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Prevention of *Chlamydia trachomatis* infections

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To my family with love

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Abstract

Urogenital chlamydia infection, caused by the bacterium *Chlamydia trachomatis* (CT), is the most common sexually transmitted bacterial infection in Sweden. In 2008 it was estimated by WHO that there were 105.7 million new cases of CT worldwide, an increase by 4.2 million cases (4.1%) compared to 2005. If untreated, CT infections can progress to serious reproductive health problems, especially in women. These complications include subfertility/infertility, ectopic pregnancy and chronic pain. The CT infection is often asymptomatic and reliable diagnostic methods and contact tracing are important tools for identifying infected individuals. CT infection is classified in the Swedish Communicable Diseases Act as a serious disease; consequently, written reporting and contact tracing are compulsory.

Previous or ongoing CT infection is not uncommon in infertile couples, especially in women with tubal factor infertility (TFI). We have tested 244 infertile couples for CT antibodies, and CT IgG positive couples were tested for CT DNA in urine. The prevalence of CT antibodies was higher in infertile men and women, and ongoing CT infection was common. Our results support a role of CT in infertility and underscore the importance of prevention of CT infection.

Contact tracing was studied by using questionnaires. A total of 544 questionnaires was sent to tracers in a Swedish county and 534 (98%) were completed. Centralized contact tracing performed by experienced tracers is effective; on average 65% of sexual contacts found by contact tracing are CT-infected. Our data show that it is worthwhile to extend the tracing period beyond 6 months as 30% of reported sexual contacts between months 7-12 were CT-infected. Contact tracing may be performed face-to-face at the clinic or by telephone.

Because of the severe consequences of CT infection there is a need for useful methods for both primary and secondary prevention of CT and other

sexually transmitted infections (STIs). An important subpopulation for CT/STI-prevention is the “core group”, i.e. a subpopulation with high incidence of STIs combined with risky sexual behaviour. This subpopulation contributes particularly to the spread of STIs in the population. Therefore, we have developed and evaluated a brief standardised but flexible manual-based single-session intervention based on motivational interviewing (MI) for the reduction of high risk sexual behaviour. Women (n=105) and men (n=119) at high risk of contracting CT infection were randomly eighter offered brief MI counselling or standard care. Our findings support the effectiveness of brief MI-based counselling in reducing high-risk sexual behaviour and incident CT infection in women ($p<0.01$) but not in men.

Our results suggest that gender aspects need to be considered and that men and women should be treated differently for achieving maximal risk-reduction. Whereas it might be sufficient to include information and motivation when performing risk-reducing counselling on women, counsellors may also add other components, such as behavioural skills and booster sessions, when counselling is performed on men.

Key Words: *Chlamydia trachomatis*, cell culture, infertility, pregnancy rate, antibodies, contact tracing, motivational interviewing

Original papers

The thesis is based on the following papers, which will be referred to in the text by their Roman numerals.

I**. Boman J**, Gaydos C, Juto P, Wadell G, Quinn TC. Failure to detect *Chlamydia trachomatis* in cell culture by using a monoclonal antibody directed against the major outer membrane protein. *J Clin Microbiol* 1997; 35:2679-2680.

II. Idahl A, **Boman J**, Kumlin U, Olofsson JI. Demonstration of *Chlamydia trachomatis* IgG antibodies in the male partner of the infertile couple is correlated with a reduced likelihood of achieving pregnancy. *Hum Reprod* 2004; 19:1121-1126.

III. Carré H, **Boman J**, Österlund A, Gärdén B, Nylander E. Improved contact tracing for *Chlamydia trachomatis* with experienced tracers, tracing for one year back in time and interviewing by phone in remote areas. *Sex Transm Infect* 2008; 84:239-242.

IV**. Boman J**, Lindqvist H, Janlert U, Brandell Eklund A, Forsberg L, Nylander E. Development and evaluation of brief manual-based single-session motivational interviewing for reducing *Chlamydia trachomatis* infection rates in women with high-risk sexual behaviour. *Submitted*.

V. **Boman J**, Lindqvist H, Janlert U, Brandell Eklund A, Forsberg L, Nylander E. Is single-session motivational interviewing effective to reduce high risk sexual behaviour in men? *Manuscript*.

List of Abbreviations

AI	Anal intercourse
BP	Baise pairs
CDC	Centers for Disease Control and Prevention
CI	Confidence interval
CMO	County Medical Officer
CT	*Chlamydia trachomatis*
DFA	Direct fluorescent assay
DNA	Deoxyribonucleic acid
EP	Ectopic pregnancy
EIA	Enzyme immunoassay
FCU	First-catch urine
HIV	Human immunodeficiency virus
HPV	Human papilloma virus
HSP	Heat shock protein
HSS	Hysterosalpingosonography
IgG	Immunoglobulin G
ICSI	Intracytoplasmic sperm injection
IVF	In vitro fertilisation
LGV	*Lymphogranuloma venereum*
LPS	Lipopolysaccharide
MI	Motivational interviewing
MIF	Microimmunofluorescence
MITI	Motivational interviewing treatment integrity
MLST	Multilocus sequence typing
MOMP	Major outer membrane protein
MSM	Men who have sex with men
NAAT	Nucleic acid amplification test
NATSAL	National surveys of sexual attitudes and lifestyles
NG	*Neiserria gonorrhoeae*
OR	Odds ratio
PCR	Polymerase chain reaction

PID	Pelvic inflammatory disease
PLH	Patients living with HIV
PPB	Proportionate population burden
RCT	Randomised controlled trial
RMO	Regional Medical Officer
RR	Relative risk
SCVS	Self-collected vaginal swab
SMI	Swedish Institute for Communicable Disease Control
STD	Sexually transmitted disease
STI	Sexually transmitted infection
TFI	Tubal factor infertility
USPSTF	The U.S. Preventive Services Task Force
WHO	World Health Organization

Sammanfattning på svenska

Klamydiainfektion orsakas av *Chlamydia trachomatis* och är den vanligaste sexuellt överförda bakterieinfektionen. WHO har uppskattat att det år 2008 var 105,7 miljoner nya fall av klamydia i världen, en ökning med 4,2 miljoner fall (4,1 %) jämfört med år 2005. Klamydiainfektion är ett folkhälsoproblem och klassificeras i den svenska smittskyddslagen som en allmänfarlig sjukdom varför det är obligatoriskt att smittspåra och göra en skriftlig anmälan till smittskyddsläkaren och Smittskyddsinstitutet.

Klamydiainfektionen ger oftast inga symtom och tillförlitliga diagnostiska metoder och smittspårning är viktiga ”redskap” för att hitta smittade personer. Om klamydiainfektionen inte behandlas kan den leda till allvarliga hälsoproblem, speciellt hos kvinnor. Bland komplikationer efter klamydiainfektion ingår ofrivillig barnlöshet, utomkvedshavandeskap och kronisk buksmärta. Tecken på tidigare eller pågående klamydiainfektion är vanliga hos ofrivilligt barnlösa par, speciellt hos kvinnor med skadade äggledare som orsak till barnlösheten. Våra resultat ger stöd för betydelsen av klamydia vid ofrivillig barnlöshet och understryker vikten av förebyggande åtgärder mot klamydia samt klamydiaprovtagning av både män och kvinnor vid utredning av ofrivillig barnlöshet.

Centraliserad klamydiasmittspårning utförd av erfarna smittspårare är effektiv och i genomsnitt är 65 % av spårade sexuella kontakter klamydiasmittade. Våra data visar att det lönar sig att förlänga smittspårningsperioden från 6 till 12 månader eftersom betydligt fler klamydiasmittade kontakter då hittas. Den så kallade ”Västerbottensmodellen” med en smittspårningsperiod på 12 månader rekommenderas nu av Socialstyrelsen. Kontaktspårning kan utföras antingen på mottagningen eller per telefon.

På grund av risk för allvarliga konsekvenser av klamydia finns det behov av metoder för att förebygga klamydiasmitta. En viktig grupp för prevention är den så kallade ”kärngruppen", alltså de personer som har en hög förekomst

av klamydia och andra sexuellt överförda infektioner i kombination med sexuellt riskbeteende. Denna grupp bidrar särskilt till spridningen av sexuellt överförda infektioner bland befolkningen. Därför har vi utvecklat och utvärderat en kort samtalsmetod som bygger på metoden motiverande samtal (MI, motivational interviewing) för att minska sexuellt risktagande. Våra fynd visar att kort MI-baserad rådgivning för att minska sexuellt riskbeteende och klamydiainfektion fungerar bra på kvinnor men inte lika bra på män. Resultaten tyder på att genusaspekter måste beaktas och att kvinnor och män ska behandlas på olika sätt för att uppnå maximal riskminskning. Det kan vara tillräckligt att fokusera på information och motivation vid rådgivning av kvinnor men för rådgivning av män kan man behöva komplettera med beteendemässiga färdigheter och/eller upprepad MI-baserad rådgivning för att nå god effekt.

1. Introduction

Sexually transmitted infections (STIs) are a major and global cause of morbidity, especially among young men and women. Urogenital tract *Chlamydia trachomatis* (CT) infection, caused by serovars D-K, is the most commonly reported bacterial STI in industrialised countries and one of the most prevalent STIs worldwide (Carey 2010). In 2008 it was estimated by WHO that there were 105.7 million new cases of CT worldwide, an increase by 4.2 million cases (4.1%) compared to year 2005 (WHO 2012). In Sweden year 2012, 37 714 CT infections were reported to the Swedish Institute for Infectious Disease Control, an increase by 10 913 cases (40.7%) compared to year 2003. The incidence rate in Sweden has increased from 157 to 395 (152%) cases per 100 000 from 1997 to 2012.

CT is a gram-negative bacterium with a unique developmental cycle; it undergoes a biphasic developmental cycle characterised by an infectious cell type known as elementary body and an intracellular replicative form called reticulate body (Omsland 2012).

CT infection is often asymptomatic, can persist for a prolonged period, and is an important preventable cause of reproductive problems, including pelvic inflammatory disease (PID), ectopic pregnancy (EP), and infertility (Fine 2008, Kortekangas-Savolainen 2012). The fact that most genital CT infections are asymptomatic increases the risk for further spread and long-term complications (CDC 2011).

The prevalence of CT IgG antibodies, as a marker of previous or ongoing CT infection, is high in women with tubal factor infertility (TFI). There is a possible etiologic role of infection with CT also in male infertility (Cunningham 2008). This might be due to a direct effect on sperm quality or to an indirect effect as a reservoir for CT bacteria that are transmitted to the female partner.

Several diagnostic methods have been used for diagnosing CT infections including cell culture, enzyme immunoassay, direct fluorescent assay, and nucleic acid amplification tests (NAATs). The use of CT cell culture from 1970s was important for performing studies to link CT to specific clinical syndromes, and the subsequent use of NAATs has been important for increasing our understanding of both the epidemiology of CT infections and approaches to prevention and control of these infections.

The incidence of urogenital CT infections reached epidemic proportions in Sweden during the 1980s. Therefore, in 1988, CT was incorporated into the Swedish communicable disease act. Since then, testing and treatment for CT are free of charge for the patient. All physicians in Sweden are obliged to report all cases of CT to the County or Regional Medical Officer (CMO/RMO) for Communicable Disease Control, and to the Swedish Institute for Communicable Disease Control (SMI); they are also obliged to perform contact tracing. Contact tracing may be delegated, usually to a counsellor. After an initial decline of the CT incidence in Sweden between 1988 and 1994, the incidence started to rise 1998 after a plateau between 1994 and 1997. The rise might be due to the introduction of highly sensitive NAATs in the mid-1990s, improved sampling techniques, an increase in number of performed tests, and changes in sexual behaviour. The CT incidence in Västerbotten has, in comparison with the other Swedish counties and regions, been low despite a high proportion of the population belonging to the "CT age group" 15-29 years. One explanation to the low CT incidence in Västerbotten could be effective highly centralised contact tracing and tracing of sexual partners 12 months back in time. Until 2006, a six month contact tracing period was the standard in most other counties and regions in Sweden but the standard was changed according to our research from Västerbotten (Carré 2008).

Individual sexual risk behaviour such as unprotected sex with many partners are among the strongest predictors of acquisition of CT and other STIs (Fenton 2005, Fenton 2010, Sonnenberg 2013). Sexual behaviour that

heightens the risk of contracting CT and other STIs appears to be increasingly common among adolescents and young adults. A 10-year follow-up study performed between 1999 and 2009 in Sweden showed that sexual lifestyles among female university students have become more risky with an increase in the number of sexual partners and first-date unprotected intercourse (Tydén 2012). Another population-based study involving Swedish men and women between the ages of 18 to 30, showed that casual sexual partners are common, that condom use with casual sexual partners is infrequent among both men and women, and that many men and women report 5 or more casual sexual partners in the previous 12 months (Leval 2011). Men and women in core groups – often defined as people reporting 5 or more sexual partners a year - are more likely to have high rates of STIs and report concurrent partnerships (Humblet 2003). Previous studies have also shown that CT re-infection rates are high in women. In a systematic review of 38 published studies, the overall median proportion of females reinfected with CT was 13.9% (Hosenfeld 2009). Recurrent CT infections increase the risk of PID and EP (Hillis 1997).

In the Swedish health care system, prevention of CT infection has so far been focused on secondary prevention through case finding and treatment (Carré 2008), rather than on primary prevention aimed at reducing risky sexual behaviour and preserving individuals from becoming infected. Thus, despite opportunistic screening and mandatory contact tracing, the incidence of CT infection in Sweden has been rising since 1997 (Bender 2011). Therefore, adding primary prevention methods for encouraging safer sex behaviour may be necessary for reducing the incidence and prevalence of CT infections. Primary prevention of STIs requires interventions targeted toward high-risk groups on a small-group or individual basis as well as different types of interventions for those at lower risk (Piper 2008). Because the acquisition of CT and other STIs is based on sexual risk behaviour, any primary prevention intervention for CT/STIs must impact individual risk behaviour. Risk reduction counselling has proved effective for preventing CT and other STIs but may be difficult to implement in the daily practices of STI care

(Rietmeijer 2007). Project RESPECT and RESPECT-2 are two important studies showing the efficacy of risk reduction counselling for the prevention of STIs (Kamb 1998, Metcalf 2005). The counselling techniques employed in these studies included both cognitive and action-oriented strategies (Hettema 2005).

Motivational Interviewing (MI) is an evidence-based method defined as a collaborative, person-centered form of guiding to elicit and strengthen motivation for change (Miller 2009). The overall spirit of MI has been described as collaborative, evocative and respectful of patient autonomy. The practice of MI has some guiding principles, viz. avoiding the righting reflex, understanding and exploring the patient's own motivations, listening with empathy, empowering the patient and encouraging hope and optimism (Rollnick 2008). MI has been successfully used in behavioural interventions for a wide variety of problems, such as substance use, risky behaviour and low patient compliance to treatment (Lundahl 2009). However, few controlled studies have use MI with the aim at reducing high-risk sexual behaviour (Lundahl 2013).

2. General background of *Chlamydia trachomatis* (CT)

Taxonomy of CT

In 1999, it was proposed to assign chlamydial strains in the single genus *Chlamydia* in the family *Chlamydiaceae* to two genera, *Chlamydia* and *Chlamydophila*. However, this proposal was rejected by many scientists (Schachter 2001). In 2009 the inclusion of all chlamydial strains within one single genus (*Chlamydia*) was suggested, based on fundamental taxonomic principles: *Chlamydia* and *Chlamydophila* strains cluster together, often have > 97% 16S rRNA gene sequence similarity, and share all the fundamental and classically defined phenotypic characteristics (Stephens 2009).

In the current taxonomy of *Chlamydia* and *Chlamydia*-like organisms, a single genus, *Chlamydia*, is now used as well as nine species: abortus, caviae, felis, muridarum, pecorum, pneumoniae, psittaci, suis and trachomatis (Bavoli 2013).

There are 17 different serotypes of CT, and the strains can be typed by use of serotyping or genotyping. High-resolution typing of CT can be achieved by sequence determination of five genomic targets and omp1 by using multilocus sequence typing (MLST). Typing of CT may be used for studying transmission patterns in sexual networks, clinical manifestations and pathogenicity, tissue and organ affinity, and may also have a role in investigation of sexual abuse or assaults. Most common types among heterosexual populations are E, D and F, and among men who have sex with men (MSM) G, D and J (Pedersen 2009).

By sequencing DNA from cases of CT infection in a Swedish county during 2001, in order to improve the efficiency of contact tracing, the omp1 gene was characterised (Lysén 2004). Approximately 990 base pairs of the omp1 gene was amplified, and sequence analysis was achieved in 678 (94%) of 725

CT-positive cases in an unselected population. The most prevalent genotype was serotype E (39%), followed by F (21%), G (11%), D (9%), K (9%), J (7%), H (2%), B (1%), and Ia (1%). Clinical manifestations were not associated with genotype. Sequence variation was linked to sexual networks identified by contact tracing and improved the epidemiological knowledge but was of limited practical benefit and use.

Biology of CT and the new variant

The genome of CT comprises a chromosome of 1.0 Mb and a plasmid of 7.5 Kb which have been found to be highly conserved among strains (Seth-Smith 2013). In October 2006, a new variant of *C trachomatis* (nvCT) was reported in Sweden (Ripa 2006). The nvCT has a 377 base pair deletion on the cryptic plasmid. The deletion includes the DNA target sequence for the earlier versions of NAATs from Roche Diagnostics and Abbott Laboratories. As a result, several thousands of false-negative CT-results were generated across Sweden. Jurstrand and colleagues examined 26 CT genotype E positive specimens collected 2002 and 25 such specimens collected 2003 (Jurstand 2013). No nvCT strain was found in 2002, but one urine specimen collected from a man in June 2003 was nvCT positive. Consequently, the nvCT strain was spreading undetected for at least 3 years explaining the high proportion of nvCT (38%) in Örebro County when it was first discovered in 2006. In Southern Sweden (Region Skåne), the longitudinal epidemiologic development of nvCT between 2007 and 2011 was studied (Persson 2013). The proportion of nvCT of all CT positive cases declined from 30% in 2007 to 6% in 2011. Among 258 CT culture positive strains collected 2000-2001 none was nvCT positive.

Epidemiology of sexually transmitted infections (STIs) including CT

General epidemiology of CT and other STIs

Several trends in CT epidemiology are emerging after more than 20 years of experience with CT control programs (Brunham 2008). The first trend

includes the development of two distinct epidemiologic profiles of reported case rates following the introduction of control programs: one U-shaped profile in Sweden, Norway, Finland and Canada, and one profile with steadily increasing case rates without an initial decline in Australia, United States and United Kingdom.

The second trend is an increase in reinfection rates that parallels increases in case rates. For example in 2008 in British Columbia, Canada, reinfections accounted for 14% of annual reported cases with most reinfections (> 90%) occurring within one year after initial infection.

Third, reproductive sequelae rates have declined where they have been measured.

Fourth, in Sweden case detection based on NAATs has been sufficiently intense to select for the emergence of a single novel genetic variant of CT (nvCT). The clone emerged in 3-4 years and was able to escape conventional NAAT detection in such a manner that it accounted for 20-65% of all identified CT strains in selected counties in Sweden 2006-2007.

Increasing reported rates of CT may be due to an increasing burden of CT infections (including both incident (new) and prevalent (existing) cases), more sensitive tests, improved sampling techniques, increasing coverage of CT screening, and better case finding. Increased incidence may be attributable to more new infections from an increase in high-risk sexual behaviour and/or more reinfections from arrested immunity due to early antibiotic therapy (Rekart 2012).

The basic reproductive number ($R_o = \beta \cdot c \cdot D$) is fundamental to understanding infectious disease epidemiology (Giesecke 2002). R_o describes the average number of secondary infections that an infected individual generates when entering a fully susceptible population, and when greater than 1, it defines ecologic success for a pathogen. There are three possible situations related to R_o: 1) $R_o < 1 \rightarrow$ the infection will eventually disappear; 2)

$R_o = 1 \rightarrow$ the infection will become endemic; 3) $R_o > 1 \rightarrow$ there will be an epidemic. R_o is determined by three parameters: β – represents the per-capita transmissibility of the agent; c – the pattern of contact between infectious and susceptible individuals; D – the average duration of infection. STI prevention programs can be aligned with each of these parameters. For example, condoms and vaccine reduce susceptibility (β); behavioural interventions may alter sexual behaviour and sexual networks (c); screening and contact tracing followed by antibiotic therapy reduces the average duration of infection (D). A distinct characteristic of infectious disease epidemiology is that incidence depends on prevalence, and therefore case detection and treatment is a major approach in bacterial STI prevention efforts (Brunham 2005).

Improvements in reproductive health can occur after the introduction of CT prevention programs, but infection rates may paradoxically rise as a result of effects on time-dependent acquisition of immunity (arrested immunity hypothesis) because individuals may re-enter unchanged sexual networks with heightened susceptibility to reinfection (Brunham 2005; 2008). This theory can explain both the raising case rates and the declining rates of sequelae as both the development of protective immune responses and the tissue damaging effects of infection appear to depend on the duration of infection. However, arrested immunity is unlikely to be the only explanation for raising case rates following the introduction of CT control programs. Changes in diagnostic test sensitivity and sampling techniques, increased accessibility to CT screening, and changes in sexual behaviours may also contribute to raising CT rates.

The outcome of public health efforts can be poorly predicted, in part, because of incomplete understanding of the forces that determine the dynamic equilibrium of a directly transmissible infection in the population. Clearly characteristics of heard immunity, infection transmission within heterogeneous social networks, and the intervention itself are all able to result in highly nonlinear outcomes (Brunham 2008).

Global STI epidemiology

STIs are a major global cause of acute illness, infertility, long-term disability and death. There are over 30 bacterial, viral and parasitic pathogens that can be transmitted sexually. In fact, more than 1 million people acquire an STI every day (Gottlieb 2013). WHO has estimated the global occurrence of four curable STIs, namely CT, *Neisseria gonorrhoeae*, syphilis, and *Trichomonas vaginalis* (WHO 2012). The total number of new cases (incidence) in 2008 in people between 15 and 49 years was estimated to be 498.9 million: 105.7 million cases of CT, 106.1 million cases of *N gonorrhoeae*, 10.6 million cases of syphilis and 276.4 million cases of *T vaginalis*. The incidence of these four STIs in Europe 2008 was estimated to be 46.8 million cases, CT 20.6 million, *N gonorrhoeae* 3.4 million, syphilis 0.2 million, and *T vaginalis* 22.6 million cases.

CT epidemiology in Europe

CT is not only the most frequently reported STI in Europe; it is the most commonly reported communicable disease in Europe (ECDC, Annual report 2012). In 2010, 24 of the 30 EU/EEA Member States together reported 344 491 cases of CT, a rate of 186 per 100 000 population. The majority of CT cases (almost 95%) were reported by only six countries (United Kingdom, Sweden, Denmark, Norway, Finland and the Netherlands), and the true incidence in Europe can be expected to be much higher (see above). Reported incidence was particularly high in Iceland (691 per 100 000), Denmark (505), Norway (464), and Sweden (386). The age category 20-24 years is the largest (42%), followed by the age category 15-19 years (33%), altogether 75%. Of the 343 280 cases with information available on gender, 140 563 (41%) were males, 202 717 (59%) were women, and gender was reported as unknown for 2 141 (0.6%) cases. Of the transmissions, approx. 95% were reported among heterosexuals, and approx. 5% among MSM.

CT epidemiology in Sweden

The reported CT incidence is increasing in Sweden, particularly among young Swedes. Between 1994 and 2012, the number of notifications for CT increased by 164%, from 14 275 to 37 691 cases (SMI 2013). The reported CT incidence in 2012 was 394 cases per 100 000, an increase by 1% compared with 2011. Adolescent and young adult Swedes appear to be particularly vulnerable to contracting CT. For example in 2012, the majority (84%) of CT notifications referred to Swedes aged 15-29 years of whom 57% were women. Median age of infected women was 21 years, and of non-MSM men 23 years (MSM 32 years). Heterosexual transmission accounted for 91% in women, and 88% in men, and in 8% gender was not reported. Non-heterosexual transmission accounted for 3% in men, and 0.3% in women.

Country where the infection was acquired: Sweden 84%, abroad 6%, country not reported 10%. Infections acquired abroad were most frequently reported from Thailand, Spain, Norway, Greece, Turkey, and the United Kingdom.

Neonatal infection: 28 cases.

Seasonality: peak August-October.

Number of reported tests 2012: 462 830, CT-positive individuals of those tested 2012: 6.7% (2011: 7.1%). Of all tested 30% were men (9.8% positive) and 69% were women (5.3% positive).

Lymphogranuloma venereum (LGV) 2012: in total 15 cases, all were MSM, mean age was 39 years, 9 were infected in Sweden, and at least 6 were also HIV-infected. Note: LGV is a variant of CT.

UngKAB09

UngKAB 09 (Tikkanen 2011) was a survey of sexual health performed 2009 among Swedish youths (aged 15-19) and young adults (aged 20-29). The

study focused on STIs, HIV in particular, and unwanted pregnancies. The majority of included subjects believed that the risk of getting CT is low or absent (women 79%; men 78%). Between ages 15-29 years 95% knew that condom is effective for protection against HIV/STI, however, only 50% reported use of condom when having sex with new or casual partners.

Recurrent CT infections

The incidence of recurrent CT infections was studied between 1995 and 2009 in Finland (Wikström 2012). The proportion of annual recurrent diagnoses of genital CT infection increased among females from 5% to 7% and among males from 4% to 5%. In 2009, 25% of the females and 20% of the males had had earlier CT infection during the follow-up time. Of all recurrent CT diagnoses, 34% occurred within 12 months.

Estimates of true CT incidence

CT is the most commonly reported notifiable disease in the United States (US). A total of 1.24 million infections were reported in 2009, but 2.25 times as many infections (2.8 million) were estimated to occur in the US (CDC 2011). Although the reported CT rates have increased steadily during the two past decades in the US, this do not necessarily reflect actual trends in CT incidence as increased case rates may be attributed to increased detection of CT-infection because of greater screening and use of more sensitive tests. Following a 9-year 60% decline, CT positivity increased 46% from 1997 through 2004 among young sexually active women screened in the US Region X (Pacific Northwest) family planning clinics (Fine 2008). The influence during this period of risk factors, changing laboratory test methods, and inter-clinic variability on CT positivity was examined systematically. A significant 5% annual increase in the risk of CT was found even after adjusting for various factors including laboratory test characteristics (OR1.05; 95% CI: 1.04, 1.06). Thus, there was most likely a true increase in CT positivity over the eight year study period (1997-2004).

Population-based studies of CT infection

The spread of CT is determined by host behaviour on one hand and prevention and control measures on the other. In the second British national surveys of sexual attitudes and lifestyles (Natsal 2000), 10.8% of men and 12.6% of women between 16-44 years reported ever having had STI (genital warts: men 3.6%, women 4.1%; CT: men 1.4%, women 3.1%). Half of all sexually experienced respondents aged 18-44 years were invited to provide a urine sample for testing CT infection and 71% agreed to participate. CT was found in 2.2% of men and 1.5% of women with age-specific prevalence being highest among men aged 25-34 (3.1%) followed by women aged 18-24 years (3.0%). Non-married status, age, unprotected vaginal and/or anal intercourse, reporting partner concurrency and increasing numbers of reported sexual partners in the past year were independently associated with CT infection (Fenton 2001). In Natsal-3 performed between 2010 and 2012, CT prevalence in individuals aged 16-24 years was 3.1% in women and 2.3% in men. In Natsal-2 compared with Natsal-3, prevelance of CT in people aged 18-24 years was similar in the two studies both for women (3.1% vs 3.2%) and men (2.9% vs 2.6%). Higher reported numbers of sexual partners in the past year, especially without use of condoms was a significant risk factor for CT infection, both in women ($p=0.01$) and men ($p<0.0001$). Also area-level deprivation was a significant risk factor for CT infection in women ($p=0.0078$) and men ($p=0.0028$) (Sonnenberg 2013).

The most influential variables on prevalence of CT are age and setting of the population tested (Adams 2004). In the United Kingdom and Ireland in general practice surgeries the < 20 year old age group had a CT prevalence of 8%, 20-24 year olds had 5%, 25-29 year olds had 3%, decreasing to 1% in those > 30 years. Overall, healthcare settings have higher prevalence estimates than found in population based studies. For example, among < 20 year olds, estimates were 17% in STD clinics, 13% in antenatal clinics, 12% in termination of pregnancy clinics, 11% in youth clinics, 10% in family

planning clinics, and 8% in general practice, compared to 5% in population based studies.

A population based cross-sectional study was conducted 2009 in five high schools in Norway using web-questionnaires and CT PCR using first-catch urine (FCU) (Gravningen 2013). The students were 15-20 years old and only sexually active students (1112 of 1554, 72%) were included in the study. CT prevalence was 7% in females and 4% in males. Previous clinic-based CT testing was reported by 56% females and 21% males with more females reporting multiple tests. Among females with pervious CT testing the prevalence was 7% compared with 7% for females with school-only test. Corresponding figures for males were 6% compared with 3%. This study shows that there might be a value of school based CT-screening because of the large pool of CT infections in both previously CT-tested and untested females and males. The lower prevalence in men with school-only testing might be due to less sexual activity of men of this age and, as a consequence, a reduced CT-infection risk.

Symptoms of CT

The majority of genital CT infections in both males and females are asymptomatic. Symptoms in women include increased/altered vaginal discharge, bleeding during/after intercourse or between menstrual periods, lower abdominal pain, and burning sensation/pain during urination. Symptoms in men include penile discharge, burning sensation/pain during urination, and tenderness or pain of the testicles. Extra genital symptoms in both men and women include red irritated eyes (eye infection) and rectal discharge, pain, bleeding and diarrhoea (rectal infection). Infection of the throat seems to rarely produce symptoms (Carré 2008). Reactive arthritis can be caused by CT including Reiter's syndrome with arthritis, conjunctivitis and urethritis (USPSTF 2007).

Risk factors of CT/STIs

The prevalence, distribution, and associated demographic and behavioural factors of self-reported STIs were examined in a population survey of sexual attitudes and lifestyles in the United Kingdom (Fenton 2005). Data were analysed from stratified probability sample surveys obtained through the British Natsal, which was undertaken in 1990 (n=13 765), 2000 (n=11 161) and 2010 (n=15 162) among men and women aged 16-44 years (16-74 years in Natsal-3). National STD surveillance data for 1999 were used in Natsal-2 to determine infection- and risk factor-specific proportionate population burden (PPB). The PPB can be defined as the proportion of all cases in the population among the fraction of the population exposed to a particular risk factor. Reported STI acquisition was independently associated with age, increasing numbers of sexual partners, male homosexual partners, and partners from abroad (for women only). Of all cases of STIs reported within the past 5 years, 10% referred to those 3% of men who had homosexual partners during the past 5 years. Of all reported STIs in the past 5 years among women, 42% (52% of genital CT and 44% of genital warts) referred to those 4% of women who had had $\geq$10 sex partners during that time. Of all reported STIs in the past 5 years among men, 58% (63% of genital CT and 63% of genital warts) referred to those 10% of men who had had $\geq$10 sex partners during that time. Overall, reported sexual partnerships remained the dominant association, with men and women who reported having $\geq$10 sex partners during the past 5 years were at greatest STI risk. Thus, the numbers and types of sexual partnerships remain the dominant individual and population risk factors for STI acquisition. In this population aged 16-44 years, 11% of men and 13% of women that had had sexual intercourse reported ever having been diagnosed with at least one of eight major STIs. Genital warts were the predominant STI, and genital CT infection the predominant bacterial STI among both men and women. An important primary prevention strategy for women would be to engaging men in CT screening interventions (Fenton 2001).

Alcohol use is an independent risk factor for intentions to engage in unprotected sex. As risky sex intentions have been shown to be linked to actual risk behaviour, the role of alcohol consumption in the transmission of STIs including HIV may be of public health importance (Rehm 2012). Consequently, CT/STI prevention interventions of individuals with risky sexual behaviour may also include interventions to reduce alcohol use, especially in individuals with heavy and problematic drinking.

Transmission of CT

The frequency of genital CT infection within sexual partnerships has been studied by Quinn et al. and high rate of concordant infection, high frequency of asymptomatic infection, and high frequency of transmission regardless of sex was found with similar frequencies (68%) of male-female and female-male transmission (Quinn 1996). Therefore, routine screening for CT in both males and females and provision of treatment to sexual partners of CT-infected individuals are important.

As non-genital CT-infection in the rectum and pharynx is not uncommon it is important to routinely question about anal and oral sex, and where indicated screen for non-genital infection. The prevalence of rectal infection in a study of 2 808 Brittish women was 7.1% compared to 6.7% for genital infection and 1.3% for pharyngeal infection (Shaw 2013).

Prevalence and risk factors for common STIs in Nordic women

The prevalence of women reporting ever having had genital CT, genital herpes, *T vaginalis*, and *N gonorrhoeae*, and identified factors associated with each of these STIs were assessed in more than 69 000 women (106 000 were invited) (Faber 2011). The overall prevalence in Denmark, Iceland, Norway, and Sweden was 1.5% for reporting ever having had *T vaginalis* (Sweden 1.1%), 1.9% for *N gonorrhoeae* (Sweden 1.5%), 4.8% for genital herpes (Sweden 5.5%), and 17.0% for genital CT (Sweden 14.3%). The

prevalence of each of these STIs varied with birth cohort and country, and was strongly associated with lifetime number of partners and having a previous diagnosis of another STI. A diagnosis of genital CT or *N gonorrhoeae* was associated with early age at first intercourse and early age at smoking initiation. Moreover, reporting previous genital CT infection was associated with early age at drinking initiation, and ever use of hormonal contraceptives and condoms. Lifetime number of sexual partners was strongly associated with all four STIs. It was most strongly associated with genital CT infection with an odds ratio (OR) of 14.9 for women with $\geq$ 10 lifetime partners compared with women with only one lifetime partner, followed by *N gonorrhoeae*, genital herpes, and *T vaginalis*. Thus, genital CT infections are common among women in the Nordic countries. As risk-taking behaviour, particularly sexual behaviour, is strongly associated with CT and other STIs, further information about STIs and their consequences may be needed, and especially targeting high-risk groups. There is also a need for continued monitoring of STIs in order to follow the prevalence and to gain further knowledge about risk factors. In this population based study, risk factors for having had multiple (> 10) sexual partners (reported by 30% of the women) were increasing age at enrolment, a higher alcohol intake, and young age at first intercourse ($\leq$ 14 years) (Jensen 2011).

STI risk factors in Swedish men and women

Condom use with temporary partners is infrequent both among men and women: only 41% of men and 34% of women report always using a condom when having intercourse with a temporary partner (Leval 2011). Further, temporary sexual partners in the previous 12 months are common and reported among 37% of men and 29% of women, with 7% of men and 4% of women reporting five or more temporary partners in the previous 12 months. Awareness and severity perceptions of human papilloma virus (HPV) or HPV-related cancer are not associated with either condom use or risk perception, whereas education level is positively associated with condom use. Women who are < 15 years at sexual debut have two-fold increased odds

of reporting non-condom use with temporary partners. Thus, efforts primarily aimed at increase STI awareness and/or perceptions of risk may not be sufficient in influencing prevention behaviour. Therefore, a deeper understanding into the actual barriers individuals experience in engaging in prevention behaviour, with a strategy to alleviate these barriers, may be necessary for achieving successful STI prevention.

Sexual behaviours that increase risk for STIs appear to be more and more common among adolescents and young adults. In a 10-year follow-up study performed between 1999 and 2009 in Sweden (Tydén 2012), it was shown that female university student's sexual lifestyle has become more risky with an increase in number of lifetime sexual partners and "first-date" unprotected intercourse. The mean number of sexual partners had increased to 11.0 in 2009, compared with 7.4 in 2004 and 5.4 in 1999. Sixty-five percent of the women reported "first-date" unprotected intercourse 2009, compared to 45% 2004 and 37% 1999. Thirty-nine percent of the women had experienced anal intercourse 2009, compared to 32% 2004 and 27% 1999. Thus, there is a continuous trend toward more risky sexual behaviours with increased number of sexual partners and increased unprotected first-date intercourse.

Complications of CT

Complications of CT in women

Both symptomatic and asymptomatic untreated CT-infection in women can ascend to the upper genital tract and can result in PID with infection and inflammation of the uterus, fallopian tubes, ovaries, and/or peritoneum. Both clinical and subclinical upper genital tract infection can result in fibrosis, scarring, and loss of tubal function, and potentially lead to serious long-term reproductive consequences such as TFI, EP, and chronic pelvic pain. It has been estimated that 10-15 % of untreated CT-infections lead to clinical PID (Haggerty 2010, Oakeshott 2010), and 10-15 % of clinical PID may result in TFI (Haggerty 2010). Also subclinical PID may lead to TFI. As

a consequence, an even greater proportion of untreated CT-infections likely lead to TFI (MMWR 2010).

Most women who are infertile due to fallopian tube occlusion have never been diagnosed as having an STI and have never had symptoms consistent with PID. Women with asymptomatic TFI have a greatly increased prevalence of antibodies to CT and Chlamydia heat shock protein 60 (HSP60). There is no association between the extent and severity of tubal damage and symptoms (Linhares 2010). Thus, a chronic CT infection may induce localised tissue damage without invoking clinical symptoms.

Infertility is a major public health problem, and in 2002 it was estimated that 7.4% of married US females aged 15-44 years were infertile (MMWR 2010). PID is the aetiology of infertility in at least 15% of infertile American women and TFI is thus an important cause of infertility among women. As mentioned above, most women with TFI do not have a history of PID despite serologic evidence of previous infection with CT or *N gonorrhoeae*. Women with subclinical PID have a 40% reduced pregnancy incidence compared with women without subclinical PID, and this information shed light on the aetiology of unexplained infertility in women (Wiesenfeld 2012). The proportion of all infertility in women due to TFI has been estimated to 45%, and CT infection is therefore the leading preventable cause of infertility (Macaluso 2010). It is not necessarily the damage caused by the CT-infection itself that leads to development of reproductive sequelae, but rather the host's immune response to the CT infection that may cause the damage (Carey, 2010).

Subfertility, defined as failure to conceive within 12 months despite regular unprotected intercourse, occurs in 10% of all couples. Tubal pathology (besides ovulation disorders and sperm defects) is one of the main causes of subfertility. The prevalence of tubal pathology in subfertile couples ranges between 10 and 30%. The accuracy of the Chlamydia IgG antibody test (CAT) in diagnosing tubal pathology has been reassessed (Broeze, 2011). Data of 14

primary studies containing information of 6 191 women (of which 3 453 women were available for analysis) showed that the prevalence of any tubal pathology was 29% (13% bilateral). In CAT-testing, MIF has a significantly (p=0.01) better accuracy than IF and ELISA for the diagnosis of any tubal pathology with moderate ability to discriminate between women with and without tubal pathology (sensitivity 74%; specificity 66%).

A positive CAT-test by MIF is both predictive of tubal damage and predictive of a reduced cumulative pregnancy rate when excluding treatment with IVF (Keltz 2013).

Clinical assessment of women with pelvic pain may be a poor indicator of disease seen at laparoscopy. In a study from London of 109 women with pelvic pain, 22 at laparoscopy had salpingitis, 19 had adhesions without salpingitis, 20 had endometriosis or ovarian pathology and 48 had no observable abnormality (Taylor-Robinson 2012). Of all micro-organisms investigated, CT had the greatest propensity for spread to the Fallopian tubes. Of 28 women who had CT organisms in the vagina/cervix, 13 had them in a Fallopian tube (ratio 2.2:1). The ratio was 6:1 for *N gonorrhoeae*, 8:1 for *M. genitalium*, 21:1 for *M. hominis* and 31:1 for *Ureaplasma* spp. Serologically, CT was also related to adhesions without salpingitis, more often (63%) than any other micro-organism.

The probability that a CT infection will cause an episode of clinical PID, and the reduction in such episodes that could be achieved by annual CT screening were estimated by using prospective data from eight published studies (Pricer 2012). The probability that a CT episode will cause clinical PID was estimated to 16%, and annual screening would prevent 61% of these CT-related episodes (Pricer MJ 2013). Also the proportion of TFI that is caused by CT has been estimated by using retrospective studies of CT antibody and adjusting for sensitivity and specificity of the tests. However, there are three important problems: 1) the antibody tests are relatively insensitive to previous infection 2) antibody levels may differ between TFI

cases whose TFI is caused by CT and previously CT infected controls, and 3) the odds ratios must be adjusted for confounding variables as the formula cannot distinguish between a TFI that is caused by a previous CT infection and a TFI caused by another organism in individuals with coincidental past exposure to CT. When adjusting for these problems the proportion of TFI episodes that were due to CT infection was estimated to be 45 % with an interval ranging from 28% to 62%.

CT infection during pregnancy is associated with adverse outcomes, including miscarriage, premature rupture of membranes, preterm labour, low birth weight, infant mortality, neonatal CT infection, and postpartum endometritis. Moreover, CT infection facilitates the transmission of HIV among both men and women in both the HIV carrier and the exposed recipient (USPSTF 2007).

Complications of CT in men

CT can cause male urethritis, epididymitis, and epididymo-orchitis but the role of CT in prostatitis is controversial. It may be an aetiologic agent with incidences up to 39.5% reported in patients with prostatitis (Cunningham 2008). CT infection of the testis and prostate is implicated in a deterioration of sperm, possibly affecting fertility. CT infection may also affect male fertility by directly damaging the sperm. CT infection in men may in rare instances result in urethral strictures and the Reiter syndrome (USPSTF 2007).

Whether serum CT IgA, CT IgM and CT HSP60 IgG are of additional value to CT IgG regarding the impact on fecundity in infertile couples has been evaluated, as well as CT serum antibodies relation to semen characteristics, diagnoses and pregnancy outcome (Idahl 2007). CT IgA in men (but not in women) correlated with reduced chances of achieving pregnancy (relative risk, RR=0.65), and in combination with CT IgG the chance was further reduced (RR=0.35). CT serum antibody positive men had reduced motility of the spermatozoa, increased number of dead spermatozoa, higher prevalence

of leucocytes in semen, and decreased sperm concentration. CHSP60 IgG correlated with reduced motility, and in women it was correlated to TFI.

Medical costs of CT

Direct medical cost estimates of eight major STIs in the USA 2008 have been calculated (Owusu-Edusei 2013). The total lifetime direct medical cost of the 19.7 million cases of eight major STIs that occurred in 2008 was 15.6 billion US dollars, and STIs continue to impose a substantial economic burden in the USA. Costs for CT were assessed for diagnosis and treatment of symptomatic cases and for treatment of asymptomatic cases; screening costs for asymptomatic cases were not included. The total costs for CT cases in the USA 2008 were estimated to 516.7 million USD with all costs adjusted to 2010 USD. Medical cost estimates for CT cases in Sweden are not available, but the average costs per CT case may be lower compared to the average costs per case in the USA because of higher prices for health care in the US compared to Sweden (Squires 2012).

Diagnosis of CT

Cell culture

After the first publication on diagnosis of CT by Gordon and Quan in 1965, CT cell culture became widely available to researchers and public health programs, and the decade from 1975 to 1985 can be considered as the CT cell culture era (Stamm 2001). Studies were undertaken to link CT to specific clinical syndromes such as nongonococcal urethritis, mucopurulent cervicitis, and PID. It was also demonstrated that CT was frequently associated with *N gonorrhoeae* infection and treatment of *N gonorrhoeae* with CT-active antibiotic was shown to reduce the occurrence of postgonococcal urethritis, cervicitis, and PID. The performance of cell culture is dependent on several factors including sampling technique, transport medium, duration of transport, cell line, culture medium and cell culture confirmation.

Nonculture tests

The development in the mid-80s of CT monoclonal antibodies resulted in development of nonculture tests for CT – direct fluorescent antibody (DFA) test and enzyme immunoassays (EIAs) – and widespread access to clinic-based testing for CT. The decade from 1985 to 1995 can be considered the CT nonculture test era (Stamm 2001).

NAATs

In the early 90s, NAATs became available and in the mid-90s they were available for routine clinical use. Unique and important characteristics of NAATs include improved sensitivity and specificity, ability to use novel types of specimen such as urine and vaginal swabs, and the ability to test for multiple pathogens simultaneously. The NAATs all have enhanced sensitivity of detecting urogenital CT infection by approximately 20% compared to former tests (Stamm 2001). Because of the ability to use different types of samples (such as swabs and urine) in combination with high sensitivity and specificity, NAATs have become the diagnostic tests of choice for diagnosing CT infections. Falk and colleagues evaluated the sensitivity of women's self-collected vaginal swabs (SCVSs), FCU, combined vaginal/FCU specimens and endocervical specimens for detecting CT infection (Falk 2010). The sensitivities calculated in 171 CT-infected women were equal for endocervical specimens (97%), vaginal specimens (96%) and combined vaginal/FCU specimens (95%). However, for FCU the sensitivity was significantly lower (88%; p=0.00024).

The performance characteristics of the Cobas CT/NG (c4800) Test from Roche Diagnostics was estimated for vaginal swabs from more than 4 000 US women (Van Der Pol 2013). In addition to Cobas CT/NG, the specimens were analysed by using the Gen-Probe APTIMA Combo 2 Assay and the BD ProbeTec CT7GC Q^x Amplified DNA Assay as comparator assays. For 248 CT infections, vaginal swabs identified 93.5%, clinician-collected endocervical swabs 92.7%, and urine 89.5% of the infections. Similarly, vaginal swabs

detected the highest proportion (98.5%) of the 65 *N gonorrhoeae* infections. Specificities ranged from 99.7% to 99.8% for CT, and for gonorrhoea the specificity was 100%.

CT organism load

CT organism load was determined in matched specimens from different anatomic sites, and its relation to clinical signs and symptoms in men and women was also examined (Michel 2007). For men urethral swab offer no yield advantage over FCU and organism load did not differ significantly between these two specimen types; the organism load of FCU specimens was positively associated with dysuria. For women, endocervical swabs contained the highest CT organism load, followed by SCVSs, urethral swabs, and FCU specimens. For matched cervical and urethral swabs the proportion of discordant pairs was 33%. Symptomatic upper genital tract infection was more likely to occur in women with a higher CT load. Based on these and other results, it is recommended that CT screening programs adopt the use of FCU specimens in men and SCVSs in women as the most appropriate noninvasive specimen types.

Rectal CT infection

Prevalence and correlates of rectal CT infection was studied among female clients at STD clinics in Los Angeles between 2008 and 2010 who reported anal intercourse (AI) within the previous 90 days (Javanbakht 2012). Among all women 12% (n = 2 084) reported AI in the past 90 days; percent CT positivity by anatomic site was 12% (n = 144) for urogenital CT, 15% (n = 171) for rectal CT with 25% (n = 44) of CT cases having rectal-only infection, 11% (n = 22) having urogenital-only infection, and 63% having both. Rectal testing increased the number of CT cases detected by 34% from 144 to 193. Thus, CT positivity was high among women reporting AI, and a large proportion of these cases would have been undetected in the absence of rectal testing. The findings highlight the importance of rectal testing and

stress the importance of specific counselling messages for women related to the risks associated with unprotected AI.

Retesting after CT diagnosis

CT-positive STI-clinic visitors were invited to participate in a two-armed intervention study for retesting after 4-5 months (Götz 2013). Interventions were either home-based sampling by mailed test-kits, or clinic-based testing without appointment. Of 216 visitors enrolled, 75 (35%) accepted retesting: 46% in the home group versus 23% in the clinic group. Men were less often retested (15% versus 43%). The overall CT positivity rate at retest was 17% compared to 12% seen at all visits at the STI clinic. Both untreated infections of current partners as well as unprotected sex with new partners contributed to recurrent infections. Persons reporting symptoms in the period since treatment had a significantly higher risk of CT infection than those without symptoms (31% versus 7%; p=0.01). Persons with a new sexual partner since treatment had a higher repeat-infection rate (24%) than persons without a new sexual partner (9%), and CT infection rates increased with the number of reported partners in the 6 months before retesting: 9% for one, 21% for two and 28% for three or more sexual partners.

Antibiotic treatment of CT infection

CT infection can be treated with azithromycin or doxycycline. In Sweden a CT infection is usually treated with oral doxycycline for 9 days (Boman 2011). Liberal use of single dose (1 g) azithromycin treatment is not recommended because of the risk of *Mycoplasma genitalium* resistance development in patients with concomitant such infection. Pregnant women may be treated with amoxicillin. All sexual partners of CT infected individuals should be tested and treated if infected, or treated presumptively (USPSTF 2007).

A meta-analysis of 12 randomised clinical trials of azithromycin versus doxycycline for treatment of genital CT infection demonstrated that these

treatments were equally efficacious with microbial cure rates of 97% and 98%, respectively (Lau 2002). Adverse events occurred in 25% and 23% of patients treated with azithromycin and doxycycline, respectively. The difference in efficacy for microbial cure and the risk difference for adverse events between the two antibiotics were not statistically significant.

To minimise transmission to sex partners, persons treated for CT should be instructed to abstain from sexual intercourse for 7 days after single-dose therapy, or until completion of a 7- or 9-day doxycycline regimen. To minimize the risk for reinfection, patients also should be instructed to abstain from sexual intercourse with an untreated sexual partner until the partner is treated. Except in pregnant women, test-of-cure is not advised, unless therapeutic compliance is in question, symptoms persist, or reinfection is suspected (Workowski 2010, Boman 2011).

Prevention of STIs

Prevention of STIs involves ensuring that uninfected individuals avoid acquiring infection (primary prevention), and that infected individuals avoid transmitting their STI to susceptible sexual partners (secondary prevention). However, neither STIs nor risky or preventive behaviour are distributed evenly through populations. As a consequence, coverage in some subpopulations is more critical to the achievement of population-level impact compared with coverage in other subpopulations. Because of limited resources, choices often need to be made about which subpopulations to target (Aral 2007). Possible alternatives are: infected individuals with high-risk behaviour, infected individuals with low-risk behaviour, uninfected individuals with high-risk behaviour, and uninfected individuals with low-risk behaviour. A sound strategy for prevention may be to first prioritise the so called “core groups”, i.e. subpopulations with high prevalence and incidence of STIs and risky behaviours and that contribute particularly to the spread of STIs in the population. A second priority may be infected individuals with low-risk transmission behaviours, followed by uninfected individuals with high-risk behaviours. For relatively uncommon STIs, a focus

on prevention of transmission from infected individuals (secondary prevention) may be the most efficient and cost-effective approach in order to decrease prevalence and incidence of STIs in the population. The population-level impact of a specific intervention is determined by three factors: the efficacy of the proposed intervention in the specific population, the contribution of the specific subpopulation to the health outcome of the whole population, and the achieved effective coverage of the intervention in the specific subpopulation. Also the prevalence in the specific population may be of importance. Biomedical interventions combined with behavioural interventions may be particularly synergistic and effective. For example, screening and treatment efforts for bacterial STIs may be combined with behavioural interventions to enhance health care seeking by at-risk populations. In contrast to the aetiology of many chronic diseases, STIs have a strong behavioural component in the causal pathway, with individual infection risk depending directly on the exposure of susceptible to infected individuals (Shiboski 1996).

Primary prevention

Outcome measures for testing of interventions for preventing STIs

When comparing trials of behavioural interventions to reduce STIs a key factor to evaluate is the outcome measure selected. Primary outcome measures in these trials can be categorized as biologic outcomes or behavioural outcomes. Biologic outcomes include documented new STIs and the presence of biomarkers for unprotected intercourse (eg. prostate-specific antigen). Behavioural outcomes include self-reported condom use, the number of current/new sexual partners, the risk status of partners, and the use of alcohol and/or drugs in conjunction with sexual activity. If the goal of the intervention is to reduce STI acquisition, direct measurement of STI acquisition rates, i.e. biologic outcome, is logically the most appropriate outcome measure (Piper 2008).

Behavioural interventions for STIs

Primary prevention of STIs requires interventions targeted toward high-risk groups on a small-group or individual basis as well as different types of interventions for those at a lower risk (Piper 2008). Many of the interventions aimed at lower-risk individuals are implemented through community-based programs. These programs include educational campaigns, assuring that condoms are widely available (and affordable), and providing access to STI testing/treatment and counselling. Because the acquisition of STIs is based on sexual risk behaviour, any primary prevention intervention for STIs must impact individual risk behaviour. Concepts that form the basis for the behavioural science behind STI prevention interventions include recognition and acknowledgement of individual risk and recognition of internal and external factors that influence an individual's ability to alter his or her risk status. A number of different theoretic models have been applied in STI prevention interventions, and they share common themes including recognition of risk, desire to reduce risk, recognition of barriers to change, and identification/implementation of ways to eliminate or reduce those barriers to facilitate change.

The information, motivation, behaviour (IMB) model

The IMB model is a model that states that STI risk-reduction interventions need to provide individuals with **information** about STI transmission and prevention, incorporate strategies that increase **motivation** to reduce STI risk, and train individuals in **behavioural skills** that will be needed to enact the specific behaviour required for successful risk reduction. The model was proposed following a thorough review of the HIV/AIDS risk-reduction research that identified intervention characteristics favouring behavioural changes (Fisher 1992). The interventions with evidence of effectiveness were characterized by providing a combination of HIV/AIDS risk-reduction information, motivation, and behavioural skills. Information regarding the means of transmission and about specific methods of preventing infection is regarded as necessary prerequisites of risk-reduction

behaviour. Motivation to change risky behaviour affects whether one acts on the knowledge regarding transmission and prevention. Finally, having the necessary behavioural skills to perform the specific preventive behaviour is a critical and important factor of whether even a knowledgeable, highly motivated individual will be able to change behaviour. This model integrates and combines educational (education and information), cognitive (attitudes, values, perceptions, intentions and beliefs) and behavioural theories (Fisher 1992, Aral 2007).

Risk reduction strategies include use of a condom during intercourse, reducing the number of sexual partners, avoiding risky sexual partners, and removing the influence of alcohol and drugs on sexual decision making. The first step in getting people to adopt such protective behaviour is to make them aware of their vulnerability to STIs (Pollack 2013). However, despite great advances in knowledge about prevention and treatment, STIs still remain an important cause of morbidity and mortality.

The evidence for behavioural counselling interventions to prevent STIs in adolescents and adults has been systematically reviewed (Lin 2008). Most evidence suggested a modest reduction of STIs at 12 months among high-risk adults receiving multiple intervention sessions and among sexually active adolescents. Evidence also suggested that these interventions increase adherence to treatment recommendations for women in STI clinics and general contraceptive use in male adolescents and decrease nonsexual risky behaviours and pregnancy in sexually active female adolescents. No low-intensity or single-visit counselling interventions were used in the highest-risk populations, i.e. trials conducted in STI clinics. No evidence of behavioural or biological harms for risk reduction counselling was found.

The efficacy of brief STI risk-reduction interventions for African American women in primary care settings has been investigated (Jemmott 2007). Primary outcomes were self-reported sexual behaviour in the previous 3 months; secondary outcome was STI incidence. At 12-month follow-up,

participants in the skill-building interventions reported less unprotected sexual intercourse than did participants in the information interventions, a greater proportion of protected sexual intercourse than did information intervention participants and control participants, and they were less likely to test positive for an STI than were control participants.

Interventions to prevent sexual transmission of STIs

Ninety-three papers reporting data from 74 randomised controlled trials evaluating 75 STI prevention interventions were identified in a systematic review (Wetmore 2010). Eight intervention modalities were used: behavioural interventions (36%), vaginal microbicides (16%), vaccines (16%), treatment (11%), partner services (9%), physical barriers (5%), male circumcision (5%), and multicomponent (1%). Two-thirds of the 27 published RCTs of behavioural interventions demonstrated significant effects, with 17 (63%) showing positive results, 9 (33%) no effect, and 1 (6%) showing increased risk for STIs (CT/*N gonorrhoea*/ *T vaginalis*) in subgroup analyses (men in the Project RESPECT-2 trial who received risk reduction counselling with rapid HIV testing). All behavioural interventions included risk reduction counselling and 56% included a skills-building component (eg. condom use, negotiation, and/or other communication skills). Only one trial (performed by Patterson et al.) tested MI. There was a wide range of effect estimates for the positive trials (9%-83%) and the greatest effect (48%-83% reduction) was against a combined endpoint of CT and/or *N gonorrhoea* infection.

STI risk-reduction programs for adolescents

DiClemente at al. claim that different STI risk-reduction programs that were developed and implemented specifically for adolescents seeking health care services at clinical venues have not yet reached a level of success that should be considered satisfactory and one reason is methodological limitations identified in several studies (DiClemente 2004). One such limitation is the lack of specification *a priori* of sample size and specified power calculation

which may be especially important in the interpretation of null results. Is the reason an insufficient sample size? The appropriateness of the control or comparison condition may be another limitation according to DiClemente et al. as it is often poorly defined and the activities poorly articulated.

Condoms and CT/STIs

Behavioural risk reduction efforts, such as promoting correct and consistent condom use, can have an impact on CT and other STIs including HIV and on unintended pregnancy (Workowski 2010). In fact, using condoms consistently and correctly is the single most important step that a sexually active person can take to prevent acquiring and transmitting STIs including HIV. For people who are sexually active, condoms remain the best solution to reducing the risks of acquiring STI (if uninfected) or transmitting these infections (if infected). Laboratory studies show that latex condoms are effective physical barriers against passage of even the smallest STIs. Other than abstinence, which is difficult to achieve, condoms are the most effective means of stopping the spread of STIs (Steiner 2008). Forty-five studies of condom use and risk of *N gonorrhoeae* and CT were reviewed (Warner 2006). All studies had one or more methodological limitations: only 28 clearly distinguished self-reported consistent from inconsistent condom use, only 2 assessed whether any type of incorrect condom use or condom use problems occurred, only 13 distinguished incident (new) from prevalent (pre-existing) infection, and only one study included a study population with documented exposure to infection. Despite these methodological limitations that all tend to underestimate condom effectiveness, most studies found that condom use was associated with reduced risk of *N gonorrhoeae* and CT in men and women. Of 8 studies that evaluated condom use and CT among males, 7 (88%) reported a protective effect ranging from 15% to 100% risk reduction. Of 21 studies that evaluated condom use and CT among females, 18 (86%) showed a protective effect ranging from 10% to 90% risk reduction. In males and females, 25 of 29 (86%) studies showed a protective effect, and in 13 (45%) of these 29 studies a statistically significant protective effect was

shown. In a study of condom effectiveness against CT/*N gonorrhoeae*, a great difference was found in incidence between individuals reporting condom use with errors and problems (16%) and those reporting condom uses without these errors/problems (0%) (Warner 2008). Warner et al. have previously reported that consistent condom use among participants with known CT/*N gonorrhoeae* exposure was associated with a significant reduction in prevalent *N gonorrhoeae* and CT infections (30% vs 43%; OR=0.42; 95% CI: 0.18, 0.99). However, among participants with unknown GC/CT exposure, consistent condom use was associated with lower reduction in *N gonorrhoeae* and CT rates (24% vs 25%). The number of unprotected sex acts was significantly associated with infection when exposure was known ($p<0.01$) but not when exposure was unknown ($p=0.73$) (Warner 2004).

Stages of change and condom use

The relationship between decisional balance and stages of change for consistent condom use with a current romantic heterosexual partner has been examined (Prat 2012). Cessation of risky behaviour and acquisition of preventive behaviour is a gradual dynamic process in which individuals move through five stages of change (Prochaska 1994). These stages are: precontemplation (not considering the possibility of change), contemplation (seriously thinking about changing within the next six months), preparation (intending to take action in the next month), action (individuals who have modified their behaviour for a period of less than six months), and finally, maintenance (starts after six months of successfully taking action). Decisional balance involves weighting up the advantages and disadvantages of the health-related behaviour. The advantages (pros) can be defined as the expected benefits of using condom (e.g. the protection from unwanted pregnancy and STI), and the disadvantages (cons) are the expected costs of condom use and include such things as a reduction in pleasure or their partner's disapproval. The sample consisted of 619 undergraduate students (35% males; mean age 20 years), and the results showed that the pros and

cons of using condoms were related to the stages of change for condom use, but that the pros were more strongly related. Therefore, the authors stated that campaigns for preventing transmission of STIs including HIV should attempt to maximize the perceived advantages of condom use, rather than refuting the cons. An interaction effect between gender and stages of change was found, suggesting that a major increase in pros is needed to progress along the stages of change, but more in males than in females.

Condom use in Sweden

Although the spread of CT can be mitigated by consistent condom usage, rates of consistent condom use in Sweden have tended to be low (Carré 2011). Consistent condom use with recent temporary sexual partners was reported by 34% of women and 41% of men aged 18-30 years (Leval 2011).

Condom protection against STIs

The protective value of consistent and correct use of latex condoms against the acquisition of CT, *N gonorrhoeae* and *T vaginalis* was evaluated prospectively for 6 months among patients attending clinics that treat STIs (Crosby 2012). Individuals who used condoms **both** correctly and consistently were estimated to have at least 59% lower risk of acquiring an STI.

Condom protection against CT infection

A disease specific estimate was provided for the effectiveness of condoms in preventing CT infection while controlling for known exposure to infection (Nicolai 2005). Among clients with known exposure, 13% of consistent condom users were diagnosed with CT infection compared to 34% of inconsistent condom users with an adjusted OR of 0.10 (95% CI: 0.01, 0.83). Thus, a significant 90% protection against CT in consistent condom user was achieved. Among clients with unknown exposure, there was no observed protective effect of condoms.

Vaccines

CT vaccine research has been ongoing for over 20 years, exploring the efficacy of subunit, cellular, and DNA vaccines (Carey 2010). However, as yet, no fully protective vaccine has been developed. Any CT vaccine candidate needs to be able to not only protect the individual from infection but also prevent the development of pathological sequelae such as infertility. This requires a vaccine that can induce CD4+ T-cell-mediated immunity, along with neutralizing antibodies and long lasting immunity. No single candidate vaccine has so far been able to do this effectively and it will probably require the development of a multi-subunit vaccine. Also, it is important to select vaccine antigens that elicit protective immunity without enhancing pathology.

Motivational interviewing (MI)

MI is an evidence-based method defined as a collaborative, person-centred form of guiding to elicit and strengthen motivation for change. The overall spirit of MI has been described as collaborative, evocative and respectful of patient autonomy. The practice of MI has some guiding principles: avoiding the righting reflex, understanding and exploring the patient's own motivations, listening with empathy, empowering the patient and encouraging hope and optimism. MI has been successfully used in many behavioural interventions for a wide variety of problems including risky behaviour (Lundahl 2013), and has been shown to be effective also when tailored to brief encounters (Rollnick 2008). In short, MI can be described as a brief, directive approach used to help patients modify unhealthy behaviour (Seng 2013).

MI is defined as a person-centered counselling style for addressing the common problems of ambivalence about change and for encouraging people to make behavioural changes to improve health outcomes (Rollnick 2008). It arouses from efforts to start difficult conversations with patients about risky alcohol intake. The inclination to confront or persuade patients was replaced

by evoking clients own reasons to change, which minimised resistance. MI can focus on a variety of problem behaviours and can be delivered in a single session or through multiple sessions together with other treatments or as a stand-alone intervention.

MI for STI and pregnancy prevention counselling

A randomised controlled trial on STI and pregnancy prevention counselling was performed using an adaptation of MI (Petersen 2007). Rates of unintended pregnancy and CT infection were assessed over the study period. At two months both intervention and control group had improved contraceptive use significantly ($p<0.001$ and $p<0.05$), but no significant differences were found between the groups at 12 months, or between baseline and 12 months. Repeated counselling sessions may be needed to improve contraceptive decision-making and to reduce the risk of unintended pregnancy and STIs. The improvements during the first two months in the control group may be related to the completion of the baseline questionnaire which included many questions about contraceptive use and the risk of unintended pregnancy and STIs. Exposure to these questions may have prompted control participants to think more about these issues and to use contraceptives more effectively.

Patterson et al. also examined the efficacy of brief behavioural intervention to promote condom use among female sex workers in Mexico (Patterson 2008). Women were randomised to a 30-minute behavioural intervention or a didactic control condition. MI techniques were used to elicit information on the participants' current situation and motivation, and to increase their motivation to practice safer sex. Four main areas were addressed: 1) motivations for practicing safer sex 2) barriers to condom use 3) techniques for negotiating safer sex with clients and 4) enhancement of social supports. A significant 40% decline in cumulative overall STI incidence in the intervention group was observed ($p=0.049$). However, significant results were not observed for individual STIs (HIV, syphilis, gonorrhoea, CT), likely because of low statistical power. There were also increases in the number

and percentages of protected sex acts resulting in decreases in the number of unprotected sex acts with clients.

A randomised controlled trial was performed for assessing the efficacy of two sessions plus booster of motivational behavioural intervention (MBI) to promote CT and *N gonorrhoeae* screening in young women (Chacko 2010). Outcome measures monitored for 12 months included: client-initiated clinic visits for STI check-ups in response to seven high-risk sexual behaviours by self-report (primary outcome), consistent condom use, number of CT and GC episodes, and movements along the stages of change obtained at baseline and at 6- and 12-month follow-up assessments (secondary outcome). At baseline, more than 70% endorsed the action stage of change for seeking STI check-ups for three of seven high-risk sexual behaviours. However, no significant differences were noted between the two groups for the primary or secondary outcomes. Across groups, having multiple partners and being pregnant or thinking one might be pregnant were associated with STI check-ups.

Core skills in MI

MI is an evolution of the client-centered therapy developed by Carl Rogers, and is a way of being with clients that seek to promote a safe, collaborative atmosphere in which clients can sort out their often conflicting feelings about change (Westra 2013). In MI, clients are regarded as the best experts on themselves, with the inherent and intrinsic knowledge of what is best for them, and the freedom to make their own choices. MI is intentionally focused on the exploration of ambivalence of change, and the core MI skills involve a highly active therapist who is deliberately listening for key process markers such as ambivalence, resistance and change talk. Core skills in MI include rolling with resistance, expanding change talk, and developing discrepancy. There are two types of resistance: resistance to change (ambivalence about change) and resistance to the therapist and/or treatment.

Therapist directiveness has been found to increase resistance whereas supportive approaches have been found to decrease it. One of the distinctive features of MI that differentiates it from client-centered therapy is its focus on the elicitation and elaboration of change talk (i.e. speech that reflects desire, ability, reasons, need and commitment to change). Empathic listening is the major method used in the elaboration of change talk. Empathy serves to amplify and extend change talk, and this paves the way to increasing commitment to change. A vital issue of MI refers to initiation of arguments, a role which shall be assigned the client and not the therapist. Systematically seeking to identify discrepancies between what the client intrinsically values and desires, and the current behaviours that are inhibiting or are inconsistent with those directions, can be powerful in building resolve to change. MI therapists seek to evoke, actively identify, and reflect such discrepancies to bring these to the client's attention. Not to confront the client but to invite the client to wrestle with and to resolve them.

MI fidelity

The term treatment fidelity broadly refers to the extent to which a behaviour change intervention, such as MI, is delivered reliably and validly (Seng 2013). For clinical MI trials, high treatment fidelity provides confidence that observed results are due to the effects of MI, rather than to other potentially unknown factors. For dissemination and implementation, measures of treatment fidelity can guide clinician training when translating effective interventions from research into clinical practice.

MITI coding

The Motivational Interviewing Treatment Integrity (MITI) Code is a brief coding system used to assess MI treatment fidelity (Seng 2013, Flickinger 2013). The MITI focuses specifically on clinician behavioural utterances and requires only a single pass through an MI session segment, making it ideal for both health researchers and clinicians who have limited coding resources

but are concerned with MI fidelity. Provider speech is coded using the MITI, originally developed to evaluate trained counsellors' adherence to MI principles. The MITI has two components: behaviour counts and global scores. The behaviour counts capture the technical elements of MI, while the global scores assess an overall impression of the relational aspect of MI.

To code behaviour counts, provider utterances are assigned to the following mutually exclusive categories: MI-adherent (advising with permission, affirming the patient, emphasising the patient's control and supporting the patient); MI-nonadherent (advising without permission, confronting the patient and directing the patient); reflections (simple or complex); questions (open or closed); giving information. A summary score of MI balance is calculated using MI-consistent talk and behaviours (reflections and MI-adherent behaviour) minus MI-inconsistent talk and behaviours.

To assess global scores, coders rate each dialog in the following dimensions: evocation (the extent to which providers elicit the patients' own motivations for change); collaboration (the extent to which providers work with patients as equal partners); autonomy/support (the extent to which providers support and actively foster patients' sense of choice and control); and empathy (the extent to which providers understand patients' perspectives). Each dimension is scored on a scale from 1 (low) to 5 (high). Consistent with the MITI, a summary score of MI spirit is calculated using the mean of scores on evocation, collaboration and autonomy/support. When used to evaluate counsellors, the MITI assigns global scores to random 20-min segments within counselling sessions.

MITI, MI and sexual risk behaviour in people living with HIV/AIDS

MITI is a reliable and valid measure of treatment fidelity for MI targeting sexual risk behaviours in people living with HIV/AIDS (Seng 2013). Greater adherence to MI, as measured by MI style (Global Spirit, Percent Complex Reflections, Percent MI-Adherent statements) and MI technique

(Reflections-to-Questions Ratio, Percent Open Questions) was associated with fewer reported unprotected sex acts by study participants after treatment. The findings support the use of the MITI coding system for both training clinicians in the faithful administration of MI to reduce sexual risk behaviours, and examination of MI treatment fidelity for research purposes in sexual risk reduction trials.

MI can promote behaviour change but HIV care providers rarely have training in MI. Therefore, the prevalence of MI-consistent behaviours and their association with patient intentions to reduce high-risk sexual behaviours were studied (Seng 2013). Audio-recorded visits between HIV-infected patients and their healthcare providers were searched for counselling dialog regarding sexual behaviours. The association of providers' MI-consistence with patients' statements about behaviour change was assessed. The odds of patient commitment to change were higher when providers used more reflections, used more MI consistent utterances, demonstrated more empathy, and spent more time discussing sexual behaviours. Patients gave more statements in favour of change (change talk) when providers used more reflections and more empathy. It was concluded that untrained HIV providers do not consistently use MI techniques when they are counselling patients about sexual risk reduction. However, when they do, their patients are more likely to express intentions to reduce sexual risk behaviours.

Computerised interventions for reducing sexual risks in patients living with HIV (PLH)

Prevention of HIV transmission from patients living with HIV (PLH) has a high priority and strategies that are easy to implement and sustain to eliminate sexual transmission acts among PLH are needed (Lightfoot 2010). Computerised motivational interventions delivered in waiting rooms at medical clinics may be an effective strategy to reduce unprotected sex acts among PLH. It can be focused on enhancing motivations and encouraging PLH to act in accordance with their values without providing the intensity of

the existing evidence-based programs for PLH. Among 566 PLH, PLH in the computerised delivery condition reported a significant decrease in the number of HIV-/unknown sexual partners compared with the provider delivery ($p=0.02$) and standard care ($p<0.01$) conditions and a significant decrease ($p<0.01$) in the number of unprotected sex acts in comparison to the standard care condition. Thus, brief motivational interventions implemented in medical care settings may be efficacious in reducing sexual transmission risk acts, particularly when delivered by a computer.

Secondary prevention of CT

According to CDC Grand Round on CT prevention, expanding CT screening of females will be critical to reducing disease burden and associated reproductive sequelae (CDC 2011). But also other prevention strategies should be used including behavioural interventions, rescreening of CT-infected persons, and partner treatment. Because recurrent CT infection is common, CDC recommends rescreening of CT-infected persons 3 months after treatment. Treating male sex partners of infected females may be critical for preventing recurrent infections in females, and may be essential for interrupting CT transmission in the population (Workowski 2010).

CT screening

Screening is defined as a procedure in which members of a defined population, who may not know they are at risk of a disease or its complications, are asked a question or offered a test to identify those who are more likely to be helped than harmed by further tests or treatment. In proactive screening, population registers are used to invite members of the population at risk for screening at appropriate intervals. In opportunistic screening a health professional offers a screening test to patients attending health care or other defined settings for unrelated reasons; the onus is on the health professional to repeat the test offer at appropriate intervals.

CT would seem to be an ideal candidate for screening: CT is a common, curable, easily diagnosed, an STI that usually causes no symptoms, and can cause devastating complications, including TFI, EP, neonatal infection, and facilitation of HIV transmission.

A program of widespread opportunistic CT screening in Sweden resulting in controlled transmission of CT infection and reduced morbidity of the female reproductive tract is often stated as fact. However, this has been questioned and the fall in CT rates between 1988 and mid-1990s in Sweden coincided with the national campaign to prevent HIV (Low 2007). Opportunistic screening of CT is widely assumed to be the only acceptable model of service delivered for CT; however, not everyone uses the services that provide testing and not every person who uses those services is offered a test. In fact, no randomised controlled trial has evaluated opportunistic CT screening as it is currently practiced, and most studies cited as showing that CT screening is cost effective do not satisfy accepted quality criteria for economic evaluations. Despite an absence of evidence of effectiveness, and increasing rates of CT in countries that are assumed to have such programs, belief in the success of opportunistic CT screening persists.

CT screening recommendations

There is good evidence that screening for CT infection in women who are at increased risk can reduce the incidence of PID (USPSTF 2007). Screening programs have been demonstrated to reduce both the prevalence of CT infection and rates of PID in women. Since most CT infections are asymptomatic screening is necessary to detect and treat infections. However, routine screening is likely only cost-effective in populations with prevalence above a minimum threshold, for example 3%, and screening criteria may be needed to identify populations most at risk.

The U.S. Preventive Services Task Force (USPSTF) makes recommendations about preventive care services for patients without recognised signs and symptoms of the target conditions. USPSTF defines CT screening of sexually

active young women age 24 years or younger who are at increased risk as an A-rated recommended preventive service, i.e. the strongest recommendation.

Centers for Disease Control and Prevention (CDC) recommends annual CT-screening for all sexually active females aged < 25 years and for females aged ≥ 25 years if they are at increased risk for infection, e.g. if they have new and/or multiple sexual partners (Workowski 2010).

Screening females aged < 25 years is ranked by the National Commission on Prevention Priorities as one of the 10 most beneficial and cost-effective prevention services. However, it is also among the most underutilised (Maciosek 2006).

In a US population-based study, factors associated with prevalent CT infection in women aged 26 to 39 years were determined (Torrone 2013). Overall, CT prevalence among the women was 1.2% and the study results support the current CDC recommendations that women above 25 years should not be routinely screened for CT. However, in women with two or more sexual partners in the past 12 months the prevalence was 3% compared to 1% in women with 0-1 partner. In widowed/divorced/separated women the prevalence was 3% compared to 1% in women who were married/living with a partner. Thus, sexual history may be useful in identifying women above 25 years at higher-risk for CT infection. Having new multiple sexual partners in the last 12 months increases a woman's risk for exposure to CT. In addition, possible partner concurrency may also increase the risk. This can be measured by the question: At any time within the past 12 months, did any of your sexual partners have sex (of any type) with someone else while they were still in a sexual relationship with you?

The possible effect of CT screening on development of sequelae in infected women

Evidence on the effect of CT screening on development of sequelae in infected women was reviewed and critically evaluated (Gottlieb 2013). The focus was primarily on the role of screening in interrupting progression to upper tract inflammation and sequelae in already infected women. Four randomised controlled trials of one-time screening for CT infection are published: one study was performed in Seattle, two in Denmark, and one in London. Taken together, these trials provide evidence that CT screening and treatment can reduce the incidence of PID over the following 12 months for individual women. For a woman with CT, the potential benefits of screening depend on how much PID would occur after the infection is detected and the potential for treatment to cure the infection. If the treatment is effective, the amount of PID that could be averted depends on the overall risk of PID if the infection is untreated, when PID occurs during the course of CT infection, and when the CT infection is detected. The randomised controlled trial (RCT) with the most rigorous methodology is the Prevention of Pelvic Infection (POPI) trial conducted 2004 to 2007 in London (Oakeshott 2010). Seven of 74 untreated control women (9.5%), who later tested positive for CT infection in stored sample collected at baseline, developed PID over 12 months compared with one of 63 (1.6%) screened and immediately treated women (RR 0.17; 95% CI: 0.03, 1.01). Screening for and treating CT reduced the risk of clinical PID by more than 80% (from 7/74 to 1/63) among CT-infected women; between 42% and 62% of all PID cases in the study were associated to CT. Thus, long-standing prevalent CT infection is still an important contributor to PID incidence.

Rescreening for recurrent CT infection

In a meta-analysis of 8 randomised controlled trials and 4 controlled observational studies rates of rescreening for recurrent CT infection between patients receiving and not receiving an intervention were compared (Guy 2012). Rescreening is defined as a test occurring 3 weeks to 12 months

subsequent to an index episode of CT infection. It was concluded that the use of mailed screening kits is an important strategy to increase rescreening, reminder systems are promising, and MI is worth investigation. Evidence is insufficient to recommend routine CT screening for males because of several factors such as feasibility, impact and cost-effectiveness. Besides, there is a critical gap in the evidence relating to whether CT screening programs that target men decrease the incidence of infection among women (USPSTF 2007, CDC 2011).

Contact tracing/partner notification of CT

Quality Improvement Scotland standards for sexual health services require that on average 0.64 contacts per case should be verified as having attended within 90 days of the first partner notification interview. Utilisation of partner for mediation of therapy results in more patients being treated than when patients are given information for partners but removes the possibility of further cases of CT being diagnosed through tracing of secondary contacts. An audit was performed to estimate the impact of removing secondary contacts on the number of CT infections identified (Forbes 2009). Patients who were not known to be contacts of CT infection were included. One hundred and twenty-seven index cases generated 189 contacts, of which 100 were confirmed as tested and treated. Sixty-four (64%) were CT positive, who in turn generated 36 new contacts. Fourteen (39%) of these were positive. Secondary contact tracing identified 22-28% more cases of CT infection than if all partners were treated without testing. In Sweden partner delivered therapy is not allowed because contact tracing is mandatory and all sexual partners have to provide samles for CT analysis prior to eventual CT treatment.

The Swedish infectious disease law

In 1988, a change in the Swedish infectious diseases law requires all physicians in Sweden to provide free CT testing, treatment, and contact tracing for anyone with a suspected or documented CT-infection, and to

report cases to the CMO or RMO and to the Swedish Institute for Communicable Disease Control (SMI). As CT infection is classified as a public health hazard under the Communicable Disease Act a person who knows or has reason to suspect that he or she carries CT is obliged to immediately consult a physician and let the physician take the necessary samples to determine whether the person is CT-infected or not. Obligation applies to both public and private medical practitioner. If the patient is suspected to carry CT and does not consent to examination and sampling, the physician should promptly notify the CMO or RMO. Examination, care and treatment of suspected or proven CT infection are still free to the patient.

3. Aims

This is a study of various aspects of primary and secondary prevention of CT infection including diagnosis, contact tracing and behavioural interventions. Our goal was to develop, evaluate and implement prevention procedures for decreasing the spread of CT infections. Initially our efforts were focused on secondary prevention strategies including improved diagnosis and contact tracing. As the CT-incidence continued to rise despite secondary prevention measures, we decided to add primary prevention strategies including counselling with MI, condom education and condom distribution.

Specific aims

1. To find a useful antibody for detection of CT in cell culture and, more specifically, by comparing anti-LPS and anti-MOMP antibodies.

2. To determine the prevalence of CT antibodies in men and women seeking for involuntary childlessness, and to prospectively evaluate the effect of previous CT infection on pregnancy rates and pregnancy outcome.

3. To examine if an extended CT contact tracing period from 6 to 12 months is of value for identifying more CT-infected sexual partners, and if contact tracing of sufficient quality can be performed by telephone.

4. To examine whether intervention with MI is more efficacious than standard STI care in reducing high-risk sexual behaviour in men and women, and whether there are differences regarding gender.

4. Materials and Methods

CT cell culture (Paper I)

In total more than 33 000 genital samples were cultured in McCoy cells and stained with two different types of monoclonal antibodies. One of the antibodies was directed against the major outer membrane protein (MOMP) and the other was directed against the lipopolysaccharide (LPS).

CT antibodies in infertile couple (Paper II)

Patients and specimen sampling

All consecutive couples attending due to unfulfilled desire for pregnancy for more than one year were included in the study. Blood was drawn from both partners and tested for the occurrence of CT IgG antibodies. Semen analysis of the man was undertaken. If at least one of the partners of the couple had detectable CT IgG antibodies a FCU sample was collected from the man and the woman for detection of CT DNA. When all test results were available routine infertility work-up was done. TFI was defined as one or both tubes occluded or dilated. Sera from pregnant women undergoing spontaneous pregnancies were used as controls for the CT antibody analyses.

CT serology and CT DNA testing

CT-specific IgG antibodies were determined by the microimmunofluorescence (MIF) test. The urine specimens were analysed for CT DNA by PCR.

Follow-up

After a follow-up period of up to 54 months the medical records of all couples were studied. Primary endpoints of the study were pregnancy and pregnancy outcome. Pregnancy data were available from 238 of the 244 couples.

Improved contact tracing for CT (Paper III)

Procedure

To evaluate the regional model for contact tracing, questionnaires were sent to contact tracers in Västerbotten. Sexual partners (contacts) were categorised into groups corresponding to time at last intercourse with the index partner. In a second part of the study the effectiveness of contact tracing face-to-face was compared with tracing performed by telephone.

MI for reducing CT infection rates (Paper IV and V)

Recruitment and procedure

Swedish-speaking women (paper IV) and men (paper V) consecutively attending the STI services reception at the University Hospital of Northern Sweden were randomly assigned to either brief MI counselling followed by STI standard care or STI standard care only. The participants were asked to complete a questionnaire before seeing a health care provider. Eligibility was limited to a core group of individuals who in the past 12 months had had at least four sexual partners and unprotected vaginal and/or anal sex. Six months after inclusion in the study the participants were invited to complete a questionnaire and provide samples for CT-testing. All additional CT-testing was monitored for at least 12 months following inclusion in the study.

Intervention

The intervention arm consisted of a single 20-30 minute, manual-based, MI counselling session.

Intervention protocol

The intervention protocol consisted of three parts; in total 14 questions were used.

Treatment fidelity assessment

To ensure treatment fidelity, MI skill was assessed using MITI 3.0.

Outcome measures

The primary study outcome measure was the acquisition of new CT infection over the 12 month follow-up period. Secondary outcome measures included behavioural factors that were recorded 6 months after inclusion in the study.

5. Results and discussion

The main findings of our studies and other efforts are reported and discussed below.

CT cell culture (Paper I)

Cell culture results

Three sets of sample were analysed: two sets in Umeå and one set in Baltimore. Difference in performance between the two antibodies was noted in all three set. Of the discrepant samples, all were positive by the anti-LPS antibody. A subset of the samples collected in Balimore were serotyped using serovar-specific antibodies or by PCR. The majority (85%) of the discrepant serotyped samples were CT serovar J. The more broadly reacting anti-LPS antibody was superior for cell culture confirmation of CT inclusions. It was more sensitive than and as specific as the anti-MOMP antibody. In addition, it produced a stronger fluorescence with more numerous and larger inclusions. Therefore, we recommend the anti-LPS antibody for cell culture confirmation of CT.

The higher sensitivity of the anti-LPS antibody may be due to a better capability to detect EBs and RBs and/or lower capability of the anti-MOMP antibody to detect all serovars of CT with the same sensitivity. Nowadays cell culture is mainly used for research and to investigate possible antibiotic resistance. Thus, it is still important to have access to cell culture as it is the only diagnostic method that can confirm the prescence of viable CT organisms. CT antibodies, CT antigens and CT DNA/RNA may be present without the presence of viable and living CT organisms. Optimised and quality assured cell culture confirmation of CT may also be important for detecting the emergence of mutant CT strains that escape NAAT testing, such as the Swedish nvCT that was detected in Sweden 2006.

CT antibodies in infertile couples (Paper II)

Clinical diagnoses, CT IgG and CT DNA

In 107 women with a principal infertility diagnosis, 37 (35%) were due to TFI. The occurrence of male factor infertility was 36 (15%). In 78 (32%) couples at least one partner was CT IgG positive; corresponding figures for CT DNA was 9 of 68 couples (13%).

CT IgG and CT DNA among women and relationship to TFI and pregnancy

In women with diagnosed TFI the prevalence of CT IgG was 17 (46%) of 37; corresponding figures in the control group of pregnant women was 38 (16%) of 244 (OR 4.6; 95% CI: 2.2, 9.6). In the 9 couples with at least one partner being CT DNA positive, 4 had TFI as the principal cause of infertility. The presence of CT IgG in women (independent of diagnosis) was not related to pregnancy.

CT IgG and CT DNA among men and relationship to pregnancy

The finding of CT IgG in the male partner was a significant outcome predictor (OR 2.6; 95% CI: 1.3, 4.9) of failure to achieve pregnancy. In multivariate analysis, this was the only parameter that was found to significantly influence the primary study outcome (achieving pregnancy).

Infertility is a major public health problem and the proportion of infertility in women related to TFI has been estimated to 45%, and CT is the leading preventable cause of infertility. Signs of previous or ongoing CT infection are common in infertile couples, especially in women with TFI. Our results with a significantly higher prevalence of CT IgG in women with TFI support the role of CT in infertility, particularly in TFI, and underscore the importance of CT primary prevention as well as identifying and treating CT infected individuals. The finding of ongoing CT infection in several couples was unexpected and our results show that NAAT-testing for CT of both men and women should be included in the standard infertility investigation. It is

possible that antibiotic treatment of these ongoing CT infections in infertile couples will increase future fertility. The interesting association between male CT IgG positivity and reduced fecundity merits further investigation.

Improved contact tracing for CT

Reported contacts and CT test results of the contacts

The 534 index cases included in the study reported a mean of 2.5 contacts per index patient. On average 0.9 CT-infected partners per index were identified and as many as 65% of all contacts with known test result were CT-infected. As expected, the frequency differed by time since last intercourse: 0-2 months = 79% (n = 340), 3-6 months = 51% (n = 96), 7-12 months = 30% (n = 27), and > 12 months = 73% (n = 11). Not unexpected, experienced tracers (counsellors and midwifes) reported on average more contacts (2.6 and 2.5, respectively) compared with inexperienced tracers (physician) who reported on average 1.8 contacts/index.

Contact tracing at the clinic or by telephone

Six experienced tracers interviewed 567 index patients by phone (n = 310, 55%) or face-to-face at the clinic (n = 257, 45%). When contact tracing was performed face-to-face at the clinic on average 3.0 contacts were reported compared to 2.3 when tracing was performed by phone. The difference was not statistically significant.

Contact tracing is one of the most important tools for finding CT infected individuals and for stopping the spread from CT infected to uninfected individuals. Centralised contact tracing performed by experienced tracers is effective as 65% of contacts in our study were CT-infected. In addition, our data show that it is worthwhile to extend the tracing period beyond 6 months as 30% of reported contacts between months 7-12 were CT-infected, and 73% of the few contacts > 12 months were CT-infected. Contact tracing may be performed at the clinic or by telephone; the somewhat higher average number of contacts reported when the tracing was performed in real life

face-to-face at the clinic compared to phone tracing may be due to the fact that index patients with high number of sexual contacts were encouraged to attend the clinic for tracing.

It is thus a necessity that tracing should be performed by educated and experienced personnel, either face-to-face or by phone.

"Västerbottensmodellen"

Our results changed the national recommendations for contact tracing from 6 to 12 months back in time. Further improvements of contact tracing would be to systematically investigate various strategies to help index patients remember more sexual contacts during the last 12 months. A challenge is to find all temporary sexual partners found through internet; sometimes the index patient only has nick names of the partners. It may be even more important in the future to perform internet tracing because internet is playing an increasing role for finding casual sexual partners (Buhi 2013).

MI for reducing CT infection rates in women (Paper IV)

Patient characteristics

Fifty-eight women participated in the MI intervention group and 47 women served as controls. Sixteen of the controls had been offered MI but had declined. Both intervention and control women reported on average 6 sexual partners in the previous 12 months.

Sexual risk behaviour after intervention

The reduction of high-risk sexual behaviour from baseline to 6 months follow-up was greater in the intervention group than in the control group as 73% of the women in the intervention group had reduced their sexual risk from high to low, compared to 57% in the control group.

CT testing results

Up to 12 months after the intervention, none of the 38 CT-retested women in the intervention group was CT-infected compared to two (7%) of the 28 CT-retested women in the control group ($p<0.01$).

Despite all efforts to stop the CT epidemic, there is still a need for implementing useful methods for primary prevention of CT and other STIs; therefore, we developed a brief manual-based MI intervention for reducing risky sexual behaviour. Our findings support the effectiveness of MI-based counselling in reducing risky sexual behaviour and CT infection in women. In our brief manual, the most important aspects of CT/STI prevention are included and we could focus on each woman's specific needs for reducing her sexual risks. The MI-intervention was perceived very positively by the women. An important factor for evaluation of the efficacy of MI is the use of MITI for assessing MI fidelity. We noted risk reduction also in the control group and this reduction may be related to the completion of the baseline questionnaire which may have prompted control participants to think more about these issues, and, as a result to reduce their sexual risks.

MI for reducing CT infection rates in men (Paper V)

Patient characteristics

Sixty-one men participated in the MI intervention group and 58 men served as controls. Twenty-six of the controls had been offered MI but had declined. Men in the intervention group reported on average 7 sexual partners in the previous 12 months compared to men in the control group who on average reported 6 sexual partners.

Sexual risk behaviour after intervention

The reduction of high-risk sexual behaviour from baseline to 6 months follow-up was similar in the intervention (63%) and control groups (61%). In the intervention group, only 2 men belonged to the MSM group. However,

both these men were highly motivated and committed to reduce risks after the MI-intervention and rated their commitment to 10 out of 10. At follow-up both men reported condom protected anal intercourse. Moreover, no CT infections were detected during the follow-up period. It may be interesting to evaluate this protocol on a larger group of MSM with high-risk sexual behaviour.

CT testing results

Up to 12 months after the intervention, 4 of 32 CT-retested men in the intervention group and 3 of 24 men in the control group were CT-infected, and the difference was no statistically significant. However, no reinfection was detected up to 12 months after inclusion in the study.

Contradictory to our findings in women these findings do not support the effectiveness of brief MI-based counselling for reducing risky sexual behaviour in heterosexual men. Our results are in line with the results reported by Kalichman et al. They have shown that men and women may react differently to similar treatment protocol and men and women should therefore be treated differently for achieving optimal sexual risk reduction (Kalichman 2005). For example, it may be necessary to focus more on improving behavioural skills during the counselling session with men. In addition, it may be necessary to also add a booster session after some weeks. Thus, it is important to take gender issues into account much more than has been done previously. One may be concerned about studies that do not take this aspect in consideration.

From theory to practice - CT epidemiology in Västerbotten and the sharp reduction in 2009

The incidence of CT has increased in Sweden since 1997 as in many other countries in Europe. The increase in CT-incidence since 1997 can be explained by several factors; for example increased number of performed CT-tests, improved sampling techniques, and more sensitive diagnostic methods. Other important factors explaining the high and increasing

incidence may be the high infectivity of CT, the absence of symptoms in the majority of CT-infected men and women, changed sexual behaviour and low condom use. The number of sexual partners has increased, which is also important for the spread. The rising CT-trend in Sweden was broken in 2009, and the county in Sweden with the lowest incidence was Västerbotten with 255 cases per 100 000 population.

The CT-incidence in Västerbotten decreased 2009 by almost 33 percent compared with 2008. The decrease in incidence could not be explained by a reduced number of analysed CT-samples: to find one CT-infected individual in Västerbotten in 2009, on average 20 samples were collected. By using this measurement, the CT-testing in Västerbotten was the most extensive in Sweden.

The STI prevention focus in Sweden has earlier been on secondary prevention, i.e. to detect those already carrying the infection by using opportunistic CT-screening, sampling of individuals with suspected CT infection and contact tracing. However, as the frequency of CT increased steadily despite this strategy we decided to focus even more on the recommendations from the National Board of Health's National Action Plan 2009-2014 for CT prevention. Thus, primary prevention procedures were added as well as improving secondary prevention strategies. One of the main objectives in the national CT prevention plan is to achieve a higher proportion of adolescents and young adults who use condoms. Another objective is to increase the proportion of adolescents and young adults who have insight into the potential negative consequences of unprotected sex. A third goal is to increase the proportion of adolescents and young adults who know when to get tested for CT, as well as the proportion of adolescents and young adults with elevated risk behaviour that get CT-tested regularly (at least every six months). By combining these measures, i.e. both primary and secondary prevention, we were able to achieve a declining CT incidence for 15 months in a row and Västerbotten reached an incidence as low as the CT incidence in Finland. Potential key factors of successful CT prevention in

Västerbotten and six other Swedish counties were studied by Deogan et al. The authors found that it is important to focus on structural factors and this include organisational structure, strong leadership, managing STI-networks, research connection, multiple local collaborations with health care and community, high testing coverage and strategic risk approach (Deogan 2013). In Västerbotten we have focused on all these aspects, i.e. on a broad approach with inclusion of several factors, and this is likely the explanation to the sharp decrease of the CT incidence in Västerbotten that started in December 2008. In the study by Deoagan et al. Västerbotten was defined as the strongest case with all investigated structural (10 of 10) and activity factors (8 of 8) identified as strengths; in fact it was the only county among the seven studied counties that demonstrated strengths in all prevention factors studied. Seven out of 10 structural and 6 out of 8 activity components were rated at the highest level (5); the remaining five factors were rated at the next highest level (4).

In conclusion, as shown in Västerbotten, it is possible to reduce the incidence and burden of CT by implementing a broad prevention program that includes several different factors and utilises both primary and secondary prevention strategies.

6. Conclusions

This thesis gives new knowledge especially on different aspects of CT prevention and counselling, and the results and conclusions will aid in the work for prevention of CT transmission. The specific conclusions and recommendations are:

- Anti-LPS antibody is more sensitive than and as specific as an anti-MOMP antibody for CT cell culture confirmation of genital swab specimens.

- Presence of CT IgG in both the male and female partner in a couple is associated to infertility, especially TFI, and it is suggested that CT testing of both partners of the couple should be included in the routine infertility work-up.

- The finding of CT IgG in the male partner of the couple is a significant outcome predictor of failure to achieve pregnancy.

- Contact tracing is important in finding asymptomatic CT-infected individuals. Centralised contact tracing performed by experienced tracers, and extending the tracing period of the index patients sexual history to at least 12 months back in time, are recommended.

- The contact tracing may be performed by telephone or face-to-face at the clinic.

- Brief manual-based single-session MI counselling aimed at assessing personal risks, resolving ambivalence, developing risk-reduction strategies and implementing safer sex behaviour seems to be effective in the reduction of incident CT infections in women with high-risk sexual practices.

- The brief MI intervention seems not to further reduce high-risk sexual practices and incident CT infection in men compared to standard care at an STI clinic. Additional components such as behavioural skills training and booster sessions may be added to the protocol for men.

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