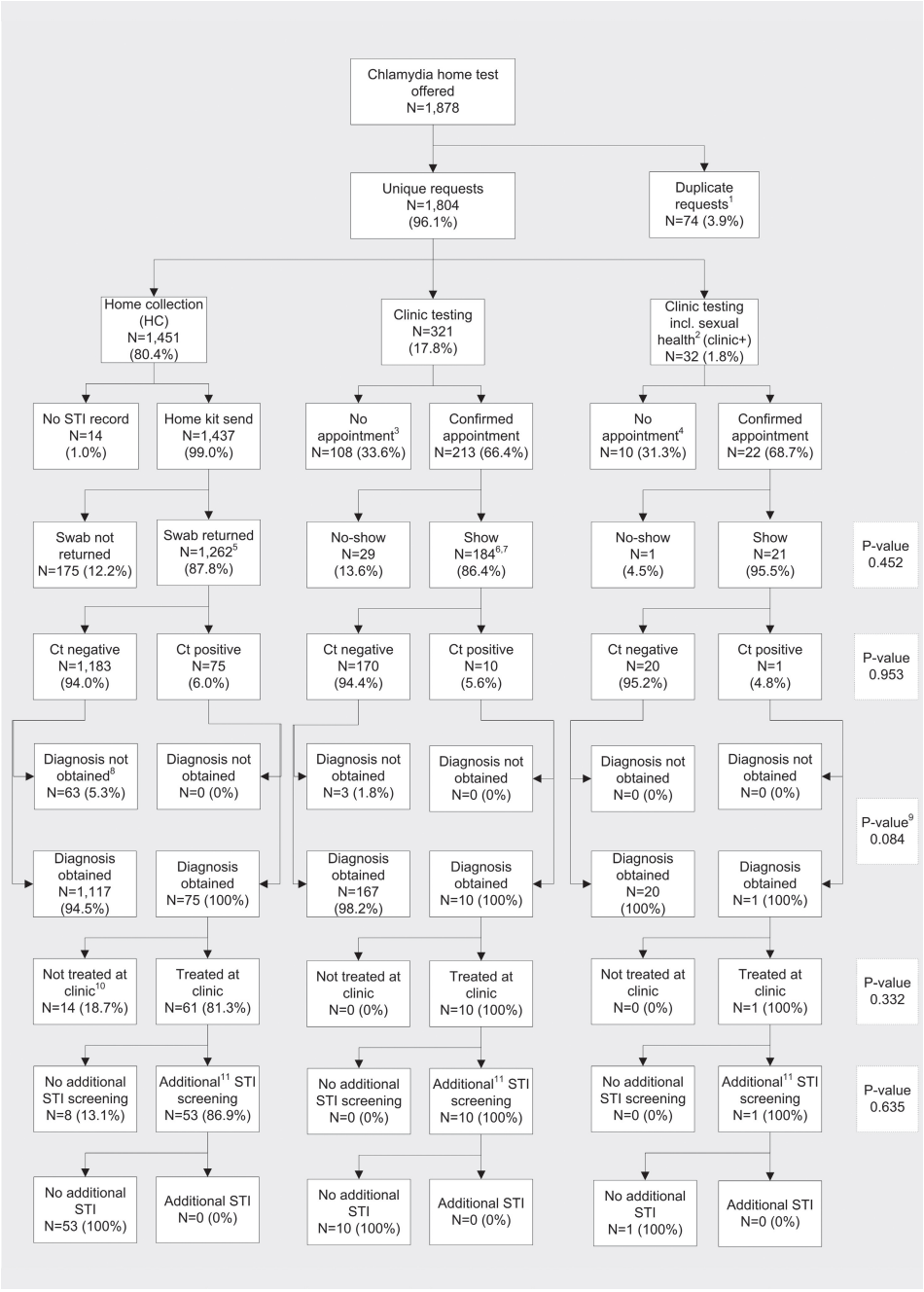

Figure 1. Flow chart of online appointment requests and testing preference at the STI clinic, September 2012 until July 2013, the Netherlands.

Abbreviations
Clinic+: clinic testing including sexual health consultation; HC: home collection; incl.: including.

[1]32 duplicate requests within 24 hours and 42 requests from clients who both requested for a home and clinic appointment within 24 hours.
[2]STI screening combined with consultation for sexual health by a trained nurse or doctor
[3]No appointment because of invalid or not reachable phone number (n=40), fully booked appointments (n=21), and unknown reason (n=47).
[4]No appointment because of invalid or not reachable phone number (n=1), fully booked appointments (n=5), and unknown reason (n=4).
[5]Initially 6 swabs were invalid; 2 of these clients came to the clinic to collect a new swab (both chlamydia negative). One received swab was lost during logistics.
[6]Initially 3 swabs were invalid; all clients came to the clinic to collect a new swab (all chlamydia negative).
[7]Because of no risk of contracting STI 4 clients who showed up at their STI consultation did not receive an STI check.
[8]From 3 clients, it was unknown whether they have received their definitive diagnosis.
[9]p Value calculated for proportion who obtained their diagnosis in chlamydia negative clients. All chlamydia-positive patients obtained their diagnosis.
[10]Six patients were treated at their general practitioner or specialist, in 2 cases a prescription was sent to local pharmacy, in 6 cases it was unknown whether treatment was given.
[11]Additional STI screening consisted of gonorrhoea, hepatitis B, syphilis and HIV.

Table 1. Baseline characteristics, sexual behaviour and chlamydia prevalence of clients who request online for an STI appointment, September 2012 until July 2013, the Netherlands[1]

Total		Home collection n=1,436	Clinic[2] n=209	Clinic+[3] n=22	
		n (%)	n (%)	n (%)	p Value
Demographics					
Sex	Male	238 (16.6)	67 (32.1)	9 (40.9)	<0.001
	Female	1,198 (83.4)	142 (67.9)	13 (59.1)	
Age in years (median, IQR)		22 (21- 23)	21 (20-22)	21 (20-22)	<0.001
Age in years (Quartiles)					
16-20.4		332 (23.1)	77 (36.8)	8 (36.4)	<0.001
20.5-21.8		345 (24.0)	59 (28.2)	7 (31.8)	
21.9-23.3		379 (26.4)	39 (18.7)	4 (18.2)	
23.4-24.9		380 (26.5)	34 (16.3)	3 (13.6)	
Ethnicity	Dutch	1,343 (93.5)	200 (95.7)	20 (90.9)	0.227
	European	74 (5.2)	5 (2.4)	2 (9.1)	
	Other/unknown	19 (1.3)	4 (1.9)	0 (0)	
Living in Amsterdam[4,5]	No	411 (28.6)	49 (29.9)	4 (22.2)	0.785
	Yes	1,025 (71.4)	115 (70.1)	14 (77.8)	
Distance from STI clinic in kilometres (median, IQR)[4,5]		3.6 (2.1-7.6)	3.7 (1.8-8.0)	3.1 (2.0-4.9)	0.506
History of HIV and STI testing					
Previously tested for HIV[4]	No	923 (64.3)	119 (56.9)	13 (59.1)	0.906[6]
	Yes	500 (34.8)	61 (29.2)	8 (36.4)	
	Unknown	13 (0.9)	0 (0)	0 (0)	
	Missing	0 (0)	29 (13.9)	1 (4.5)	
Previous visit at our clinic (self-reported)	No	1,111 (77.4)	153 (73.2)	19 (86.4)	0.235
	Yes	325 (22.6)	56 (26.8)	3 (13.6)	

Table 1. Continued

Total		Home collection n=1,436	Clinic[2] n=209	Clinic+[3] n=22	
		n (%)	n (%)	n (%)	p Value
Sexual behaviour					
Number of sexual partners in previous 6 months	1	636 (44.3)	85 (40.7)	6 (27.3)	0.183
	2	800 (55.7)	124 (59.3)	16 (72.7)	
Unprotected sex previous 7 days	No	774 (53.9)	97 (46.4)	9 (40.9)	0.068/0.024[7]
	Yes	662 (46.1)	112 (53.6)	13 (59.1)	
Reporting anal sex (female participants) in previous 6 months[4,8]		135/1,198 (11.3)	7/128 (5.5)	1/13 (7.7)	0.113/0.042[9]
New STI diagnosis at current visit					
Urogenital chlamydia[10]		75/1,258 (6.0)	10/180 (5.6)	1/21 (4.8)	0.953
Anorectal chlamydia[11]		7/99 (7.1)	1/7 (14.3)	0/1 (0)	0.475

Abbreviations:
CT: *Chlamydia trachomatis*; IQR: interquartile range; STI: sexually transmitted infection

[1]Appointment requests without linkage to the electronic patient file (14 home collectors, 108 clinic and 10 clinic+ testers) are not included in this table.
[2]Clinic visit for a chlamydia test only.
[3]Clinic visit for a chlamydia test combined with sexual health counselling.
[4]This item is requested online for home collectors and during STI consultation for all clinic testers.
[5]Known for 1,436 (100%) home testers, 164 (78.5%) clinic testers, and 18 (81.8%) clinic+ testers.
[6]Previous HIV-tested versus those with no previous HIV test.
[7]P=0.024 when comparing home with all clinic testers.
[8]Anal sex variable known for all female home and clinic+ testers; missing for 14 female clinic testers who did not show up at their appointment.
[9]P=0.042 when comparing home with all clinic testers.
[10]Known for 1,258 (87.6%) home testers, 180 (86.1%) clinic testers, and 21 (95.5%) clinic+ testers who returned their home collection kit or attended their STI clinic appointment and had a valid test result.
[11]Known for 99 (73.3%) female home testers, 7 (100%) female clinic testers, and 1 (100%) female clinic+ tester who reported anal sex and returned their home collection kit or attended their STI clinic appointment and had a valid test result.

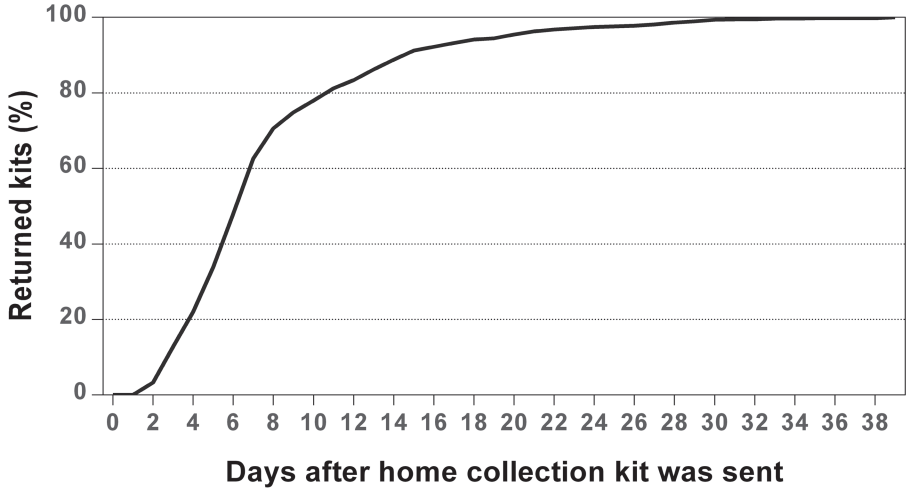

Figure 2. Time between sending the home collection kit and receiving the mailed self-taken specimens for chlamydia testing at the STI clinic, September 2012 until July 2013, the Netherlands. Only the n=1263 returned home collection kits were included in this figure.

DISCUSSION

Most young women and men with low-risk of STI - females more than males - prefer to collect samples for chlamydia testing at home. Compliance to collect and return the samples is high (87.8%) and is comparable to the proportion of clients attending their clinic appointment. Compared with the general STI clinic population, urogenital chlamydia positivity is relatively low among these low-risk clients (overall 5.7%). Among clients with a chlamydia diagnosis, no infection with gonorrhoea, syphilis, hepatitis B, and HIV was found during additional STI screening.

Many screenings programs had disappointing participation rates and relatively low chlamydia prevalences were found.[3 15] In this study, compliance in home collectors to return samples was high, and the chlamydia positivity was comparable to the positivity among those who opted to visit the clinic. Three studies in which home collection kits were actively requested by the client through the Internet or by phone - as in our study - testing rates were lower (between 31.1% and 62.5%), and chlamydia positivity was comparable or higher (between 5.2% and 12.8%).[16-18] Moreover, our study results are in line with a review concluding that home-based STI screening is feasible, well accepted, and for many patients, the preferred mode to test for STIs compared with testing at

a traditional clinic venue.[7] Next to the use of home collection kits in clients actively requesting for an STI test, the use of home kits to facilitate retesting in chlamydia-positive cases resulted in substantial improvements in chlamydia retesting rates.[19] An observational study from the United States among women from a contraception study showed that 75.7% chose to screen for STI at home.[20] This is comparable to our finding that most young women prefer to screen for STI at home. Interestingly, clinic testers were younger than home collectors. This might be due to the limitation to receive the package at home.

A randomised controlled trial in young women in the United States showed that offering home collection to increase STI testing was most effective in women who did not routinely use clinical care.[8] Because we only offered home collection to clients who actively searched for STI care, we cannot determine whether home collection lowered the barrier for STI testing. We assume that most clients who requested an STI consultation and chose to collect at home would otherwise have visited our clinic. An STI screening using home collection was not actively advertised but our appointment website included text about the possibility of home collection. Word-of-mouth dissemination and earlier experience with home collection might have encouraged clients to seek care who otherwise would not have performed an STI test.

Testing for chlamydia using home collection can save both direct and indirect costs.[21] A chlamydia retest study showed that the home test pathway was cheaper than the clinic-based pathway.[22] However, about 12% of the collection kits were not returned. In comparison, in clients who opted for a clinic appointment, approximately 13% did not show up. A cost-effectiveness study using local costs could be performed to assess the added value of implementing home collection at an STI clinic.

Only a small number (0.5%) of self-taken swabs from home collectors had an invalid test result possibly caused by incorrect sampling. We therefore assumed that the sample instructions were comprehensible. Although a preference for urine collection above a self-administered vaginal swab has been shown in adolescent women,[23] we do not consider urine collection in women suitable because first-void urine has shown to be less sensitive than self-collected vaginal swabs.[24]

A relatively large number (13%) of female home collectors did not return their anorectal swab. This might be explained by the finding that home collectors more often reported

anal sex than women who came to the clinic, or by a barrier to collect anorectal swabs. Possibly, home collectors wrongly interpreted the question about anal sex. The possibility of a barrier would be in contrast to a previous clinic-based study where no barrier to self-collection of anorectal swabs was found.[25]

Although the geographical distance from the clinic did not differ between home collectors and clinic clients, less home collectors were eventually treated at our clinic. Offering the option of treatment closer to their home address, for example, at the general practitioner or pharmacy, might improve the therapy uptake in home-based testers.

Based on the results from this study, we have continued home-based chlamydia testing for low-risk heterosexual clients at our clinic. Free home-based testing might result in an increasing demand from "worried well" clients and clients who repeatedly request tests even in the absence of risk behaviour. To prevent this, the number of repetitive requests for home screening, and the chlamydia positivity will be monitored. In addition, we might develop online sexual health counselling. This will give clients who opted to come to the clinic for both chlamydia testing and a sexual health consultation, the opportunity to perform both at home.

A limitation of this study was that - because of limited time slots and unreachable - clients about one third (n=118) of all clinic requests did not result in an appointment, and they were excluded from the analysis. Those who initially preferred to come to the clinic might have changed their preference and chosen to collect at home. Although this will have caused an overestimate of the proportion with preference for home collection, the absolute number of identified clients with both a clinic and a home collection request was low. Clients might misuse the system by ordering several home test kits using different email addresses and phone numbers. Other limitations were that we did not have a control group in which no home collection was offered and the selected group of young low-risk clients might be difficult to generalise to other health care settings.

In conclusion, home collection is the preferred method for most young, low-risk clients who seek STI care. With a high compliance to collect and return the samples, home collection can be used as a tool to increase efficiency and dedicate STI clinic workers efforts to those at highest risk.

Conflicts of interest and source of funding
None declared.

Contributors
M.v.R., R.K., and M.v.V. designed the study protocol, supported by P.v.L. and A.H. R.K. and A.H. were responsible for implementation at the STI clinic. M.v.R. did the data management. M.v.R. completed the data analysis supported by R.K. and M.v.V. M.v.R., R.K., and M.v.V. drafted the article. All authors commented on draft versions, and all approved the final version.

Ethics approval
Since all interventions were routine procedures, ethical approval was deemed unnecessary for this study.

Previously presented
Information from this paper has been orally presented at the STI & AIDS World Congress (17 July 2013, Vienna, Austria; abstract number O09.5).

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5.1

CHAPTER 5.2

Initial evaluation of use of an online partner notification tool for STI, called 'suggest a test': a cross sectional pilot study.

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Sexually Transmitted Infections 2014 May;90(3).
Erratum in: Sexually Transmitted Infections 2015 Feb;91(1):74.

ABSTRACT

Objectives

Partner notification is crucial for sexually transmitted infection (STI) control. We developed suggestatest.nl (SAT), an Internet-based notification system for verified diagnoses of STI/HIV.

Methods

SAT uses email, short message service, postal letter or a gay dating site to notify sexual contacts. SAT was piloted at the Public Health STI clinics in two major cities in the Netherlands. We evaluated SAT from March to July 2012 by analysing SAT notifications linked with epidemiological data. Determinants for SAT use were assessed using multivariable logistic regression analysis.

Results

Of 988 index clients receiving a SAT code, overall 139 (14%) notified through SAT, sending 505 notifications (median 2), 84% by text messaging and 15% by email; 88% anonymously. Of those intending to use SAT, 23% notified with SAT. Intention to use SAT was the only independent determinant of SAT use in heterosexuals and men who have sex with men. Among the 67 SAT users in Rotterdam, 56% (225/402) of their partners at risk were contactable, and 95% (213/225) of those were notified using SAT. 58% of SAT-notified partners accessed the SAT-website and 20% of them subsequently consulted the STI clinics. STI positivity in partners was lower in those notified by SAT (28% (32/116)) than in those with contact cards (45% (68/152); $p<0.001$).

Conclusions

Although the challenges posed by non-contactable partners are not solved by SAT, it is a valuable novel tool for notification of verified STI diagnoses by index patients and providers. In addition to current standard partner notification practice it suits a small number of clients, especially those reporting more than one partner.

INTRODUCTION

A cornerstone in the control of sexually transmitted infections (STIs) is notification, testing and treatment of (asymptomatic) sexual partners of patients diagnosed with treatable STIs, referred to as partner notification (PN).[1 2] Testing of notified partners generally yields a higher rate of STIs compared with individuals unselectively screened for STIs.[3] Furthermore, treatment of infected partners reduces the likelihood of index patients being re-infected, complications from untreated infections, and, most likely, further transmission.[4] Many index patients find it difficult to notify their partners.[5] PN can be assisted by contact cards mentioning the verified STI and advice. The index patient hands out these cards to partners personally.

In the past few years, Internet-based PN services, such as InSPOT, Let Them Know and The Drama Down Under, have been developed in the USA and Australia, which allow individuals to send either named or anonymous e-cards, emails or short message service (SMS) messages to their partners.[6-9] This way of informing partners is seen as less confronting, more convenient, and less time-consuming than informing partners face-to-face or by phone.[9 10] Although website user statistics (with high absolute numbers) suggest frequent visits to these services,[6-9] evaluations of these publicly accessible websites show limited use and effectiveness.[11-13] These Internet services are not based on verified diagnoses and not developed for use by providers.

We developed 'suggestatest.nl' (SAT), an Internet-based notification system for verified diagnoses of STI/HIV, to support the individual (index patient) and nurses in the process of PN. SAT was piloted at the Public Health STI clinics in Amsterdam and Rotterdam, the Netherlands. In this paper we aim to evaluate SAT use and partner response during the pilot, by linking SAT data to clinical data. Acceptability of SAT in index patients and notified partners will be reported in a separate paper.

METHODS

Suggest-a-test

After counselling the index patient in the STI clinic, the nurse logs in into the SAT website and enters the specific STI, sex and sexual preference details of the index patient; SAT then creates a personal login code for the index patient with these data encrypted. The nurse enters this code and the patient's PN preference (SAT or non-SAT)

manually in the electronic patient system (EPS). The index patient receives the index code automatically in print (Amsterdam) or by SMS (Rotterdam). Even when patients prefer to notify partners in person, they are supposed to receive a SAT code to enable them to use SAT in case they reconsidered. SAT also allows provider referral, where the nurse can login into SAT with the index code and inform partners anonymously with data received from the index patient.

Index patients can login at http://www.suggestatest.nl with their code at any convenient time. For each sexual partner they can choose to send a notification via email, SMS, a postal letter, or a personal message on a gay dating site (gay.nl). STI notifications are standardised texts, which can be sent anonymously (default) or non-anonymously. The SAT code can only be used for 10 logins where after it is blocked. Partners then receive a notification from the STI clinic via SMS, email, postal letter or to their inbox at http://www.gay.nl. This message consists of a personal partner code and a short text saying that they have had sex with somebody who has recently been diagnosed with an STI and advising them to read their online notification and to get tested.

With this partner code, partners can login into SAT to find out more about the notified STI, possible treatment and how to make an appointment at the STI clinic. They are asked to print this personal notification and bring it to the STI clinic or their general practitioner (GP) for testing and treatment. The verified diagnosis notification through SAT is comparable with the existing method of contact tracing cards: if indicated, treatment will be started immediately.

In SAT, patient information is dealt with the highest confidentiality. The SAT codes provided to the index patients and the notified partners are unique, randomly created letter and number combinations. A secure server is used and data are encrypted. After sending a notification to partners, all personal data of notified partners are automatically removed leaving an anonymous database. To assure confidentiality, no connection between notified partners and the index patient is possible. To stress authenticity, we made a clear link at suggestatest.nl to the STI clinic websites, as well as to the STI Aids helpline in the Netherlands.

This study was waived by the Medical Ethical Committee of the Erasmus University of Rotterdam, because SAT is an extension of standard care.

Data analysis

The evaluation period for this pilot was from 1 March to 30 June 2012 in Amsterdam and from 23 April to 20 July 2012 in Rotterdam. Study size was determined by resources and feasibility. PN data from the Amsterdam and Rotterdam EPS were linked to data from the SAT database by the SAT code given to the index patient. Due to initial problems of registering the SAT code in the EPS, a few codes could not be linked to EPS data.

From the SAT database we assessed number of codes created, used for login, and used to send notifications; method and anonymity of notifications; and number of notifications by sex, sexual preference, STI and by clinic. We calculated the percentage of partners logging in by all partners notified by SAT. Lead time between creating the index code and login of the index patient and between sending a notification and login of the partner was calculated.

From the linked SAT and routinely collected EPS data, we assessed the percentage of index patients not receiving a code and the reason for this. The percentage of patients with STI using SAT to notify partners, as well as those intending to use SAT, was calculated using all patients with STI as denominators. Percentages were compared using the $\chi 2$ test, considering $p<0.05$ as statistically significant.

Determinants for using SAT such as age, sex, sexual preference, ethnicity, STI and number of sex partners in the previous 6 months were assessed. We categorised the STI/co-infections into STI groups: chlamydia including lymphogranuloma venereum (CT); gonorrhoea (Go); CT/Go co-infections; new HIV infections including co-infections like CT, Go or syphilis; and syphilis including co-infections except HIV. We performed univariable and backward multivariable logistic regression analysis to asses determinants of SAT use. Analyses were performed using SPSS V.19 (SPSS, Chicago, Illinois, USA).

It was not possible to compare effectiveness of PN (i.e., number of notified partners per index patient) during the pilot with the months preceding the pilot, as pre-pilot registration of PN was found to be poor. Therefore, we used three proxy measures to evaluate SAT.

RESULTS

SAT codes provided and SAT use by index cases and notified partners

During the intervention period, 1717 patients were diagnosed with STI in the Amsterdam clinic, versus 578 in the Rotterdam clinic. In Amsterdam, 61% of patients with STI did not receive a SAT code, mostly because they preferred another PN method (57%) or because PN was not necessary or already done (27%). In Rotterdam, 44% of patients with STI did not receive a SAT code, mainly because PN was already done (52%) or for unknown reasons (34%).

During the evaluation period, 1184 SAT codes were provided to index patients; 988 of these could be linked to medical records in the EPS (figure 1).

Of these 988 index patients, 17% logged in into SAT, although 14% actually used SAT to notify partners. SAT use was higher in Rotterdam than in Amsterdam (21% vs 11%, $p<0.001$, results not shown). Of the 457 index patients who expressed the intention to use SAT, 23% actually used SAT, compared with 6% not intending to use SAT.

The percentage using SAT did not differ between men who have sex with men (MSM), heterosexual men and women ($p=0.7$). The median number of notified partners per index patient was 2 (IQR 1–4). This was 4 for MSM (IQR 1–7; maximum 40), 1 for heterosexual men (IQR 1–3; maximum 21) and 2 for women (IQR 1–3; maximum 7). Provider notification was done in 17% (8/47) of MSM, in 11% (7/62) of women and none in heterosexual men.

Most notifications were done anonymously (88%, figure 1). The percentage anonymous notifications did not differ between MSM, heterosexual men and women ($p=0.15$). Of the index patients using SAT, 26% (36/138) sent at least one notification non-anonymously. SMS was the most used method (84%), followed by email (15%); three letters were sent (1%) and http://www.gay.nl was only used twice. Of the 505 notified partners, 294 (58%) logged in into SAT to read their STI notification. This percentage did not differ for anonymous versus non-anonymous notifications ($p=0.3$), nor for notifications sent by MSM, heterosexual men or women ($p=0.11$). Ninety per cent of index patients notified within 1 week, and 98% within 2 weeks; for the log in time of notified partners this was 84% and 95%, respectively.

SAT use was 13% in CT cases, 15% in gonorrhoea cases, 13% in CT/Go cases, 26% in syphilis cases and 20% in HIV cases (p=0.14). For HIV, SAT use was 10% (2/21) in Amsterdam versus 44% (4/9) in Rotterdam. In Rotterdam, all HIV notifications were sent by the nurses (provider notification). Nurses reported that some patients with co-infections were willing to notify via SAT for the STI but not for HIV.

Univariable analysis of SAT use for heterosexual men and women in both cities showed that the number of sex partners in the past 6 months (1, 2, 3–4, 5+) was correlated with SAT use, with patients reporting only one partner using SAT less often (8%) than those with 2 (15%; OR 2.2 (95% CI 1.0 to 4.7)), 3–4 (19%; OR 2.9 (95% CI 1.4 to 6.1)) and 5+ partners (14%; OR 2.1 (95% CI 0.9 to 4.8)). Although not significant, native Dutch patients used SAT slightly more often (18%) than patients of non-Dutch origin (12%; OR 0.7 (95% CI 0.4 to 1.0)). Patients from the STI clinic of Rotterdam used SAT more often (20.5%; OR 2.2 (95% CI 1.4 to 3.4)) than those from Amsterdam (10.6%). Patients who preferred to use SAT notified more often using SAT (21.4%; OR 3.4 (95% CI 2.0 to 5.6)) than patients who did not prefer or from whom preference was unknown (7.4%). Age, sex and STI category were not correlated with SAT use. In multivariable analysis, only preference for SAT use remained significant in the final model (OR 3.4 (95% CI 2.0 to 5.6)).

For MSM, only STI category was significantly associated with SAT use; MSM diagnosed with syphilis used SAT more often than MSM having CT (27% vs 9%, respectively, OR: 3.9 (95% CI 1.5 to 9.9)). MSM from the STI clinic of Rotterdam used SAT more often (23.3%; OR 2.5 (95% CI 1.3 to 4.8)) than those from Amsterdam (10.9%). MSM who preferred to use SAT notified more often using SAT (27.5%; OR 8.7 (95% CI 4.0 to 18.7)) than MSM who did not prefer or from whom preference was unknown (4.2%). Age, number of sex partners in the past 6 months and ethnicity were not correlated with SAT use. In multivariable analysis, only preference for SAT use remained significant in the final model (OR 8.7 (95% CI 4.0 to 18.6)).

SAT EVALUATION

The percentage of all reported, eligible (at risk) and contactable partners notified through SAT by clinic

SAT users notified 51–56% of their partners in the previous 6 months in SAT (see table 1). As the number of partners eligible for PN may be lower than the total partners in the last 6 months, a different picture arises taking this into account. Of the 67 index

patients who used SAT in Rotterdam, 96% of their partners were eligible, but only 56% (225/402) of these were reported to be contactable. This percentage was 36% for MSM, 70% for heterosexual men and 99% for women. Comparing these percentages to those of patients not using their SAT code, patients who used SAT overall have a lower proportion of partners that are contactable (56% vs 65%, $p<0.001$). In contrast to MSM and heterosexual men, women who used SAT reported 99% of their eligible partners to be contactable versus 51% for women who did not use SAT. When looking in more detail into the EPS files of the women who did not use SAT, we found that six of them were sex workers with around 100 sex partners per 6 months, of whom the vast majority was not contactable. The remaining 110 women (who did not use SAT) reported that 95% of their eligible partners are contactable.

Of the contactable partners, 95% were actually notified through SAT; this percentage was lowest for women (79%) and highest for MSM (111%). This means that MSM notified more partners than that they reported to be contactable during consultation at the clinic. Looking in detail into this we found that 29% of individual MSM notified more partners than identified at first, versus 25% of heterosexual men and 21% of women (data not shown). Compared with users of the SAT code, index patients not using the SAT code had less partners at risk (73% (1229/1693) versus 96% (402/419); $p<0.001$) and a slightly higher percentage of partners at risk was contactable (65% (802/1229) versus 56% (225/402); $p<0.001$). In total, of the 1027 contactable partners that were reported by index patients with STI who received a SAT code in Rotterdam, 213 (21%) were notified through SAT. However, we do not know whether the other contactable partners were not notified at all or notified using a different method.

Partners notified in SAT and tested in the two clinics

Of the total number of SAT-notified partners, 56% read their notification, and 20% of all visited one of the two STI clinics with a SAT notification. This was similar for the various STIs ($p=0.5$) (see figure 2).

STI positivity in all notified partners by STI category, clinic and notification method

Of all notified persons visiting the STI clinics, 5% (59/1255) were notified via SAT in Amsterdam versus 17% (57/342) in Rotterdam, whereas 11% (143/1255) were notified by contact card in Amsterdam versus 3% (9/342) in Rotterdam. Of all notified persons presenting, 143 (11%) had been notified by contact card, 59 (5%) via SAT and 1053 (84%) through other ways without a verified diagnosis of the index patient; see table 2. In

notified partners who visited the clinics during the intervention period, the percentage positive for the STI they were notified for was calculated. Overall STI positivity was lower in those notified by SAT (28%, n=116) than in those with contact cards (45%, n=152; p<0.001).

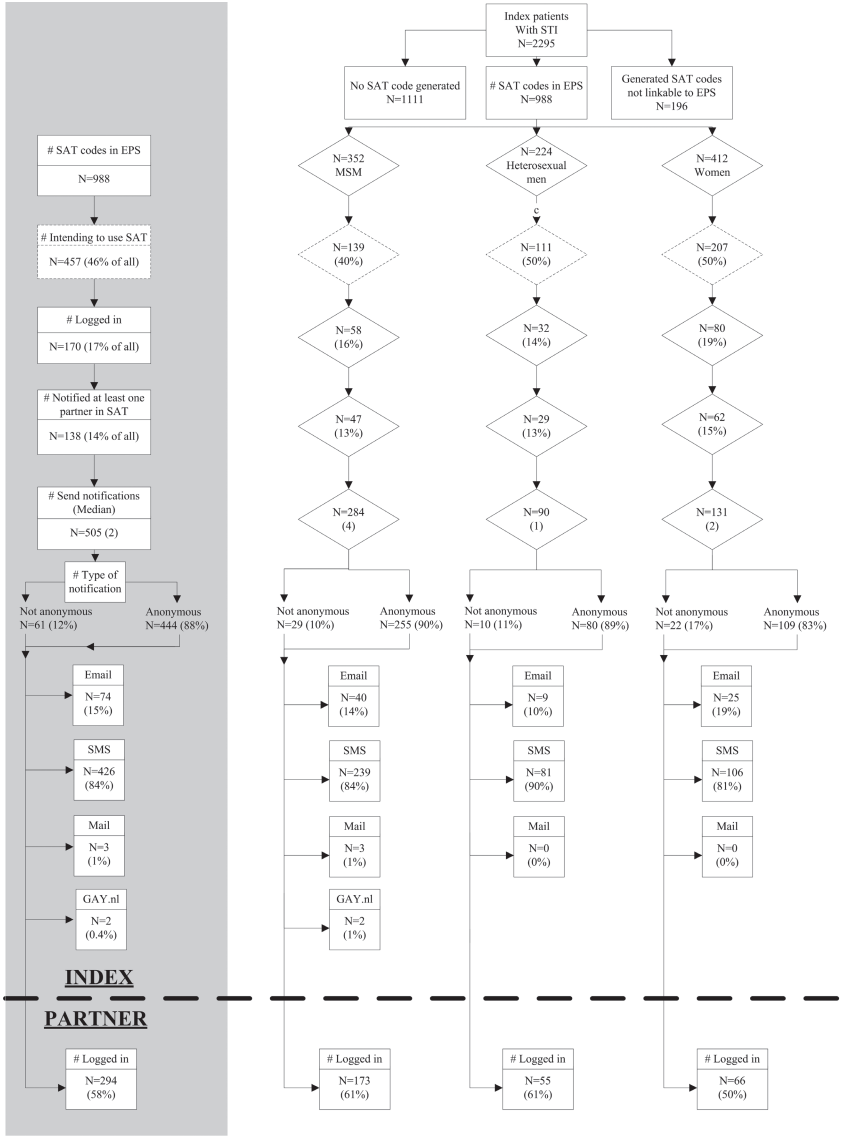

Figure 1. Use of suggestatest.nl (SAT) of index patients and notified partners, overall and by sex and sexual orientation.

Abbreviations: EPS, electronic patient system; MSM, men who have sex with men.

Table 1. Percentage of all reported, eligible (at risk) and contactable partners notified by clinic and by using SAT

	# Index cases	# Partners last 6 months	# Partners eligible	% Eligible of partners last 6 months	# Contactable partners	% Contactable of partners eligible	# Notifications through SAT	% Notifications through SAT of partners contactable	% Notifications through SAT of partners 6 months
SAT code used									
MSM	48	604					284		47
Heterosexual men	28	168					90		54
Women	62	166					131		79
Total	138	938					505		54
Amsterdam: SAT code used									
MSM	31	357					187		52
Heterosexual men	12	84					38		45
Women	28	78					67		86
Total	71	519					292		56
Rotterdam: SAT code used									
MSM	17	247	239	97	87	36	97	111	39
Heterosexual men	16	84	81	96	57	70	52	91	62
Women	34	88	82	93	81	99	64	79	73
Total	67	419	402	96	225	56	213	95	51
Rotterdam: SAT code not used									
MSM	55	381	343	90	196	57			
Heterosexual men	84	504	469	93	395	84			
Women	116	808	417	52	211	51			
Total	256	1693	1229	73	802	65			

Abbreviations:
MSM, men who have sex with men; SAT, suggestatest.nl.

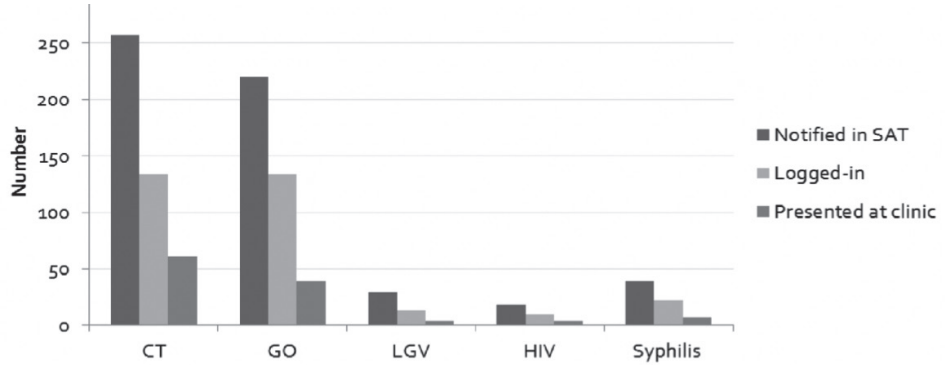

Figure 2. Number of all partners who were notified by suggestatest.nl (SAT), who read their online notification, and who visited the STI clinics of Amsterdam and Rotterdam, by STI.

Abbreviations:
CT, Chlamydia; GO, gonorrhoea; LGV, lymphogranuloma venereum.

Table 2. STI test outcomes by STI specific notification method among notified persons presenting to STI clinics

STI	Method of notification	Test positive / N notified (% positive)
Chlamydia trachomatis	Contact card	48/93 (52)
	SAT	23/63 (37)
	Other	275/899 (31)
	Total	**346/1055 (33)**
Gonorrhoea	Contact card	17/42 (40)
	SAT	8/41 (20)
	Other	74/242 (31)
	Total	**99/325 (30)**
Syphilis	Contact card	3/16 (19)
	SAT	0/8 (0)
	Other	20/94 (21)
	Total	**23/118 (19)**
HIV	Contact card	0/1 (0)
	SAT	1/4 (25)
	Other	8/94 (9)
	Total	**9/99 (9)**
Total	Contact card	68/152 (45)
	SAT	32 / 116 (28)
	Other	377 / 1329 (28)
	Total	**377 / 1329 (28)**

Abbreviations:
SAT, suggestatest.nl.

Note: STI diagnoses are shown for patients who were notified for this specific STI.

DISCUSSION

This is the first study to demonstrate substantial use of online PN by index patients, particularly by those with intention to use it. Almost one in four of the heterosexual and MSM index patients who preferred to use SAT actually notified partners in SAT. Innovative in our service is the provider-led creation of a code which limits misuse by sending verified STI-specific information to partners.[13]

Strengths and limitations

To our knowledge this is the first study with clinic-based follow-up of use of Internet-based PN that evaluates use, by index patients and by notified partners, in combination with epidemiological data. Moreover, we could monitor notified partners presenting at the clinic and their test outcome.

A main limitation of our project is that this was not a research project but a pilot implementation in daily practice with all the associated constraints. We would preferably have compared PN outcomes during the pilot with the period before. However, poor registration impaired this and proxy measures were used instead for evaluation.

Interpretation and comparison with international studies

The provided SAT codes were used by 14% of index patients. This may seem modest when compared with the high absolute numbers of people who used some other Internet-based PN programmes. However, evaluation of inSPOTLA (Internet PN Los Angeles) found very limited evidence of programme effectiveness among MSM in Los Angeles County.[12] Awareness of that open Internet site was around 15% among MSM and reported use of the site was less than 2%. Another study also shows low recognition and use of inSPOT by heterosexual STI clinic attendants (6% and 2%, respectively).[13] In 3 years, only two visitors of a high-volume sexual health clinic stated having received an inSPOTLA e-card as reason for their visit[12] compared with 7% (116/1597) of all notified partners presenting at our clinics. Thus, SAT use is not that low. In a substantial number of index patients, provider referral was done through SAT, which alludes to the additional value of the system for STI health professionals.

The plan was that nurses would create a SAT code for all patients with STI, but this was clearly not done in either clinic. Some nurses found that SAT interrupted their motivational counselling process too much. Strikingly, issuing and use of SAT codes was

higher in the Rotterdam clinic than in the Amsterdam clinic. This may be related to the more frequent provision of contact cards to patients with STI in Amsterdam compared with Rotterdam. As providing a SAT code was slightly more manual work in Rotterdam, this cannot explain higher use.

SAT was designed to enable anonymous PN by the index patient, and 88% of the notifications were sent anonymously This shows that anonymous PN is preferred by those using SAT. However, also non-anonymous notifications were done, which is valuable for clinical practice, as notified partners can provide specific information about time of sexual contact during the consultation at the clinic.

SMS was used most widely (82%), followed by email (16%). The option of using chat addresses in the dating site was only used twice. Users of these sites may want to use the site exclusively for dating and not STI-related issues. Also they may have mobile numbers of their dates and notify by SMS. Further research is needed before we can decide whether or not to add other dating sites.

It is unknown for whom Internet PN may be most beneficial.[14] We found that heterosexuals with multiple recent partners were more likely to use SAT, suggesting that SAT is used more for notification of ex- and non-regular partners than for current partners. The intention to use SAT was the only independent factor for SAT use in heterosexuals and MSM.

We noticed that patients were reluctant to use SAT for HIV notifications. Most of the HIV notifications in SAT were provider referral, which demonstrates the advantage of the system for this purpose. On the other hand, there is also a danger in providers preferring to use SAT over phoning partners to notify of HIV, knowing that many notified partners do not read their online notification. It is still preferable to do provider notification personally, and use SAT additionally. We agree with others that an online PN system is a supplemental tool to existing PN services[15] and cannot replace counselling.

The major challenge in PN remains the high number of non-contactable partners in MSM due to anonymity. We do not know whether we have reached more anonymous partners than without SAT. However, 29% of MSM notified more partners in SAT than they identified first-hand in the clinic and some of these partners may not have been

notified if SAT had not existed. Only 20% of the SAT-notified partners were tested at our two clinics, but partners may have been screened at their GP instead.

As with contact cards, SAT-notified partners receive a notification with a verified diagnosis, which may motivate STI testing and enable timely partner treatment. The lower positivity in SAT notifications compared with contact cards may be due to use of SAT in ex-partners and contact cards in current partners, who may still be infected.

To measure effectiveness of SAT, a randomised controlled trial comparing SAT with other PN methods with biological outcomes (partner testing and treatment) would be necessary. In practice this would be very challenging. In a project aiming at better registration of PN and training of nurses, we have shown that registration by itself improved PN and case finding in partners; of all detected new HIV cases among MSM in the Netherlands, 19% were detected through PN in 2010 versus 35% in 2012.[16]

Strikingly the majority of notified patients still arrive without a verifiable notification, leaving room for improvement in STI clinics (providing all index patients with either a contact card or a SAT code) as well as in GP practices. At this moment we are piloting SAT and other tools to improve PN in GP practices.

CONCLUSION

Although the challenges posed by non-contactable partners are not solved by SAT, it is a valuable novel tool for notification of verified STI diagnoses by index patients and providers. In addition to current standard PN practice it suits a small number of clients, especially those reporting more than one partner.

Key messages

- In suggestatest.nl (SAT), 84% of notifications with verified diagnosis were sent by text messaging, 15% by email; 88% anonymously.
- Of 998 index patients with a verified STI diagnosis, overall 14% notified through SAT, and of those intending to use SAT 23%.
- Fifty-eight per cent of SAT-notified partners checked their notification. STI positivity in SAT-notified clients was 28%.
- SAT is a valuable tool for notification of verified STI diagnoses by index patients and providers; it suits especially those reporting more than one partner.

Contributors

HMG, PV, HACMV designed the intervention and study protocol, supported by MSvR, EOdC, TH, RK. MH and FvdH were responsible for implementation and data collection at the STI clinics. HMG, MSvR did the analyses, HMG, MSvR and HACMV drafted the paper, all authors have commented on draft versions and approved the final version.

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Competing interests

None.

Ethics approval

This study was waived by the Medical Ethical Committee of the Erasmus University of Rotterdam.

Provenance and peer review

Not commissioned; externally peer reviewed.

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CHAPTER 5.3

Sender and receiver acceptability and usability of an online partner notification tool for sexually transmitted infection in the Netherlands.

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ABSTRACT

Users (index patients with a verified sexually transmitted infection and notified partners) rated the health care provider–initiated Internet-based partner notification application suggestatest.nl acceptable and usable. Both groups were less positive about suggestatest.nl to notify/get notified of HIV than other sexually transmitted infection. An anonymous notification was perceived less acceptable.

INTRODUCTION

Partner notification (PN) is the process whereby the sexual partner(s) of a patient diagnosed as having a sexually transmitted infection (STI) are identified and informed of their exposure to an STI.[1] Many studies show a preference to notify partners face-to-face or by telephone rather than with technologies such as short message service (SMS) or email.[2-5] However, Internet-based PN might be an additional method to reach more partners.[2]

To assist PN at the STI clinics of Rotterdam and Amsterdam, the Netherlands, an online tool called suggestatest.nl was developed explicitly for patients who were diagnosed as having an STI or HIV infection. Using this tool, index patients could send an anonymous or non-anonymous notification message by email, SMS, or postal mail, or - with the username of their partner - to a gay social network account. A general evaluation of the use of suggestatest.nl showed that this novel tool suits a small number of index clients, mainly by sending anonymous text messaging.[6 7] Of those intending to use suggestatest.nl, 23% notified a partner through suggestatest.nl and 58% of the partners notified through suggestatest.nl logged-in to read their notification online.

To date, suggestatest.nl and CheckOUT (Portugal) are, to our knowledge, the only published health care provider–initiated Internet-based notification systems that are designed for patients with a verified STI only.[8] Less is known about the acceptability of these tools for both the sender (index patient) and the receiver (notified partner). In addition, much of the published acceptability research relied on hypothetical scenarios of accessing options for PN.[9]

In this study, we evaluated the acceptability and usability of suggestatest.nl in both index patients and notified partners who have used this PN tool.

MATERIALS AND METHODS

Study setting

The STI outpatient clinics of Rotterdam and Amsterdam perform, respectively, approximately 12,500 and 40,000 STI consultations annually, free of charge and anonymous. In case an STI is diagnosed, the health care professional discusses the PN options and registers the patient's preference. These options consist of patient referral

(supported with a contact card or - from March 2012 onward - with suggestatest.nl), provider referral, or contract referral.

Suggestatest.nl

Patients with a confirmed STI diagnosis (chlamydia, lymphogranuloma venereum, gonorrhoea, syphilis, HIV, and/or trichomoniasis) received a nurse-generated code when they preferred to use suggestatest.nl for PN. To notify, the index patient had to log in to suggestatest.nl using the nurse-generated code. For each partner, the patient had to select the method (SMS/ email/postal/gay dating site) and the mode (anonymous/ non-anonymous) of sending the notification. All partners - irrespective of the previously mentioned selected method - received a standardised message with a unique partner code and had to log in to the website to read about the notified STI or HIV, possible treatment, and how to make an appointment at the STI clinic.

Theoretical framework from the technology acceptance model was used to develop the questionnaires for index patients and notified partners.[10] The 2 factors that determine the technology acceptance model are "perceived usefulness" (referred to as acceptability) and "perceived ease of use" (referred to as usability).[10] Questionnaires on acceptability and usability to notify/be notified through suggestatest.nl of STI and HIV were offered online to all participants regardless of their diagnosis/received notification. After the index patient had sent a suggestatest.nl notification, an invitation window popped up to complete an online questionnaire. Partners were recruited for an online questionnaire after reading their STI notification online. After completing the questionnaire, participating partners were asked to fill in their email address to receive an additional online questionnaire after 2 weeks. The online questionnaires were collected from March 2012 to June 2013 (supplementary tables 1 and 2). Because the online response of partners was low, partners visiting the STI clinics and notified through suggestatest.nl (who had not yet filled in an online questionnaire) were recruited from July 2012 to June 2013 to fill in a paper-and-pencil questionnaire.

Statistical analysis and data collection

All questionnaire data were analysed in IBM SPSS Statistics, version 21 (IBM Corporation, Armonk, NY). The acceptability and usability scores were constructed from the mean of the items included. Constructs were only calculated if none of the items for this construct had a missing value. For each construct, the reliability was calculated using

the Spearman-Brown statistic (2 items) or the Cronbach coefficient α (≥3 items).[11] Reliability values of at least 0.7 were assumed acceptable and all were 0.75 or greater. Frequency of Internet use for arranging personal matters was categorised in less frequent (scores 1–3) and frequent (scores 4–5). Respondents and non-respondents were compared using the $\chi 2$ test or Fisher exact test and the Mann-Whitney *U* test. Using the independent *t* test, the mean scores of notified partners who responded to the online and those who responded to the paper-and-pencil questionnaires were compared. The paired *t* test was used to compare scores on different items within the same group. p Values of less than 0.05 were considered statistically significant.

Ethics

This study was waived by the Medical Ethical Committee of the Erasmus University of Rotterdam, because suggestatest.nl was an extension of standard care.

RESULTS

Index patients

During the study period, 112 (19.8%) of 565 suggestatest.nl users completed the questionnaire (supplementary figure 1). Response was higher among men who have sex with men (MSM; 27.7%) compared with heterosexual men (13.1%) and women (17.0%; p=0.002), and responders notified a higher median number of partners than did non-responders (supplementary table 3). Four responders were newly diagnosed as having HIV.

Most index patients reported that they were able to notify more partners than without the existence of suggestatest.nl (table 1). The acceptability and usability to use suggestatest.nl to notify sexual partners of HIV were rated significantly less acceptable and usable (3.0 and 3.6, respectively) than notifying of another STI (4.4 and 4.7, respectively; p<0.001; table 1). Among MSM, the overall acceptability was higher (4.4) than among non-MSM (4.1; p=0.007), whereas the overall usability was not different (4.5 vs 4.4, respectively; p=0.28).

Notified partners

Of 2030 notified partners, 163 (8.0%) responded to the questionnaires (53 online and 110 offline at the STI clinic; supplementary figure 1). Notified partners who filled in the questionnaire were comparable with those who did not respond (supplementary

table 1). The acceptability and usability scores of online and offline responders were not significantly different. Of the 106 partners who were notified of HIV exposure, 3 responded to a questionnaire.

Most notified partners preferred to receive a non-anonymous notification via SMS (table 2). Partners who were notified anonymously rated their notification less acceptable (2.7) than did partners who were notified by name (4.4; $p<0.001$; table 2). The acceptability and usability to be notified of HIV through suggestatest.nl were rated significantly less acceptable and usable (3.3 and 3.2, respectively) than being notified of another STI (both 4.4; $p<0.001$). The overall acceptability and usability scores of suggestatest.nl (4.1) did not differ between MSM and non-MSM ($p=0.28$ and $p=0.50$).

Table 1. Acceptability and usability scores and PN-related answers of index patients who used SAT to notify sex partners, the Netherlands, March 2012 to June 2013

	Acceptability* (n=112), Mean (sd)	Usability* (n=112), Mean (sd)
Arrange personal matters via Internet	4.0 (0.8)	4.6 (0.7)
Notify sex partners via Internet	4.0 (0.9)	4.6 (0.8)
Notify sex partners with SAT while at home	4.4 (1.0)	4.6 (0.7)
Notify with SAT compared with former performed notification method (n=52)	4.0 (1.0)	4.5 (0.7)
Notify of STI with SAT†	4.4 (0.8)	4.7 (0.7)
Notify of HIV with SAT†	3.0 (1.5)	3.6 (1.4)
The STI clinic offering SAT	4.8 (0.4)	4.8 (0.4)
Willingness to receive notification through SAT‡	4.4 (1.0)	NA§
Recommend SAT	4.6 (0.6)	NA§
Overall¶	**4.2 (0.6)**	**4.4 (0.5)**
	Yes, n (%)	**No, n (%)**
Experience with notifying partners	53 (47.3)	59 (52.7)
Able to fill in contact information of all partners at the STI clinic	41 (36.6)	71 (63.4)
Notified more partners with SAT than without the existence of SAT	88 (78.6)	24 (21.4)

Abbreviations:
NA indicates not applicable; SAT, suggestatest.nl.

*Acceptability and usability scores ranged from 1 to 5.
†Because most participants did not notify of HIV, questions about using SAT to notify of STI or HIV exposure were asked regardless of type of notification sent. Four index patients were newly diagnosed as having HIV. Three rated SAT as very acceptable and usable to notify partners of both HIV and STI exposure (all scored 5). The other patient was less positive (HIV, 2 and 3.5; STI, 3 and 4, respectively).
‡This is not based on experience but on the index patient's opinion.
§Usability was not applicable for these items because the questionnaires focused on the acceptability of SAT only.
¶Overall acceptability and usability are based on all items mentioned in the table, except "Notify with SAT compared with former performed notification," because of a relative high number of missing values.

Table 2. Acceptability and usability scores and PN-related answers of partners who were notified through SAT, the Netherlands; March 1, 2012, to May 31, 2013

	Acceptability* (n=163)† Mean (sd)	Usability* (n=163)† Mean (sd)
Arrange personal matters via Internet	4.1 (0.8)	4.5 (0.8)
Enter a personal code online to view detailed notification	4.0 (1.2)	4.3 (1.1)
Read the STI-specific notification using the Internet	4.0 (1.0)	4.4 (0.9)
Receive an anonymous or non-anonymous notification‡		
Anonymous	2.7 (1.5)	NA§
Non-anonymous	4.4 (0.9)	NA§
Receive notification via SAT compared with previously received notification¶	3.6 (1.0)	3.6 (1.0)
Receive notification of STI via SAT‖	4.4 (0.9)	4.4 (0.9)
Receive notification of HIV via SAT‖	3.3 (1.5)	3.2 (1.5)
The STI clinic offering SAT	4.4 (0.9)	4.5 (0.8)
Willingness to send notification through SAT**	4.1 (1.2)	NA§
Recommend SAT	4.4 (0.9)	NA§
Overall††	**4.1 (0.8)**	**4.1 (0.7)**

	n (%)
Received in the past an STI notification through a method other than SAT (36 missings)	
Yes	64 (50.4%)
No	63 (49.6%)
Preferred method of receiving a notification through SAT (50 missings)	
SMS, anonymous	31 (27.4%)
SMS, non-anonymous	56 (49.6%)
E-mail, anonymous	11 (9.7%)
E-mail, non-anonymous	14 (12.4%)

Table 2. Continued

	n (%)
Preferred method of receiving a notification through SAT (50 missings)	
Postal, anonymous	0
Postal, non-anonymous	1 (0.9%)
Gay dating site, anonymous	0
Gay dating site, non-anonymous	0

Abbreviations:
NA indicates not applicable; SAT: suggestatest.nl.

*Acceptability and usability scores ranged from 1 to 5.
†Total number of questionnaires were n=163: n=53 were filled in online after sending a notification and n=110 offline when visiting the STI clinic. None of the scores were statistically different between those who responded online and those who responded offline. Because of missing answers, single items and constructs (only calculated if all items were available) were not available for all participants; for acceptability, the items were complete for (from the table) 118, 150, 150, 90, 32, 47, 146, 144, 147, 128, 127, and 133 participants, respectively, and for usability, the items were complete for (from the table) 119, 157, 149, 46, 145, 141, 146, and 136 participants, respectively.
‡Opinion about (non)anonymous notification was only measured for the type of received notification (n=90 anonymous, n=32 non-anonymous, n=41 missing).
§Usability was not applicable for these items because the questionnaires focused on the acceptability of SAT only.
¶Only asked to n=64 partners who were notified before.
‖Questions on acceptability and usability of SAT to notify for STI and HIV were offered to all participants regardless of the type of received notification. Three partners were notified of HIV exposure. They rated SAT as acceptable (mean, 4.7; individual scores 4, 5, and 5) and usable (mean, 4.2; individual scores 3.5, 4, and 5) to notify of STI. The acceptability and usability to receive an HIV notification through SAT were rated 4.3 (individual scores 3, 5, and 5) and 3.2 (individual scores 3, 5, and 1.5), respectively.
**This is not based on experience but on the opinion of the notified person.
††Overall acceptability and usability based on items mentioned in the table, except—because of a relative high number of missing values—"Arrange personal matters via Internet," "Receive an anonymous or non-anonymous notification," "Receive notification via SAT compared with previous received notification," "Preference to send notification through SAT," and "Recommend SAT." For online respondents, the first item and latter 2 items were asked only in the follow-up questionnaire participants received 2 weeks after completing the first one (23/53 online responders filled in).With the latter 2 items included, the mean (SD) acceptability score was 4.1 (0.7), and the total number of completed questionnaires was n=103.

DISCUSSION

Statement of principal findings

The online PN tool suggestatest.nl was rated acceptable and usable by both senders (index patients) and receivers (notified partners). Both groups were less positive about suggestatest.nl to notify/get notified of HIV than of another STI. Partners notified anonymously perceived their mode of notification less acceptable compared with those notified by name.

Strengths and weaknesses of the study

Although most articles on acceptability of electronic PN relied on hypothetical scenarios, we measured acceptability and usability in a real setting, in both patients and partners who used suggestatest.nl.[9] Moreover, we measured the opinion of both MSM and heterosexuals who used suggestatest.nl. Patients who chose to use suggestatest.nl may be more enthusiastic about suggestatest.nl than STI patients in general. However, their partners who did not have any choice in the method of how they received a notification were also generally positive about suggestatest.nl.

For our study, we recruited notified partners when they visited the website to read their notification or during the resulting consultation at the STI clinic. Unfortunately, the overall participation rate of notified partners was low (8%). This might have resulted in overestimated acceptability and usability scores, making it difficult to generalise the measured opinion to the general STI clinic population. Because of missing notification codes of 43 notified clients, no information of the received notification was known.

The questions concerning the acceptability and usability of using suggestatest.nl to notify of HIV exposure were mainly answered by patients and partners who notified or were notified of an STI other than HIV. As a consequence, the lower acceptability and usability to notify of HIV through suggestatest.nl were mainly hypothetical. Theoretically, the usability to notify partners of STI or HIV exposure through suggestatest.nl should be comparable because it uses the same system with identical actions. However, the construct of usability was rated lower for HIV than for other STI, indicating that it probably did not measure usability only.

Comparison with other studies

A study among Peruvian MSM and transgender women diagnosed as having STI showed that the introduction of a hypothetical Internet-based PN system resulted in a dramatic increase in anticipated notification of secondary partners.[12] In our study, almost 80% of the index patients reported that they had notified more partners than they would have done without the existence of suggestatest.nl.

A study among Spanish MSM of their anticipated notification behaviour showed that face-to-face or a telephone call was the preferred method to notify of STI or HIV for both stable and casual partners.[13] An identifiable SMS was the next most popular method to notify stable and casual partners of STI or HIV. The preference for sending an identifiable SMS contradicts our findings: most patients notified their partners anonymously.[6 7] A similar effect was seen in a UK study: the preference of respondents for a PN method was dependent on whether they see themselves as index patients or contacts.[14] Another possibility is that patients in our study who were willing to send an identifiable SMS or email have used their own mobile or email, and only those with interest in sending an anonymous notification have used suggestatest.nl.

In a review of the acceptability of electronic PN, a pattern emerged across studies showing that anonymity was less acceptable than the electronic delivery method itself.[9] In our study, the same effect was seen: notified partners were less positive about the fact that their suggestatest.nl notification was anonymous but were still content about suggestatest.nl.

Implications for clinicians and policymakers

It seems that, according to the opinion of our patients, STI clinics should offer an online PN tool like suggestatest.nl. As stated by Hottes and Gilbert,[15] a web-based PN service like inSPOT should be supplementary to traditional PN tools. After developing a PN website, the costs of facilitating online PN are relatively low and it can easily be offered as an addition to already existing traditional tools. On the basis of our findings, we would recommend to incorporate the possibility to notify anonymously.

Patients could be asked to immediately start filling in the contact information of their partners in suggestatest.nl when they are at the STI clinic for a treatment consultation. Possibly, patients are then more motivated to notify their partners than later at home, and public health nurses could assist with this process. However, it is also important to

offer suggestatest.nl use at a later stage, because at the STI clinic, most participants reported that they were unable to fill in contact details of all partners.

Unanswered questions and future research

We recognise that there is a possible trade-off between reaching more partners by the implementation of a low-threshold online PN tool and the quality of the sent notification: because many partners do not read their online notification (42%; e.g., because they think that it is an unsolicited message/spam), the sent notification might not have resulted in health care seeking.[7] Future research should focus on the most suitable ways of directing online notified partners into care. After the inclusion period of this study, the tool was renamed to "partnerwaarschuwing.nl" (partnernotification.nl in English) because some notified partners reported that they were confused about the name suggestatest.nl.

Our study mainly focuses on patients who chose to use suggestatest.nl and their partners in which participation was low. For generalizability, more research that measures the opinion of all notified STI-clinic clients regarding online PN is necessary.

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Conflict of interest and sources of funding

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Contributors

M.v.R. and R.K. designed the study protocol supported by H.G., T.H., and H.V. P.V. was responsible for the development of the suggestatest.nl website and the implementation of the online questionnaires. M.v.R. performed the statistical analyses supported

by H.G., M.v.V., and H.V. M.v.R., H.G., and H.V. drafted the manuscript, all authors commented on draft versions, and all approved the final version.

Previously presented

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SUPPLEMENTARY TABLES

Supplementary table 1. Individual questionnaire items for index patients who used suggestatest.nl to notify, the Netherlands, 01-03-2012 until 31-05-2013

	Acceptability items	Reliability acceptability construct	Usability items	Reliability usability construct
Questions:				
1. How do you find it to use the Internet for arranging personal matters (e.g. making appointments, searching for information, arrange travel, tax return etc.)?	Unreliable – Reliable Unsafe – Safe	r_{SB}: 0.83	Difficult - Easy Hard – Handy	r_{SB}: 0.92
2. How do you feel about notifying sexual partners over the Internet?	Unreliable – Reliable Unsafe – Safe	r_{SB}: 0.91	Difficult - Easy Hard – Handy	r_{SB}: 0.91
3. How do you feel to notify sexual partners using suggestatest.nl while at home?	Disagreeable – Agreeable	Na	Difficult - Easy Hard – Handy	r_{SB}: 0.87
4. How did you experience notifying your sex partners through suggestatest.nl compared to your previous performed STI notification?	More unreliable – More reliable More unsafe – Safer	r_{SB}: 0.87	More difficult - Easier Harder –Handier	r_{SB}: 0.89
5. How do you feel about using suggestatest.nl to notify of STI?[1]	Unacceptable – Acceptable	Na	Difficult - Easy Hard – Handy	r_{SB}: 0.87
6. How do you feel about using suggestatest.nl to notify of HIV?[1]	Unacceptable – Acceptable	Na	Difficult - Easy Hard – Handy	r_{SB}: 0.87
7. How do you feel that the STI clinic offers suggestatest.nl?	Useless – Useful Meaningless – Meaningful	r_{SB}: 0.94	Hard – Handy	Na
8. Would you like to be notified via suggestatest.nl?	Certainly not – Certainly	Na	-	-
9. Would you recommend people to use suggestatest.nl?	Certainly not – Certainly	Na	-	-
10. Overall score[2]	Items 1-3, 5-9	α: 0.83	Items 1-3, 5-7	α: 0.83

Abbreviations:

α: Cronbach Alpha; na: not applicable; r_{SB}: Spearman-Brown coefficient

[1]Items 5 and 6 were offered to all participants independent of their STI and HIV diagnoses.

[2]Because of higher number of missing values, items from question 4 were omitted from the overall acceptability and usability constructs.

Supplementary table 2. Individual questionnaire items for partners who were notified through suggestatest.nl, the Netherlands, 01-03-2012 until 31-05-2013

	Acceptability items	Reliability acceptability construct	Usability items	Reliability usability construct
Questions[1]:				
1. How do you find it to use the Internet for arranging personal matters (e.g. making appointments, searching for information, arrange travel, tax return etc.)?	Unreliable – Reliable Unsafe – Safe	r_{SB}: 0.91	Difficult - Easy Hard – Handy	r_{SB}: 0.93
2. I experienced entering the received code to view my detailed warning as:	Unpleasant – Pleasant	Na	Hard – Handy	Na
3. How do you feel about checking your notification over the Internet?	Unreliable – Reliable Unsafe – Safe Unpleasant – Pleasant	α: 0.83	Difficult - Easy Hard – Handy	r_{SB}: 0.84
4. How do you feel that this notification was anonymous / non-anonymous?	Unpleasant – Pleasant	Na	Hard – Handy	Na
5. How did you experience being notified through suggestatest.nl compared to your previous received STI notification?	More unreliable – More reliable More unsafe – Safer	r_{SB}: 0.79	More difficult - Easier Harder – Handier	r_{SB}: 0.75
6. How do you feel about being notified of STI through suggestatest.nl?[2]	Unacceptable – Acceptable	Na	Difficult - Easy Hard – Handy	r_{SB}: 0.89
7. How do you feel about being notified of HIV through suggestatest.nl?[2]	Unacceptable – Acceptable	Na	Difficult - Easy Hard – Handy	r_{SB}: 0.87
8. How do you feel that the STI clinic offers suggestatest.nl?	Unpleasant – Pleasant Unacceptable – Acceptable	r_{SB}: 0.85	More difficult - Easier Harder – Handier	r_{SB}: 0.86
9. Now that you've experienced to be notified via suggestatest.nl, would you use suggestatest.nl to send notifications to sex partner(s)?	Certainly not – Certainly	Na	-	-
10. Would you recommend people to use suggestatest.nl?	Certainly not – Certainly	Na	-	-
11. Overall score[3]	Items 2-3, 6-8	α: 0.83	Items 2-3, 6-8	α: 0.83

Abbreviations:
α: Cronbach Alpha; na: not applicable; r_{SB}: Spearman-Brown coefficient
[1]In the online questionnaires, question 8 was only asked in the questionnaire at day 0 and questions 1, 4, 5, 9, and 10 in the additional questionnaire after 2 weeks. Questions 2, 3, 6, and 7 were asked in both questionnaires and only the results from the questionnaire at day 0 were used in the analysis.
[2]Items 6 and 7 were offered to all participants independent of notified STI
[3]Because of higher number of missing values, items from questions 1, 5 and 9-10 were omitted from the overall acceptability and usability constructs. Item 4 was not included because it was dependent on the type of notification.

Supplementary table 3. Main characteristics of index patients sending and partners receiving an online notification trough suggestatest.nl; comparing those who did and those who did not respond to the questionnaire, the Netherlands, March 2012 - June 2013

Index patient	**Responded to questionnaire**		
	Yes n=112	**No[1] n=453**	**p Value[2]**
	n (%)	**n (%)**	
Sexual behaviour/sex[3]			0.002
Heterosexual male	16 (14.3)	106 (23.7)	
MSM	56 (50.0)	146 (32.7)	
Female	40 (35.7)	195 (43.6)	
STI[4]			
HIV	4 (3.6)	23 (5.1)	0.5
Syphilis	12 (10.7)	31 (6.8)	0.2
Chlamydia incl. LGV[5]	78 (69.6)	320 (70.6)	0.8
LGV	4 (3.6)	6 (1.3)	0.1
Gonorrhoea[6]	29 (25.9)	132 (29.1)	0.5
Trichomoniasis	0 (0)	3 (0.7)	0.4
Used notification method[7]			
SMS	101 (90.2)	405 (89.4)	0.8
Email	28 (25.0)	91 (20.1)	0.3
Postal mail	2 (1.8)	3 (0.7)	0.3
Gay.nl	1 (0.9)	5 (1.1)	0.8
Median number of partners notified (IQR)	3 (IQR 1-6)	2 (IQR 1-4)	<0.001
Has sent at least 1 non-anonymous notification	30 (26.8)	121 (26.7)	1.0

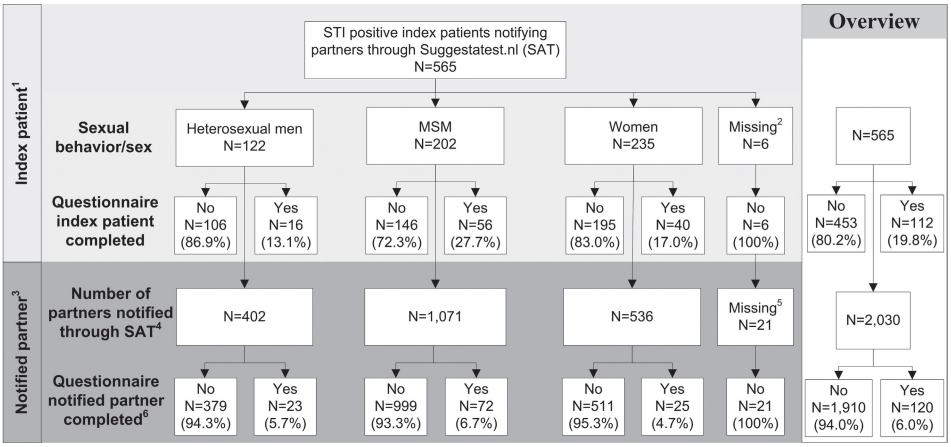
Notified partner	**Responded to questionnaire**		
	Yes[8] n=120	**No[9] n=1,910**	**p Value[2]**
	n (%)	**n (%)**	
Sexual behaviour/sex of index patient[10]			0.4
Heterosexual male	23 (19.2)	379 (20.1)	
MSM	72 (60.0)	999 (52.9)	
Female	25 (20.8)	511 (27.1)	
STI notification[4]			
HIV	3 (2.5)	103 (5.4)	0.2
Syphilis	19 (15.8)	222 (11.6)	0.2
Chlamydia incl. LGV[5]	77 (64.2)	1,178 (61.7)	0.6
LGV	0 (0)	51 (2.7)	0.07
Gonorrhoea[6]	34 (28.3)	673 (35.2)	0.1
Trichomoniasis	0 (0)	3 (0.2)	1.0

Supplementary table 3. Continued

Notified partner	Responded to questionnaire		
	Yes n=120	No[1] n=1,910	p Value[2]
	n (%)	n (%)	
Notification method			0.7
SMS	103 (85.8)	1,676 (87.7)	
Email	17 (14.2)	215 (11.3)	
Postal mail	0 (0)	11 (0.6)	
Gay.nl	0 (0)	8 (0.4)	
Received a non-anonymous notification	21 (17.5)	284 (14.9)	0.4

Abbreviations:
Incl.: including; IQR: interquartile range; LGV: lymphogranuloma venereum; NA: not applicable; MSM: men who have sex with men.

[1]41 of the non-responders were index patients for whom the nurse performed the notification.
[2]The chi-squared or the Fisher's Exact test was used for categorical variables and the Mann-Whitney U test for continuous variables. Because the notified STI and the type of notification were not mutually exclusive, the comparison is per STI and per type of notification.
[3]Sexual behaviour/sex is unknown for 6 index patients who did not respond to the questionnaire.
[4]Because of co-infected persons, the total number of STI sums up to more than the group total.
[5]Being diagnosed with urogenital and/or anorectal and/or oropharyngeal chlamydia.
[6]Being diagnosed with urogenital and/or anorectal and/or oropharyngeal gonorrhoea.
[7]Because some index patients used multiple methods to notify, method of notification sums up to more than 100%.
[8]43 notified partners were excluded because their questionnaires could not be linked to the SAT database due to missing notification codes.
[9]Due to 43 questionnaires with missing notification codes, the notified partners were not identifiable in the database and consequently included in the group who did not respond to the questionnaire.
[10]Sexual behaviour/sex of the index partner is unknown for 21 notified partners who did not respond to the questionnaire.



Supplementary figure 1. Flowchart showing response rates of STI positive index patients and their notified partners to complete the questionnaire concerning acceptability and usability of the online notification tool "suggest a test", the Netherlands, March 2012-June 2013.

Abbreviations:
MSM: men who have sex with men; SAT: suggestatest.nl.

[1]These patients were diagnosed with an STI or HIV infection at the Public Health Service of Rotterdam and Amsterdam and they used suggestatest.nl to notify partners.
[2]From 6 patients who used suggestatest.nl no background information was available.
[3]Notified partners could respond to the questionnaire online (after reading their detailed notification) or offline during their STI clinic visit at the Public Health Service of Rotterdam and Amsterdam. As a consequence, those partners who did not read their online notification and did not come to the clinic were not invited.
[4]Sexual behaviour/sex only known for the index patient and not for the notified partner. Presumably, when the index patient was a heterosexual man, the questionnaire was filled in by a woman, in case of an index MSM by an MSM or woman (out of 1,071 notified partners 25 were sent by bisexual men), and in case of an index women by a heterosexual or bisexual man.
[5]Background information of 21 sent notifications was unavailable in the database.
[6]Identification code was missing for 43 questionnaires; 6 online questionnaires due to a technical issue and 37 offline questionnaires due to a missing or incorrect code. This code was necessary to know the sexual behaviour/sex and diagnosed STI of the index patient. Consequently, these 43 questionnaires are included in the groups that are stated as not-responders to the questionnaire.

5.3

CHAPTER 6

General discussion

In this thesis, the accuracy of diagnostic tests for sexually transmitted infections (STI) and the related costs were assessed. Furthermore, the STI positivity at anatomical sites that were previously not routinely tested, was evaluated. Next, we described the outcome of surveillance activities in three groups: hepatitis C virus (HCV) infection among HIV-positive MSM, early incubating chlamydia and gonorrhoea infections among MSM requesting for PEP, and STI among victims of a sexual assault. Finally, we evaluated the use and clients' preference of two innovations: chlamydia home sampling and an online partner notification tool.

In the Introduction, four different research approaches were mentioned. Below, per approach the outcomes of the studies are discussed.

EVALUATION OF DIAGNOSTIC TESTS: IMPLICATIONS FOR CLINICAL PRACTICE

False-negative type-specific HSV-1 and HSV-2 serology

Only recently, commercially available type-specific herpes simplex virus (HSV) serology assays became available that were helpful to accurately differentiate antibodies against HSV-2 from those to HSV-1.[1] In chapter 2.1 we measured the performance of three different type-specific serologic tests (glycoprotein G directed) in patients with a recurrent HSV episode that was proven with PCR. Relatively often, false-negative results were observed in both HSV-1 and HSV-2 patients. In 11.8% (2/17) and 9.1% (3/33) of the patients with HSV-1 and HSV-2 respectively, none of the three tests detected antibodies against the recurrent HSV type. False-negative HSV type-specific serological test results can have far-reaching consequences for pregnant women (erroneous indication for caesarean delivery), for HSV serodiscordant sexual couples (unnecessary prophylactic measures) and for sero-epidemiological studies (biased proxy for sexual risk behaviour).[2]

The Amsterdam STI clinic does not use type-specific serology for clinical purposes and does not have the intention to implement this in the near future. In contrast, type-specific molecular tests for HSV are performed, but only in case of clinical symptoms suggestive of herpes. The rationale for serological testing is to identify asymptomatic HSV infection.[1] When considering HSV type-specific serology in asymptomatic patients, one should take into account the value of the results for clinical decision making versus the impact of this knowledge on the client's sex life. Especially with HSV-1, seropositivity in asymptomatic clients does not clarify whether they have a genital or

orolabial infection or an infection at both anatomical sites. The discomfort of episodes of HSV induced lesions should be outweighed by the lifetime distress someone might experience after being diagnosed positive for HSV antibodies. Additionally, a false-negative test will result in an incorrect perception of the risk of HSV transmission. More research is needed to measure the benefit of knowledge of serostatus and the impact on someone's life.

Urethral Gram-stained smear evaluation as presumptive test for chlamydia

In chapter 2.2, the outcome of urethral Gram-stained smear evaluation was used as a point-of-care test for urethral chlamydia. Next to a Gram stain, all STI clinic clients were tested for chlamydia with a nucleic acid amplification test (NAAT). Unfortunately, some chlamydia cases – as proven with NAAT - had a negative Gram-stain (false-negative result). The sensitivity was 83.8% when applied to all high-risk patients and 91.0% when applied to symptomatic patients only. Patients with a false-negative Gram-stain result might continue transmitting chlamydia for a prolonged period until they receive treatment. Possibly, those with a false-negative Gram-stain result are less motivated to abstain from unprotected sex, because they assume that the negative Gram-stained smear evaluation implies that they are not infected with an STI.

The specificity was 74.1% when applied to all high-risk patients, and 53.1% when applied to symptomatic patients only. In both cases, the relatively high number of false-positive outcomes for chlamydia resulting in overtreatment, is a disadvantage of using a Gram-stained smear as a point-of-care test for chlamydia. With the current development of antibiotic resistance, overtreatment might be a concern for the application of this point-of-care test.[3]

The cost per consultation was lower when performing Gram-staining only in high-risk-patients with urethral symptoms, compared to when offered to all high-risk patients. This benefit was based on the perspective of consultation cost; possible transmission in the period between initial consultation and treatment consultation was not accounted for. Next to using a Gram-stained smear to test for *Chlamydia trachomatis*, this test can also detect *Neisseria gonorrhoeae*. A mathematic modelling study for the use of Gram-stained smears to test for *N. gonorrhoeae* among MSM, accounted for possible transmission until treatment was administered.[4] This study showed that downscaling Gram-staining to symptomatic clients only, had a marginal effect on the gonorrhoea prevalence in MSM.[4] Because chlamydia was not included in this model, further

research is needed to elucidate whether performing Gram-stained smear evaluation in symptomatic clients only – on the short term a reduction of costs - does not result in an increase in the chlamydia prevalence.

Although recently progress has been made in the development of point-of-care diagnostics for chlamydia, more studies are needed into the acceptability, feasibility, and the cost involved, especially for low and middle income countries.[5] Moreover, data on the sensitivity and specificity among low-risk populations are needed.[5] In the absence of accurate, fast and affordable point-of-care diagnostics for chlamydia, Gram-staining in symptomatic high-risk patients seems to be an interesting alternative for STI clinics. However, offering Gram-staining will not be possible for all STI clinics: if financial resources are limited, required laboratory facilities may be too costly.

CLINICAL MANAGEMENT: IMPLICATIONS FOR TESTING PROTOCOLS

Positivity and spontaneous clearance of chlamydial RNA in the pharynx

Among high-risk women who reported fellatio and among MSM (regardless of sexual behaviour) the positivity rate of pharyngeal chlamydial RNA was 2.3% and 1.1%, respectively. This is low compared to the observed prevalences of anogenital chlamydia, respectively 11.1% and 10.3%. The majority of MSM with pharyngeal chlamydia did not have anogenital chlamydia; this was also observed in other studies.[6 7] In contrast, the majority of women with pharyngeal chlamydia did have a concurrent anogenital chlamydia infection. Thus, without routine screening of the pharynx (and treatment if positive), more than half of the MSM and about one third of the women with pharyngeal chlamydia would have left the STI clinic untreated.

Spontaneous clearance of chlamydial RNA in the time period between test and treatment (and a repeat test) was lower than hypothesised: with a median follow-up of 10 days, spontaneous clearance was 37% in MSM and 36% in women. In the scarce literature available, spontaneous clearance appears to be low.[8 9] A systematic review estimated from the prevalence and incidence rate that pharyngeal chlamydia has an infection duration of 667 days.[10] Proof of pharyngeal chlamydia persistence was also found in a retrospective study at the Amsterdam STI clinic.[11] Sixteen patients with untreated pharyngeal chlamydia were pharyngeal chlamydia positive at consecutive consultations.[11] Out of these 16 patients, all samples from four patients could be

genetically typed: in all cases the genotypes in the coupled samples were identical with an interval of 112, 168, 207, and 268 days, respectively.[11]

The combination of persistence, the absence of pharyngeal symptoms, a high proportion of patients without a concurrent anogenital chlamydia infection and a low clearance rate, could make the pharynx a possible reservoir for the ongoing transmission of chlamydia. Little is known about the contribution of pharyngeal chlamydia to the epidemic at large. Some case reports have described patients with a urogenital chlamydia infection who reported passive oral sex only.[12-14] Interestingly, pharyngeal chlamydia has also been proposed as a cause of descending infections along the gastrointestinal tract.[15]

A mathematical model for gonorrhoea showed that the pharynx is a reservoir for gonorrhoea and pharyngeal infections contribute to the ongoing transmission more so than rectal infections.[16] The Centres for Disease Control and Prevention (CDC) does not recommend screening for *C. trachomatis* pharyngeal infection in MSM who report receptive oral sex; they should be screened for pharyngeal *N. gonorrhoeae* only.[17 18] More research is needed to elucidate the role of the pharynx as a potential reservoir for chlamydia.

Urethral lymphogranuloma venereum

In chapter 3.2, a selected group of MSM at high risk for urethral lymphogranuloma venereum (LGV) is described. Out of 33 MSM with anorectal LGV and urethral chlamydia, the urethral chlamydia infection was caused by *C. trachomatis* biovar L, the chlamydia strain causing LGV, in seven cases. Four out of 10 MSM with urethral chlamydia who reported an LGV infected partner were infected with *C. trachomatis* biovar L. This high proportion of patients with a biovar L positive urethral chlamydia infection, raised questions about offering routine screening for urethral LGV to MSM. Without screening for urethral LGV, patients with urethral chlamydia who are treated with azithromycin are possibly treated sub-optimally.[19] Sub-optimal treatment might have consequences for the individual (continuation of infection and possible sequelae) and for public health (transmission of infection).

After the study of urethral LGV in the selected group of MSM, a prospective study was started at the Amsterdam STI clinic to estimate the urethral LGV prevalence in all MSM.[20] Systematic screening revealed only a small number of urethral *C. trachomatis* biovar

L infections. In addition, a discrepancy between the number of anorectal and urethral infections of *C. trachomatis* biovar L was found. This cannot be explained by ano-genital transmission only. Possibly, *C. trachomatis* biovar L is less successful in establishing a urethral infection. Another explanation might be the role of descending infections of chlamydia via the gastrointestinal tract.[15 21] In chapter 3.1 two cases of pharyngeal LGV were described, and this finding supports a possible intra-patient ora-anal route via the gastrointestinal tract.

Prevalence of MRSA among men who have sex with men

In contrast to earlier reports, mainly from the USA and the UK, the positivity of methicillin-resistant *Staphylococcus aureus* (MRSA) was rare among MSM visiting the Amsterdam STI clinic. MRSA was diagnosed in two out of 211 MSM (0.9%) and none had a community-associated-MRSA (CA-MRSA) strain.

Among Spanish HIV-positive patients, the prevalence of nasal MRSA colonization was 1% and pharyngeal colonization 2% and none of the isolates belonged to a typical CA-MRSA lineage.[22] Among French MSM no CA-MRSA carriage or active infection was found.[23] Next to these European studies, in Canadian MSM the prevalence of CA-MRSA nasal or rectal carriage (1.6%) did imply that colonization with this organism had not occurred in this population to a significant extent.[24] This is in contrast with US MSM in whom outbreaks of CA-MRSA skin infections have been reported.[25 26]

In a British case study, MRSA was found as the cause of genital ulcer-adenopathy syndrome.[27] Although MRSA was rare in MSM attending the Amsterdam STI clinic, clinicians should be aware of the possibility of MRSA infections.

PUBLIC HEALTH SURVEILLANCE

Hepatitis C screening among HIV-positive MSM: added value of screening at an STI clinic

When HIV-positive MSM were screened for HCV at the Amsterdam STI clinic, 6.4% were HCV antibody positive at their first STI consultation. During follow-up consultations of those tested negative at first visit, 32 HIV-positive MSM became anti–HCV-positive (HCV incidence rate: 2.35/100 person years). The high prevalence and incidence support routine HCV screening of HI- positive MSM who visit the STI clinic. However, the majority of these men are frequently consulting an HIV specialist for HIV treatment and monitoring. During follow-up at the HIV specialist, HCV infections are routinely

monitored either via alanine transaminase (ALT), anti-HCV or HCV RNA screening. In our study we showed that about half (n=28) of HCV diagnoses were first detected at the STI clinic. Of these 28, seven were newly diagnosed with HIV at the same visit and therefore not yet in care of an HIV specialist.

In HIV-HCV co-infected patients, progression to liver disease is more rapid and more common, and treatment response rates with the first choice treatment at that moment (pegylated interferon alfa and ribavirin), were lower, especially when HCV was diagnosed late in infection.[28] The poor treatment outcome motivated us to start HCV screening in HIV-positive MSM to diagnose HCV infection as early as possible. However, since 2015 highly successful direct acting antivirals (DAAs) became available to all patients with chronic HCV. Nowadays, early detection with the intention to start early treatment for higher treatment success is no longer a motivation to screen for HCV at STI clinics. However, from a public health perspective it is still valuable to diagnose as early as possible: early detection and treatment lowers the community viral load, thus limiting the transmission of HCV.

From May 2014 HCV screening in HIV-positive MSM was ceased, because of budget restrictions and redundancy since HCV screening was routine at HIV treatment centres. From the beginning of 2017, the Amsterdam STI clinic resumed HCV screening of HIV-positive MSM. Based on the outcome of this study, the test algorithm was changed to test only those HIV-positive MSM who had not had a check-up at their HIV specialist in the preceding 3 months. In this way, HIV-positive MSM are tested up to twice a year for HCV at the STI clinic, and twice at the HIV treatment centre.

Until May 2014, MSM were tested at the Amsterdam STI clinic for anti-HCV only. The disadvantage of antibody screening is the likelihood of missing early infections, due to HIV associated delayed antibody maturation.[29 30] In some cases, elevated ALT associated with an acute HCV infection was detected by the HIV treatment specialist, while around the same time the infection was missed due to a false negative anti-HCV test at the STI clinic. Another disadvantage of anti-HCV screening is the impossibility to detect reinfections. Since the restart of HCV screening at the STI clinic, HIV-positive MSM who report a history of HCV are tested for HCV RNA. The outcomes of this screening algorithm and the resources used will be evaluated in the near future.

Before 2014, HCV screening was not only offered to HIV-positive MSM, but also offered to MSM who opted-out of HIV testing. Only a small percentage of these men were anti-HCV-positive at first consultation (0.7%) and during follow-up no seroconversions were observed. Based on this finding, the Amsterdam STI clinic ceased HCV screening in this group.

STI in MSM requesting for post-exposure prophylaxis for HIV

In MSM requesting for PEP, routine STI screening revealed that 16.5% had a *C. trachomatis* and/or *N. gonorrhoeae* infection. Although a very sensitive NAAT was used during this first screening, 4.1% of those previously negative, proved to be positive for *C. trachomatis* and/or *N. gonorrhoeae* when tested during a follow-up visit, at a median of 14 days after the initial PEP consultation. Possibly, these infections were missed because they were in the incubation phase at the time of the initial visit.

To limit expenditures, STI clinics providing PEP could choose to screen for chlamydia and gonorrhoea after the incubation period, e.g. at the 2 week follow-up screening. However, they should be cautious because a relative high prevalence was detected at the initial visit and transmission of STI could occur in the period between initial visit and follow-up. In addition, one third of the clients who started PEP did not return for their follow-up visit after two weeks.

A limitation of this study is the absence of routinely collected data on sexual risk behaviour in the period between first and repeat screening. Nowadays, the Amsterdam STI clinic recommends to test for STI at least 7 days after the last high-risk sexual contact. Clients with possible ongoing exposure to STI like sex workers and MSM, and clients who are symptomatic do not need to wait for an appointment until the end of their window phase. If during consultation recent (within 7 days) sexual risk behaviour is reported by asymptomatic patients not being MSM or sex workers, they are recommended to postpone their appointment.

Unfortunately, there is no sound scientific evidence to establish the optimal time-point to test for chlamydia using NAAT following potential exposure to infection through unprotected sex.[31] Based on expert opinion, it is recommended that patients are encouraged to undergo testing for chlamydia with NAAT when they first opt for screening and, if they are concerned about exposure which has occurred within the preceding two weeks, a repeat NAAT two weeks after the last exposure.[31] Data from

a study about bacterial load of chlamydia and time between testing and date of last unsafe sex provided new evidence that justifies that the 2 week limit before tests - as recommended by many guidelines - might be abandoned.[32] Further research should focus on the implication of possible recent exposure and the detection of *C. trachomatis* and *N. gonorrhoeae* with modern highly sensitive NAAT.

STI in victims of a sexual assault

In a period of just over 11 years, a relatively small number of sexual assault victims (SAV) were tested at the Amsterdam STI clinic. Apparently, SAV do not easily access STI care or do not disclose their assault. Nowadays, nationwide coverage of centres for victims of a sexual assault provide care for SAV. Individuals who have recently experienced sexual violence are supported with medical, forensic, and psychological help.[33] Since 2016, this collaboration also started in the Amsterdam region.[34] However, some SAV do not seek care at these specialised centres but apply for an appointment at the STI clinic. Especially among male SAV, a large proportion did not report the assault to the police, and did not undergo forensic examination. Possibly, these clients only opt for STI care and are not willing to be confronted with conversations about their assault. A British study showed that, compared to female SAV, male SAV were more likely to access the routine walk-in genito urinary medicine clinic than a specialised sexual assault clinic.[35]

The STI positivity rate was 11.2% among female SAV and 12.6% among male SAV. In the multivariable analysis, non-SAV and SAV females had a comparable risk to be diagnosed with an STI. Although male SAV had a significantly lower odds of an STI diagnosis compared to non-SAV males, STI positivity was considerable. Moreover, STI screening is worthwhile because in many cases – next to their assault - victims reported consensual but unprotected sexual contacts. Based on the above findings, it remains important that STI clinics continue to provide low access STI care for SAV.

In the literature, low return rates of SAV for treatment are reported.[36] Based on this and in view of the high positivity rate of STIs among SAV, the 2015 CDC STD treatment guidelines recommend empiric presumptive antimicrobial therapy (before test results are available) targeted towards gonorrhoea, chlamydia, and trichomoniasis at the initial evaluation.[36] The policy of the Amsterdam STI clinic deviates from this guideline: SAV are not routinely (and blindly) treated at the initial consultation. The considerable proportion of SAV who returned to the STI clinic for treatment (female SAV: 89.0%; male SAV: 92.0%) does not support presumptive treatment as recommended by the CDC

guideline in our setting. Moreover, presumptive treatment may contribute to increasing antimicrobial resistance. Nevertheless, transmission of STI could have occurred in the period until treatment or in those who did not return for treatment.

PUBLIC HEALTH INTERVENTIONS

Chlamydia testing at home

Since 2002, improvements in *C. trachomatis* and *N. gonorrhoeae* NAAT technologies have enabled implementation and significant expansion of screening programs using less invasive specimen collection.[37] At the Amsterdam STI clinic, low-risk young clients were given the choice to collect specimens at the clinic with or without sexual health counselling, or at home. The vast majority chose to collect specimens at home and the chlamydia positivity was 6%.

Although the Dutch National Institute for Public Health (RIVM) regards being young (below 25 years) as an indication to be tested at an STI clinic, the urogenital chlamydia prevalence in young clients without any other risk indicators was low compared to the positivity rate among all STI clinic clients. Next to STI testing, the Amsterdam STI clinic also provides sexual health counselling. Clients who were eligible to self-collect samples at home were asked whether they would like to have a sexual health consultation. Less than 2% chose this option. Based on the high proportion of low-risk youngsters who chose to collect at home, the low chlamydia positivity rate, and the low number seeking face-to-face care by a health provider, offering home collection seems acceptable.

A possible drawback of home collection could be that the threshold is too low; patients who could benefit from sexual health counselling might choose the home option as this is experienced as an easier – but not necessarily the best - alternative. Offering all young clients a face-to-face consultation by a counsellor might lower the barrier to disclose any need for sexual health counselling. However, a qualitative study among young clients at the Amsterdam STI clinic showed that most youngsters did not have any interest in counselling concerning sex, STI or testing.[38] Those with a need for counselling would prefer a combination of online and face-to-face. The STI clinic does provide face-to-face counselling only; online counselling is offered by Sense – a nationwide program for youngsters – by email or with a chat function.[39]

The identity of clients opting for home collection could not be verified as these patients did not come to our clinic and anonymous care is provided. Every time a client requested specimen home collection, a new patient record was created in our electronic patient database. Due to this duplication of patient records, it is difficult to follow-up on home collectors. Therefore, clients with many duplicate requests – those who will possibly benefit from sexual health counselling - could not be easily identified. Due to budget restrictions, the STI clinic ended the home collection option in the beginning of 2015. From this moment on, all clients need to visit the STI clinic and identify themselves to obtain their collection materials and collect their specimens at the sampling toilets. In future, the STI clinic aims to offer self-sampling in low-risk young clients at most twice a year. At the third request within a year, a consultation with a trained nurse will be planned.

At the Amsterdam STI clinic, youngsters are only screened when they actively request an STI test. This mode of STI care resulted in a high proportion (88%) of clients who returned their specimens. From 2008-2011, the Chlamydia Screening Implementation (CSI) study invited youngsters from two Dutch cities and one region to test for chlamydia. Participants were invited offline but could request the chlamydia test online and also the results could be retrieved online.[40] Specimens were self-collected at home and sent to a laboratory for testing. Participation declined from 16.1% at the first invitation to 9.5% at the third.[40] Although the return rate at the STI clinic was considerably higher than the proportion who participated in the CSI study, the threshold to participate in the CSI screening was probably lower than that for actively seeking STI care at the STI clinic.

Commercial providers have also started to offer anonymous STI and HIV testing. In two Dutch pharmacies clients can buy STI and HIV tests from a vending machine located outside.[41] In addition, an increasing number of websites offer STI self-tests.[42] Two types of tests exist: the so-called home implementation tests, which people can perform at home (similar to a pregnancy test), and home collection tests, for which they collect a self-sample at home and send it to a laboratory for testing.[42] Limited knowledge is available on the preference and use of home tests for STI and HIV. Among the general population of Amsterdam STI/ HIV home test usage was low but an increase over time was observed for chlamydia and syphilis home test usage.[43] An evaluation of Dutch commercial providers of STI tests showed that all home implementation tests had unacceptably low test performances.[42] Some of those offering reliable home collection tests did not offer proper after care and follow-up required for those who

tested positive.[42] At STI clinics, next to providing information and treatment to patients with an STI, discussing partner notification is part of routine care. The expertise of STI clinics and their collaboration with specialised laboratories could be ideal to serve also clients without a test indication who are willing to pay for low threshold anonymous care. Unfortunately, STI clinics are only allowed to serve patients selected on the basis of risk prioritization.[44] In the future, more individuals may buy their own tests from a health care provider that is not collaborating with an STI clinic. For this, it is very important that reliable and affordable home tests are offered and follow-up care is provided to those with a positive test. In the light of the developments, STI clinics should focus more on individuals at high-risk for STI and those in need of sexual health counselling.

Online partner notification tool

Suggestatest.nl, a website designed to notify sexual partners of possible STI exposure, was implemented in 2012 at the STI clinics of Rotterdam and Amsterdam. The website was used by a relatively small proportion of STI clinic patients who were diagnosed with an STI. More than three-quarters of those who used this tool, reported that they notified more partners than they would have done if the Suggestatest.nl option had not been available. From the four notification methods offered – SMS, e-mail, postal letter, and through profiles of a gay dating site – SMS was by far the method most opted for.

Patients had the option to send a notification with (non-anonymous) or without (anonymous) their name. Although most notifications were sent anonymously, about a quarter of the users also sent a non-anonymous notification. It is recommended that STI clinics should offer both options but should encourage non-anonymous notifications, because notified partners rated the acceptability of receiving an anonymous notification lower than a non-anonymous notification. Another reason to encourage non-anonymous notifications is the window period for STI: if the sender of the notification is known, partners can report the date of the last sexual exposure.

Surprisingly, the STI positivity rate was lower in partners notified through Suggestatest.nl than in partners notified with contact cards. Because of a lower threshold, digital notifications are possibly also sent to partners who are marginally at risk. Another explanation might be that those patients who use Suggestatest.nl are more concerned about their own and their partners health.

With the implementation of a tool like Suggestatest.nl, a possible trade-off appears between the lower threshold to notify partners and the STI risk of notified partners. Another possible disadvantage is the reliability: notified partners need to trust the notification they received. If these partners do not recognize the name of the sender of the notification (Suggestatest.nl) some will probably hesitate to open the notification message and likely will not click on the link to read the details. Concerns about confidence and emails ending up in spam folders might have impeded STI care seeking behaviour. Forty per cent of the partners notified through Suggestatest.nl did not log-in to read their notification. Some of them may have sought STI care without reading their detailed notification, but the low percentage suggests that in some cases the notification did not result into seeking adequate care.

One year before Suggestatest.nl was implemented, a privately initiated website called "soaalarm.nl" was launched.[45] To send a notification, users had to fill-in their own e-mail address. Because everyone could use this tool, fear for misuse of soaalarm.nl has been reported in the media.[46] To limit misuse with Suggestatest.nl, only persons verified by healthcare providers – patients diagnosed with an STI – could use this tool. Since 2015, a nationwide online tool called "partnerwaarschuwing.nl" has been developed. Both STI clinics and general practitioners can use this tool. To limit efforts of the health care professionals and to lower the threshold for patients, the process of generating and disseminating notification codes has been automated at the Amsterdam STI clinic. STI positive patients at our clinic who read their test results online can directly click on a personal link to partnerwaarschuwing.nl and start notifying partners instantaneously.

The experience with Suggestatest.nl has shown the difficulty in following trends in communication. In the development phase of Suggestatest.nl, sending notifications to profiles of a gay dating website was seen as a potential method to reach partners who were previously difficult to trace. After Suggestatest.nl was developed – including the option to send notifications to someone's gay dating profile - new gay dating websites and app's became popular. STI clinics should follow trends to actualize their working methods.

Concluding remarks

Many questions are answered in this thesis and many new research questions were raised. The attributable fraction of pharyngeal chlamydia in the ongoing transmission of chlamydia needs to be addressed e.g. by modelling studies. Next, there is only limited

knowledge of the impact of missed chlamydia and gonococcal infections due to the window phase after recent sexual behaviour. The window phase of NAAT tests should be established and implemented in guidelines and in practice.

Since LGV is less often detected in the urethra than in the anorectal tract, it is important to do further research to elucidate the transmission route. If oro-anal gastrointestinal LGV infections play a role, pharyngeal testing in at risk clients should be implemented at STI clinics and general practitioners. The same possibly applies for non-LGV chlamydia infections. As the majority of the MSM and women at the Amsterdam STI clinic did not use any protection against STI during oral sex, the risk of fellatio for pharyngeal and possibly anal chlamydia should receive more attention in testing algorithms and prevention messages.

STI clinics can play an important role in certain populations by providing specialised STI care and sexual health counselling. For HIV positive MSM in care at an HIV specialist, STI clinics could have added value by offering additional diagnostics like HCV testing. For victims of a sexual assault, STI clinics could offer counselling and dedicated STI care.

The development of new diagnostic tools and digital options offer many opportunities for STI clinics. Internet, email and the use of smartphones facilitates public health interventions that were formerly not possible. The emergence of Internet and the development of sensitive diagnostic tests including self-sampling options, enabled home-based sample collection. The high sample return rate and relatively low chlamydia positivity rate show that among young low-risk clients home-based sample collection is a feasible approach. Diverting low-risk clients to home-based testing made it possible to allocate trained personnel to clients who need specialised, face-to-face care most. The implementation of an online partner notification tool seemed to increase the number of notified partners. However, STI clinics should be aware of notified partners who do not trust their digitally received notification.

To incorporate the diagnostics and innovations evaluated in this thesis into clinical practice, financial resources are needed. STI care providers with limited budgets will have to make decisions which individuals should be screened for, which pathogens, and at which anatomical locations. Therefore, additional testing is limited by resources available. Evaluations as described in this thesis are invaluable for STI clinics to make the right evidence-based decisions.

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APPENDIX

SUMMARY

In **Chapter 1** background information is given on how STI care in the Netherlands is organised and which risk groups are indicated to be served by STI clinics. Next, a description is given of the process of the STI outpatient clinic of the Public Health Service of Amsterdam, where most of the studies were conducted. In addition, all studies in this thesis are briefly introduced.

EVALUATION OF DIAGNOSTIC TESTS

In **Chapter 2**, two types of diagnostic tests are evaluated: herpes simplex virus (HSV) type-discriminating antibody tests and Gram-stained urethral smears to diagnose chlamydia.

Commercially available type-discriminating glycoprotein G (gG) directed HSV antibody tests for genital herpes infections

At the STI clinic of Amsterdam, clients with symptoms that are suggestive of genital herpes were tested with a polymerase chain reaction (PCR) to investigate whether herpes is the cause of the symptoms and if so, what type of herpes (type 1 or 2). Herpes simplex virus (HSV) type-specific antibody tests can be used to identify individuals who have never had a herpes infection and to distinguish individuals with a first episode of HSV from those with a repeated episode. In another study, we discovered that in some HSV cases - contrary to expectations - no herpes antibodies could be detected. To investigate this remarkable finding, three commercially available type-discriminating HSV antibody tests - HerpeSelect immunoblot, HerpeSelect ELISA, and the Liaison indirect chemiluminescence immunoassay - were evaluated in sera from 17 patients with a recurrent genital HSV-1 and 33 patients with a recurrent genital HSV-2 episode. Recurrence of HSV-1 or HSV-2 was established by viral PCR tests. The time between the first and the recurrent HSV episode was at least 3 months.

For HSV-1, the immunoblot was HSV-1 positive in 70.6%, the ELISA in 82.4%, and the LIAISON in 88.2%. The sera from patients with a recurrent HSV-2 episode proved HSV-2 positive in 84.8%, 84.8%, and 69.7% of those tested with the immunoblot, the ELISA, and the LIAISON respectively. Among 15/17 (88.2%) patients with HSV-1 and 30/33 (90.1%) patients with HSV-2, HSV-1 or HSV-2 antibodies, respectively, were detected in at least one of the three antibody tests. This study showed that none of the commercial type-specific gG HSV-1 or HSV-2 antibody assays were able to detect antibodies in all

selected sera. The clinical and epidemiological use of type-specific HSV serology may be limited by false-negative results, especially if based on a single test.

Gram-stained urethral smears to diagnose chlamydia

The Amsterdam STI clinic performs Gram-stained urethral smears as a point-of-care (POC) test. Based on the result, patients could be treated immediately, for example because of a suspicion of a chlamydia infection. The infection is confirmed by a sensitive laboratory test, the results of which are available after a maximum of one week. The effect of changing the Gram stain testing algorithm of urogenital chlamydia - from all male high-risk (male sex partner, symptoms, notified of an STI and/or sexwork) patients irrespective of symptoms (2008-2009) to Gram stain testing only for those with symptoms (2010-2011) - was assessed with respect to: diagnostic accuracy, loss to follow-up, correctly managed consultations and costs. The sensitivity of the Gram stain analysis was 83.8% in the 2008-2009 period and 91.0% in the 2010-2011 period. The specificity was respectively 74.1% and 53.1%. The positive predictive value was low in both periods, respectively 31.7% and 35.6%, whereas the negative predictive value was high, respectively 97.0% and 95.4%. The loss to follow-up rate in 2008–2009 and in 2010–2011 was, respectively, 1.8% vs 2.3%. Overtreatment was high in both periods, 68.0% vs 64.1%. The cost per correctly managed consultation was 14.3% lower in the 2010–2011 period (€80.82 vs €94.31 in 2008-2009). The percentage of infections treated with delay was significantly lower in the 2008–2009 period (10.5%) compared with the 2010–2011 period (22.8%). In conclusion, the change in the testing algorithm policy resulted in a higher sensitivity of the Gram-stained urethral smears, less overtreatment, and lower costs per consultation. Next to these favourable outcomes, the specificity decreased and the percentage of chlamydia patients with delayed treatment increased. In the absence of highly sensitive and specific POC tests for chlamydia, Gram stain analysis seems an appropriate alternative to detect urogenital chlamydia in symptomatic males.

CLINICAL MANAGEMENT

Chapter 3 includes three prevalence studies on: pharyngeal *Chlamydia trachomatis* among men who have sex with men (MSM) and at-risk females, urogenital lymphogranuloma venereum among MSM, and methicillin-resistant *Staphylococcus aureus* (MRSA) among MSM.

Pharyngeal *Chlamydia trachomatis*

Until 2011, patients were not screened at the Amsterdam STI clinic for pharyngeal *C. trachomatis*. In this study, the prevalence, spontaneous clearance, and genotypes of pharyngeal *C. trachomatis* among STI clinic patients were examined. Female patients at high risk for an STI (symptoms, notified of an STI, and/or sexwork) who reported active oral sex and all MSM were screened for pharyngeal chlamydial RNA. At treatment visit – median of 10 days after the first test – a repeat swab was obtained to evaluate spontaneous clearance in untreated patients with pharyngeal chlamydia. Pharyngeal chlamydia was detected in 148/13,111 (1.1%) MSM and in 160/6915 (2.3%) women. 53% of MSM and 32% of women with pharyngeal chlamydia did not have a concurrent anogenital chlamydia infection. In 16/43 (37%) MSM and in 20/55 (36%) women, the repeat pharyngeal swab was negative. Of 23 MSM with pharyngeal chlamydia who had sex with a lymphogranuloma venereum (LGV)-positive partner recently or in the past, two were LGV biovar positive (8.7%). The pharynx is a reservoir for *C. trachomatis* including LGV, and may play a role in the ongoing transmission of these pathogens. In high-risk patients, testing the pharynx for chlamydia should be considered.

Urethral lymphogranuloma venereum

Patients are not routinely screened for urethral LGV. To establish the positivity rate for urogenital LGV and to find potential evidence for the possibility of LGV transmission from the urethra to the rectum, a selection of MSM with urogenital chlamydia was tested for urogenital LGV. We found that in 341 MSM with anorectal LGV, 7 (2.1%) had concurrent urethral LGV. Among 59 partners of anorectal LGV positive MSM, 4 (6.8%) had a urethral LGV infection. Urethral LGV was common in the selected MSM population, probably key in transmission, and is missed in current routine LGV screening algorithms.

MRSA among men who have sex with men

Community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) is common among MSM in the USA. The aim of this study was to assess the CA-MRSA prevalence in MSM at the Amsterdam STI clinic. At the STI clinic 74 MSM with clinical signs of a skin or soft tissue infection (symptomatic group) and 137 MSM without clinical signs of such infections (asymptomatic group) were included. MRSA was diagnosed in two cases (0.9%; one symptomatic and one asymptomatic), neither had CA-MRSA strains. In contrast to MSM in the USA, in the period 2008 until 2010, CA-MRSA among MSM at the Amsterdam STI clinic was rare.

PUBLIC HEALTH SURVEILLANCE

In **Chapter 4**, STI testing outcomes among three different populations at the Amsterdam STI clinic are evaluated. First the yield of hepatitis C screening in HIV-positive MSM and MSM opting-out of HIV testing is discussed. Next, the outcomes of STI screening in MSM requesting post-exposure prophylaxis (PEP) for HIV and finally, in victims of a sexual assault are evaluated.

Hepatitis C screening among HIV-positive MSM and MSM opting-out of HIV testing
In 2007, routine hepatitis C virus (HCV) antibody testing was introduced at the STI clinic for MSM with a HIV-positive or unknown status. We evaluated whether this screening resulted in additional and earlier HCV diagnoses among MSM who also attend HIV clinics. One hundred twelve (6.4%) HIV-positive and three (0.7%) HIV-status-unknown MSM tested anti–HCV-positive at first consultation. During follow-up consultations, 32 HIV-positive (incidence 2.35/100 person years) and none of the HIV-status-unknown MSM became anti–HCV-positive. HCV diagnosis data at the HIV clinic were requested for the remaining 85 MSM and were available for 54 MSM. Of the 54 MSM with HIV clinic data available, 28 (51.9%) had their first HCV diagnosis at the STI clinic, of whom 7 concurrently with HIV. Three HCV cases probably would have been missed at their subsequent scheduled HIV clinic consultation. The introduction of routine anti-HCV testing at the STI outpatient clinic resulted in additional and earlier HCV detection among HIV-positive MSM.

STI in MSM requesting for post-exposure prophylaxis for HIV
MSM with an indication for post-exposure prophylaxis for HIV were screened for STI and after 14 days, chlamydia and gonorrhoea screening was repeated. At initial visit, an STI was found in 16.5% of the MSM and among those who were initially STI negative, 4.1% had chlamydia or gonorrhoea after 14 days. In men with an indication for HIV post-exposure prophylaxis, repeat chlamydia and gonorrhoea screening is advised to diagnose infections not present at baseline screening.

STI in victims of a sexual assault
Sexual assault victims (SAV) were tested for STI and in case of an STI they had to return to the STI clinic to obtain their antibiotics. We assessed the STI positivity rate and treatment uptake of female and male SAV. From January 2005 to September 2016, 1,066 and 135 consultations involved female and male SAV, respectively. The STI positivity

rate among female SAV was comparable with female non-SAV (11.2% versus 11.6%). Among male SAV, the STI positivity rate was 12.6% versus 17.7% among non-SAV (not significant). Male SAV had lower odds for having a bacterial STI than male non-SAV, when adjusting for confounders. The return rate of SAV for treatment was high (females: 89.0%; males: 92.0%); this high return rate does not support recommendations for presumptive therapy.

PUBLIC HEALTH INTERVENTIONS

Evaluations of two new interventions – chlamydia testing at home and an online partner notification website - are presented in **Chapter 5**.

Chlamydia testing at home

The efficiency of chlamydia home collection kits for low-risk heterosexual persons under 25 years was studied. During an online appointment request, they were offered 3 different ways of chlamydia testing: (1) receiving a home collection kit or (2) coming to the clinic without sexual health counselling or (3) coming to the clinic with sexual health counselling. The collection kit was sent to the client by surface mail and the self-collected vaginal swab (all women), anal swab (women reporting receptive anal sex) or urine sample (men) were sent back to the laboratory for testing and the results could be retrieved online. Between September 2012 until July 2013, from 1804 online requests, 1451 (80%) opted for the home collection kit, 321 (18%) chose an appointment at the clinic without, and 32 (2%) an appointment with sexual health counselling. Of the requested home collection kits, 88% were returned. Chlamydia was diagnosed in 6.0% of the clients receiving a home collection kit, in 5.6% of the clients at the STI clinic without and in 4.8% of the clients with sexual health counselling. Home collection was the most often chosen method for young low-risk heterosexual clients who seek STI care. The compliance to collect and return the samples was high.

Online partner notification tool

Suggestatest.nl, an internet-based notification system, was developed for patients with a verified diagnosis of STI/HIV. Suggestatest.nl uses email, short message service, postal letter or a gay dating site to notify sexual contacts. Suggestatest.nl was piloted in two major cities in the Netherlands: at the STI clinics of the Public Health Services of Rotterdam and Amsterdam. We evaluated the use of Suggestatest.nl and among those who used Suggestatest.nl (both index patients with a verified STI and notified partners) the acceptability and usability were assessed. Of 988 index patients receiving

a code to use Suggestatest.nl, 14% notified partners through Suggestatest.nl: 84% by text messaging and 15% by email; 88% anonymously. Of those intending to use Suggestatest.nl, 23% notified with Suggestatest.nl. 58% of Suggestatest.nl-notified partners accessed Suggestatest.nl and 20% of them subsequently attended the STI clinic of Rotterdam or Amsterdam. STI positivity in partners was lower in those notified by Suggestatest.nl (28%) than in those with conventional contact cards (45%). Suggestatest.nl users rated the online tool acceptable and usable. Both groups were less positive about Suggestatest.nl to notify/be notified of HIV than of other STI. An anonymous notification was perceived as less acceptable. Suggestatest.nl is a valuable novel tool for notification of verified STI diagnoses by index patients and providers. Although the user ratings were in general favourable about Suggestatest.nl, STI clinics willing to implement an online notification tool should be aware of the expressed concerns of using Suggestatest.nl to notify of HIV and receiving anonymous notifications.

GENERAL DISCUSSION

A general discussion of the results of chapter 2 through chapter 5 is given in **Chapter 6.**

The benefit of using type-specific HSV serology – with the occurrence of false-negative results as shown in this thesis – needs to be critically assessed before being implemented. For the use of Gram-stained smears as a point-of-care test for urogenital chlamydia the same applies: false-negative and especially false-positive results are relatively often found. In STI clinics with resources available for rapid nucleic acid amplification testing (NAAT), Gram-stained smears could be omitted.

The prevalence of pharyngeal chlamydia among high-risk women and MSM was relatively low. However, the high proportion of pharyngeal chlamydia positive patients without anogenital chlamydia implies that without routine screening, they would have left the STI clinic untreated. In addition, spontaneous clearance was lower than expected a priori. More research is needed to elucidate the role of pharyngeal to urethral transmission to the epidemic at large. Although the prevalence of urethral LGV was relatively high among a selection of high-risk MSM, routine screening of urethral LGV among MSM did not reveal a high prevalence. More research is needed to study the transmission of LGV. Community-associated-MRSA, often found among US MSM, was not shown to be prevalent among MSM at the Amsterdam STI clinic.

Although hepatitis C infection was relatively often diagnosed (high prevalence and incidence) among HIV-positive MSM at the STI clinic, in many of those men their HIV specialist made the HCV diagnosis around the same time. STI were often diagnosed at initial visit of MSM requesting for post-exposure prophylaxis (PEP) for HIV. Among a considerable group of MSM using PEP and testing STI negative at initial visit, chlamydia and gonorrhoea were diagnosed after two weeks. More research is needed to elucidate the role of recent exposure and the ability of screening tools to detect early infections. STI were also often diagnosed among victims of a sexual assault; the vast majority of victims returned to the STI clinic for treatment. Unfortunately, the absolute number of victims who attended the STI clinic was relatively small. The considerable proportion of SAV who returned to the STI clinic for treatment does not support presumptive treatment in our setting as recommended by the CDC guideline.

Most young low-risk clients chose to collect specimens for chlamydia testing at home and the chlamydia prevalence among them was relatively low. Next to this interesting way of offering low-threshold STI care, the disadvantage of not having a conversation - including the possibility of counselling - with the client, should be taken into consideration. A website to notify partners - mainly via anonymous SMS texting and email - was used by a selection of patients. More research is needed to elucidate the lower STI prevalence among those notified through this website, compared to those notified through traditional partner notification cards. STI clinics willing to implement a partner notification website should consider the lower acceptance of users to notify partners about their HIV exposure or to receive anonymous notifications.

NEDERLANDSE SAMENVATTING

Hoofdstuk 1 beschrijft hoe de soa-zorg in Nederland is geregeld en aan welke risicogroepen soa-poliklinieken zorg bieden. Het werkproces van de Soa-polikliniek van de GGD Amsterdam, waar de meeste studies zijn uitgevoerd, wordt daarbij belicht. Daarnaast worden alle studies die in dit proefschrift aan bod komen, kort geïntroduceerd.

EVALUATIE VAN DIAGNOSTISCHE TESTEN

In **hoofdstuk 2** worden twee verschillende soorten laboratorium testen geëvalueerd: testen om antilichamen tegen herpes simplex virus (HSV) te bepalen en een microscopie onderzoek om chlamydia van de plasbuis vast te stellen.

Commerciële type-specifieke glycoproteïne G gerichte antilichaam testen voor genitale herpes infecties

Op de Amsterdamse Soa-polikliniek worden cliënten met verschijnselen die wijzen op een genitale herpes, getest met een polymerasekettingreactie (PCR) om te onderzoeken of herpes de veroorzaker van de symptomen is en indien het geval, welk type herpes (type 1 of 2) dit is. Herpes simplex virus (HSV) type-specifieke antilichaam testen kunnen gebruikt worden om personen te identificeren die nog nooit een herpes infectie hebben doorgemaakt en om personen met een eerste episode van HSV te onderscheiden van die met een herhaalde episode. In een ander onderzoek kwamen we tot de ontdekking dat in sommige HSV gevallen - tegen verwachting in - geen herpes antistoffen aangetoond konden worden. Om dit nader te onderzoeken zijn drie verschillende, commercieel beschikbare type-specifieke HSV antilichaam testen - HerpeSelect immunoblot, HerpeSelect ELISA, Liaison indirect chemiluminescence immunoassay – geëvalueerd met sera van 17 patiënten met een herhaalde genitale herpes episode op basis van HSV-1 en van 33 patiënten met een herhaalde genitale herpes episode op basis van HSV-2. De tijd tussen de eerste en de terugkerende herpes episode was ten minste 3 maanden. Tijdens de terugkerende episode was de immunoblot HSV-1 positief in 70,6%, de ELISA in 82,4% en de LIAISON in 88,2% van de patiënten met HSV-1. De sera van patiënten met een terugkerende HSV-2 episode bleken HSV-2 positief in 84,8%, 84,8%, en 69,7% in respectievelijk de immunoblot, de ELISA en de LIAISON. In 15/17 (88,2%) van de patiënten met HSV-1 werden HSV-1 antilichamen aangetoond in minstens 1 van de 3 gebruikte antilichaam testen. In

30/33 (90,1%) van de patiënten met HSV-2 werden HSV-2 antilichamen aangetoond in minstens 1 van de 3 gebruikte antilichaam testen. Deze studie liet zien dat geen van de commercieel beschikbare type-specifieke HSV antilichaam testen in staat was om antilichamen in alle geselecteerde sera aan te tonen. Het gebruik van deze testen in de kliniek of voor epidemiologische doeleinden kan belemmerd worden door fout-negatieve uitslagen, met name als de uitslag slechts op één enkele test wordt gebaseerd.

Urogenitale Gram-diagnostiek voor chlamydia

De Amsterdamse Soa-polikliniek verricht microscopisch onderzoek, ook genoemd Gram-diagnostiek, als point-of-care (POC) sneldiagnostiek. Op basis van deze uitslag kan er vervolgens al dan niet direct worden behandeld, bijvoorbeeld vanwege een vermoeden van een chlamydia infectie. De infectie wordt bevestigd met een gevoelige laboratorium test waarvan de uitslag na maximaal een week beschikbaar is. Het algoritme voor de Gram-test voor urogenitale (urineweg en geslachtorgaan) chlamydia is aangepast: voor 2010 werd bij alle hoog risico (seks met mannen, klachten, gewaarschuwd voor een soa en/of sekswerk) mannen ongeacht lichamelijke klachten de Gram-test gedaan, terwijl vanaf 2010 dit alleen werd gedaan bij diegenen met specifieke lichamelijke klachten. Het effect van deze aanpassing werd gemeten aan de hand van vier maten: de diagnostische precisie, loss to follow-up, het aandeel correct behandelde infecties en de bijbehorende kosten. De sensitiviteit (gevoeligheid van de test) van de Gram was 83,8% in 2008-2009 en 91,0% in 2010-2011. De specificiteit was respectievelijk 74,1% en 53,1%. De positief voorspellende waarde was in beide periodes laag: 31,7% en 35,6%. De negatief voorspellende waarde was wel hoog: 97,0% en 95,4%. Het aandeel patiënten dat de medicatie niet ophaalde (loss to follow-up) was 1,8% in 2008–2009 en 2,3% in 2010–2011. Overbehandeling (wel behandeld, maar geen chlamydia infectie) was in beide periodes hoog (68,0% en 64,1%). De kosten per correct behandeld consult waren in de 2010–2011 periode 14,3% lager (€80,82 versus €94,31 in 2008-2009). Het percentage patiënten met chlamydia dat op een later moment behandeld werd, was significant lager in de 2008–2009 periode (10,5%) dan in de 2010–2011 periode (22,8%). Samenvattend, het aanpassen van het test algoritme heeft geleid tot een hogere sensitiviteit van de urogenitale Gram-test, minder overbehandeling en lagere kosten per consult. Naast deze gunstige uitkomsten verminderde de specificiteit en zorgde het nieuwe algoritme ervoor dat meer patiënten met chlamydia pas op een later moment behandeld werden. Zolang er geen betrouwbare POC testen voor

chlamydia zijn, kan Gram-diagnostiek een geschikt alternatief zijn voor het vaststellen van chlamydia in de plasbuis bij mannen met lichamelijke klachten.

KLINISCH MANAGEMENT

In **hoofdstuk 3** komen drie prevalentie (het percentage personen met de betreffende aandoening) studies aan bod die uitgevoerd zijn op de Amsterdamse Soa-polikliniek: de prevalentie van chlamydia in de keel bij mannen die seks hebben met mannen (MSM) en hoog risico (klachten, gewaarschuwd voor een soa en/of sekswerk) vrouwen, de prevalentie van urogenitale lymphogranuloma venereum (LGV; een agressieve chlamydia variant) bij MSM en de prevalentie van meticilline-resistente *Staphylococcus aureus* (MRSA) bij MSM.

Chlamydia in de keel

De prevalentie, het spontaan klaren en het type chlamydia in de keel is onderzocht bij patiënten van de Soa-polikliniek. Vrouwelijke patiënten met een hoog risico op soa die actief orale seks rapporteerden en alle MSM werden getest op chlamydia RNA in de keel. Tijdens het behandel bezoek– een mediaan van 10 dagen na de eerste test – werd een herhaal swab (uitstrijkje) afgenomen om inzicht te krijgen in het spontaan klaren van onbehandelde keel chlamydia. Chlamydia in de keel werd bij 148/13 111 (1,1%) MSM en bij 160/6915 (2,3%) vrouwen gevonden. Van de MSM en vrouwen met keel chlamydia hadden 53% en 32% op dat moment geen anogenitale chlamydia infectie. Spontane klaring van keel chlamydia werd gezien bij 16/43 (37%) van de MSM en 20/55 (36%) van de vrouwen. In totaal hadden 23 MSM met keel chlamydia recent of in het verleden een sekspartner met LGV. In twee van deze gevallen bleek dat de keel chlamydia veroorzaakt werd door LGV (8,7%). Samenvattend, de keel is een reservoir voor chlamydia waaronder de LGV veroorzakende L-biovar, en speelt mogelijk een rol in de voortdurende transmissie van chlamydia. Het testen van de keel zou daarom overwogen moeten worden bij hoog risico cliënten.

Urogenitale lymphogranuloma venereum

In de dagelijkse praktijk worden cliënten op de Soa-polikliniek niet routinematig op urogenitale LGV getest. Een selectie van MSM met urogenitale chlamydia is getest op LGV om te onderzoeken welk percentage hiervan urogenitale LGV heeft en om bewijs te vergaren of LGV overgebracht wordt van de urethra naar het rectum. Van de 341 MSM met anorectale LGV, hadden 7 (2,1%) tegelijkertijd urogenitale LGV. Bij 4 (6,8%)

van de 59 partners van MSM met een anorectale LGV werd een urogenitale LGV infectie gevonden. Mogelijk spelen deze urogenitale LGV infecties een rol in de transmissie. Met de huidige LGV screening richtlijnen worden deze urogenitale LGV infecties gemist.

MRSA bij mannen die seks hebben met mannen
Community-associated (buiten het ziekenhuis opgelopen) meticilline-resistente *Staphylococcus aureus* (CA-MRSA) komt vaak voor onder MSM in de Verenigde Staten. Het doel van deze studie was om de CA-MRSA prevalentie onder MSM op de Soa-polikliniek te meten. Op de Soa-polikliniek zijn 74 MSM met een aanwijzing voor een huidinfectie (symptomatische groep) en 137 MSM zonder zulke verschijnselen (asymptomatische groep) getest op MRSA. In twee gevallen werd MRSA vastgesteld (0,9%; 1 symptomatisch en 1 asymptomatisch). Geen van de twee MRSA gevallen werd veroorzaakt door CA-MRSA. In tegenstelling tot Amerikaanse MSM was CA-MRSA in de periode 2008 tot 2010 zeldzaam bij MSM op de Amsterdamse Soa-polikliniek.

SURVEILLANCE VAN DE PUBLIEKE GEZONDHEID

De soa-testresultaten van drie groepen bezoekers van de Soa-polikliniek van Amsterdam komen in **hoofdstuk 4** aan bod. Als eerste wordt de toegevoegde waarde van hepatitis C screening in hiv-geïnfecteerde MSM en in MSM die een hiv-test weigerden, geëvalueerd. Daarna worden de resultaten beschreven van de soa-testen bij MSM die voor post-expositie profylaxe (PEP) voor hiv naar de Soa-polikliniek kwamen. Als laatste worden de uitkomsten van soa-screening bij slachtoffers van seksueel geweld beschreven.

Hepatitis C screening in hiv-geïnfecteerde MSM en MSM die een hiv-test weigeren
In 2007 werd op de Soa-polikliniek gestart met het routinematig testen van hepatitis C virus (HCV) in hiv-geïnfecteerde MSM en MSM die een hiv-test weigeren. Bij MSM die ook onder behandeling waren van een hiv-internist is gekeken of de screening op de Soa-polikliniek tot extra HCV diagnosen leidde en of deze infecties eerder opgemerkt zijn dan zonder screening op de Soa-polikliniek het geval zou zijn geweest.

Tijdens het eerste bezoek waarin op HCV werd getest, bleken 112 (6,4%) hiv-geïnfecteerde MSM en 3 (0,7%) MSM die een hiv-test weigerden, antilichamen tegen HCV te hebben. Tijdens follow-up bezoeken testten 32 hiv-geïnfecteerden (incidentie 2,35/100 persoonsjaren) en 0 hiv-weigeraars positief voor HCV antilichamen. We

hebben de datum waarop bij de hiv-behandelaar de HCV diagnose was gevonden, opgevraagd voor 85 MSM en verkregen voor 54 MSM. Bij 28 (51,9%) van deze 54 MSM werd HCV als eerste op de Soa-polikliniek gevonden; bij 7 tegelijk met een nieuwe hiv-diagnose. Tijdens het geplande bezoek aan de hiv-behandelaar zouden waarschijnlijk 3 van deze 28 gevallen met HCV gemist zijn. Het invoeren van de HCV test bij hiv-geïnfecteerde MSM op de Soa-polikliniek heeft dus geresulteerd in extra en vroegere HCV diagnosen.

Soa bij MSM met een verzoek voor post-expositie profylaxe voor hiv

MSM met een indicatie voor post-expositie profylaxe voor hiv werden op soa's getest en na 14 dagen werd de chlamydia en gonorroe test herhaald. Tijdens het eerste bezoek bleek 16,5% van de MSM een soa te hebben. Van diegenen die initieel geen soa hadden, had 4,1% na 14 dagen chlamydia en/of gonorroe. Om infecties op te kunnen sporen die tijdens baseline screening nog niet aangetoond kunnen worden, zouden MSM met een indicatie voor post-expositie profylaxe een herhaaltest voor chlamydia en gonorroe moeten krijgen.

Soa bij slachtoffers van een zedendelict

Slachtoffers van een zedendelict krijgen op de Soa-polikliniek naast counseling ook een soa-test aangeboden. Wanneer de test positief blijkt te zijn, moeten zij op een later moment terugkomen voor hun behandeling. In deze studie is gekeken naar het percentage mannelijke en vrouwelijke slachtoffers met een soa en naar het aandeel dat terugkwam voor behandeling. In de periode van 2005 tot en met september 2016 zijn op de Soa-polikliniek 1066 consulten bij vrouwelijke en 135 consulten bij mannelijke slachtoffers uitgevoerd. Het percentage vrouwelijke slachtoffers dat een soa had, bleek vergelijkbaar met dat van de algemene vrouwelijke bezoeker van de Soa-polikliniek (11,2% versus 11,6%). Bij mannelijke slachtoffers had 12,6% een soa en bij de algemene mannelijke bezoeker van de Soa-polikliniek was dit 17,7% (dit verschil was niet significant). Mannelijke slachtoffers hadden een lagere kans op een bacteriële soa dan mannen die geen slachtoffer waren (gecorrigeerd voor mogelijke factoren die het effect verstoren: confounders). Het percentage dat terugkwam naar de Soa-polikliniek voor behandeling was hoog (vrouwen: 89,0%; mannen: 92,0%). Het hoge percentage slachtoffers dat terugkwam voor behandeling is geen ondersteuning voor de aanbeveling in sommige richtlijnen om te behandelen voordat de test resultaten bekend zijn (zogenaamde presumptieve therapie).

INTERVENTIES IN DE PUBLIEKE GEZONDHEID

In **hoofdstuk 5** worden twee nieuwe interventies geëvalueerd: het thuis afnemen van testmateriaal voor een chlamydia test en een website voor het waarschuwen van sekspartners.

Chlamydia thuisafname

In deze studie werd bij laag risico (geen klachten, niet gewaarschuwd voor een soa, geen sekswerker en niet afkomstig uit een soa-endemisch land) heteroseksuele personen onder de 25 jaar onderzocht of het thuis afnemen van testmateriaal voor chlamydia efficiënt is. Tijdens het online maken van een afspraak hadden deze jongeren drie verschillende opties om op chlamydia getest te worden: (1) het ontvangen van een thuisafname kit, (2) naar de kliniek komen zonder een gesprek omtrent seksuele gezondheid, of (3) naar de kliniek komen met een gesprek omtrent seksuele gezondheid. De thuisafname kit werd per post naar de cliënt gestuurd. De door de cliënt zelf afgenomen vaginale swab (alle vrouwen), anale swab (vrouwen die anale seks rapporteerden) of urine monster (mannen) werd per post teruggestuurd naar het laboratorium, waar deze op chlamydia werd getest. De uitslagen konden online opgevraagd worden. Tussen september 2012 en juli 2013 was bij 1804 online afspraakverzoeken 1451 keer (80%) gekozen voor de thuisafname kit, 321 (18%) voor een afspraak op de Soa-polikliniek zonder en 32 (2%) met een gesprek over seksuele gezondheid. Van de aangevraagde thuisafname kits werd 88% teruggestuurd en bij 6,0% van de cliënten werd chlamydia gevonden. Bij respectievelijk 5,6% en 4,8% van de cliënten die kozen voor een afspraak op de Soa-polikliniek zonder of met een gesprek over seksuele gezondheid, werd chlamydia vastgesteld.

De meeste laag risico heteroseksuele personen onder de 25 jaar die op soa getest willen worden hadden de voorkeur om thuis testmaterialen voor chlamydia af te nemen. De overgrote meerderheid van de aangevraagde thuisafname kits werd teruggestuurd naar het laboratorium.

Online partnerwaarschuwingstool

De website genaamd Suggestatest.nl was ontwikkeld voor het versturen van geverifieerde soa en/of hiv waarschuwingen naar sekspartners. Patiënten waarbij een soa was gevonden kregen toegang tot deze website om partners te waarschuwen. Binnen Suggestatest.nl kon gekozen worden om seksuele partners te waarschuwen via

email, SMS, briefpost of een gay dating website. Suggestatest.nl was als proef opgezet bij twee GGD'en in Nederland: Rotterdam en Amsterdam. In twee studies is het gebruik van deze website geëvalueerd en hebben we de mening van de gebruikers gevraagd (zowel index patiënten met een geverifieerde soa als de door hun gewaarschuwde partners). Van de 988 index cliënten die na het krijgen van een soa/hiv diagnose een Suggestatest.nl code hadden ontvangen, had 14% een waarschuwing via Suggestatest.nl gestuurd. SMS (84%) en email (15%) waren de meest gebruikte methoden. Veruit de meeste waarschuwingen werden anoniem verstuurd: 88%. Van de cliënten die aangegeven hadden dat ze Suggestatest.nl wilden gaan gebruiken, had 23% een waarschuwing via Suggestatest.nl gestuurd. Van de gewaarschuwde partners had 58% de Suggestatest.nl website bezocht om te zien voor welke soa ze gewaarschuwd waren. Op de twee soa-poliklinieken kwam uiteindelijk 20% van de gewaarschuwde partners voor een soa-test. Het percentage gewaarschuwde partners met een soa was lager bij diegenen die via Suggestatest.nl gewaarschuwd waren (28%) dan bij diegenen die middels een traditionele waarschuwingsstrook gewaarschuwd waren (45%). Suggestatest.nl gebruikers vonden de tool acceptabel en bruikbaar. Echter, zowel de index patiënten als de gewaarschuwde partners waren minder positief over het gebruik van Suggestatest.nl voor het waarschuwen voor een hiv infectie. Een anonieme waarschuwing werd als minder acceptabel ervaren. Suggestatest.nl is een waardevolle nieuwe methode voor het versturen van geverifieerde soa/hiv waarschuwingen door index patiënten en is ook te gebruiken door zorgverleners. Alhoewel de gebruikersbeoordelingen over Suggestatest.nl in het algemeen positief waren, moeten soa-poliklinieken bewust zijn dat gebruikers terughoudender zijn om Suggestatest.nl te gebruiken om te waarschuwen voor hiv en voor het ontvangen van anonieme waarschuwingen.

ALGEMENE DISCUSSIE

Hoofdstuk 6 geeft een algemene discussie van de resultaten zoals beschreven in hoofdstuk 2 tot en met 5.

Het resultaat van type-specifieke HSV serologie bleek in bepaalde monsters fout-negatief. Voordat dit type testen ingevoerd wordt in de zorg dient men dit goed te overwegen. Voor het gebruik van Gram-diagnostiek als point-of-care test voor urogenitale chlamydia geldt dezelfde terughoudendheid: resultaten zijn relatief vaak fout-negatief en nog vaker fout-positief. Soa-poliklinieken met genoeg financiële

middelen zouden in plaats Gram-diagnostiek het gebruik van snelle nucleïnezuur-amplificatie testen (NAAT) kunnen overwegen.

Relatief weinig hoog risico vrouwen en MSM testten positief voor chlamydia in de keel. Een relatief groot deel van diegenen met een chlamydia in de keel bleek negatief te zijn voor anogenitale chlamydia. Zonder routine screening op keel chlamydia zou een groot deel daarom zonder behandeling de Soa-polikliniek hebben verlaten. Daarnaast bleek dat patiënten minder vaak spontaan keel chlamydia klaarden dan we van te voren hadden gedacht. Daarom is meer onderzoek nodig om uit te zoeken welke rol de overdracht van chlamydia van de keel naar de plasbuis speelt.

In een selectie van hoog risico MSM werd relatief vaak urogenitale LGV gevonden. Routinematig screening van MSM op urogenitale LGV liet echter zien dat in de totale groep MSM op de Soa-polikliniek urogenitale LGV niet vaak voorkomt. Daarom is meer onderzoek nodig om de transmissie van LGV te bestuderen. Community-associated MRSA, dat vaak werd gevonden bij Amerikaanse MSM, bleek weinig voor te komen bij MSM die de Amsterdamse Soa-polikliniek bezochten.

Alhoewel op de Soa-polikliniek redelijk vaak een hepatitis C infectie gevonden werd bij hiv-geïnfecteerde MSM (hoge prevalentie en incidentie), was rond dezelfde tijd bij veel van deze mannen ook door hun hiv-behandelaar een hepatitis C infectie vastgesteld. Tijdens het initiële bezoek van MSM met een verzoek voor post-expositie profylaxe (PEP) voor HIV werd frequent een soa gediagnosticeerd. Bij een aanzienlijk deel van de MSM die PEP gebruiken en geen soa hadden tijdens het initiële bezoek werd na 2 weken alsnog chlamydia of gonorroe gevonden. Meer onderzoek is nodig om antwoord te geven op de vraag welke rol recente blootstelling speelt en wat de mogelijkheid van laboratorium testen is om vroege chlamydia en gonorroe infecties aan te tonen. Bij slachtoffers van een zedendelict werd frequent een soa gevonden en de overgrote meerderheid kwam terug naar de Soa-polikliniek voor behandeling. Helaas komt slechts een relatief kleine groep slachtoffers in zorg bij de Soa-polikliniek. Het hoge percentage slachtoffers dat voor behandeling terugkwam suggereert dat in onze setting het geven van presumptieve therapie, zoals dat door de CDC aanbevolen wordt, niet zinvol is.

De meeste laag risico jongeren kozen ervoor om thuis testmaterialen voor chlamydia af te nemen en de chlamydia prevalentie onder hen was relatief laag. Bij deze interessante,

laagdrempelige manier van aanbieden van soa-zorg valt mogelijk wel een kanttekening te maken: soa-hulpverleners hebben dan maar weinig mogelijkheden tot het aangaan van een motiverend gesprek. Een website waarmee patiënten met een soa sekspartners konden waarschuwen– dit gebeurde met name anoniem via een SMS of email – werd door een relatief klein deel gebruikt. Meer onderzoek is nodig om uit te zoeken waarom de soa prevalentie lager is bij partners die via deze website gewaarschuwd waren dan diegenen die via traditionele waarschuwingsstroken gewaarschuwd werden. Soa-poliklinieken die van plan zijn om met een partnerwaarschuwswebsite te gaan werken moeten zich ervan bewust zijn dat gebruikers dit minder geschikt vinden om anderen voor hiv te waarschuwen, of om anoniem gewaarschuwd te worden.

ABOUT THE AUTHOR

Martijn van Rooijen was born on December 03, 1980 in Woerden, the Netherlands. He spent his youth in the city of Montfoort were he had his first part time job at the apple and pear orchard of his uncle. In 2007 he started a part time job at Schaap Foodservice were he also started his experience with driving trucks.

After high school at the Minkema college in Woerden he started in 1999 the study Biomedical Sciences in Leiden. During the Master phase, in 2003 he went for his internship to Ghana where he tried to elucidate the transmission route of *Oesophagostomum bifurcum*. This nematode can cause in humans the parasitic disease oesophagostomiasis. Interestingly, human infection with this nematode is largely localized to northern Togo and Ghana in western Africa. He performed both fieldwork (collection of baboon stools and grass from sleeping areas of the baboon) and experimental work, including molecular detection of the pathogen. During the last part of the Master period, he chose the pedagogic specialisation. During two internships of half a year each he provided biology lessons to high school students. In 2005 he obtained the Master of Science teacher training in biology degree. Next to this degree he obtained the Master of Science degree in Biomedical Sciences. In 2005, he also obtained his truck license which he used after graduation at Linde Gas Therapeutics (formerly known as Hoekloos Medical) delivering oxygen and giving instructions to patients at home. From October 2005 he went by car from Montfoort to Ghana on a 5-month road trip. After returning home, he continued at Linde Gas Therapeutics and in 2006 he worked as a temporary teacher at the Stedelijk Gymnasium in Utrecht. In April 2007 he was appointed in a permanent position at the Public Health Service of Amsterdam as data manager at the STI clinic and the Public Health Laboratory.

Currently he is supervising two PhD students and he is working on the project "Amsterdam Anders". This multidisciplinary project aiming to make the STI clinic future-proof and efficient is a very challenging task.

Martijn is married with Anne Dekkers. Together with their daughters Roos (7) and Femke (3) they live in Hollandsche Rading.

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7

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Chapter 2.2. Point-of-care management of urogenital *Chlamydia trachomatis* via Gram-stained smear analysis in male high-risk patients. Diagnostic accuracy and cost-effectiveness before and after changing the screening indication at the STI Clinic in Amsterdam.
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Chapter 3.1. Spontaneous pharyngeal *Chlamydia trachomatis* RNA clearance. A cross-sectional study followed by a cohort study of untreated STI clinic patients in Amsterdam, the Netherlands.
Conceived and designed study: M.S. van Rooijen, M.F. Schim van der Loeff, A.P. van Dam, A.G.C.L. Speksnijder, and H.J.C. de Vries. *Contributed to data collection*: M.S. van Rooijen. *Laboratory analyses*: A.P. van Dam, A.G.C.L. Speksnijder, and S.A. Morré. *Conducted statistical analyses*: M.S. van Rooijen and M.F. Schim van der Loeff. *Drafted the manuscript*: M.S. van Rooijen, M.F. Schim van der Loeff, and H.J.C. de Vries. All authors participated in review of the manuscript, and read and approved the final version.

Chapter 3.2. Urethral lymphogranuloma venereum infections in men with anorectal lymphogranuloma venereum and their partners: the missing link in the current epidemic?
Conceived and designed study: M.S. van Rooijen, N.H. de Vrieze, and H.J.C. de Vries. *Contributed to data collection*: N.H. de Vrieze, M.S. van Rooijen. *Conducted statistical analyses*: N.H. de Vrieze, and M.S. van Rooijen. *Drafted the manuscript*: N.H. de Vrieze. All authors participated in review of the manuscript, and read and approved the final version.

Chapter 3.3. Low prevalence of methicillin-resistant *Staphylococcus aureus* among men who have sex with men attending an STI clinic in Amsterdam: a cross-sectional study.
Conceived and designed study: A.P. van Dam and H.J.C. de Vries. *Contributed to data collection*: M.S. van Rooijen. *Laboratory analyses*: A.J. de Neeling and A.P. van Dam. *Conducted statistical analyses*: M.S. van Rooijen, I. Joore, and M.F. Schim van der Loeff. *Drafted the manuscript*: I. Joore, A.P. van Dam, M.F. Schim van der Loeff, and H.J.C. de Vries. All authors participated in review of the manuscript, and read and approved the final version.

Chapter 4.1. Earlier detection of hepatitis C virus infection through routine hepatitis C virus antibody screening of human immunodeficiency virus-positive men who have sex with men attending a sexually transmitted infection outpatient clinic: a longitudinal study.
Conceived and designed study: M.S. van Rooijen, T. Heijman, N.H. de Vrieze, and M. Prins. *Contributed to data collection*: M.S. van Rooijen and N.H. de Vrieze. *Conducted statistical analyses*: M.S. van Rooijen and M. Prins. *Drafted the manuscript*: M.S. van Rooijen, T. Heijman, H.J.C. de Vries, and M. Prins. All authors participated in review of the manuscript, and read and approved the final version.

Chapter 4.2. Additional gonorrhea and chlamydia infections found with rapid follow-up screening in men who have sex with men with an indication for HIV post-exposure prophylaxis.
Conceived and designed study: M.S. van Rooijen, N.H. de Vrieze, M.F. Schim van der Loeff, and H.J.C. de Vries. *Contributed to data collection*: M.S. van Rooijen. *Conducted statistical analyses*: N.H. de Vrieze, and M.F. Schim van der Loeff. *Drafted the manuscript*: N.H. de Vrieze, M.F. Schim van der Loeff, and H.J.C. de Vries. All authors participated in review of the manuscript, and read and approved the final version.

Chapter 4.3. Sexually transmitted infection positivity rate and treatment uptake among female and male sexual assault victims attending the Amsterdam STI clinic between 2005 and 2016.
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Chapter 5.1. Young low-risk heterosexual clients prefer a chlamydia home collection test to a sexually transmitted infection clinic visit in Amsterdam, the Netherlands, a cross-sectional study.

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Chapter 5.2. Initial evaluation of use of an online partner notification tool for STI, called 'suggest a test': a cross sectional pilot study.

Conceived and designed study: H.M. Götz, and H.A.C.M. Voeten supported by M.S. van Rooijen, E. Op de Coul, T. Heijman, and R.H. Koekenbier. *Implementation at the STI clinic and data collection*: M. Hamers, and F. van den Heuvel. *Conducted statistical analyses*: H.M. Götz, and M.S. van Rooijen. *Drafted the manuscript*: H.M. Götz, M.S. van Rooijen, and H.A.C.M. Voeten. All authors participated in review of the manuscript, and read and approved the final version.

Chapter 5.3. Sender and receiver acceptability and usability of an online partner notification tool for sexually transmitted infection in the Netherlands.

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PORTFOLIO

Oral presentations	Year
High persistence of pharyngeal chlamydia in high-risk visitors at the STI clinic, Amsterdam. 14th Annual scientific meeting of the Nederlandse Vereniging voor Experimentele Dermatologie, Lunteren, the Netherlands	2013
High persistence of pharyngeal chlamydia in high risk visitors at the STI clinic, Amsterdam. ISSTDR, Vienna, Austria	2013
POC-management & gonorroe. Regio-middag, Amsterdam, the Netherlands	2013
Oropharyngeal chlamydia infections and persistence. 9th Annual Amsterdam Chlamydia Meeting, Amsterdam, the Netherlands	2014
Persistence of pharyngeal *Chlamydia trachomatis* among STI clinic patients, Amsterdam, the Netherlands. Thirteenth International Symposium on Human Chlamydial Infections, Pacific Grove, CA, US	2014
Spontane klaring van *Chlamydia trachomatis* RNA in de pharynx van onbehandelde soa-poli patiënten. Nascholing anticonceptie en Soa, Amsterdam, the Netherlands	2014
Koppeling online partnerwaarschuwing met elektronisch patiënten dossier. Dragons Den pitch cluster sociaal gemeente Amsterdam, the Netherlands	2015
Soa-incidentie in de Amsterdamse regio. Nederlandse Vereniging voor Dermatologie en Venereologie (NVDV) Amsterdam, the Netherlands	2015
Soa/hiv-testgedrag en positivity rate in de Amsterdamse regio. Workshop "Onze soa/hiv-bestrijding begint met GIS-werk…!" Soa*hiv*seks congres, Amsterdam, the Netherlands	2015
Implementation, use, acceptability and usability of the online partner notification tool for STI (Suggest-A-Test). Department of Dermatology, AMC, Amsterdam, the Netherlands	2016
Privacy en good clinical practice bij onderzoek binnen de seksuele gezondheidszorg. Regiomiddag Noord-Holland/Flevoland, the Netherlands	2016
SOA-polikliniek Amsterdam ONLINE, Tools voor efficiënte bedrijfsvoering, patiënt-gemak en het doorbreken van de transmissieketen. Inspiratiebijeenkomst GGZ NHN – GGD Amsterdam, the Netherlands	2017
SOA-polikliniek Amsterdam ONLINE, Tools voor efficiënte bedrijfsvoering, patiënt-gemak en het doorbreken van de transmissieketen. Werkgroep algemene medische microbiologie (WAMM) Nieuwegein, the Netherlands	2017
Workshop: Een nieuw tijdperk voor de soa-polikliniek van de GGD Amsterdam. Nationaal Congres Soa*Hiv*Seks, Amsterdam, the Netherlands	2017
Van Londen naar Amsterdam. Department of Dermatology, AMC, Amsterdam, the Netherlands	2018
Sexual risk behavior and substance use among male and transgender women sex workers at the prostitution centre Amsterdam, the Netherlands. IUSTI 2018 World & European congress	2018

Poster presentations	Year
Development and validation of high resolution typing methods for *Chlamydia trachomatis*. 25th IUSTI European Conference on STI&HIV/AIDS, Tbilisi, Georgia	2010
Earlier HCV diagnosis by the introduction of routine HCV testing for HIV-positive and MSM opting out for HIV in a large STI outpatient clinic. NCHIV, Amsterdam, the Netherlands	2012
Earlier HCV diagnosis by the introduction of routine HCV testing for HIV positive and MSM opting out for HIV in a large STI outpatient clinic. ISSTDR, Vienna, Austria	2013
Acceptance of an online partner notification tool for STI, called Suggest-A-Test. ISSTDR, Vienna, Austria	2013
Identical multilocus sequence typing (MLST) analysis in sequential samples from patients with pharyngeal chlamydia infections. ISSTDR, Vienna, Austria	2013
STI prevalence and follow-up among female victims of a sexual assault tested at the STI clinic in Amsterdam, the Netherlands. ISSTDR, Rio de Janeiro, Brazil	2017
STI prevalence among male victims of a sexual assault: data from 12 year period, STI clinic Amsterdam, the Netherlands. ISSTDR, Rio de Janeiro, Brazil	2017
Chemsex among men who have sex with men; a sexualised drug use survey among clients of the STI outpatient clinic and users of a gay dating app in Amsterdam, the Netherlands. IUSTI, Dublin, Ireland	2018

General courses	Year
Microsoft SQL Server 2005 Querying (CT2778), Computrain, Utrecht, the Netherlands	2008
Statistics in GLIMS – MIPS, Amsterdam, the Netherlands	2008
Regression Analysis, Boerhave, Leiden, the Netherlands	2009
GCP Training Penthecilia, Amsterdam, the Netherlands	2011
Infectious diseases, AMC Graduate School, Amsterdam, the Netherlands	2011
Conceptual Foundation of Epidemiologic Study Design, Erasmus Summer Programme, Rotterdam, the Netherlands, 2012	2012
Health Economics, Erasmus Summer Programme, Rotterdam, the Netherlands	2012
Weekly in-house training in epidemiology, Department of Infectious Diseases Research and Prevention, Public Health Service of Amsterdam, Amsterdam, the Netherlands.	2013-2016
The AMC World of Science, AMC Graduate School, Amsterdam, the Netherlands	2014
Introduction to QGIS, Public health Service of Amsterdam, the Netherlands	2015
Basiscursus Regelgeving en Organisatie voor Klinisch onderzoekers (BROK), NFU BROK Academie, AMC Graduate School, Amsterdam, the Netherlands	2016
Using Geographic Information Systems (GIS) in Disease Control Programmes, Royal Tropical Institute (KIT) Amsterdam and the International Institute for Geo-Information Science and Earth Observation (ITC) Enschede, the Netherlands	2016
Didactical Skills, AMC Graduate School, Amsterdam, the Netherlands	2017
Verandermanagement, Amsterdamse School/Twynstra Gudde, Amsterdam, the Netherlands	2018

Conferences attended	Year
Nationaal Congres Soa*Hiv*Aids, Amsterdam, the Netherlands	2007
The 1st Netherlands Conference on HIV Pathogenesis, Prevention and Treatment (NCHIV 2007), Amsterdam, the Netherlands	2007
Nationaal Congres Soa*Hiv*Aids, Amsterdam, the Netherlands	2008
The 2nd Netherlands Conference on HIV Pathogenesis, Prevention and Treatment (NCHIV2008), Amsterdam, the Netherlands	2008
The 3rd Netherlands Conference on HIV Pathogenesis, Prevention and Treatment (NCHIV2009), Amsterdam, the Netherlands	2009
Nationaal Congres Soa*Hiv*Seks, Amsterdam, the Netherlands	2009
25th IUSTI European Conference on STI&HIV/AIDS, Tbilisi, Georgia	2010
4th Netherlands Conference on HIV Pathogenesis, Prevention and Treatment (NCHIV), Amsterdam, the Netherlands	2010
Nationaal Congres Soa*Hiv*Seks, Amsterdam, the Netherlands	2010
7th Annual Amsterdam Chlamydia meeting, Amsterdam, the Netherlands	2010
Expertmeeting soa & hiv, RIVM, Bilthoven, the Netherlands	2012
6th Netherlands Conference on HIV Pathogenesis, Prevention and Treatment (NCHIV), Amsterdam, the Netherlands	2012
Nationaal Congres Soa*Hiv*Seks, Amsterdam, the Netherlands	2012
Expertmeeting soa & hiv, RIVM, Bilthoven, the Netherlands	2013
World STI & HIV Congress, Vienna, Austria	2013
9th Annual Amsterdam Chlamydia meeting, Amsterdam, the Netherlands	2014
Thirteenth International Symposium on Human Chlamydial Infections, Pacific Grove, CA, US	2014
Nationaal Congres Soa*Hiv*Seks, Amsterdam, the Netherlands	2014
10th Annual Amsterdam Chlamydia meeting, Amsterdam, the Netherlands	2015
Nationaal Congres Soa*Hiv*Seks, Amsterdam, the Netherlands	2015
P&G292 in the picture, Amsterdam, the Netherlands	2016
Expertmeeting soa & hiv, RIVM, Bilthoven, the Netherlands	2017
World STI & HIV Congress, Rio de Janeiro, Brazil	2017
Nationaal Congres Soa*Hiv*Seks, Amsterdam, the Netherlands	2017
Expertmeeting soa & hiv, RIVM, Bilthoven, the Netherlands	2018
IUSTI 2018 World & European congress, Dublin, Ireland	2018

Supervising	Year
I. Joore, scientific internship	2010
L. van Kempen, scientific internship	2013
M. Kroone, data management	2016-ongoing
S. Drückler, PhD student	2017-ongoing
R. Achterbergh, PhD student	2018-ongoing
J. Speulman, scientific internship	2018-ongoing

Other activities	Year
Dragon's Den Contest of the Municipality of Amsterdam; application for means to link the electronic partner notification website "Partnerwaarschuwing.nl" to the electronic patient file.	2015
Transition to transgender friendly STI clinic. Consulting patient organisation of transgender people and coordinating the implementation at the clinic.	2016
Amsterdam Anders: project to increase the efficiency of the STI clinic.	2016-ongoing
Member of the SOAP registration commission.	2018-ongoing

DANKWOORD

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SOA-POLI