Dissertation

Prevalence, diagnosis, and treatment of urethritis, cervicitis and genital ulcer diseases.

submitted by

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Declaration

I hereby declare that this dissertation is my own original work and that I have fully acknowledged by name all those individuals and organizations that have contributed to the research for this dissertation. Due acknowledgement has been made in the text to all other material used. Throughout this dissertation and in all related publications I followed the guidelines of 'Good Scientific Practice'.

Graz, March, 2022

Birgit Sadoghi, eh

Disclosures

Parts of this thesis have already been published in the "*Journal of the European Academy of Dermatology and Venereology (JEADV)*". Therefore, some parts of the doctoral thesis are similar to the following published manuscript:

Ulcus vulvae acutum Lipschütz: a systematic literature review and a diagnostic and therapeutic algorithm. 2020; doi: 10.1111/jdv.16161

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All co-authors have explicitly agreed to the use of their data in the thesis.

Parts of the introduction are currently submitted as a mini-review including a newly developed diagnostic and therapeutic algorithm concerning urethritis.

Parts of the prospective part will be submitted as original paper, including a pretest-probability-score for detection of sexually transmitted infections.

The tables and figures were created by the authors themselves and those acknowledged in the acknowledgement section.

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1. Abbreviations

ANA	antinuclear antibodies
AMR	antimicrobial resistance
AYA	adolescents and young adults
CDC	centers for disease control and prevention
CMV	cytomegalovirus
CR	case report
CRP	C reactive protein
CS	case series
CT	chlamydia trachomatis
DIG	disseminated gonococcal infection
EBV	Epstein-Barr virus
e.g.	exempli gratia
ENA	extractable nuclear antigen
FSF	females who have sex with females
GUD	genital ulcer disease
HAV	hepatitis A virus
HBV	hepatitis B virus
HCV	hepatitis C virus
HD	Haemophilus ducreyi
HIV	human immunodeficiency virus
HPF	high power field
HSV	herpes simplex virus
IgA	Immunglobulin A
IgG	Immunglobulin G
IgM	Immunglobulin M
i.m.	intramuscular
IRB	institutional review board
i.v.	intravenous
IUSTI	International Union against sexually transmitted infections
LGV	lymphogranuloma venereum
LMA	labia majora
LMI	labia minora
MEDLINE	Medical Literature Analysis and Retrieval System Online
MG	Mycoplasma genitalium

MH	Mycoplasma hominis
MSM	men who have sex with men
NA	not available/ applicable
ND	not done
NG	Neisseria gonorrhoeae
NGU	non gonococcal urethritis
NSAIDS	non-steroidal anti-inflammatory drugs
OT	others
OEGGG	Österreichische Gesellschaft für Gynäkologie und Geburtshilfe
ÖGSTD	Österreichische Gesellschaft für sexually transmitted diseases und dermatologische Mikrobiologie
PCR	polymerase chain reaction
PID	pelvic inflammatory disease
p.o.	per os
POCT	point of care testing
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PVB19	parvovirus B19
SA	systemic antibiotics
SH	spontaneous healing
SS	systemic steroids
STD	sexually transmitted disease
STI	sexually transmitted infection
TA	topical antibiotics
TNF	tumor necrosis factor
TS	topical steroids
TP	treponema pallidum
TOC	test of cure
TV	trichomonas vaginalis
UP	Ureaplasma parvum
UU	Ureaplasma urealyticum
UVAL	ulcus vulvae acutum Lipschütz
VZV	varicella zoster virus
WHO	world health organization
YMSM	young men who have sex with men

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4. Zusammenfassung

Sexuell übertragbare Infektionskrankheiten (sexually transmitted infections= STIs) erleben zurzeit einen massiven Anstieg der globalen Inzidenz, wobei die meisten Patient*innen aufgrund von Symptomen einer Urethritis, Cervicitis oder aufgrund des Auftretens genitaler Ulzera in entsprechenden Spezialambulanzen vorstellig werden.

Das primäre Ziel dieser Dissertationsschrift war die exakte Beschreibung und wissenschaftliche Beurteilung von Prävalenz, Diagnostik und Therapie der durch sexuell übertragbare Erreger ausgelösten Urethritis und Cervicitis von Patient*innen, welche sich in der Ambulanz für sexuell übertragbare Erkrankungen der Universitätsklinik für Dermatologie und Venerologie der Medizinischen Universität Graz zwischen September 2019 und September 2021 vorstellten, zumal diesbezüglich valide Daten aus Österreich bis dato weitestgehend fehlen.

Sekundäres Ziel waren das Verfassen eines systematischen Review sowie die Durchführung einer Metaanalyse, um einen diagnostischen Algorithmus für ein bislang möglicherweise unterdiagnostiziertes dermatologisches Krankheitsbild eines genitalen Ulcus, namentlich das sog. Ulcus vulvae acutum Lipschütz (UVAL) zu entwickeln und exakt zu definieren. Die wesentliche klinische Relevanz liegt diesbezüglich darin, dass die meisten genitaler Ulzera histologisch, serologisch oder molekularbiologisch eindeutig identifiziert werden kann, nicht jedoch das vorgenannte Ulcus vulvae acutum Lipschütz.

Bezugnehmend auf das primäre Ziel dieser Dissertation führten wir eine prospektive Studie an insgesamt 178 Patient*innen durch, wobei die Prävalenz, Diagnostik und Therapie von Urethritis und Cervicitis evaluiert wurde.

Zusätzlich wurden demographische Daten, (Alter, Geschlecht) und für die Fragestellung relevante Informationen wie etwaige Koexistenz anderer sexuell übertragbarer Erkrankungen (u.a. HIV, Hepatitis B und C sowie Syphilis), sexuelle Orientierung, Anzahl der Sexualpartner in den letzten 6 Monaten und während des gesamten bisherigen Lebens, Partnerschaftsstatus, Verwendung von Sexspielzeug, Impfstatus (Meningokokkenimpfung, HPV-Impfung) sowie Behandlungsschemata und die Effektivität der Behandlung einer etwaigen Gonorrhoe erhoben.

Die Prävalenz der STIs in der untersuchten Studienkohorte war am höchsten für *Chlamydia trachomatis* (CT) mit 17%, zahlenmäßig gefolgt von Infektionen mit *Neisseria gonorrhoe* (NG) (12%). Infektionen mit *Mykoplasma genitalium* (MG) und *Trichomonas vaginalis* (TV) wurden in 8% bzw. 1% der Fälle detektiert. 22,5% Fälle von STI-bedingter Urethritis und 20% Fälle von STI-bedingter Cervicitis wurden dokumentiert.

Unsere Daten zeigten keine statistisch signifikanten Unterschiede zwischen den Gruppen der asymptomatischen und symptomatischen Patient*innen. Wir konnten jedoch 3 statistisch signifikante Risikofaktoren für den Erwerb einer STI verifizieren: Alter jünger als 25 Jahre (p: 0,042), nicht-heterosexuelle sexuelle Orientierung (p: 0,027) und Vorliegen von Ausfluss (p: 0,001).

Die STI-Diagnostik erfolgte mittels eines neu eingeführten Multiplex DNA-Microarray von Euroimmun® (Lübeck, Germany) zur PCR-basierten Auswertung relevanter sexuell übertragbarer Pathogene. Dieser Array ermöglicht eine direkte und simultane Detektion von 11 relevanten sexuell übertragbaren Keimen in nur einem Testkit. Diese Pathogene sind: CT, NG, Herpes- Simplex-Virus (HSV)-1 und -2, Haemophilus ducreyi (HD), MG, Mycoplasma hominis (MH), Treponema pallidum (TP), TV, Ureaplasma parvum (UP) und Ureaplasma urealyticum (UU).

Die jeweilige Therapie erfolgte fachspezifisch und leitliniengerecht entsprechend Österreichischer bzw. Europäischer Empfehlungen.

Die Resistenzbestimmung der NG infizierten Personen zeigte ein 100%-iges Ansprechen auf die empfohlene Therapie mit Ceftriaxon (Cephalosporin der 3. Generation/ß-Laktam-Antibiotikum).

Zudem erfolgte eine weltweite systematische Online-Literaturrecherche und Metaanalyse in Bezug auf das selten diagnostizierte genitale Ulcus vulvae acutum Lipschütz. Bislang fehlten eine standardisierte Diagnostik und Behandlung für dieses Krankheitsbild in Literatur wie klinischer Praxis. Dies steht im klaren Gegensatz zu nahezu allen übrigen beschrieben genitalen Ulzera, welche allesamt durch ihre Klinik, Serologie, Histologie und/oder molekulare Diagnostik klar abgegrenzt bzw. verifiziert werden konnten.

Anhand der extrahierten Daten aus der Metaanalyse wurde ein Diagnose- und Therapiealgorithmus für UVAL formuliert, welcher aus 2 Haupt- und 2 Nebenkriterien besteht. Die Hauptkriterien sind (I) akuter Beginn einer oder mehrerer schmerzhafter Ulzerationen in der Vulvaregion und (II) der Ausschluss von infektiösen oder nicht-infektiösen Ursachen ebendieser Ulzerationen. Nebenkriterien sind (I) die Lokalisation der Ulzerationen am Vestibulum oder den Labia minora und (II) kein Sexualverkehr innerhalb der letzten 3 Monate sowie (III) Grippeähnliche Symptome und/oder systemische Infektion innerhalb von 2 bis 4 Wochen vor Beginn der vulvären Ulzerationen. Wir sind der Meinung, dass eine konsequente Anwendung dieses symptomorientierten Behandlungskonzeptes der von uns vorgeschlagenen Kriterien zu einer deutlich rascheren Diagnostik und Therapie von UVAL beitragen und somit potenziell einen hohen klinischen Nutzen darstellen könnte.

5. Summary

Currently, sexually transmitted infections (STIs) are globally on the rise, whereby most of affected patients present either with signs of urethritis, cervicitis, or a genital ulcer. Some STIs differ significantly from each other regarding their prevalence and treatment modalities, depending on sex, certain risk groups, various geographic regions, and their antimicrobial resistance profiles of certain bacteria.

The first aim of this doctoral thesis was to accurately describe and evaluate the prevalence, diagnosis, and therapy of sexually transmitted pathogens causing urethritis, as well as cervicitis of patients that presented at the STI outpatient clinic of the Department of Dermatology and Venereology of the Medical University of Graz between September 2019 and September 2021. This evaluation gains particular scientific importance since no published data from Austria are available up to date yet.

The second aim was to perform a systematic review and meta-analysis to be able to define a diagnostic algorithm for an underdiagnosed genital ulcer disease, named ulcus vulvae acutum Lipschütz (UVAL). This became significant as we realized that almost all variants of genital ulcers can be clearly identified via histology, serology or molecular methods, which is not the case concerning UVAL.

A prospective study evaluating the prevalence, diagnosis and therapy of urethritis and cervicitis was performed, including 178 individuals.

In addition, we extracted information with respect to patient characteristics, including age distribution, co-existence of other STIs (including HIV, hepatitis B and C, syphilis), sexual orientation, number of sexual partners within the last 6 months and during lifetime, partnership status, usage of sex toys, vaccination status (against meningitis and human papilloma virus), as well as various treatment regimes, and test of cure (TOC) outcomes in cases of gonorrhea.

The prevalence of STIs in our study cohort was highest for CT with 17%, followed by infections with NG (12%); MG and TV infections were detected in 8% and 1% of the cases, respectively. We observed 22.5% cases of STI-related urethritis and 20% cases of STI-related cervicitis.

No statistically significant differences between the groups of asymptomatic versus symptomatic patients were detected. However, we found 3 statistically significant risk factors for acquisition of a STI [(Age younger than 25 years (p: 0.042), non-heterosexual sexual orientation (p: 0,027) and presence of discharge (p: 0,001)].

Sexually transmitted pathogens were detected via a newly introduced multiplex DNA microarray from Euroimmun® (Lübeck, Germany) at the Department of Dermatology and Venereology of the Medical University of Graz. This panel includes a PCR-based direct and simultaneous detection of the most important elven sexually transmitted pathogens within just one test kit. Testable pathogens were *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Herpes- Simplex-Virus* (HSV)-1 und -2, *Haemophilus ducreyi* (HD), *Mycoplasma genitalium* (MG), *Mycoplasma hominis* (MH), *Treponema pallidum* (TP), *Trichomonas vaginalis* (TV), *Ureaplasma parvum* (UP), and *Ureaplasma urealyticum (*UU).

The respective therapy was performed in accordance with Austrian and European Guideline recommendations.

The resistance profiles of NG infected individuals showed a 100% response rate towards the recommended therapy with ceftriaxone.

Moreover, a worldwide systematic online literature review and meta-analysis with respect to the rare genital ulcer disease of UVAL was performed, as a systematic description, as well as standardized treatment options are scarce for this disease. This finding is clearly in contrast to almost all other known genital ulcers, where an accurate diagnosis is feasible in a standardized fashion via serology, histology, or established molecular diagnostic methods.

According to the extracted data, we formulated a diagnostic algorithm for UVAL, which is defined by two major and four minor criteria. The major criteria were (i) acute onset of one or more painful ulcerous lesions in the vulvar region and (ii) exclusion of infectious and non-infectious causes for the ulcer. The minor criteria were (i) localization of the ulcer at the vestibule or labia minora, (ii) no sexual intercourse ever (i.e. patient was a virgin) or within the last 3 months, (iii) flu-like symptoms and/or (iv) systemic infection within 2–4 weeks prior to onset of vulvar ulcer. We sincerely believe that the developed algorithm might significantly improve the diagnosis and management of this seldom genital ulcer disease in the future.

6. Introduction

General introduction- STIs

At present, STIs are highly on the rise in a global context. Focussing on the four curable STIs gonorrhoea, trichomoniasis, syphilis and infections with *chlamydia trachomatis (CT)*, the world health organization (WHO) estimated an incidence of more than one million infections per day in people aged 15-49 years [Rowley 2019]. Moreover, several reports argued that due to the launch of pre-exposure prophylaxis (PrEP) for HIV, an even higher incidence and prevalence of STIs can be observed. This is of importance, especially in the first year of PrEP usage, probably due to a higher number of condom-less sex within that group [Jansen 2020, Nguyen 2018, Unemo 2019]. Furthermore, recently the age group of adolescents (15-24 years of age) was called a "neglected population", as the incidence and prevalence of STIs within this particular age group is rising dramatically [Shannon 2018]. It is estimated that in the United States alone this cohort seems to be responsible for just about the half of all new STIs each year [Shannon 2018]. Particularly in the young, STIs currently represent a serious concern [Shannon 2018]. For various reasons they often tend not to present themselves to a clinician easily and taking a detailed anamnestic history must been done with outmost sensitivity. Adequate screening, as well as consecutive education and treatment are of great importance with respect to that generation, not only because of possibly extensive consequences regarding reproductive health [Shannon 2018].

The reasons for increased recent incidence rates of STIs are manifold and partly believed to lay in a changed sexual behaviour of the younger generation within the last years, the frequent change of sexual partners, concurrent partners, the introduction of post-exposure prophylaxis for HIV (PEP) and PrEP (and therefore more frequent condom-less sex), the ease to find sexual partners e.g. via dating apps, sexual 'networks', the higher frequency of sexual practices that are known as risk factors for STIs, as well as the usage of 'party' drugs (so called 'chem-sex') [Unemo 2019, Shannon 2018, Jansen 2020].

In general, men are significantly more often affected by STIs (most likely due to the group of men who have sex with men (MSM)), albeit women do suffer from much more sequelae and morbidity that significantly affect not only the quality of life but particularly the reproductive health [Wiesenfeld 2017]. In this regard, STIs do not only represent a serious and immediate health concern, but they might also result in significant consequences like pelvic inflammatory disease (PID), infertility or ectopic pregnancy, or preterm birth , amongst others [Wiesenfeld 2017, Unemo 2019]. For instance, about 1/4th of infertility is estimated

to be due to tubal occlusion, occurring after genital upper tract infections with *Neisseria gonorrhoeae* (NG) and *Chlamydia trachomatis* [Wiesenfeld 2017|. An ectopic pregnancy might be due to damaged cilia, whereby both, *Neisseria gonorrhoeae* and *Chlamydia trachomatis*, are well known causative pathogens [WHO 1995]. Additionally, preterm delivery (delivery of baby before week 37 of gestation) is linked to infectious reasons in about one third of females [Goldenberg 2008]. This background information is disappointing, as the majority of STIs seem curable, or at least treatable, if detected in time. Therefore, it is absolute essential to detect and treat STIs rapidly and effectively.

A majority of STIs seem to follow an asymptomatic course, which makes it of the outmost relevance to detect infections sufficiently early, since otherwise the likelihood of transmission and consecutive morbidity is obligatorily increased.

Furthermore, antimicrobial resistance issues in some pathogens cause serious global concerns, especially in gonorrhea and infections with *Mycoplasma genitalium* (MG), evolving high levels of antimicrobial resistance [Unemo 2020, Unemo 2019, Gnanadurai 2020]. In gonorrhea up until today, the development of a resistance towards every single first or second line antibiotic that has been given to combat the infection, was observed [Unemo 2019].

Screening of STIs necessarily should be performed in those at risk, like younger age, certain sexual orientation, high number of sexual contacts, high number of changing partners, in those who have concurrent partners, experienced a STIs previously, or tend to risky behavior, among others [Bachmann 2017, Unemo 2019, Unemo 2020].

In the majority of STI's the commonest reason for presentation in men are symptoms of urethritis, in females – if they are present- symptoms of urethritis or cervicitis, as well as genital ulcers in both sexes.

6.1. Urethritis

Urethritis, the inflammation of the urethra, is predominantly caused by sexually transmitted pathogens [Moi 2015, Horner 2016]. In most patients, the responsible agents include *Chlamydia trachomatis (20* to 50% of non-gonococcal urethritis (NGU*)), Neisseria gonorrhoeae (5-20% of urethritis cases), Mycoplasma genitalium (15 to 25%), Trichomonas vaginalis* (TV) *(1 to 20%) or adenovirus* except for a few others *[Moi 2015, Rossignol 2019, Bachmann 2015, Gottesmann 2017].*

Urethritis is verified by the evidence of a causative pathogen (verified via microscopy or nuclein amplification test) or an objective inflammation (verified via clinical symptoms including discharge and microscopy via an increased amount of polymorphonuclear leucocytes (PMNL) at stain of exudate) [Bachmann 2015].

Clinical symptoms encompass mainly discharge, dysuria, urethral irritation, pruritus, or erythema of the meatus urethrae ext. among some others [Moi 2015, Bachmann 2015, Horner 2017, Horner 2016].

As depicted above, urethritis is defined as being either gonococcal (if *Neisseria gonorrhoeae* is the causative pathogen) or non-gonococcal [Horner 2016]. Nongonococcal urethritis (NGU) is defined as urethritis that is caused by any other pathogen than *Neisseria gonorrhoeae*.

In males, nongonococcal urethritis is the most common sexually transmitted disease [Moi 2015]. It is believed that in up to 50% of all cases CT, and in up to 30-50% MG is causative, however in 30-60% of all patients no contributing pathogen can be found [Moi 2015, Horner 2016]. Therefore, in all individuals that suffer from urethritis, appropriate tests for the suspected pathogenes should be performed [Moi 2015, Horner 2016].

In addition, appropriate treatment is performed according to the underlying causative pathogen and is depicted below.

6.1.1. Diagnostic considerations of urethritis

Microscopy

Diagnosis is performed via evaluating the amount of PMNL of the stained smear [Moi 2015, Bachmann 2015]. Staining may be performed with Gram stain or methylene blue [Moi 2015]. Urethritis is defined microscopically, if ≥ 5 PMNL per high power (x1000) microscopic field are present [Horner 2017]. The average of 5 fields (with highest amount of PMNLs) shall be considered [Moi 2015, Bachmann 2015]. Some authors divide into low cut-off (≥ 5PMNLs) and high cut-off urethritis (≥10 PMNLs) [Randjelovic 2018].

Patients should avoid urinating at least >1, (better) 2 hours before the smear, as bacteria otherwise might be rinsed out [Moi 2015, Unemo 2020]. The smear should be taken with a 1-5 mm plastic loop from the anterior urethra (first 0.5-1 centimeter of the urethra), in circular movements [Moi 2015, Bignell 2006, Horner 2016].

Nuclein amplification tests

NAATs have been added as a diagnostic method in a wide range of medical fields and significantly improved the sensitivity for the detection of STIs. Tests that amplify certain nucleic acid sequences can find even non-viable organisms [Bignell 2006]. That is the reason why test of cures (TOCs) shall not be performed in a shorter period than about 3 weeks after completion of treatment. A major advantage of the usage of NAATs is the possibility of non-invasiveness sampling, as urine or even self-taken vaginal swabs are suitable for diagnosis [Bignell 2006]. Moreover, NAATs are the test of choice for not only symptomatic, but especially asymptomatic patients, that otherwise might end in a delayed diagnosis and therefore treatment [Moi 2015, Unemo 2020].
If urine is used for a NAAT, not more than ten to twenty ml of first void urine shall be used [Nenoff 2017, Moi 2015, Unemo 2020]. Pooling of specimens is not recommended, due to loss of sensitivity [Unemo 2020].

6.1.2. Gonococcal urethritis

6.1.2.1. Neisseria gonorrhoeae

Gonorrhea currently represents the second commonest bacterial STI in European countries [Unemo 2020]. It is caused by a gram-negative, obligate human pathogenic, kidney-shaped bacterium called *Neisseria gonorrhoeae* [Unemo 2019, Bignell 2006, Unemo 2020]. Gonorrhea is a highly infectious STI with an estimated global incidence of nearly 90 million adults per year, and its incidence has risen by about 240% since 2008 [Unemo 2019, Unemo 2020].

Transmission occurs via unprotected sexual intercourse and direct inoculation of infected secretions at the mucosal epithelium of the specific anatomical site, like vagina, rectum, urethra or during birth [Unemo 2019, Bignell 2006, Unemo 2020].

In most infected males (nearly 90%), gonorrhea causes symptomatic urethritis, including discharge (more than 80%) and dysuria (more than 50%) [Unemo 2019, Unemo 2020]. As the incubation period is very short (from one day to about one week), symptoms start very quickly, usually two to eight days after the sexual contact [Unemo 2020]. An asymptomatic infection from the male's urethra is very uncommon [Unemo 2020]. Ascending infections in men may result in prostatitis, epididymitis or even epididymo-orchitis [Unemo 2019, Wiesenfeld 2017, Unemo 2020].

In females, epithelial cells of the cervix and urethra may be infected [Unemo 2019, Unemo 2020]. Symptoms comprise altered vaginal discharge (about half of infected females), lower abdominal pain (about 1/4th), seldom dysuria (10-15%) or bleeding [Unemo 2020]. In women ascending bacteria may lead to cervicitis, salpingitis, pelvic inflammatory disease and their consequences, like ectopic pregnancy and even infertility [Unemo 2019, Wiesenfeld 2017, Unemo 2020]. During pregnancy, an infection with NG may cause adverse birth outcomes, like premature delivery and the well-known perinatal infection resulting in ophthalmia neonatorum in the newborn [Unemo 2019].

In both sexes, extragenital sites, like pharynx or rectum among others, might be affected and are in most of the cases completely asymptomatic [Unemo 2019, Unemo 2020]. In extragenital sites, microscopy is not recommended for detection of NG due to low sensitivity and specificity [Unemo 2020]. Sensitivity of a culture is significantly lower at

oropharyngeal and rectal sites either, which must be considered for the correct diagnosis [Unemo 2020].

Diagnostic considerations of gonococcal urethritis

Detection of *Neisseria gonorrhoeae* is performed microscopically, via culture or by direct detection using NAATs [Unemo 2020]. Samples that are suitable for diagnosis are urethral smears or first void urine.

Microscopy and culture

Microscopy was established decades ago, is not expensive and acts as a point of care testing (POCT) in gonococcal urethritis, as in symptomatic men; it has a sensitivity of up to 95 % [Bignell 2006, Unemo 2019, Unemo 2020]. However, in asymptomatic males' the sensitivity of the urethral smear is lower, ranging from 50 to 75% [Bignell 2006, Unemo 2020]. Specificity can be very high, if educated personnel performs microscopy (up to 99%) [Bignell 2006]. In ideal settings and trained personnel, the sensitivity for urethral and cervical smears is believed to reach up to 85% [Bignell 2006].

The level of laboratory infrastructure must not be very specific, a microscope only is used for instrumentation [Unemo 2019]. In case of microscopic detection of intracellular gram-negative diplococci within polymorphonuclear leukocytes, diagnosis of gonorrhea can be made [Unemo Guideline 2020].

As NG is known as a multi-resistant agent, starting a culture on enriched medium, a compatible agar, e.g. a Thayer-Martin Agar is inevitable [Nenoff 2017, Unemo Guideline GO 2020, Unemo 2019, Bignell 2006]. Susceptibility testing of the isolate is highly recommended in all cases of gonorrhea [Nenoff 2017, Unemo 2019]. Inoculating the material at the most rapid timepoint is important. Incubation is done for at least 2 days at 36° of Celsius in a CO2-free or microaerophilic environment [Bignell 2006, Unemo 2019]. If gonococci grow, they show a characteristic appearance, like grey or opaque colonies of kidney shaped coffee beans [Nenoff 2017, Unemo 2019]. Specimens from all sites (cervical, urethral, pharyngeal and rectal) can be used for culture and the highly recommended susceptibility testing [Bignell 2006, Unemo 2019]. However, swabs from urine and vagina seem not to be appropriate [Unemo 2020].

Nucleic acid amplification test:

Clearly, NAATs nowadays represent the recommended state-of-the-art testing method for gonorrhea [Nenoff 2017, Unemo 2019, Unemo 2020]. However, antimicrobial resistance (AMR) testing is not possible with the usage of NAATs alone [Unemo 2019]. Concerning NAATs, urethral smear or first stream urine samples are suitable for diagnosis. The first 15-30 ml of urine are collected after a micturition abstinence for at least 1 hours [Bignell 2006, Unemo 2020]. A very high sensitivity is guaranteed (>95%) using this method [Bignell 2006].

Table 1- Suitable testing methods for *Neisseria gonorrheae*

specimen type	sex	microscopy	culture	NAAT
first void urine	female	-	-	+
	male	-	-	+
urethral swab	female	+	+	+
	male	+	+	+
penile swab	male	-	-	-
endocervical smear	female	+	+	+
ectocervical smear	female	+	+	+
vaginal smear	female	-	-	+
extragenital sites (be aware that especially at the oro-pharyngeal site apathogenic *Neisseria* species may be present)	both sexes	-	+/-	+

6.1.3. Non-gonococcal urethritis

6.1.3.1. Chlamydia trachomatis

Chlamydia trachomatis is a very small, obligate intracellular bacterium that only replicates in living bacteria [Witkin 2017, Bebear 2009, Lanjouw 2015, Nenoff 2017]. It represents the most common bacterial STI in European countries, whereby it is estimated that there are more than 100 million of people infected with CT every year [Bebear 2009, Lanjouw 2015]. Females are affected twice as often as men, and the group of adolescents is most infected [Nenoff 2017].

Chlamydia trachomatis has a very complex reproductive cycle [Witkin 2017, Bebear 2009]. Elementary bodies, which are infectious, attach to receptors on target epithelial cells,

and are taken up via endocytosis [Bebear 2009, Witken 2017]. In the host cell elementary bodies transform to metabolically active reticulate bodies that can multiply [Bebear 2009]. After about one day, reticulate bodies transform back into elementary bodies, infecting neighbor cells. Immunity is not possible after an expired infection [Lanjouw 2015].

To the best of our knowledge, fifteen different serovars of CT exist that are differentiated due to the cell wall surface and are uptaken by different cells. They are the causative agents of trachoma (serovars A-C), classical urogenital STIs (D-K) and the invasive variants causing lymphogranuloma venereum (LGV) (L1-3) [Witkin 2017, Lanjouw 2015]. Its exclusive natural host is humans [Witkin 2017]. Transmission of serotypes D to L occurs by direct mucosal contact via sex and perinatally [Lanjouw 2015]. The incubation period is 1 to 3 or 4 weeks [Bebear 2009, Horner 2017]. Autoinoculation may play a role in both sexes, as conjunctival irritation may be due to CT from the urogenital tract [Bebear 2009]. CT predominantly infects mainly mucosal epithelium of the cervix, the urethra and the rectal area [Nenoff 2017].

In men, if a CT infection is symptomatic, they present themselves with dysuria and a moderate clear or whitish urethral discharge [Bebear 2009]. In cases of a rectal infection, signs of proctitis might be present [Bebear 2009]. In case of severe proctitis and lymphadenopathy one must keep in mind the invasive variants of CT L1-L3, causing lymphogranuloma venereum. Reactive arthritis or sexually acquired reactive arthritis (SARA) is highly associated to a CT infection [Bebear 2009]. Asymptomatic courses in men are not uncommon and range from 25 to 100% [Lanjouw 2015].

In females, cervicitis might present itself with vaginal discharge, mucopurulent discharge from the cervix, cervical friability, edema or bleeding [Bebear 2009, Lanjouw 2015]. According to Bebear and colleagues, a urethral infection can be linked to a cervicitis [Bebear 2009], and might present itself due to dysuria or bleeding [Bebear 2009, Lanjouw 2015].

Persisting and ascending infections may result in epididymitis and orchitis in men, whereas there could be found no clear hint for prostatitis [Bebear 2009, Lanjouw 2015]. In women, endometritis, salpingitis, tubal infertility (due to scarring), ectopic pregnancy and pelvic inflammatory disease are well known sequelae [Witkin 2017, Bebear 2009].

Pelvic inflammatory disease (PID) is a clinical syndrome based on chronic inflammation of the female upper reproductive tract [Brunham 2015, Wiesenfeld 2017]. It is

believed that at least 60% of acute PID are caused by a CT infection [Bebear 2009]. Diagnosis and consecutive effective treatment are of outmost relevance, as otherwise it may result in long-term reproductive disability, including not only ectopic pregnancy but infertility [Brunham 2015].

Diagnostic considerations of *Chlamydia trachomatis* urethritis

Chlamydia trachomatis urethritis is diagnosed by NAATs [Lanjouw 2015, Babaer 2009]. NAATs are the recommended method of choice and shall be positive within the first 3 up to 14 days of exposure [Lanjouw 2015, Babaer 2009]. In case that NAATs are not available, culture may be performed [Lanjouw2015]. However, culture of CT is challenging, as enough vitals cells are necessary for CT replication, removal of enough material is painful and consumes too much time until diagnosis [Babaer 2009,AWMF guideline Chlamydia]. Culturing shall only performed if NAATs are not available. Microscopy can only show urethritis, based on the amount of PMNLs of the smear [Moi 2015], but not differentiate the various causative pathogens of NGU.

Samples that are suitable for the diagnosis of urethritis are urethral smears or first void urine. In men, first void urine (up to 20 ml, after 1 h of micturition abstinence) is enough and non-invasive [Bebear 2009]. In women, first void urine or vaginal self-samples might be used. Vaginal self-collected specimens show a smaller bacterial load than endocervical swabs, but a higher load than first void urine [Bebear 2009], Lanjouw 2015.

Table 2- Suitable testing methods for *Chlamydia trachomatis*

specimen type	sex	microscopy	culture	NAAT (recommended test of choice for CT)
first void urine	female	-	-	+
	male	-	-	+
urethral swab	female	-	+/-	+ (swab is preferred)
	male	-	+/-	+
penile swab	male	-	-	-
endocervical smear	female	-	+/-	+ (endocervical is preferred)
ectocervical smear	female	-	+/-	+
vaginal smear	female	-	-	+ (self-collected appropriate)
extragenital sites	both sexes	-	+/-anorectal -orophayngeal	+
serology	both sexes	not recommended as only invasive CT infections might lead to detectable levels of antibodies		

6.1.3.2. Mycoplasma genitalium

Mycoplasma genitalium (MG) is an independently reproducing, very small, slow growing, flask-shaped, sexually transmitted bacterium [Gnanadurai 2020, Horner 2017, Jensen 2016]. It causes acute and (more importantly) chronic NGU in men and seems to be another causative agent for cervicitis and PID in women [Gnanadurai 2020, Horner 2017, Jensen 2016]. MG lives parasitically, can replicate intra- and extracellular and provokes inflammation due to an adhesion to epithelial cells, resulting in high activation of pro-inflammatory signals [Gnanadurai 2020, Horner 2017]. Prevalence seems to range from 1 to 4% in the general population [Gnanadurai 2020, Horner 2017]. Incubation time is believed to take a little bit longer than in CT infections (where we have a 1–4-week incubation period) [Horner 2017, Jensen 2016], while some experts believe that the incubation period might last up to 2 months [Horner 2017]. Transmission occurs via unprotected sexual intercourse, a perinatal transmission is not clearly identified [Jensen 2016].

Male infection can result in acute and chronic nongonococcal urethritis, proctitis and epididymitis [Gnanadurai 2020, Jensen 2016]. Symptoms encompass urethral discharge, dysuria or signs of proctitis, like rectal tenderness or pain or discharge [Jensen 2016].

In females, cervical and urethral infections are possible, and symptoms encompass vaginal discharge, dysuria or bleeding, and cervicitis [Jensen 2016]. Moreover, in females it seems to be associated with PID and cervicitis mainly [Gnanadurai 2020, Wiesenfeld 2017]. The correlation with PID seems to be very high, and in those suffering from endometritis, MG is found in a similar number like CT or NG [Wiesenfeld 2017].

Diagnostic considerations of Mycoplasma genitalium urethritis

Testing is recommended in symptomatic males (NGU- especially recurrent/persistent NGU, and probably epididymo-orchitis) and PID (and probably cervicitis) in women and proctitis in both sexes [Gnanadurai 2020, Jensen 2016]. The only method of choice is NAATs [Horner 2017, Jensen 2016]. First void urine, vaginal swabs and anorectal swabs are appropriate [Jensen 2016]. However, extragenital routine testing is not recommended [Workowski 2021].

Table 3- Suitable testing methods for *Mycoplasma genitalium*

specimen type	sex	microscopy	culture	NAAT (recommended test of choice for MG)
first void urine	female	-	-	+
	male	-	-	+
urethral swab	female	-	-	+
	male	-	-	+
penile swab	male	-	-	-
endocervical smear	female	-	-	+
ectocervical smear	female	-	-	+
vaginal smear	female	-	-	+ (self-collected appropriate)
extragenital sites	both sexes	-	-	+
serology	both sexes	not recommended, cross reactivity		

6.1.3.3. Trichomonas vaginalis

Trichomonas vaginalis (TV), the causative pathogen for most common parasitic STI in the world (trichomoniasis), is a flagellated, primarily anerobic extracellular protozoan [Kissinger 2015, Van Gerwen 2019, Sherrard 2018]. According to the WHO, in 2008 around 276 million people became infected with TV worldwide that year, so the number of TV cases are higher than CT, NG and syphilis cases in total [Kissinger 2015].

Its only host are humans [Kissinger 2015]. In most cases, it infects squamous epithelia of the urogenital tract [Kissinger 2015]. The incubation period is believed to range from 4 to 28 days [Kissinger 2015]. *Trichomonas vaginalis* may persist for a long time in females at the lower genital tract and in males at the urethra and prostate gland [Kissinger 2015]. Transmission occurs via direct mucosal contact, predominantly via unprotected sexual intercourse Kissinger 2015]. However, nonsexual transmission, i.e. smear infection has been described, as well as perinatal infection and is linked to adverse birth outcomes, like low birth weight or preterm delivery [Crucitti 2011, Kissinger 2015, Van Gerwen 2019, Arabi 2018].

An infection with TV can proceed symptomatically or asymptomatically, which is the case in most individuals (f>m; >85% vs >77%) [Kissinger 2015]. Nevertheless, a screening of asymptomatic patients seems to be of no major advantage [Van Gerwen 2019].

In males, TV can cause urethritis (which is uncommon in western countries) [Moi 2015], with a mostly milky-white discharge, dysuria, and may end in epididymitis and prostatitis [Kissinger 2015, Van Gerwen 2019].

In females, it may infect the urethra, vagina and cervix. Symptoms encompass discharge, dysuria, itching and even abdominal pain [Kissinger 2015]; an ascending infection may lead to infestation of the adnexa or the endometrium [Kissinger 2015].

Diagnostic considerations of Trichomonas vaginalis urethritis

Microscopically motile parasites can be detected in acute trichomoniasis without staining, however, even though this could represent a POCT, due to its low sensitivity (50-70%) it is not recommended as the diagnostic test of choice [Kissinger 2015, Van Gerwen 2019]. A culture in women might be discussable (sensitivity 81-94%), but not in men due to the low sensitivity [Kissinger 2015, Van Gerwen 2019]. Consequently, the diagnostic method that is recommended are NAATs with a sensitivity up to 100% [Kissinger 2015, Van Gerwen 2019]. They are best performed on vaginal or endocervical swabs or from urine of both sexes [Kissinger 2015, Van Gerwen 2019].

Table 4- Suitable testing methods for *Trichomonas vaginalis*

specimen type	sex	microscopy (if performed rapidly; but low sensitivity)	culture (only if NAATs are not available, for females)	NAAT (recommended test of choice for TV)
first void urine	female	-	-	+
	male	-	-	+
urethral swab	female	+/-	-/+	+
	male	+/-	-	+
penile swab	male	-	-	-
endocervical smear	female	+/-	-	-/+
ectocervical smear	female	+/-	-	-/+
vaginal smear (recommended site for testingin females)	female	+/-	-/+	+
extragenital sites (unlikely that extragenital infection ispossible)	both sexes	-	-	-
serology	both sexes	detection not possible		

6.1.4. Treatment and management of sex partners

6.1.4.1. Treatment of urethritis caused by either Neisseria gonorrhea, Chlamydia trachomatis, Mycoplasma genitalium or Trichomonas vaginalis

The choice of treatment is based on national guidelines (and based on the country of the acquired infection). Infected individuals must be educated precisely and abstain from sexual intercourse for a certain number of days, concerning the respective infection. If one STI is found in an individual, she/he must be offered a check for the most relevant STIs (of the respective country).

Herein we present treatment recommendations of the International Union against sexually transmitted infections (IUSTI) and compare them to national guidelines of the 'Österreichische Gesellschaft für sexually transmitted diseases und dermatologische Mikrobiologie" (ÖGSTD)'.

Gonococcal urethritis

Treatment recommendations for the treatment of gonorrhea differ worldwide. They are based on therapeutic availabilities, resistance profiles and national conditions. In the pity of the absence of a sufficient vaccine, clinicians call for early diagnosis, effective and guideline-based treatment modalities, antimicrobial testing and a mandatory partner notification and education concerning the prevention of acquiring this and other STIs [Unemo 2019].

The European (IUSTI) guideline recommends in case of unknown antimicrobial susceptibility, ceftriaxone 1g plus azithromycin 2g as single doses OR ceftriaxone 1g monotherapy as single dose [Unemo 2020]. The dual therapy shows very high cure rates, aims to decrease resistance levels, treats simultaneously CT infection and to a certain proportion also MG infection [Unemo 2020]. The Austrian (ÖGSTD) guideline recommends ceftriaxone 1 g i.v. or i.m. as monotherapy, which was adapted in late 2021 [ÖGSTD update on gonorrhea].

Infected individuals should be educated precisely and abstain at least 7 days (if ceftriaxone monotherapy was administered) to 14 days (if dual therapy was administered) after they and their partners are asymptomatic and completed treatment [Unemo 2020].

A test of cure to ensure an eradication of the infection and to identify resistance should be performed, with a NAAT taking place not earlier than 2-3 weeks after completing treatment [Unemo 2020, ÖGSTD].

A rare but scaring complication is gonococcal bacteremia or disseminated gonococcal infection (DIG) and must be kept in mind, at least, if gonorrhea cases rise [Unemo 2020, Birell 2019]. In individuals with disseminated gonococcal infection, hospitalization, especially in the beginning, is recommended for intravenous treatment and controlling potential septicemia [Unemo 2020].

Nongonococcal urethritis

Experts recommend that, in case of clinical symptoms and clear diagnosis of urethritis, treatment shall be started without final lab results [Moi 2015]. In those who have symptoms, but no signs for urethritis (diagnosed microscopely), no treatment should be initiated [Horner 2016]. If NAATs are negative and symptoms do not fade, individuals are asked to present themselves for a new early morning smear [Horner 2016].

Up until today the treatment of choice used by the majority of clinicians for treating nongonococcal urethritis is either azithromycin 1 g orally as single dose or doxycycline 100 mg orally twice (BID) a day for a week [Bachmann 2017].

Chlamydia trachomatis urethritis

The European (IUSTI) guideline and the ÖGSTD guideline both recommend either doxycycline 100 mg twice a day for one week or azithromycin 1 g p.o. as single dose for the treatment of uncomplicated CT infections [Bebear 2009, Lanjouw 2015, ÖGSTD]. In the case of a complicated CT infection, like ascending infections including epididymitis or PID among others, the antibiotic treatment duration should be extended to 14 days according to the ÖGSTD [ÖGSTD]. In pregnancy and during breast feeding, treatment with azithromycin 1g p.o. as single shot is recommended in both guidelines [Lanjow 2015, 22ÖGSTD].

Individuals with CT infection should abstain from sexual contact for 7 days after they and their partners completed treatment and symptoms have resolved [Lanjouw 2015].

A test of cure is not routinely recommended if patients received the above mentioned 1st--line treatment [Lanjouw 2015], but might be performed either 3 weeks or 3 months after treatment [ÖGSTD].

Mycoplasma genitalium urethritis

According to the current European (IUSTI) guideline and the ÖGSTD guideline, the treatment of uncomplicated MG infections (in absence of macrolide resistance) consists of either azithromycin 500 mg on day one, then 250 mg od days 2–5 (oral) or Josamycine 500 mg three times daily for 10 days [Jensen 2016, Horner 2017, ÖGSTD].

Individuals with MG infection should abstain from sexual contact after they and their partners completed treatment, symptoms have resolved, and a negative test of cure is obtained [Jensen 2016].

There is a strong urge for a testing method that simultaneously describes resistance profiles [Gnanadurai 2020, Jensen 2016]. Macrolide resistance is believed to range from 30 to 100% worldwide [Gnanadurai 2020]. A TOC is recommended by IUSTI guidelines 3 weeks after ending of treatment [Jensen 2016].

Trichomonas vaginalis urethritis

According to the current European (IUSTI) guideline and the ÖGSTD guideline, Metronidazole 400-500 mg BID for 5- 7 days should be administered as treatment of first choice [Kissinger 2015, ÖGSTD, Sherrad 2018]. It is important that patients are informed to abstain from alcohol consumption for at least 24 hours after treatment [Kissinger 2015, ÖGSTD].

Individuals with TV infection should abstain from sexual contact after they and their partners completed treatment and symptoms have resolved [Sherrard 2018, Kissinger 2015].

A test of cure can be performed, and if, not earlier than 3 weeks after ending the treatment [Kissinger 2015]. According to the IUSTI guideline, a TOC is not necessarily found in asymptomatic patients [Sherrad 2018].

Recurrent or persistent nongonococcal urethritis

Quite often patients present themselves with recurring or persisting NGU. Persistent NGU is defined as persisting symptoms of urethritis, despite a prior treatment and has a prevalence of 15-25% [Horner 2016]. Recurrent NGU is defined as recurring symptoms within 30-90 days after treatment and occurs in 10-20% of individuals [Horner 2016].

However, first, an exact history taking must be done, and one has to evaluate whether there has been non-compliance or re-exposure/ risky behavior to an untreated or new partner, drug-resistance or misunderstanding in treatment, or complicated/ ascending infection [Bachmann 2015, Moi 2015, Horner 2016]. Up to 40 % of NGUs are believed to not respond to 1st-line treatment, which is a terrifyingly high number, and in about the half od persistent NGUs no causative agent is to be found [Moi 2015, Horner 2016]. Up until today, the pathogenesis and course of persistent NGU are not fully elucidated and are likely to be multifactorial [Moi 2015].

Persistent or recurrent NGU seems to be more often the case in infections with *Mycoplasma genitalium* (up to 40 %), however it is also known to possibly happen through infections with CT and TV among others [Bachmann 2015, Horner 2017, Moi 2015].

6.1.4.2. Management of sex partners

Partner notification should be performed and documented at the very first timepoint possible. Partner notification is necessary to prevent reinfection, preventing complications, and preventing onward transmission [Horner 2017].

Partners should be offered testing for not only the specific, but all STIs relevant in the respective country [Unemo 2020, Tiplica 2015]. It is inevitable to interrupt the transmission to more individuals and to prevent reinfection, long-term persisting infections and possible sequelae [Tiplica 2015]. Furthermore, education of individuals is highly recommended as part of every prevention [Tiplica 2015]. However, it must be stated that partner notification is voluntary and, in many cases, not all sexual partners can be identified due to various reasons [Tiplica 2015]. Notification can be done by the index patient or a health professional [Tiplica 2015]. In the latest IUSTI guideline a very informative table is presented concerning the recommended time frame and need concerning epidemiological treatment and is adopted concerning the relevant pathogens and thus presented here.

Table 5- Partner notification for relevant STI pathogens

Disease or causative pathogen	Time frame to trace contacts (beginning with symptoms)	Epidemiological treatment (treatment before lab results arrive)
Chlamydia trachomatis [Lanjouw 2015, Tiplica 2015]	6 months	yes
Neisseria gonorrheae [Unemo 2020, Tiplica 2015]	3 months	yes
NGU [Tiplica 2015]	4 weeks	yes
Trichomonas vaginalis [Tiplica 2015]	2 months	Yes
Mycoplasma genitalium [Jensen 2016]	Only current partner [Gnanadurai 2020], 3 months [Jensen 2016]	yes

6.2. Cervicitis

Cervicitis is the inflammation of the cervix and already in the early 1980s Brunham et al. depicted cervicitis as 'the ignored counterpart in women of urethritis in men' [Brunham 1984].

Unfortunately, the objective diagnosis of cervicitis differs from author to author, from clinician to clinician [Wiesenfeld 2017, Brunham 1984], and already in 1984 Brunham et al. claimed that there are no objective criteria defined [Brunham 1984]. The majority of authors define cervicitis according to at least two of the following criteria (A) visible mucopurulent discharge from the cervix, (B) ≥10, ≥20, or ≥30 PMN leucocytes/HPF (C) easily induced cervical bleeding also called 'friability'(D) oedema of the cervical ectropion (oedematous ectopy) or (E) more PMNs than epithelial cells [Wiesenfeld 2017, Brunham 1984, Marazzo 2007]. Moreover, detailed information concerning the exact infected part, i.e. ecto- or endocervix, that clearly differ in many ways, are missing in a lot of studies [Marazzo 2007].

However, in 2018 Randjelovic et al. suggested that for the diagnosis of cervicitis either (or both) of the following criteria should be present according to the Center for disease control and prevention (CDC) (A) (muco)purulent exudate of the endocervix and (B) easily induced cervical bleeding (induced by e.g. a cotton swab) [Randjelovic 2018, Workowski 2021]. Other authors proposed that a cervicitis shall be defined by >30 PMNLs/HPF in combination with one of the two latest mentioned clinical criteria [Lusk 2008, Randjelovic 2018].

Causative pathogens for cervicitis are in the vast majority infections with CT and NG *[*Marazzo 2007, Brunham 1984*],* but include others like TV, *herpes simplex virus*, or MG *[*Workowski 2021*].* According to a meta-analysis from Lis et al. the latter is associated with a 70% increased risk for the development of a cervicitis [Lis 2015, Marazzo 2007, Brunham 1984]. However, almost up to the half of all patients show no detectable pathogen and an unspecific cervicitis is also common [Randjelovic 2018, Marazzo 2007, Workowksi 2021].

Clinically, cervicitis is most commonly asymptomatic, although sometimes altered vaginal discharge or intermenstrual bleeding or lower abdominal pain might be present [Workowski 2021].

6.1.1. Diagnostic considerations

Microscopy

Via a gram-stain the amount of PMNLs can be defined. However, up to this day authors are discussing the cut-off for the definition of cervicitis (≥10, ≥20, or ≥30 PMN leucocytes/HPF) controversially. The smear should be taken with a 1-5 mm plastic loop from the endocervix in circular movements.

Nuclein amplification tests

Nowadays, NAATs are highly recommended to detect the causative pathogen of cervicitis, due their high specificity and sensitivity [Marazzo 2007]. Females shall be tested for CT, NG and considered being tested for MG and TV in case of suspicion [Workowski 2021]. Especially in those who have recurrent cervicitis and who were tested for NG/CT only, MG testing shall be performed consecutively [Workowski 2021]. Testing for Ureaplasma parvum, Ureaplasma analyticum and Mycoplasma hominis is not recommended due to CDC [Workowski 2021].

6.1.2. Gonococcal cervicitis

Clinically, gonorrhoeic cervicitis might be associated with hyperaemia and contact bleeding of the endocervix [Unemo 2020].

A significant number of infected females remain asymptomatic (about 50%) or show unspecific symptoms only, either at the urethra or cervix [Unemo 2019, Bignell 2006, Unemo 2020]. In case of a delayed diagnosis, an ascension of the infection is possible [Unemo 2019]. In those cases, a significant morbidity might be the result as the infection might spread via the endometrium and tubes to the ovaries. Salpingitis might end in extrauterine pregnancy, even infertility or chronic pain and PID (PID) [Unemo 2019, Bignell 2006].

Diagnostic considerations of *Neisseria gonorrheae* cervicitis

While microscopy of urethral fluor has great sensitivity, cervical smears show a sensitivity of 16–50% only [Marazzo 2007, Bignell 2006, Unemo 2020], and do therefore not represent the test of choice. NAATs on the other hand provide a very high sensitivity (>95%) [Bignell 2006]. Using a NAAT, sensitivity is lower in urine testing than endocervical samples [Bignell 2006].

Table 6- Testing methods for *Neisseria gonorrheae* cervicitis

specimen type	sex	microscopy	culture	NAAT
Endocervical smear	Female	+	+	+
Ectocervical smear	Female	+	+	+
Vaginal smear	Female	-	-	+

6.1.3. Chlamydia trachomatis cervicitis

Diagnostic considerations of *Chlamydia trachomatis* cervicitis

In women first void urine or vaginal self-samples might be used, as well as endocervical smears [Marazzo 2007]. Vaginal self-collected specimens show a smaller bacterial load than endocervical swabs, but a higher load than first void urine and are therefore recommended as first choice [Bebear 2009, Lanjouw 2015]. NAATs are the method of choice for diagnosis [Lanjouw 2015]. It is believed that despite an incubation period of 1-3 weeks, a positive NAAT might be observed within three days of contact with CT [Lanjouw 2015].

Table 7- testing methods for CT cervicitis

specimen type	sex	microscopy	culture	NAAT
Endocervical smear	Female	-	+/-	+ (endocervical is preferred)
Ectocervical smear	Female	-	+/-	+
Vaginal smear	Female	-	-	+ (self-collected appropriate)

6.1.4. Mycoplasma genitalium cervicitis

Diagnostic considerations of Mycoplasma genitalium cervicitis

Clinically, an MG cervicitis cannot be diagnosed. Via microscopy, MG cannot be detected as they have no cell wall and can therefore not be verified in gram stainig. A culture would take up to months to grow and is therefore no suitable method of testing. Up to this day, the only meaningful diagnostic method are NAATs [Jensen 2016].

As up to now, no association from MG with vulvovaginitis is to be proven, positive MG results from the vaginal region are therefore believed to represent contaminations from a cervical infection [Wiesenfeld 2017].

Table 8- testing methods for MG cervicitis

specimen type	sex	microscopy	culture	NAAT
Endocervical smear	Female	-	-	+
Ectocervical smear	Female	-	-	+
Vaginal smear	Female	-	-	+ (self-collected appropriate)

6.1.5. Trichomonas vaginalis cervicitis

Diagnostic considerations of Trichomonas vaginalis cervicitis

Clinically, a red cervix with punctate yellow lesions may be called as 'colpitis macularis' or 'strawberry cervix', that may hint to TV infected females; in colposcopy this observation can be found in close to 50%. [Marazzo 20017; Kissinger 2015].

Microscopy from wet mount is not recommended due to low sensitivity (ranging from 50 to 70%, in women, lower in men), being highly dependent not only on the time when they are looked at (should be performed within 10 minutes) but also on the expertise of the examiner [Kissinger 2015]. As well as in urethritis, cultures are not recommended, thus in European countries the diagnosis is performed via NAATs, providing excellent sensitivity and specificity (both 95-100%) [Kissinger 2015, Sherrad 2018]. However, in some countries where the prevalence is very high, POCTs on vaginal secretions are used with an acceptable sensitivity of about 83% and a specificity of at least 97% [Kissinger 2015]. However, it must be emphasized that in the scientific community only vaginal specimens are recommended to be tested for TV; endocervical samples seam suitable with a lower sensitivity (88%) and nearly equal specificity (99%) [Kissinger 2015].

Table 9- testing methods for TV cervicitis

specimen type	sex	microscopy	Culture	NAAT
Endocervical smear	Female	+/-	-	-/+
Ectocervical smear	Female	+/-	-	-/+
Vaginal smear (recommended site of choice)	Female	+/-	-/+	+

6.1.6. Treatment of cervicitis and management of sex partners

For the ease of reading, treatment recommendations are presented in the very same table, including the ÖGSTD guideline recommendation, as well as the latest European IUSTI recommendations [ÖGSTD, Sherrad 2018, Jensen 2016, Lanjouw 2015, Unemo 2020].

Table 10- treatment and partner management of those suffering from cervicitis due to a STI

Pathogen	ÖGSTD Guideline	IUSTI Guideline	Management of sex partners	Test of cure	Comment
Neisseria gonorrheae	Ceftriaxone 1g i.v. OR Cefixime 800 mg p.o. as single shot	Ceftriaxone 1g i.m. plus azithromycin 2 g p.o. as single shot OR Ceftriaxone 1g i.m.	All sexual partners of the last 60 days have to be tested	Necessary, 3 weeks after treatment	i.m. injection of ceftriaxone in Austria is off label, therefore i.v. administration is possible
Chlamydia trachomatis	Doxycycline 100 mg 1-0-1 for 7 days OR Azithromycin 1g p.o. as single shot	Doxycycline 100 mg 1-0-1 for 7 days OR Azithromycin 1g p.o. as single shot	Sexual partners of the last 6 months shall be informed and invited for testing	Recommended, 3-6 months after treatment	
Mycoplasma genitalium	Azithromyzin 500 mg on day 1, 250 mg on days 2-5 OR Josamycin 500 mg TID for 10 days	Azithromyzin 500 mg on day 1, 250 mg on days 2-5 OR Josamycin 500 mg TID for 10 days	Sexual partners of the last 3 months shall be informed and invited for testing	Necessary, 3 weeks after treatment	The recommendations are given in the absence of macrolide resistance
Trichomonas vaginalis	Metronidazol 400-500 mg p.o. BID for 5-7 days OR Tinidazole 2 g p.o. as single shot	Metronidazol 400-500 mg p.o. BID for 5-7 days OR Tinidazole 2 g p.o. as single shot	Current sexual partners should be treated, regardless of results	TOC/ Follow up is not necessary in asymptomatic women after treatment	

6.3. Genital ulcers

Aside from urethritis or cervicitis, the onset of genital ulcer(s) is the second commonest reason for presentation for patients at STI outpatient clinics.

The etiology of genital ulcer disease (GUD) is very broad, including various infectious and non-infectious diseases, and in many cases no causative pathogen is to be found [Maliyar 2019, Workowski 2021]. Due to the demanding field of exploring GUD, a correct diagnosis and therefore treatment might be significantly delayed [Laetsch Semadeni 2009, Maliyar 2019, DiCarlo 1997]. In many cases, the treatment is based on the clinical picture alone at the very first presentation of the patient [DiCarlo 1997, Rosen 1998].

An ulcer is defined as focal breakage of the full epidermis and deeper to dermis or subcutaneous fat [Maliyar 2019].

Since most genital ulcers are caused by an STI, it is of outmost importance that primarily, STIs such as syphilis I and genital herpes in most cases, but also chancroid or lymphogranuloma venereum, are excluded [Maliyar 2019, Rosen 1998, Workowski 2021]. Taking a detailed and tactful history is very important, including not only a patients sexual but also the vacation history. The STIs that cause a genital ulcer can be diagnosed either via their typical clinical picture (all), serology (syphilis, in some extent herpes and LGV), or NAATs (lues, chancroid, *herpes simplex viruses* and LGV- including subtyping of CT).

Other infectious, non-sexually transmitted causes include EBV or CMV-associated ulcers and leishmaniasis [Laetsch Semadeni 2009]. Non-infectious causes include diseases like Behçet‘s disease, hidradenitis suppurativa, pyoderma gangrenosum or malignancies [Latesch Semadeni 2009, Huppert 2010, Vieira-Baptista 2016].

Less frequent causes of genital ulcers may include trauma, fixed-drug eruption, Steven-Johnson syndrome, fungal infections, insect bites, pemhigus or herpes zoster, as well as tropical skin diseases, like amoebiasis, among many others [Mroczkowski 1994]

Many of these above-mentioned diseases show typical and unique clinical features or can be verified or excluded by, serology or molecular examinations, like NAATs (for syphilis, herpes simpex viruses or haemophilus ducreyi), helping the clinician in identifying a correct diagnosis and therefore correct treatment [Maliyar 2019, Rosen 1998, Mroczkowski 1994, Workowski 2021]. Moreover, it should be kept in mind that in up to 10 % of those suffering from a STI-related ulcer, more than one STI is to be found [Rosen 1998].

In 1997, DiCarlo and colleagues reported a study of 446 patients who had a genital ulcer. They tried to evaluate sensitivity and specificity of physical exams for the diagnosis of syphilis, chancroid and genital herpes. The authors found that only a minimal fraction of these genital ulcers can be diagnosed with a typical clinical picture/ physical examination alone [DiCarlo 1997]. This finding clearly supports the urgent need of additional tests, including serology, histology or molecular examinations [Workoksi 2021].

6.3.1. Genital ulcers caused by a sexually transmitted infection

6.3.1.1. Syphilis – Overview, diagnosis, and treatment

Syphilis, also called Lues, represents a systemic, curable STI [Janier 2020, Workowski 2021]. It is caused by the spirochaete *Treponema pallidum subsp. pallidum* (in short *Treponema pallidum*, or TP) [Janier 2020, Maliyar 2019]. This bacterium gains access to the dermis and subcutaneous fat or mucosa via small cutaneous/mucosal injuries [Maliyar 2019].

Syphilis runs along in various stages. Early syphilis (within 1 year of infection) encompasses primary and the highly infectious secondary syphilis, as well as early latent syphilis [Janier 2020]. Late syphilis is divided into late latent and tertiary syphilis [Janier 2020]. The transmission occurs via unprotected sex, may be congenital and even a transmission via kissing has been described [Janier 2020, Yu 2016].

The typical sign of primary syphilis is the chancre, a superficial, solitary, painless ulcer with indurated edges and regional lymphadenopathy [Janier 2020, Maliyar 2019, DiCarlo 1997, Rosen 1998, Workowski 2021]. However, the chancre may present itself in a multitude of clinical variations, and thus a correct diagnosis might be hampered [Janier 2020, Maliyar 2019]. Therefore, it is of outmost importance that any anogenital ulcer should be suspicious of syphilis until this diagnosis is excluded [Janier 2020]. In inconclusive clinical settings serology shall be performed after weeks 1, 2, and 6 [Janier 2020]. Even without treatment, this ulcer will heal within weeks [Maliyar 2019]. Due to a bacteraemia, the highly infectious stage of secondary syphilis presents itself as a ‘chameleon’ concerning clinical presentation, including disseminated exanthema, generalized lymphadenopathy, luetic alopecia or ‘angina specifica’, among many others [Janier 2020, Maliyar 2019, Workowski 2021]. Latent stages are defined as stages without clinical signs, but serological positivity [Janier 2020]. Tertiary syphilis is syphilis involving various organs [Janier 2020].

Diagnosis is based on clinical presentation (as it is in many cases typical), serology (which is of outmost importance), NAAT or dark field exam [Janier 2020]. Incubation time ranges from 10 to 90 days between the infectious event and the occurrence of the chancre [Janier 2020]. However, it must be stated that serology might take up to some weeks before it becomes reactive after the onset of the very first symptoms [Rosen 1998].

In all stages of syphilis, up until today, penicillin G is regarded as the only treatment of choice [Moliyar 2019, Workowski 2021]. Interestingly, it seems that there is no development of resistance taking place in this disease [Rosen 1998]. Treatment consists of an intramuscular injection of benzathine penicillin 2.4 million units once (for early syphilis) or every week for 3 successive weeks (for late syphilis) [Janier 2020, Workowski 2021].

6.3.1.2. Genital herpes – Overview, diagnosis, and treatment

Genital herpes is caused by the DNA viruses *herpes simplex virus 1* ('oro-labial variant', HSV1) and *herpes simplex virus 2* ('genital variant', HSV2), which both present clinically identical [Patel 2017, Maliyar 2019].

The prevalence of the disease is extremely high, as *herpes simplex virus 1* is believed to be present in close to 70% of all adults younger than 50 years [Maliyar 2019]. *HSV2* seems to have a little bit lower infection rates, ranging from 10 to 60% in the overall population [Maliyar 2019]. Genital herpes is believed to be the most common cause for a genital ulcer (at least in the United States) [DiCarlo 1997, Rose 1998].

Incubation ranges from two days to three weeks after exposure to the virus [Rosen 1998]. The very first, also called primary infection, occurs at the site of viral entry and presents itself as flat-bottomed, papular microvesicels that rapidly erode and seldomly perform real ulcers on erythematous ground [Patel 2017, Maliyar 2019, Rosen 1998]. In some cases, even systemic symptoms are possible [Patel 2017]. However, in general, clinical pictures may vary broadly [DiCarlo 2019, Rosen 1998]. After one- or two-weeks re-epithelization starts, and the lesions heal [Maliyar 2019, Rosen 1998]. After the primary infection, the virus will remain in the individual for a lifetime, with courses of reactivation and viral shedding, primarily in times of stress or sickness, menstruation or local trauma [Maliyar 2019, Rosen 1998]. Those episodes are normally shorter in duration, less painful and less severe in their clinical presentation [Maliyar 2019]. Viral shedding may happen in times of absolute clinical unaffectedness of the skin, possibly infecting another person after direct contact [Maliyar

2019]. Transmission primarily happens in times of clinical apparent herpes lesions and during prodromes, like itchiness or painfulness skin [Patel 2017]. In that particular time frame, sexual intercourse or close skin- to- skin contact shall be avoided [Patel 2017].

Diagnosis is done clinically, seldomly (and no longer recommended) via the so called Tzanck test, but culture and primarily via DNA detection by PCR [Patel 2017, Maliyar 2019, Workowski 2021], whereby NAATs are currently the most sensitive tests (up to 100%) [Workowski 2021].

Management includes systemic antivirals for about one week, especially in symptomatic primary infections [Patel 2017, Maliyar 2019]. Systemic treatment can milder the symptoms and shorten the duration of the disease, whereas a healing is not possible [Workowski 2021].

6.3.1.3. Chancroid – Overview, diagnosis, and treatment

Ulcus molle, or chancroid is a STI caused by the gram-negative bacterium *Haemophilus ducreyi* (HD) [Maliyar 2019, Lautenschlager 2017]. Typically, a chancroid presents itself as a painful, soft, anogenital ulcer accompanied of lymphadenitis [Maliyar 2019, Lewis 2006, Lautenschlager 2017, Workowski 2021]. In a fourth of cases a formation of buboes might be possible, ending in rupture of the tissue, leaving behind deep inguinal wounds [Rosen 1998]. The occurrence of not only one, but several ulcers is not uncommon [Rosen 1998]. Incubation time is quite short, ranging from 3 to 7 days [Maliyar 2019, Lautenschlager 2017, Rosen 1998]. It is considered as a 'tropical' STI, occurring mainly in Africa, Asia or Latin America [Maliyar 2019, Rosen 1998, Workowski 2021]. Similar to TP, *Haemophilus ducreyi* gets human access through disrupted skin or mucosa [Maliyar 2019].

Clinically, the initial papule(s) become pustular and then form a painful, well demarcated ulcer, possibly progressing to buboes if untreated [Maliyar 2019, Lautenschlager 2017].

Diagnosis is done clinically, via culture and NAATs [Lautenschlager 2017, Workowski 2021]. Moreover, histological analysis via Giemsa staining may be performed [Rosen 1998].

The European IUSTI guideline and latest CDC guideline recommend either Ceftriaxone as a single intramuscular injection of 250 mg or Azithromycin, as a single 1 g oral dose [Lautenschlager 2017, Workowski 2021].

6.3.1.4. Lymphogranuloma venereum (LGV) – Overview, diagnosis, and treatment

The endemic STI called Lymphogranuloma venereum (LGV) is caused by the invasive serotypes L1, L2, and L3 of *Chlamydia trachomatis* [Maliyar 2019, de Vries 2019, Workowski 2021]. They are entering via breaks in skin and mucosa [Maliyar 2019]. It is a relatively common cause of proctitis, especially in the MSM group, whereas heterosexual transmission is rare [de Vries 2019]. In some regions it is believed to be responsible for up to 2-10% of GUD [de Vries 2019]. Incubation time is very short, about some days to 4 weeks [Maliyar 2019, de Vries 2019, Rosen 1998].

This STI represents a disease of three stages, however, asymptomatic courses in up to a 4th of MSM are possible [de Vries 2019]. At the primary stage ('ulcerative stage') it presents initially as a self-limiting small papule, resulting in a painless ulcer [Maliyar 2019, de Vries 2019,Workowski 2021]. Afterwards, a bacterial spread to local inguinal or femoral lymph nodes ('inguinal stage') is taking place, that – in the last stage- may transform to buboes and rupture in persisting, untreated disease cases [Maliyar 2019, de Vries 2019, Workowski 2021]. Proctitis is the main syndrome in infected patients. Individuals suffering from anorectal LGV often complain about rectal discharge, pain or constipation [Maliyar 2019, de Vries 2019]. Sequelae due to untreated disease may result in fistulas, strictures and elephantiasis [Maliyar 2019, de Vries 2019]. Genital or oral- pharyngeal infections are known albeit rare [de Vries 2019].

Diagnosis is done clinically and confirmed via PCR and subsequent genotyping [[de Vries 2019, Workowski 2021]. A chlamydia serology shall not be used because its interpretation and validation are not standardized [Workwoski 2021].

According to the latest European IUSTI Guideline, ÖGSTD guideline and the CDC, treatment consists of doxycycline 100 mg orally twice daily for 21 days [de Vries 2019, Workowski 2021].

6.3.2. Non-infectious genital ulcers

6.3.2.1. Behçet‘s Disease – Overview, diagnosis, and treatment

Behçet’s disease is a chronic, autoinflammatory, systemic disease, characterized by the onset of mainly oral and genital aphthous and ulcerous lesions, as well as ocular involvement [Bulur 2017, Malinar 2019]. More seldom it shows an involvement of the cutis, the gastrointestinal tract and the joints among others [Bulur 2017, Malinar 2019]. Typically, adults from 20 to 40 years of age are affected [Bulur 2017, Malinar 2019]. Prevalence varies significantly, the highest to be found worldwide in Turkey [Bulur 2017|. Etiopathogenesis is not fully elucidated [Rosen 1998].

Clinically, Behçet’s disease presents itself in the shape of recurring and very painful oro-genital ulcers in most affected individuals [Bulur 2017, Malinar 2019, Rosen 1998]. Diagnosis is made using the international criteria point score system [Davatchi 2017]. A score of greater or equal 4 points hints to Behçet’s disease. Two points can be given for oral and genital aphtosis respectively, as well as for ocular lesions. One point can be reached if a skin lesion, neurologic or vascular manifestations, or a positive pathergy test are present [Davatchi 2014]. The pathergy test is positive when after a small trauma, a papule or pustule at erythematous ground arises [Bulur 2017].

Treatment consists of local and systemic corticosteroids, topical calcineurin inhibitors, antimicrobial and nonsteroidal anti-inflammatory drugs (NSAIDs) or various anti-inflammatory medications, as well as immunosuppressants [Bulur 2017, Maliyar 2019].

6.3.2.2. Hidradenitis suppurativa – Overview, diagnosis, and treatment

Hidradenitis suppurativa, also called acne inversa, is a chronic, inflammatory disease with an estimated prevalence of about 1 % [Zouboulis 2019, Maliyar 2019]. Risk factors and comorbidities known for hidradenitis suppurativa include smoking, metabolic- and cardiovascular disorders, adipositas, diabetes, hypertension, and hypertriglyceridemia, among others [Zouboulis 2019].

Clinically, it presents itself with painful tender subcutaneous papules, nodules and ruptured deep dermal abscesses in the axillar or genital region, as well as in the buttocks or upper thighs [Zouboulis 2019, Maliyar 2019]. Chronic disease results in sinus tracts, or fistula-like appearance, fibrosis, induration of the cutis and massive scarring [Zouboulis 2019, Maliyar

2019]. The severity is described by the so called Hurley stage (I-III, mild to severe, respectively).

Treatment consists of weight reduction due to body mass index, systemic antibiotics (e.g. a triple regime, including rifampicin, moxifloxacin and metronidazole for up to 6-12 weeks must be considered. Another approach is the systemic administration of clindamycin and rifampicin for 10 weeks. In moderate to severe hidradenitis suppurativa, biologic therapy with the TNF inhibitor adalimumab must be considered. Second- and third-line options would be infliximab and anakinra [Zouboulis 2019]. Surgical options must be discussed not only in cases of limited disease, but in a chronic stage to prevent recurrences [Zouboulis 2019].

6.3.2.3. Pyoderma gangraenosum – Overview, diagnosis, and treatment

Pyoderma gangraenosum is a rare neutrophilic, chronic relapsing disease [Quist 2016; Maliyar 2019]. It is characterized by chronic inflammatory ulcers that progress despite classic treatment and respond to anti-inflammatory immunosuppressive therapeutic options [Quist 2016]. Clinically it starts most frequently with a tender papule that rapidly progresses to an ulcer. Diagnosis is done in concordance of the clinical picture and histology.

Due to a pathergy phenomenon, the surgical treatment must be postponed [Quost 2016, Maliyat 2019]. Treatment options include topical, intralesional or systemic corticosteroids, topical calcineurin inhibitors or immunosuppressive medication, like azathioprine or biologics [Quist 2016].

6.3.2.4. Malignancies – Overview, diagnosis, and treatment

Genito-anal malignancies include mainly the intraepithelial neoplasia forms bowenoid papulosis, Bowen's disease, or erythroplasia Queyrat, and the progressed forms of squamous cell carcinoma, paget's disease, melanoma, and basal cell carcinoma. Most malignant tumors of the genito-anal region belong to the types of squamous cell carcinoma [Rosen 1998].

The clinical presentation is broad, including erythematous, livid patches or plaques or up to bleeding persisting ulcers, or dark pigmented patches, papules or nodules.

Diagnosis is done by histological evaluation after clinical suspicion. Treatment consists of surgical removal and depending on the underlying tumor further local or systemic medication (in case of progressed disease).

6.3.2.5. Ulcus vulvae acutum Lipschütz – Overview, diagnosis, and treatment

One ulcer that is highly linked to infectious- but not sexually transmitted- pathogens is UVAL. This term has been coined by the Austrian dermatologist Lipschütz in 1913 [Lipschütz 1913]. The lesions of UVALare the most common cause for painful ulcer(s) at the female genitals, however an exact incidence is unknown [Maliyar 2019]. Usually they arise in young females, most of them being sexually inactive [Maliyar 2019, Vieira-Baptista 2016, Sadoghi 2020].

The etiopathogenesis of UVAL is not fully elucidated yet [Sadoghi 2020, Maliyar 2019]. However, a bunch of bacteria and viruses has been linked to the occurrence of UVAL, including *salmonella parathyphii, Epstein–Barr virus* (EBV), c*ytomegalovirus* (CMV), or *adenovirus*, among others [Sadoghi 2020, Maliyar 2019]. Similarly to its etiopathogenesis, no clear diagnostic or therapeutic algorithm is existing yet.

Diagnosis is done by exclusion of other causes usually resulting in GUD [Sadoghi 2020, Maliyar 2019]. Histology is of no major help in that specific disease [Sadoghi 2020]. However, until 2019, an accurate diagnostic and therapeutic algorithm was missing. Therefore, as one important aim of this thesis, we performed a systematic literature review on UVAL, to be able to formulate algorithms that might facilitate correct diagnosis and treatment in the respective disease in the future [Sadoghi 2020].

Ulcer(s) can heal without treatment; however, at least supportive aid, like a correctly performed wound hygiene and care should be recommended. Pain medication and sometimes local or systemic corticosteroids, as well as systemic antibiotics might be useful [Sadoghi 2020, Maliyar 2019].

PART A PROSPECTIVE STUDY on urethritis and cervicitis

A 7. Rationale and aims

The first aim of this doctoral thesis was to conduct a clinical study to describe the prevalence, diagnosis, and treatment of urethritis and cervicitis from our own department and outpatient clinic of STIs at the Department of Dermatology and Venereology of the Medical University of Graz. Since no published data on the Austrian prevalence of certain STIs, as well as their accurate diagnosis and treatment, are available yet, this research topic gains particular importance.

A 8. Patients and methods

Between September 2019 and September 2021, we conducted a prospective, single-center study to estimate the prevalence and to evaluate diagnostics and treatment possibilities of STIs causing urethritis and cervicitis. According to our IRB approval from the ethical committee of the Medical University of Graz (31-539 ex 18/19), we included 178 patients (110 symptomatic, 68 asymptomatic) into the study protocol. All patients were invited to participate in this study regardless of the presence of symptoms or not. Unfortunately, due to the outbreak of the COVID-19 pandemic during the study recruitment period, a significantly smaller number of patients than expected finally counselled our STI outpatient clinic. No financial incentives whatsoever were given to the study personnel nor participants.

A 8.1. Patients

Inclusion criteria were

- Patients gave their oral and written consent (ICF version 1 from 20.8.2019).
- patients with an age of 18 years and above
- patients that presented themselves at our STI outpatient clinic for a
    - pharyngeal,
    - urethral,
    - cervical,
    - vaginal or
    - anal swab

Exclusion criteria were:

- patients under the age of 18
- patients that got no swab at the above-mentioned locations

- patients that present themselves at the outpatient clinic for other diseases than STIs that might be detected via NAATs
- patients that present only for a TOC
- patients who did not speak/read German fluently
- Moreover, patients who presented at the outpatient clinic due to sexual assault, were not asked to participate in this study for ethical reasons.

Patients were asked the mandatory questions concerning STI history, including all those below mentioned.
Demographic variables included sex and age.
STI related symptoms were specifically asked: Do you have any complaints (yes/no): (1) discharge, (2) burning sensation, (3) itchiness, (4) an erosive/ulcerous lesion?
Patients could also report other symptoms despite the four above mentioned, but in a not standardized fashion.
Since when do you have these complaints (duration in days).
When did the last unprotected sexual intercourse (in days) take place?
Was this sexual intercourse with a random person or in an existing partnership?
A partnership was defined as a stable partner, whereby subjects were free to define their relationship as they wanted. A stable partner was e.g. a husband, wife, boyfriend or girlfriend.
Which sexual orientation fits best for you: MSM, FSF, bisexual, heterosexual
Number of sexual partners within the last 6 months?
Number of sexual partners in total?
Do you use sex toys that are inserted?
Did you get vaccinated against meningococci?
Did you get a vaccination against HPV?
Did you previously experience an STI?
Moreover, females were asked if they were pregnant.

A 8.2. Clinical examination

All patients were clinically examined in terms of classical signs of STIs, including the enoral, as well as the palmoplantar region, the genitals (with a special focus on the meatus urethrae), all lymph node stations and the trunk. All regions examined were assessed for clinical apparent pathologies. If there were pathologic findings, this was documented in electronic patient files and in some cases also photo-documented after oral and written consent of the patient.

A 8.3. Sample collection and diagnostic tests

Biological samples were obtained using pharyngeal, anal, vaginal, cervical and urethral swabs. Cervical, urethral and anal samples were collected by medical doctors working in the STI outpatient clinic, whereby no samples were pooled.

A 8.3.1. Microscopy

Smears were taken with Greiner 1 µl plastic disposable eyeletes (Greiner® Kremsmünster, Austria) from the patients urethra. If no discharge was present or not enough material could be collected, this was defined as not representing an urethritis (concerning missing clinical and microscopical criteria). If material could be obtained, a microscopic smear on a slide (Epredia®, Braunschweig, Germany) was performed followed by Gram staining (including crystal violet solution, Lugol's solution, Gram's decolorizing solution, Gram's safranine solution [reagents all from Merck®, Darmstadt, Germany]) and evaluation of the amount of PMNLs on a Nikon, Eclipse E200.

We defined Urethritis with a cut off 5 PMNLs/ HPF. This was performed by medical staff and in terms of uncertainty a medical doctor was asked for evaluation.

In female patients, the cervix was furthermore evaluated clinically after a speculum was positioned (whether mucopurulent discharge or friability were present or not). After cleansing with a cotton swab, an endocervical smear was taken with a Greiner 1 µl plastic disposable eyelete (Greiner® Kremsmünster, Austria). If no material could be obtained, this finding was noted as 'no cervicitis'. We defined cervicitis as >30 PMNLs/HPF. Microscopic analysis was performed by medical staff and in terms of uncertainty, a medical doctor was asked for a second evaluation.

A 8.3.2. Molecular biology

After the disposable eyelet, (for urethra and/or cervix) DNA was isolated from 1ml of medium using a PCR swab ('eNAT'- collection and preservation for nucleic acids, Copan® Brescia, Italy). For the urethra, pharynx and cervix, the eNAT® 608CS01M (medium size) and for vaginal and anal sites, the eNAT®608CS01R (regular size) was used.

Due to a global high rise of STIs over the last years, an adapted and especially expanded diagnostics in the field of venereology was necessary, and a multiplex PCR for rapid and sensitive testing became necessary in late 2019. We therefore adapted our analytics to the usage of NAATs, which are highly recommended in all guidelines. The Euroimmun® (Lübeck, Germany) EUROArray test system is based on PCR amplification of defined gene sections. Via a hybridization reaction with immobilized DNA probes, detection is possible. The

molecular array provides detection of the following eleven sexually transmitted pathogens from urine and smear samples: *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG), *Herpes simplex virus* (HSV)-1 and -2, *Haemophilus ducreyi* (HD), *Mycoplasma genitalium* (MG), *Mycoplasma hominis* (MH), *Treponema pallidum* (TP), *Trichomonas vaginalis* (TV), *Ureaplasma parvum* (UP) or *Ureaplasma urealyticum* (UU). This is the so-called 'STI 11 panel'. Moreover, a smaller test array including the two most frequent sexually transmitted bacteria (NG and CT) is to be used in the case of no need for testing for the above-mentioned pathogens, e.g., on mainly extragenital samples or in case of a TOC. In both tests, an additional PCR-system that detects human DNA, is included to assure a high quality of the samples.

In case of detection of NG in the pharyngeal swab, a second in-house, real-time PCR (GeneProof®, Brno, Czech Republic) was performed according to the recommendation of guidelines, due to the high number of false positive results due to apathogenic *Neisseria species* [AWMF guideline gonorrhea 2018]. DNA in each sample was extracted and PCR was performed according to the recommendations of the manufacturer. The detection was based on the amplification of a multi-copy sequence of the gene encoding for *16S rRNA* and *porA*pseudogene and in measurement of fluorescence increase. *porA*pseudogene and its mutants can be detected and they are both highly specific for *Neisseria*.

A 8.3.4. Microbiology

In case of a suspected NG infection, culture for propagation of *Neisseria gonorrhoeae* was carried out. The isolates were cultured on a selective gonococcal agar (chocolate agar + PolyViteX VCAT3 [Biomerieux®, Marcy-l'Ètoile, France]) and a non-selective chocolate agar (GC II with IsoVitaleX [Becton Dickinson®, New Jersey, USA]). NG was confirmed by either (A) the presence of gram-negative diplococci in microscopy, (B) the typical clinical picture of greyish, opalesque colonies of kidney shaped 'coffee beans', or (C) a positive oxidase reaction. If a growth on the agar was observed, AMR was performed. Molecular resistance testing for NG was done via the above-mentioned selective agar, and only in the case of a lack of growth on that agar, AMR was performed on a non-selective agar. The minimum inhibitory concentrations (MIC; mg/L) of cefixime, ceftriaxone, penicillin G, azithromycin, doxycycline, ciprofloxacin (and in some patients spectinomyzine) were analyzed using BD BBL™ Sensi-Disc™ antimicrobial susceptibility test discs (Becton Dickinson®, New Jersey, USA).

Results (susceptibility and resistance) were interpreted in concordance with the latest European Committee in antimicrobial susceptibility Testing (EUCAST recommendations); (last check: https://www.eucast.org/clinical_breakpoints/ - 24.11.2021)

A 8.3.5. Serology

Blood samples were drawn by medical staff only and serological analysis was performed including a testing for HBV, HCV, HIV, and syphilis at the central laboratory (clinical institute for medical and chemical laboratory diagnostics [KIMCL]) of the Medical University of Graz. The serological tests were all chemiluminescent microparticle immunoassays (Abbott®, Chicago Illinois, USA) performed at the Alinity analyzer (Abott®, Chicago Illinois, USA).

A 8.4. Patient Parameters

Data on the demographics (sex, age), sexual orientation, last unprotected sexual intercourse, number of sexual partners (within the last 6 months, as well as during lifetime), usage of sex toys, previous STIs, pregnancy status, clinical presentation and smear results, serological results and vaccination status (against meningococci and HPV), and the resistance profile of patients suffering from gonococcal-caused urethritis and cervicitis, were extracted.

A 8.5. Data protection

For the reason of identification, a barcode including name and birth date of the respective patient was used to allocate samples. The source data, namely demographic and clinical data were documented in the electronic patient file MEDOCS program of the Medical University of Graz. The testing laboratory received the samples and the obtained results were provided electronically. An excel table was secured at a locked folder, only four people had access to the folder and only two of them had access to the excel file itself, which was protected electronically via a password. A statistician received the anonymized dataset for all analyses. All patients that were tested positive for any STI, received treatment according to the current valid national treatment guidelines.

A 8.6. Statistical analysis

All statistical analyses were performed with MS Excel (Windows version 16.56) and Stata (Windows version 17.0, Stata Corp., Houston, TX, USA). For descriptive statistics, means, medians, minimum and maximum Excel was used. The level of significance for all valuations was set at 5%. Continuously coded variables were reported as medians [25th-75th] percentiles, and count data as absolute frequencies (%). The distribution of variables between patients with and without STI were evaluated with ranksum-tests, $\chi 2$-tests, and Fisher's exact tests, as appropriate. Uni- and multivariable logistic regression models were fitted for modeling the prognostic association between baseline variables and subsequently confirmed STIs. The data set had few missing values, due to no information given by patients. The missing values are depicted in the tables below.

A 9. Results

A 9.1. Demographics (sex, age)

In total, we screened 178 individuals (148 males, 30 females) between 7/10/2019 and 24/9/2021. There was a long period of one year (12/2019-12/2020) with no inclusion of subjects due to the COVID-19 pandemic.

The majority of patients was aged between 20-39 years (n=118), two subjects were younger than 20 years, and 49 patients were aged between 40 and 59 years; nine patients were older than 60 years. Mean age was 35.7 years (SD ± 12.9), median age was 32 years. Minimum age was 18 maximum was 79 years. The subgroup of adolescents (15-24 years) revealed 37 individuals.

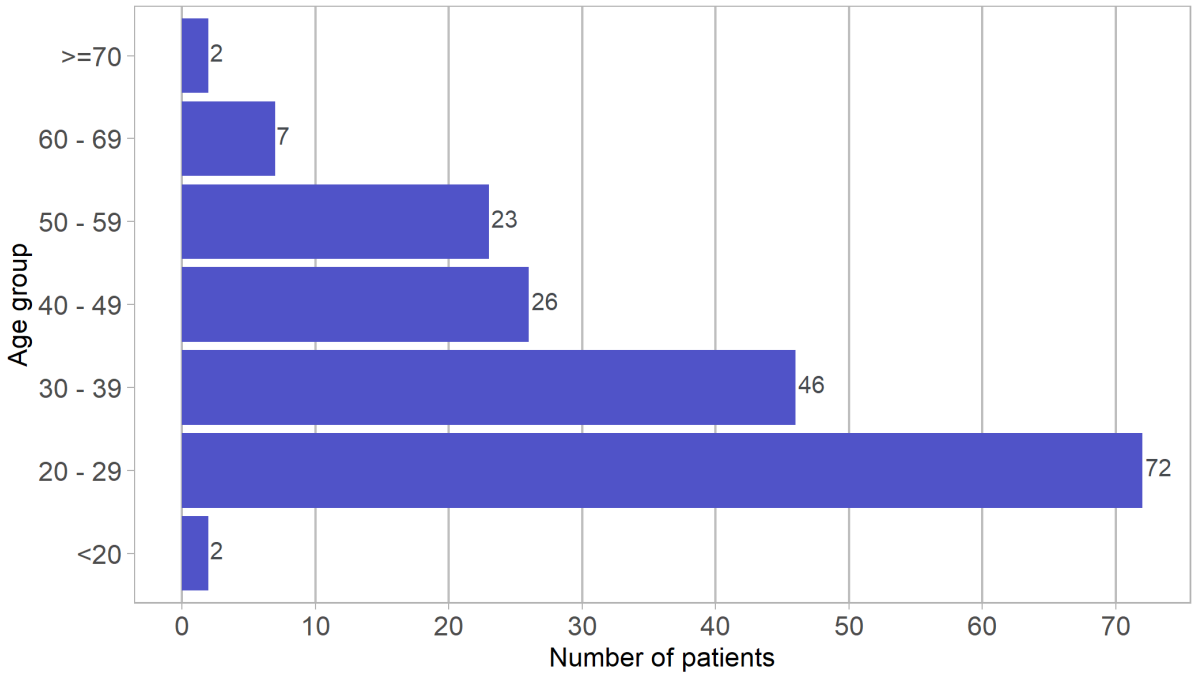

Figure 1- Number of included patients (x-axis), age of patients in years (y-axis).

A. 9.2. Sexual partners

There was a wide range concerning sexual partners of the study participants.
Included individuals reported numbers of sexual partners during lifetime ranging from one to 600, with a median of 12 (mean: 34.6; SD ± 70.6).

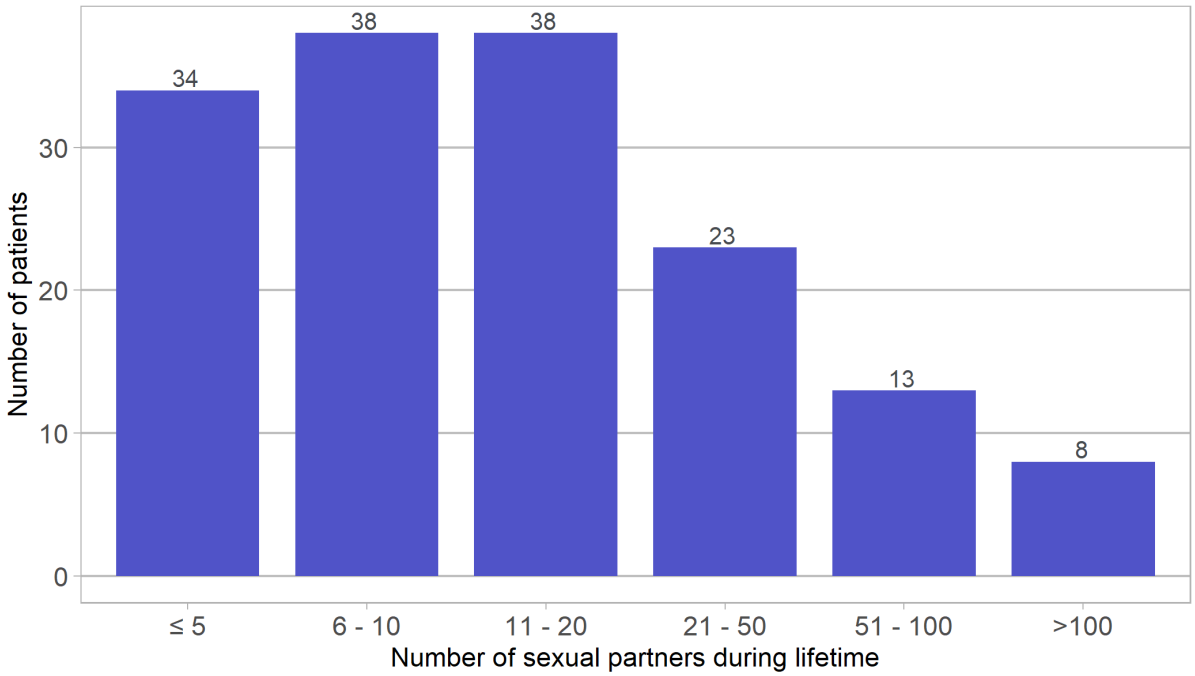

Figure 2- Number of sexual partners during lifetime (x-axis), number of patients (y-axis)

Within the last 6 months numbers of sexual partners differed between 0 and 60, with a median of two partners (mean: 3.2, SD ± 5.5). Most patients (n=73/176) reported about only one sexual contact within the last 6 months, followed by two (n=32), three (n=24), four (n=7), five (n=12), six to ten (n=12), 11 to 19 (n=5) or more than 20 (n=2) or 60 (n=1) contacts, respectively. Eight individuals reported to have had no sexual contact within the last 6 months.

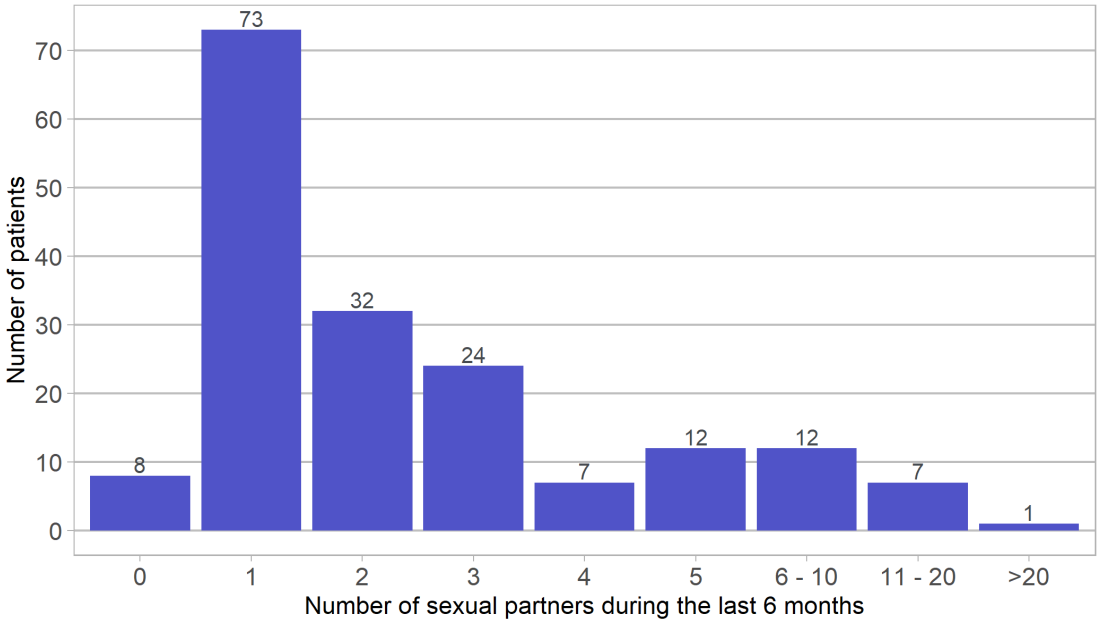

Figure 3- Number of sexual partners during the last 6 months (x-axis); number of patients (y-axis).

A 9.3. Relationship

Of all patients, 88 (77 heterosexual, 10 MSM, 1 bisexual) reported to have had the last unprotected sexual contact with their partner they were in a relationship with. Ninety patients (52 heterosexual, 21 MSM, 17 bisexual) reported having a history of a recent random sexual contact.

A 9.4. Sexual orientation

The majority of participants were heterosexual (129 patients [102 males/27 females]), followed by MSM (31 patients), and 18 patients with bisexual orientation (15 males/ 3 females).

A 9.5. Vaccination status (meningococci, HPV)

Seventeen patients reported to be vaccinated against meningococci, from 28 , an information was not applicable, 133 stated that they did not receive a vaccination against meningococci. Of those 17, who were vaccinated, two suffered from a NG infection (oral and pharyngeal).

Only nine (five males, four females) patients were vaccinated against HPV, in 18 patients this information was not applicable, and 151 patients defined their HPV vaccination status as being negative. Of those who were vaccinated, three were bisexual, two were MSM and four were heterosexual. Their age ranged from 21 to 29 years with a mean of 25.2 years.

A 9.6. Previous STI status

73 (62 males, 11 females) patients had a previous STI, whereby 40 of those were heterosexual, 21 were MSM, and 12 were bisexual.
Of 70 patients in whom a current STI was detected, 34 reported a previous STI (15 heterosexual, 14 MSM, five bisexual).

A 9.7. Symptoms

As mentioned above, patients were asked specifically for relevant symptoms, namely discharge, dysuria, pruritus and epithelial defects.

In total, 111 patients were symptomatic and 67 patients asymptomatic. Symptoms were reported by 93 males and 18 females. The majority that claimed about symptoms were heterosexuals (79 individuals), followed by MSM and bisexuals (21 and 11 patients, respectively). Fiftytwo of the symptomatic patients were in a relationship, whereas 59 had sexual contact with a random acquaintance.

The most commonly reported symptom was dysuria (61 patients), followed by discharge (50 patients), pruritus (17 patients), and the report of an epithelial defect (15 patients).
Of all patients that had a verified STI detected by multiplex PCR (64), 51 were found to be symptomatic. Sixty reported about symptoms, but no relevant STI could be detected via PCR. Symptoms were most frequently linked to presence of CT, followed by NG, MG, TP, HSV1, and HSV2. Moreover, symptoms were most likely in the case of urethral infection, followed by vaginal/cervical infection.

A 9.7.1. Dysuria

Of those patients who reported dysuria, 14 had a CT infection at the urethra, ten had an infection with NG, one had vaginal CT, one had a cervical CT infection, one had HSV1 in the urethra and in three patients MG was detected.
Several co-infections were observed: one patient had simultaneously CT and NG in the urethra, one had CT and HSV 1 in the urethra, and one patient had a simultaneous detection of CT at the urethra and at an epithelial defect.

A 9.7.2. Discharge

Of those patients who reported discharge, 11 had a urethral infection due to NG (one had simultaneously CT), one was vaginal positive for NG (and had a simultaneous vaginal CT infection).
At the cervix, two females were positive for CT, and one for HSV1 and HSV2, respectively.
Three patients were urethral positive for MG, and 13 were positive for urethral CT (one had a simultaneous CT infection at the cervix and NG in the pharynx; two patients were positive for CT at the vagina or cervix too, and in one patient CT was found at an epithelial defect).

A 9.7.3. Epithelial defect

Thirteen patients reported an epithelial defect, in three patients TP was verified, all others had no relevant STI to be proven at the site of the lesion. In one patient CT was found in the epithelial defect, however this respective patient had a concurrent urethral CT infection, and thus the result is most likely contaminated.
Additionally, in those patients who recognized a defect, we found urethral CT in one patient, and urethral CT and HSV 1 infection in another patient. We detected no cervical or vaginal pathologies in those patients, and at the anal region one coinfection of CT/NG (that patient was also positive for TP at the epithelial defect).

A 9.7.4. Pruritus

Pruritis was reported in 17 patients. Of those, in two patients CT and in one patient MG was found in the urethra, whereas in one patient simultaneously CT and NG were found vaginal. Thirteen patients that experienced itchiness, had no relevant STI.

A 9.7.5. Symptoms without detection of STI

Onehundredeleven (93 males/18 females) patients reported about subjective symptoms, in 53 (47.7%) of these a STI could be verified. Forty-nine males had complaints but no STI, whereas nine females had complaints but no STI.

A 9.8. Detection of STI

A 'real' STI could be verified in 70 (58 males, 12 females) patients. A real STI was defined as new occurrence of relevant bacteria or viruses, including positive swab results for NG, CT, MG, HD, HSV1/2, TV, TP (in 63 patients) using the Euroimmun® (Lübeck, Germany) panel or a positive new serological detection of HBV, HCV, HIV, or syphilis (15 patients). The detection of MH, UU or UP was not defined as representing a real STI.

Of all patients (n=178), in 70 (39.3%) we diagnosed a STI.
Regarding the subgroup of adolescents and young adults [(AYA) = 15- 24 years of age)] (n= 37), 20 (54%) patients had a STI.

In 70 patients who had a STI (43 heterosexual participants, 18 MSM, and nine with bisexual orientation), 18 had less than ten sexual partners during lifetime, 28 had 10-49 partners, nine had 50-99 partners, and six had more than 100 partners. Nine patients did not respond to the question how many sexual contacts they had during lifetime.

Among the group where a STI was detected by the multiplex PCR only (and not verified via microscopy) (n=64), 41 heterosexual, 15 MSM, and eight bisexual patients were recorded, respectively.

A 9.8.1. Characteristics of the included patients- Distribution overall and by STI status

In Table 11 baseline characteristics of the study cohort are depicted, including two columns for patients who suffered from no STI and those who got infected with a STI.

Table 11- Baseline characteristics of the study population – Distribution overall and by STI status (n=178)

Variable	n (% miss.)	Overall (n=178)	No STI (n=108)	STI (n=70)	p
Age (years)	178 (0%)	32 [25-44]	34 [27-43]	30 [24-44]	0.325
AYA (i.e. age < 25 years)	178 (0%)	37 (21%)	17 (18%)	20 (29%)	**0.039**
Female sex	178 (0%)	30 (17%)	18 (17%)	12 (17%)	0.934
Symptoms	178 (0%)	111 (62%)	58 (54%)	53 (76%)	**0.003**
Duration of symptoms (days)	110 (<1%)*	10 [5-24]	14 [5-50]	10 [4-21]	**0.053**
Discharge	178 (0%)	50 (28%)	20 (19%)	30 (43%)	**<0.0001**
Dysuria	178 (0%)	61 (34%)	34 (31%)	27 (39%)	0.330
Pruritus	178 (0%)	17 (10%)	13 (12%)	4 (6%)	0.198
Ulcer	178 (0%)	13 (7%)	8 (7%)	5 (7%)	0.947
Last unprotected sex (days)	177 (<1%)	18 [7-45]	18 [7-60]	17 [7-37]	0.915
Index sex contact with random acquaintance	178 (0%)	90 (51%)	48 (44%)	42 (60%)	**0.043**
Pregnancy	178 (0%)	2 (1%)	1 (1%)	1 (1%)	0.999
Sexual orientation	178 (0%)	/	/	/	**0.025**
---Heterosexual	/	129 (72%)	86 (80%)	43 (61%)	/
---MSM	/	31 (17%)	13 (12%)	18 (26%)	/
---Bisexual	/	18 (10%)	9 (8%)	9 (13%)	/
Number of sexual partners in the last 6 months	176 (1%)	2 [1-3]	1 [1-3]	2 [1-4]	**0.044**
Lifetime number of sexual partners	154 (13%)	12 [6-30]	10 [5-30]	15 [8-23]	0.190
Anal use of sextoys	177 (<1%)	35 (20%)	21 (20%)	14 (20%)	0.951
Prior meningococcal vaccination	150 (16%)	17 (11%)	12 (13%)	5 (9%)	0.405
Prior HPV vaccination	160 (10%)	9 (6%)	6 (6%)	3 (5%)	0.742
History of STI	178 (0%)	73 (41%)	39 (36%)	34 (49%)	0.099

Table 11. Baseline characteristics of the study population – Distribution overall and by STI status (n=178). Reported data are medians [25th-75th percentile] or absolute frequencies (column %). n (%miss.) indicates the number of observations with fully observed variable (% missing). *Only reported for those n=111 patients who had symptoms. Abbreviations: STI – Sexually transmitted infection, p – p-value, AYA – Adolescents and Young Adults, MSM – Men who have Sex with Men, HPV – Human Papilloma Virus.

A 9.9. Symptomatic versus asymptomatic individuals

Table 12- Baseline characteristics of the study population comparing symptomatic with asymptomatic patients – Distribution overall and by STI status (n=178).

Variable	n (% miss.)	Overall (n=178)	No symptoms (n=67)	Symptoms (n=111)	p
Age (years)	178 (0%)	32 [25-44]	34 [26-44]	32 [25-44]	0.578
AYA (i.e. age < 25 years)	178 (0%)	37 (21%)			
Female sex	178 (0%)	30 (17%)	12 (18%)	18 (16%)	0.770
Last unprotected sex (days)	177 (<1%)	18 [7-45]	21 [7-60]	14 [7-37]	0.177
Index sex contact with random acquaintance	178 (0%)	90 (51%)	31 (46%)	59 (53%)	0.373
Pregnancy	178 (0%)	2 (1%)	2 (3%)	0 (0%)	0.140
Sexual orientation	178 (0%)	/	/	/	0.793
---Heterosexual	/	129 (72%)	50 (75%)	79 (71%)	/
---MSM	/	31 (17%)	10 (15%)	21 (19%)	/
---Bisexual	/	18 (10%)	7 (10%)	11 (10%)	/
Number of sexual partners in the last 6 months	176 (1%)	2 [1-3]	1 [1-3]	2 [1-3]	0.264
Lifetime number of sexual partners	154 (13%)	12 [6-30]	12 [7-50]	12 [6-22]	0.364
Anal use of sextoys	177 (<1%)	35 (20%)	13 (20%)	22 (20%)	0.984
Prior meningococcal vaccination	150 (16%)	17 (11%)	10 (17%)	7 (8%)	0.070
Prior HPV vaccination	160 (10%)	9 (6%)	6 (9%)	3 (3%)	0.085
History of STI	178 (0%)	73 (41%)	29 (43%)	44 (40%)	0.632

Table 12. Baseline characteristics of the study population comparing symptomatic with asymptomatic patients – Distribution overall and by STI status (n=178). Reported data are medians [25th-75th percentile] or absolute frequencies (column %). n (%miss.) indicates the number of observations with fully observed variable (% missing). Abbreviations: STI – Sexually transmitted infection, p – p-value, AYA – Adolescents and Young Adults, MSM – Men who have Sex with Men, HPV – Human Papilloma Virus.

A 9.10. Risk factors and predictors for STIs

Table 13- Univariable predictors of STI

Variable	Odds ratio	95% CI	p
Age (per 5 years increase)	0.96	0.86-1.08	0.539
AYA (i.e. age < 25 years)	2.14	1.03-4.46	**0.042**
Female sex	1.03	0.46-2.31	0.934
Symptoms	2.69	1.38-5.22	**0.004**
Duration of symptoms (per 7 days increase)*	0.91	0.83-1.00	**0.047**
Discharge	3.30	1.68-6.50	**0.001**
Dysuria	1.37	0.73-2.57	0.331
Pruritus	0.44	0.14-1.42	0.170
Ulcer	0.96	0.30-3.07	0.947
Last unprotected sex (per 4 weeks increase)	0.94	0.87-1.03	0.181
Index sex contact with random acquaintance	1.88	1.02-3.45	**0.044**
Pregnancy**	N/A	N/A	N/A
Sexual orientation	/	/	**0.027**
---Heterosexual	Ref.	Ref.	Ref.
---MSM	2.77	1.24-6.17	0.013
---Bisexual	2.00	0.74-5.40	0.172
Number of sexual partners in the last 6 months (per 1 partner increase)	0.99	0.94-1.05	0.779
Lifetime number of sexual partners (per 1 partner increase)	1.00	0.99-1.00	0.636
Anal use of sextoys	1.02	0.48-2.18	0.951
Prior meningococcal vaccination	0.63	0.21-1.89	0.408
Prior HPV vaccination	0.74	0.18-3.06	0.675
History of STI	1.67	0.91-3.08	0.100

Table 13. Univariable predictors of STI. Reported data are from univariable logistic regress ion models. *Only modelled in in those n=110 patients who had symptoms and their sympto m duration observed.
**As we observed only two pregnancies, no formal statistical modeling was performed for this variable. Abbreviations: 95%CI – 95% confidence interval, p – p-value, AYA – Adolescents and Young Adults, MSM – Men who have Sex with Men, HPV – Human Papilloma Virus, STI – Sexually Transmitted Infection.

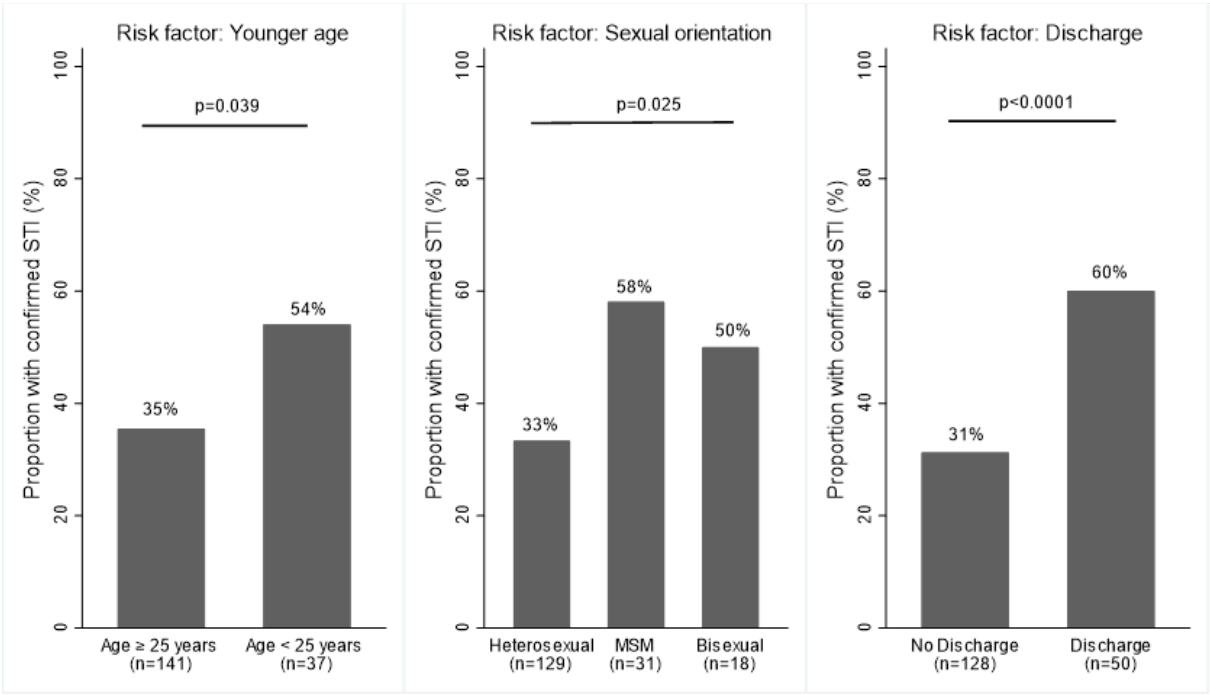

Figure 4- Bar graph for risk factors (AYA, sexual orientation, discharge) for acquisition of a STI

Figure 4. Bar graph for risk factors (AYA, sexual orientation, discharge) for acquisition of a STI. Reported data are absolute frequencies (n in total and %). P value is depicted for each parameter above bar graphs. X axis shows risk factor, Y axis shows proportion of conformed STIs (in %) Abbreviations: STI – Sexually transmitted infection, p – p-value, MSM – Men who have Sex with Men,

A 9.11. Urogenital STIs

Among the study participants that were checked for relevant STIs (n= 178) via multiplex PCR, in 44 (24.7%) patients a relevant urogenital STI could be detected.

A 9.11.1. Urethra

Urethral infections were most often caused by CT (23 patients- 20 males/three females), followed by NG (13 patients -12 males/one female), MG (seven patients- six males/one female), and HSV1 in in one male patient.

A 9.11.2. Female genital tract

At the female genital tract (vagina and cervix), an infection with CT showed the highest prevalence (n=4), followed by *N. gonorrhoeae*, HSV1 and HSV2, respectively (n=1).

At the vagina (19 females were tested), one female had NG to be detected, and two were positive for CT. No infection with HD, MG TV, HSV1, HSV2 or TP at the vaginal region was observed.

In total, 13 females were tested at the cervix. Hereby, *Chlamydia trachomatis* prevalence was highest (n=2), followed by HSV 1 and HSV 2 (n=1, respectively). No infection with NG, HD, MG, TV, or TP at the cervix was observed.

A 9.12. Extragenital STIs

An extragenital (oropharyngeal and rectal) STI was verified in 29 individuals.
At an ulcerous lesion in 7 patients a STI was detected.

A 9.12.1. Oropharyngeal region:

170 patients were tested at the pharyngeal region (51 of those via the *STI 11 panel* of Euroimmun® (Lübeck, Germany).

In 15 swabs (14 patients, one had co-infection of NG/MG) at the enoral region, a STIs was verified via multiplex PCR. *Neisseria gonorrheae* was the commonest pathogen (seven patients, three females/four males), followed by *Treponema pallidum* (one female, two males), *Trichomonas vaginalis* (one female/one male), HSV1 (two males), and MG (one male patient).

A 9.12.2. Anorectal region

Fiftyeight patients were tested at the anorectal region (in three additional patients the obtained results were not valid, 16 of 58 got tested via the 11panel of multiplex PCR, the other cohort with a CT/NG panel).

Fourteen positive results regarding an anorectal infection were recorded. In detail, it were 13 pateints (one had NG and CT infection simultaneously). Anorectal infection was most commonly caused by CT (six patients), followed by anal gonorrhea (four patients), HSV 2 and TP (two patients each, respectively).

A 9.13. Genital ulcer

An epithelial defect/ulcerous lesion was most commonly caused by treponema pallidum (4 patients), followed by CT (two pateints), and HSV2 (one patient).

Thirteen patients reported an epithelial defect/ulcer, however, 21 were tested at an ulcerous lesion. Of those, four were tested positive for TP, two were positive for CT (most likely due to contamination), and one for HSV2.

A 9.14. Serological results

Complete serological results (including hepatitis B, C, HIV, and syphilis) were available for 169 patients. Onehundredthirtyeight patients were negative for HIV and syphilis. Three individuals were HIV+ (two MSM, one bisexual male) and equally positive for syphilis. There was no newly detected HIV+ case within this patient cohort and timeframe.

Seropositivity for syphilis was found in 34 (19.7%) patients (18 MSM, 11 male bisexuals and five heterosexual patients- three females/ two males). In 15 patients (one female heterosexual, 14 males- nine MSM, four bisexuals, two heterosexuals) we found a newly detected early syphilis. A late latent was detected in one female heterosexual patient. No cases of tertiary syphilis or neurosyphilis were detected.
Thirty-eight% (13/out of 34) patients positive for syphilis had a concurrent STI. We had four simultaneous anal CT and one urethral infection, three urethral MG infections, one urethral and three anal NG infections, and two anal HSV2 infections. One of those patients had simultaneously a NG and CT infection anally.

Seroprevalence for hepatitis B was 2.9% (5/169), and for hepatitis C it was 0.6% (1/170). We did not detect any new cases of a hepatitis B or C virus infection.

Serology is inevitable, as we had eight patients with early syphilis that were not positive for TP in the swabs, and would otherwise, probably not be diagnosed. Four patients were completely asymptomatic. However, equally four patients demonstrated clinical signs of secondary syphilis (all four patients had a maculous exanthema, one had an angina specifica).

A 9.15. Confirmed pathogens among the study cohort

A 9.15.1. Overall prevalence of STI pathogens in the study cohort

Table 14- Overall prevalence of STI pathogens in the study cohort

Pathogen	Number of confirmed infections (%)
C. trachomatis	30 (17%)
N. gonorrhoeae	22 (12%)
HSV-1	4 (2%)
HSV-2	3 (2%)
H. ducreyi	0 (0%)
M. genitalium	8 (4%)
M. hominis	20 (11%)
T. pallidum	7 (4%)
T. vaginalis	2 (1%)
U. parvum	28 (16%)
U. urealyticum	35 (20%)

Table 14. Overall prevalence of STI pathogens in the study cohort at any site.
Reported percentages have the total number of n=178 subjects in the denominator

In those 51 patients who were tested microscopically, we observed 32 (62.7%) cases of microscopically identified urethritis. Of those 32 patients who had urethritis, we had eleven cases of CT and NG infection, one infection with MG, and one simultaneous mixed infection with CT and NG. In eight cases, no relevant pathogen could be verified.
Of those 19 patients who had no microscopically identified urethritis, two infections with MG, one infection with CT, and one mixed infection of CT and NG were recorded.
In total (n=178), 40 subjects (prevalence: 22.5%) who suffered from a PCR-verified urethritis of relevant pathogens, including CT, NG, MG, HSV1, HSV2 and TV, were identified.

Of those eight females, whose swabs were additionally evaluated microscopically, we detected two microscopically verified cases of cervicitis, in one case HSV2 was causative, in the other one no relevant pathogen could be detected. In six female patients whose swabs were additionally microscopically evaluated, no cervicitis was found, however, in one case CT was detected by PCR.
In total (n= 30 females), six vaginal/cervical verified cases (prevalence: 20%) of STI related cervicitis were observed.

A 9.15.2. Distribution of CT/NG/MG and TV by anatomic site of infection among all participants.

Table 15- Confirmed pathogens among the study cohort (n=178) by site

Pathogen	Oral (n=51)*	Urethral (n=177 tests)	Vaginal (n=19 tests)	Cervical (n=13 tests)	Anal (n=16 tests)**	Ulcer (n=21 tests)
C. trachomatis	n=0	n=23	n=2	n=2	n=6	n=2
N. gonorrhoeae	n=7	n=13	n=1	n=0	n=4	n=0
HSV-1	n=2	n=1	n=0	n=1	n=0	n=0
HSV-2	n=0	n=0	n=0	n=1	n=2	n=1
H. ducreyi	n=0	n=0	n=0	n=0	n=0	n=0
M. genitalium	n=1	n=7	n=0	n=0	n=0	n=0
M. hominis	n=2	n=15	n=3	n=0	n=5	n=1
T. pallidum	n=3	n=0	n=0	n=0	n=2	n=4
T. vaginalis	n=2	n=0	n=0	n=0	n=0	n=0
U. parvum	n=0	n=26	n=9	n=4	n=0	n=1
U. urealyticum	n=0	n=31	n=7	n=4	n=3	n=1

Table 15. Confirmed pathogens among the study cohort (n=178).
The table reports the number of positive tests for each of the tested pathogens at the relevan t sites. *Oral result: *N. gonorrhoeae* and *C. trachomatis* were tested in n=170 patients. **Anal result: *N. gonorrhoeae* and *C. trachomatis* were tested in n=58 patients.

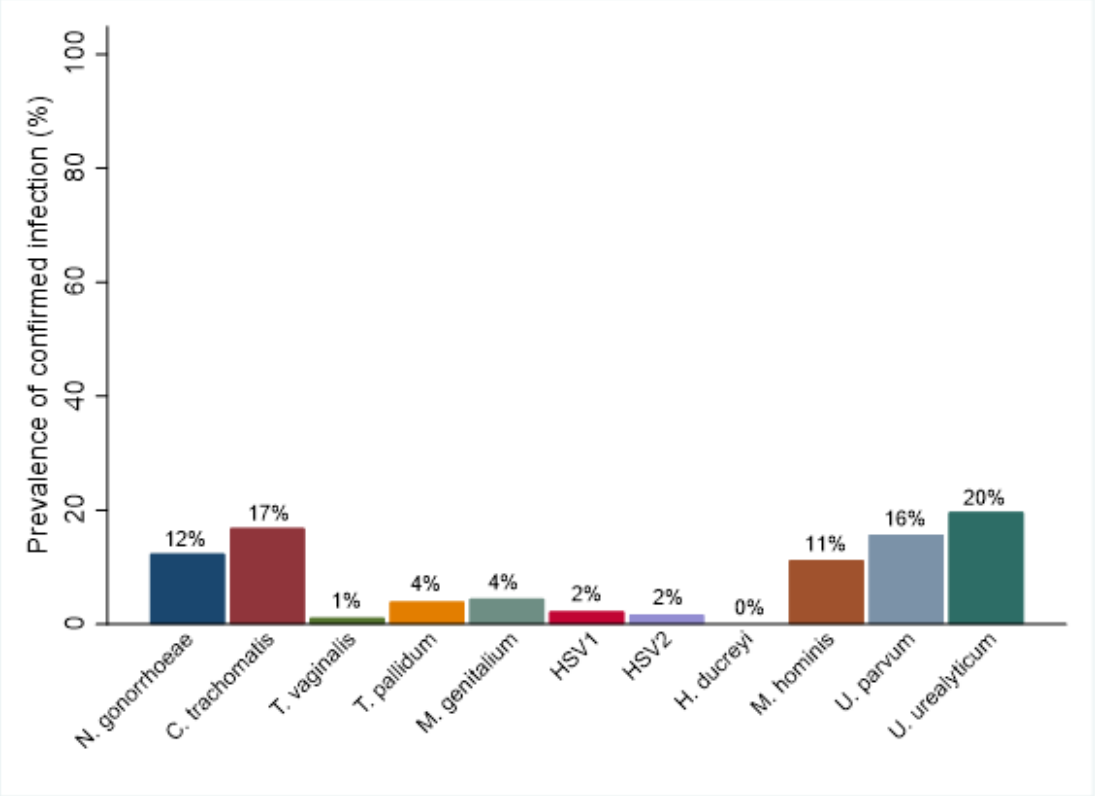

Figure 5- Prevalence of STI pathogens in the cohort

Figure 5. Prevalence of sexually transmitted pathogens among the study cohort (n=178). The table reports the number of positive tests for each of the tested pathogens at the relevant sites.

A 9.15.3. Prevalence of CT/NG/MG and TV

The overall prevalence for CT was 17%. The localization of CT was mostly urethral (23 patients), followed by anorectal site (six patients), vaginal, cervical, and ulcerous lesion (two patients, respectively). No oral CT infection was detected. All vaginal or cervical CT infected patients (100%) reported about either discharge, or dysuria, or both. Of all 23 urethral CT infections, 17 (74%) reported complaints. Sixteen reported about either discharge (13 patients), or dysuria (14 patients), or both (11 patients). Only one patient reported about an exanthema which was due to a Lues II infection.

The overall prevalence for NG was 12%. The localization of NG was mostly urethral (13 patients), followed by oral region (seven patients), anorectal site (four patients) and vaginal (one patient). No cervical NG infection was detected.

Of the 13 urethral infections, 12 (92%) had complaints. Eleven patients reported about discharge, ten about dysuria, and nine complained about both symptoms.

The overall prevalence for MG was 4%. MG was detected in one male patient pharyngeal and in seven patients (six males, one female) at the urethra. None of them reported complaints.

The overall prevalence for TV was 1%. TV was verified in only one male and in one female patient at the oropharynx. Both had no complaints at the oropharyngeal region.

A 9.15.4. Prevalence of TP, HSV1, HSV2 and HD

Among the study participants, 4%, 2%, 2% and 0% were tested positive for TP, HSV1, HSV2, and HD.

The overall prevalence of TP was 4%. The localization of TP was mostly an ulcer (four patients), followed by three oral and two anal cases. No vaginal, cervical, or urethral cases of TP infection could be detected.

The overall prevalence for HSV1 and HSV2 were 2% each, respectively.

HSV1 was most frequently found at the oral region (two patients), followed by urethral and cervical sites (one patient each). HSV2 was most frequently found at the anal region (two patients), followed by cervical and ulcerous lesions (one patient each). We did not verify any infection with HD.

A 9.15.5. Prevalence of mollicutes (MH/ UU/UP), despite MG

UU, UP and MH were frequently detected, as evidenced by 20%, 16%, and 11%, respectively.

Urethral mollicutes colonization.

Of those patients who had a UP mono-infection, one reported about discharge, five about dysuria and four about both; two patients had discharge and pruritus.

Of those patients who had a UU mono-infection with UU, one experienced discharge, dysuria, and pruritus (three patients in total).

Only one patient had a MH mono-infection, he experienced pruritus.

Vaginal or cervical mollicutes colonization

Of those patients who had a UP mono-infection, one female reported about pruritus and discharge. The only one who had a UU mono-infection at the cervix, experienced discharge.

A 9.16. *Neisseria gonorrheae* and antimicrobial resistance

Out of 22 subjects suffering from an infection with NG, a culture and consecutive antimicrobial resistance testing was performed in 11 patients; unfortunately, in two of these patients their urethral swabs material was not sufficient for analysis.
In nine patients, a culture was not created or did not grow, which was the case in five patients' material from the oropharynx, in three from the anorectal region, and in one from the vaginal region.

NG showed sensitivity to ceftriaxone in every single subject. Cefixime and azithromycin were resistant in one patient each. NG was resistant to ciprofloxacin in eight patients, in one it was unclear, and in two it might be sensitive. NG showed a resistance towards doxycycline in nearly all patients (in one it was intermediary). Spectinomycin was taken off the AMR as it is not available in Austria.

Table 16- resistance and susceptibility testing of *Neisseria gonorrheae* infections

	ID 13	ID 17	ID 37	ID 72	ID 80	ID 100	ID 106	ID 127	ID 131	ID 170	ID 175
Penicillin	+/-	+/-	+/-	+/-	*/-	+	-	+/-	+/-	-	-
Azithromyzine	+	+	-	+	+	+	+	+	+	+	+
Doxycycline	+/-	-	-	-	-	-	-	-	-	-	-
cefixime	+	-	+	+	+	+	+	+	+	+	+
Ciprofloxacin	+	+	+/-	-	-	-	-	-	-	-	-
Ceftriaxone	+	+	+	+	+	+	+	+	+	+	+
Spectinomycin	+	+	ND	ND	ND	ND	ND	ND	ND	ND	ND

Table 16. Table shows resistance and susceptibility results of those patients infected with NG. Of 22 infected individuals, we had eleven antimicrobial testing results.

A 10. DISCUSSION

It seems inevitable that regional recommendations and guidelines were developed to combat STIs, including the important reduction of a significant morbidity and the transmission of highly relevant infectious diseases, like HIV [Kent 2005]. Nevertheless, such recommendations must be based on regional data. However, up until today, such data concerning prevalence, diagnosis, and treatment of the relevant STI pathogens in Austria are scarce or even not available.

To the best of our knowledge, we herein present the first prospectively assessed study showing prevalence data regarding the STI epidemiology of patients in Austria.
In summary, we were able to identify a large number of STIs in our patient cohort with an overall STI prevalence of 39%, despite the fact that we did not focus on various risk groups, like MSM or HIV+ individuals exclusively. Out of 178 patients in total, we had 129 (72.5%) heterosexual subjects, and only 49 (27.5%) non-heterosexuals. Three patients were HIV positive and 175 were HIV negative.

We report moreover a prevalence of urethritis of 22.5%, and a prevalence of cervicitis of 20%. Regarding pathogenic agents, the prevalence of STIs in our cohort was highest for CT with 17%, followed by infections with NG (12%). *Mycoplasma genitalium* and TV infections were detected in 8%, and 1% of the cases, respectively. The most commonly diagnosed STI emerged to be urethral CT infection (n=23), followed by urethral NG infection (n=12). In total, we observed 35 infections with CT and 23 with NG. Only eight patients suffering asymptomatically from MG, and two who were positive for TV at the pharynx, were recorded, which is discussed below.

A high STI prevalence rate could be observed in the groups of bisexuals, MSM, those of young age (>age of 25) and females. Out of 18 bisexual patients (three females, 15 males), ten (55.5%) developed a new STI. Out of 31 MSM cases, 18 developed a new STI (58.0%). The subgroup of adolescents (15-24 years) included 37 individuals. Of those, 21 showed a STI (56.8%). This finding is in concordance with a reportedly high number of adolescents and young adults that are believed to account for about the half of newly acquired STIs [Skaletz 2020]. However, it has to be emphasized that according to our inclusion criteria, only patients older than 18 years were to be included into analyses, and an even higher number of STIs might have been reported, if individuals aged younger than 18 years would have been included. Of all included female patients (30), 12 (40%) developed a new STI.

A 10.1. URETHRITIS

In most STIs, urethritis represents the predominant syndrome [Rossignol 2019]. This is in line with our study population, as the predominant symptoms were dysuria (reported by 60 patients, 33%) and discharge (reported by 50 patients, 28%).

The prevalence of urethritis data differ widely in the global context, based on various populations, sex, and regions, from about 2% to 34% [CDC 3/12/2021, Nenoff 2017, Khatib 2015, Jordan 2020]. The prevalence of urethritis was 22.5% in our own study cohort. This is quite a high number and of special interest, since the study cohort included a significant number of heterosexual individuals (129/178; 72.5%), while most studies that report on STIs, focus on the group of non-heterosexual individuals only. In the United States alone, it is estimated that 2.8 million urethritis cases are prevalent each year [Jordan 2020].

For most countries, prevalence data regarding relevant sexually transmitted pathogens, are published. For example, in Portugal, in a MSM cohort, the overall prevalence of CT or NG infections was 16.05%, (14.23% had a coinfection) and 40.73% of subjects who were infected, were asymptomatic [Ribeiro 2019]. The prevalence for CT was 7.59%, and for gonorrhea 10.75% [Ribeiro 2019]. In Sweden, women were tested for CT and MG, whereby the prevalence of CT was 10%, and for MG 6%. A co-infection was found in four women [Falk 2005]. In 2016, a study was published from Germany, where women, heterosexual men, and MSM were studied. The CT prevalence ranged from 3.2 % (in heterosexual men) to 3.5% in MSM, and 5.3% in females [Lallemand 2016]. In the United Kingdom in 2014, a study was performed in males suffering from urethritis, and the authors reported CT in 33.7%, NG in 16.8%, MG in 12%, and TV in 3.6% in the urine [Khatib 2015]. In a recent and prospective study from Tel Aviv, males were screened for relevant pathogens and showed a prevalence for NG of 23.1%, for CT 12.7%, for MG 6.6%, and no infection with TV was to be proven [Gottesmann 2017]. In the United States in 2020, a study was published that examined urine of symptomatic and asymptomatic males presenting in a STI clinic; the authors reported in those suffering from NGU, in 34% CT, in 17% MG, and in 2% TV [Jordan 2020].

We observed an overall prevalence of 17% regarding CT, and 12% regarding NG in our study population, which represents a relatively high number of infections, underlining the need of better screening programs and rapid treatment possibilities.

Non-gonococcal urethritis

In about a half of patients CT is known to be the causative pathogen of NGU, followed by MG (15-25%), TV (10-20%), HSV and adenovirus [Nenoff 2017, Horner 2016, Jordan 2020].

We examined material from the urethra of 51 patients via staining and microscopy, and found urethritis in 32 patients (31 males, one female). In total, 20 cases of NGU (eleven had a CT infection (55% causative agent), and one a MG infection (5%), three were positive for UP. This finding is in accordance with the above-mentioned literature. In five additional patients we did not find a causative STI agent by our panel. This is in line with the literature , as about one half of all NGU cases show no identifiable pathogen [Jordan 2020, Moi 2015, Horner 2016, Nenoff 2017].

In total, out of all 178 patients, we did not have that many NGU cases diagnosed via staining, which might be in part explained by the generally used cut-off value of five PMNLs/HPF. The CDC already recommended a lower cut-off (two PMNLs/HPF; regardless of the presence of symptoms) to define NGU in males, not to miss patients with a low grade urethritis [Workowski 2021]. One study even showed that close to 1/6 of men suffering from CT infection, did not have microscopically defined urethritis [Rietmeijer 2012]. Our own study population showed a low detection rate of microscopically detected NGU (32 cases). In our study population, we recorded 23 cases of urethral CT infection. In 12 of those, non-gonococcal urethritis cases could be verified microscopically, in three we did not find a NGU after staining. In eight patients no POCT was performed due to too few material of the urethra. In total, in about the half of patients (11/23) suffering from CT urethritis, we were not able to verify microscopically defined urethritis, hinting to the need for NAAT testing and not only relying on microscopic analysis.

A check for urinary tract infection is routinely not necessary in young males, as this diagnosis is very rare in that particular age group [Moi 2015]. If indeed a urinary tract infection is diagnosed, patients should be presented to a urologist searching for possible anatomical abnormalities [Horner 2016].

A urinary dipstick might be considered in cases with severe clinical symptoms, like hematuria, urinary urgency among others, and moreover, a mid-stream urine might be analyzed via culture [Moi 2015].

An early morning smear can be recommended, as well as micturition abstinence from at least 2 hours to guarantee a higher bacterial load prior to the extraction of material [Moi 2015]. Staining of urethral smear should only be performed in patients who are symptomatic [Moi 2015].

Chlamydia trachomatis

According to the ECDC, infections with CT are rising and globally, there are more than 250.000 new infections reported each year, additionally large numbers of unreported cases are suspected. In the latest surveillance report, published in 2020 (containing epidemiological data from 2018), more than 406.000 cases of CT infections were reported in the European Union alone [ECDC 2020].

In this regard, prevalence data from Austria are scarce, as there are no epidemiological studies published, and moreover, governmental reporting of CT infections is not mandatory in our country.

In our study population we found 23 cases of urethral CT infections, 20 (out of 148) males and three (out of 30) females. This might represent a lower number than expected and thus be biased, since in this study information about condom usage was not asked for. As condoms are well known to be able to reduce the risk of certain STIs, including CT, this might be one reason [Warner 2004]. Seventeen individuals had symptoms, two of them were heterosexual women, one was a bisexual male, and 11 were heterosexual men. Six reported having no complaints at the urethra (one female, five males). Asymptomatic male CT infection is not uncommon [Jordan 2020]. In total, six subjects (out of 23) with urethral CT infection were asymptomatic. This finding is in concordance with the literature, since ¼ up to 100% of men are believed to have asymptomatic courses [Lanjouw 2015] and underlines the urgent need for enhanced STI prevention and screening approaches.

Interestingly, we found only three CT infections at the urethra in women. This finding is not in concordance with the published literature, as it is known that CT is found in women twice as often as in men [Nenoff 2017]. However, two important factors must be kept in mind: First,a very low number of females were included into our study cohort. This might be most likely due to the fact that women do not seek for examination at the venereologist but at the gynecologist first, who will definitely treat a CT infection if detected. Second, CT shows relatively high clearance rates [Lanjouw 2015]. In untreated women, clearance rates reach about 80% after two years; however, an ascending and persisting infection is feared [Lanjouw 2015]. Therefore, testing for those at risk (mainly women under the age of 25) is inevitable. In 2009, Bebear et al. called for a high awareness of CT infections in women, as 2/3rd of all cases of tubal factor infertility and 1/3rd of ectopic pregnancies might be due to this disease [Bebear 2009]. Skaletz-Rorowski and colleagues even stated that CT is regarded as the commonest cause of infertility of women [Skaletz 2020]. CDC and OEGGG recommend screening for CT in all sexually active females (aged under 25 years) annually [Workowski 2021].

In 2016, Lallemand et al. published results from Germany, that studied the prevalence of CT in women, heterosexual men and MSM, presenting themselves to HIV counselling institutions. The CT prevalence ranged from 5.3% in females, 3.5% in MSM, to 3.2% in heterosexual males [Lallemand 2016]. The highest prevalence was found in the age group of 18-24 year old females and heterosexual males (9%, 5.7%, respectively) [Lallemand 2016]. The CT prevalence of a study on swedish female STD clinic attendees was 10% [Falk 2005]. In our recent study, we detected a 16% CT prevalence in females (5/30), 18.6% in heterosexual males (19/102), and 13% in MSM (6/46). These numbers are of the outmost importance, since they underline a high need for testing in Austria.

Another issue is the asymptomatic course of many infections. The majority of infected females (76.7%), heterosexual males (70%) and MSM (84.2%) were completely asymptomatic [Lallemand 2016]. This was not the case in our study cohort, as we had only 26% of asymptomatic patients that were infected with CT at the urethra. All four females that had a CT infection at the vagina/cervix, were symptomatic and four out of six that were infected at the anal region, reported about symptoms.

According to a study of Kent et al., CT was the commonest bacteria found in the urethra in their studied group of MSM [Kent 2005]. This report is in concordance with our data, as we found CT in 23 subjects, followed by 13 infections with NG in the urethra.

Some authors believe that CT causes almost always a microscopically detectable urethritis, at least in men [Jordan 2020]. Twenty males out of 148 (13.5%) demonstrated a urethral CT infection in our study cohort, whereby 12 of those showed a microscopically defined urethritis. However, there are other studies claiming CT infection rates of approximately 15%, diagnosed by a Gram staining of 2-5 PMNL/ HPF [Rietmeijer 2012]. In the recent study, a higher cut-off value was used, which might explain the observed differences regarding this important outcome.

Mykoplasma genitalium and other mollicutes

Our data showed a prevalence for MG of 4%, and an overall prevalence of 51% of all mollicutes, including MG, MH, UP, and UU. According to the published literature, MG seems to have a prevalence of about 1.2-1-3% in sexually active males and females, respectively [Nenoff 2017], which is in line with our own data. *Mykoplasma genitalium* was prevalent in eight individuals only, 4 of these were heterosexual, and 4 MSM. In one patient, MG was detected pharyngeally and in seven patients (6 males, 1 female) located at the urethra. We were not able to detect MG at any extragenital sites.

The prevalence of MG in cases of symptomatic urethritis is believed to range from 15 to 25% [Spornraft 2020]. In the overall population, the prevalence is believed to be much lower, ranging from 1.1 to 3.3 or 3.9% [Spornraft 2020, Jensen 2016]. This finding seems to be in concordance with our own study population. Since our study cohort included a low number of patients suffering from a microscopically confirmed urethritis, this observed low prevalence is not unexpected. It is known that MG is to be found mainly in patients with symptomatic urethritis, contributing up to 35% in these cases (at least in males) [Jensen 2016].

Gottesmann et al., who focused a prospective study on MG, were able to show that the presence of this bacterium is highly linked to symptomatic urethritis [Gottesmann 2017]. In our recent study, none of MG infections turned out to be symptomatic, a finding that is particularly important concerning treatment options, and will be discussed at a later point in this thesis.

Gottesmann and colleagues even found a statistically significant association regarding MG urethritis and male heterosexuality [Gottesmann 2017]. Interestingly, Bing-jie et al. depicted that MG was more prevalent in males with bisexual orientation than in homosexually oriented men only [Bing-jie 2014]. In our own study cohort, out of eight MG positive patients, 4 were heterosexual and 4 MSM, whereas no bisxeual person with a positive MG result was detected.

For decades, MG was believed to be of no major importance in the large field of STIs. However, that impression changed within the last ten years, as evidenced by the expression of a 'true STI' [Horner 2018]. Even though there are more than 100 different types of mycoplasma known, only a few seem to be pathogenic to humans [Nenoff 2017]. Mollicutes encompass the well-known bacterium MG, as well as MH, UP, and UU [Spornraft Ragaller 2020].

Other Mycoplasma subspecies than MG, including MH, UU, and UP are still under investigation concerning their potential pathogenicity [Spornraft- Ragaller 2020]. In 2020 Spornraft-Ragaller et al. published data about the prevalence of mollicutes in HIV-infected MSMS. The authors report the highest prevalence for Ureaplasma species, followed by MG, MH, CT, and NG [Spornraft- Ragaller 2020]. An anorectal colonization in such patients was not uncommon and mostly asymptomatic. The authors propose to keep MH or Ureaplasma species to keep in mind only in cases of persisting anorectal symptoms [Spornraft- Ragaller 2020].

However, it seems that there is no distinct hint for the pathogenicity of MH causing urethritis, despite a quite high colonization rate of up to 20% is described [Horner 2018].

In 2020, Jordan et al. published a study, where the authors investigated mixed- and mono-infections in NGU [Jordan 2020]. They found that only in 14/113(12.3%) patients, mixed infections are prevalent in NGU cases and in control groups (defined as < 1 PMNL/HPF). Ureaplasma urealyticum was as twice as prevalent as in NGU cases. Furthermore, UU mono-infection was not found to be related to NGU [Jordan 2020]. In those 14 cases (total n =113), the vast majority (close to 80%) were co-infected with UU, and MG was the most frequent co-pathogen

Ureaplasma urealyticum was found to be able to colonise the urethra, but is no obligate pathogen, causing urethritis, despite the fact that there are substantial hints that it might also be a sexually transmitted bacterium [Jordan 2020]. As these authors used a very low cut-off value for the definition of 'no urethritis' of <1 PMNL/HPF and moreover, they had no urethritis in those showing a presence of UU (about 20%), this fact underlines the concept of a potentially non-obligate pathogen [Jordan 2020].

Trichomonas vaginalis

In our study cohort, we recorded two patients in whom a TV infection was detected by multiplex PCR. Both, however, were located at the oral region. One was a male heterosexual and one a female heterosexual patient. In both patients all other sites were negative for TV, and a false positive PCR result is likely according to our opinion and must be kept in mind. The male patient did not present for a re-test and the female patient was treated by an extramural gynecologist who seeked the results. However, as early as in 2018, in the IUSTI guideline for vaginal discharge it is stated that due to site specificity, an infection only follows vaginal and urethral inoculation of this flagellated protozoon [Sherrard 2018].

The observed low rate of TV in our study cohort is not unexpected, as Austria is historically not a country with a high TV prevalence. In general, it is believed that the TV prevalence ranges from 1-2% in western countries [Jordan 2020]. However, in certain countries like Iran, the prevalence ranges are extremely high and differ from 1 up to 42% [Arabi 2018]. Most infections usually run asymptomatically (85%) [Kissinger 2015]. Both of our patients had no pharyngeal complaints.

Gonococcal urethritis

It is well known that the big majority of gonococcal urethritis leads to discharge (80%) and dysuria (50%) in males [Unemo 2019, Unemo 2020]. This is in concordance with our own findings, as we detected NG in 12 patients at the urethra, 11 male (three MSM, eight heterosexual) and one female heterosexual patient. Of those 12, all but the female patient presented with dysuria, or discharge, or both. Asymptomatic courses of gonococcal urethritis

in men are not common [Unemo 2020]. These findings are in line with the literature, as we had no asymptomatic urethral NG infection.

We had one female urethral gonorrhea case, and that patient was completely asymptomatic. In women symptoms are less typical and may include altered vaginal discharge, abdominal pain, and more seldom dysuria (10-15%) [Unemo 2020]. Like in other STIs, women tend to have more asymptomatic courses, which highlights the importance of screening even in asymptomatic patients, and especially in those at risk.

Most of our patients who suffered from gonococcal urethritis developed symptoms after 5.4 days (range 1-35 days). This is in concordance with the literature, as symptoms appear very quickly after two to eight days after sexual intercourse [Unemo 2020].

Clinical symptoms caused by *Neisseria gonorrheae*

It is believed that about 0.5 to 3% of NG infected patients might develop a disseminated gonococcal infection [Birell 2019]. A disseminated gonococcal infection (DIG) is usually defined (A) by a triad, including arthralgia, tenosynovitis and skin lesions (additionally often associated with fever) or (B) purulent arthritis [Unemo 2020, Birell 2019]. A persisting and untreated disseminated gonococcal infection might end in joint destruction and sepsis [Birell 2019]. In 2019, Birell et al. retrospectively analyzed 106 cases of disseminated gonococcal infection. Interestingly they found the classical "'triad' in only one patient [Birell 2019]. Most patients had arthritis (88.7%), affecting the knees, wrists, ankles, and elbows in descending order, respectively [Birell 2019]. A dermatitis (mainly pustular) occurred in just ten patients [Birell 2019]. Culturing the synovial fluid and/or detection of NG with a NAAT was positive in 54.7% [Birell 2019]. Notably, in 43.5% of patients with proven disseminated gonococcal infections, a positive mucosal result could be found [Birell 2019]. In the examined region, Birell et al. stated that gonococcal infection is believed to be causative for about ten percent of septic arthritis cases [Birell 2019].

In our own study cohort, no hint for a disseminated gonococcal infection did emerge.

A 10.2. CERVICITIS

We examined the stained swabs of eight females of the cervix and found only two microscopically diagnosed cases of cervicitis. In one female, HSV 2 was the causative pathogen, in the other female patient we could only detect UP, which most probably does not represent the causative agent. In six females we did not find cervicitis in the microscope, however one of those had a CT infection at the cervix, and in one patient we found both, CT and NG at the urethra. These findings underline the need of not only microscopically defined cervicitis but the usage of NAATs at the urethra and the cervix or vagina.

In total, we diagnosed infections of the vagina/cervix via PCR method in 5 female patients. Two CT infections at the cervix and one HSV1- and HSV2- infection, respectively ocurred. Via vaginal swabs we detected a CT/NG co-infection in one female patient, whereas another one developed a CT monoinfection.

In a study from Falk et al., the authors did not differentiate between urethritis and cervicitis in females but called it an infection of urethritis and/or cervicitis [Falk 2005]. This is discussable in females, as a cervicitis was called the 'ignored female counterpart in women of urethritis in men' [Brunham 1984]. Since both infections are anatomically located at very close sites, it might indeed be better not to differentiate in cervicitis or urethritis, but to sum up these two inflammations in females.

The CT prevalence (urethritis and/or cervicitis) of a study on swedish female STD clinic attendees was 10%, compared to MG with 6% [Falk 2005]. A co-infection rate was low and found in only four patients. This scarcity is in line with our own findings. We observed 16% CT infections at the urethra/cervix or vagina in our female patients and 3% MG infections (only one urethral female infection). Falk et al. defined cervicitis as more PMNL than epithelial cells in wet smear (of the endocervix), and urethritis as > 10 PMNLs/HPF [Falk 2005]. Interestingly, these authors chose a higher cut-off value regarding urethritis. Since in general, STIs in females are known to be much more often asymptomatic than in their male counterparts, we recommend to stick to the contemporary definition of urethritis at least of >5 PMNLs/HPF. In Falk et al.'s study, urethritis or cervicitis was found in about half of patients attending the STD clinic [Falk 2005]. This finding is similar to our findings, as we had 40% of female patients that were positive for a STI. Out of 30 females, in 12 (40%) we detected a STI, and in eight a microscopically analysis of the urethra and cervix was performed. Of the 12 STI positive females, in two cases syphilis was detected (one early onset (orally transmitted, PCR-verified TP at the pharynx) in a heterosexual patient, and one late latent) syphilis in a pregnant heterosexual female. In total, we diagnosed 23.3% genito-urethral infections in females (7/30). Two female patients had a urethral and simultaneous cervical infection with CT. One patient had a co-infection of CT/NG at the urethra, and one female showed the very same co-infection at the vagina. One female patient was positive for CT at the vagina exclusively, and one had a urethral infection with MG. One female patient developed an infection with HSV2 at the cervix.

Interestingly, in Falk et al.'s study, the authors report quite a high number of pathogen-negative subjects but a presence of symptoms (40%) [Falk 2005]. In our study, this number was even higher (52.3%), as we had 111 symptomatic subjects, but 58 had no STIs.

We detected no cases of MG infections at the vagina or the cervix. In the meta-analysis from Lis et al., 174 studies were screened concerning the relationship between MG and the occurrence of cervicitis. This analysis showed a significantly increased risk of cervicitis (pooled odds ratio 1.66 (95% CI, 1.35-1.04)) [Lis 2015].

A 10.3. EXTRAGENITAL INFECTIONS

Extragenital sites that might be infected include the pharynx and the rectum. In such cases, most of the patients report a completely asymptomatic course [Unemo 2019, Unemo 2020]. On the other hand, some may report about symptoms like sore throat, anal -discharge or -discomfort [Unemo 2020]. In our study population the extragenital infection rate was 16.3% (29). According to the literature it is well known that in the subgroup of MSM, rectal STIs are more common than urethral STIs [Kent 2005]. This phenomenon is in line with our findings. In our study cohort of 46 MSM (15 of those were bisexual males), 12 had an anorectal infection, while eight had an urethral infection. In only one patient, a coinfection of the urethra and an anorectal site was present.

The prevalence of extragenital infections is believed to range from 1-2% in the case of CT infections, and rises up to 8% in the case of NG at the oro-pharynx [Dudareva 2014]. No infection of CT at the pharynx was observed. This finding is equally not uncommon, as it is known that CT predominantly infects the mucosal epithelium of genital sites and the rectal area [Nenoff 2017]. Studies that focused only on the group of MSM had prevalence data for CT of 1.5% (95% CI 1.0-2.0%) [Dudareva 2014]. We had a prevalence of 4% regarding NG at the oral region (n=7). Three patients were female (one heterosexual, one bisexual) and four male (one MSM, three heterosexual patients). Interestingly, five patients showed urethritis-like symptoms, but only in one patient a simultaneous NG infection at the urethra could be found. No specific pharyngeal complaints were reported by patients of our study cohort.

The prevalence of CT and NG at the anorectal region ranges from 6-9% and 2-7%, respectively [Dudareva 2014]. However, most of these data are based on the group of MSM only [Dudarava, Kent 2005]. We found a prevalence of 10% for CT (n=6) at the anorectal region. One was a female heterosexual person, four were bisexual men and one was MSM. All but one patients had a previous STI. In two patients proctitis-like symptoms (diarrhea, tenesmus) were reported and both were therefore also tested for LGV. However, LGV could not be verified. In total, four patients reported symptoms, however, in one female patient urethritis-like symptoms were reported and another patient reported an epithelial defect (and was diagnosed with primary syphilis). Ribeiro et al. even report anorectal infections in 51.3%

of patients suffering from NG, and in 67.3% of those suffering from a CT infection [Ribeiro 2019]. The anorectal region was mostly infected by CT (67.3%), followed by a CT urethritis (24.8%) and pharyngeal infection (17.5%) [Ribeiro 2019]. In the study from Kent et al., CT was more often found in the rectum than in the urethra, however, they analysed exclusively MSM patients in their study population, and therefore results are limited to that specific patient cohort [Kent 2005]. This finding is comparable to our study cohort, as we found one urethral MSM CT infection, compared to five anorectal MSM CT infections.

Dudareva and colleagues report a prevalence for rectal CT infections of 8% in their study MSM group [Dudareva 2014]. A lower prevalence was found in a study from 2020 that screened HIV+ MSM and the authors found a positive swab in 5.1% [Spornraft 2020]. Of our patients, three had co-infections at other sites, one of them was positive for TV at the pharynx. We do believe that this might have been due to false positive laboratory results, however, unfortunately the respective patient did not present for a re-test. It is of note to emphasise that at least 50% of infections would have been not detected, when the testing of subjects would have been restricted to the urethra solely.

We detected two patients that were positive for CT at an epithelial defect, however, both were positive for CT in the urethra, and thus were treated according to the guideline recommendations.

We diagnosed a NG infection in four patients examined at the anorectal region (6%). All were MSM. We observed no female patient who was tested positive for NG. Three had symptoms, but no proctitis-like symptoms (one had urethritis symptoms and the two other an epithelial defect, both diagnosed with primary syphilis). Spornraft et al., who focused on a cohort of 227 HIV positive MSM, reported about symptomatic gonococcal infections at the anorectal region in only about a third of patients (33%) [Spornraft 2020].

We did not detect any extragenital infection of MG in our study cohort.

In one male and one female patient TV was found at the pharynx. This was interpreted as most likely false positive laboratory result, as it is known that TV typically and predominantly infects the squamous epithelium of the uro-genital tract of males and females, including urethra, vagina and cervix typically, but not extragenital sites [Kissinger 2015, Arabi 2018]. However, the respective male patient did not present for a re-test, and the female patient was treated by a gynecologist. No rectal infections with TV were observed in our study cohort.

A 10.4. SEROPREVALENCE

We tested 169 patients for HBV and verified an infection in five patients, however, in all of those the diagnosis was already established before study entry, and no new infections were to be detected afterwards. However, two of these patients developed an early syphilis.

We tested 170 patients for HCV and HIV. In one patient, an already known HCV infection was verified. Three HIV+ patients (2 MSM, 1 bisexual male) were recorded. In all of them a new STI was diagnosed. These patients had a mean of 6.6 partners over the last 6 months and the last sex was with a random acquaintance in all cases. In two patients we found a MG infection in the urethra, and in one an anal CT infection. Two patients had a sero-scar for syphilis, one had an early syphilis (detected by serology only).

In 34 (19.7%) of 173 patients tested, syphilis was detected via serology. In 15 patients an early syphilis could be verified. In one patient we diagnosed a late latent syphilis. In 18 patients a sero-scar was verified. Of all patients who needed treatment, 15 received benzathine penicillin i.m. injections and one got treated with doxycycline according to the current ÖGSDT guidelines. Interestingly, Spornraft et al. stated recently that the seropositivity of syphilis shows an elevated risk (47.1%) of the development of at least another STI [Spornraft 2020]. This finding was comparable with our own study population. Out of the 34 patients with a seropositivity of syphilis, 24 (70.6%) had a new STI to be detected (including those 15 cases of early syphilis).

A 10.5. DIAGNOSIS

Urethritis in males

In our study, the diagnosis of urethritis was performed via microscopic evaluation and confirmation of the presence of PMNLs. Moreover, and according to the recent literature, we would like to underline and recommend the confirmation of a urethritis via microscopy in all symptomatic males, as in those it has shown a sensitivity of up to 95% [Bignell 2006, Unemo 2019, Unemo 2020, Randjelovic 2018].

In asymptomatic males, the sensitivity of microscopy and staining is known to be relatively low (ranging from 50 to 75%) [Bignell 2006, Unemo 2020], and thus representing the reason why we would like to recommend NAAT testing in first void urine (the first 15-30ml are collected after a micturition abstinence for at least 1 hour [Bignell 2006, Unemo 2020].) from now on. According to the CDC, first-void urine from men is optimal for the screening of uro-

genital gonococcal infections, and CT infections can be diagnosed via NAATs of first-void urine or urethral swab [Workowski 2021]. In our study, the diagnosis was additionally performed via NAATs in all gonorrhea cases, which currently represents the recommended test of choice [Nenoff 2017, Unemo 2019, Unemo 2020]. It must be kept in mind that urgent susceptibility testing is not possible via usage of NAATs alone, and moreover, the rapidly achieved diagnosis of urethritis (via staining) is missed. In those cases where a culture was possible, we performed the highly recommended susceptibility testing [Bignell 2006, Unemo 2019].

Urethritis in females

In female patients, only a minority of hospitals perform microscopic diagnostics of the urethra routinely, whereby it has not a high predictive value as in males [Randjelovic 2018]. Microscopy might be discussed in patients who are symptomatic. It must be stated that even staining and at least interpretation of smears depends highly on the observer and her/his respective experience. Additionally, the success rate depends much on the microscope that is used, the instrument and the amount of sampling, and thus can never be standardised [Falk 2005]. These findings might explain why we do not recommend staining in asymptomatic females.

Cervicitis

Until today, universally accepted and specific criteria regarding cervicitis are missing. After a careful and thorough assessment of the published literature, we sincerely believe that no clinical assessment is fully able to verify or to exclude STI-related cervicitis.

We decided to define cervicitis as follows: (a) Proof of NG/CT/MG or TV via NAAT from the vaginal or endocervical smear or (b) ≥30, PMN leucocytes/HPF of the endocervical swab.

This definition is in concordance with Marazzo et al., as well as Lanjouw et al., who both highly recommended NAATs for the detection of the causative agent in cervicitis cases only [Marazzo 2007, [Lanjouw 2015]. A lot of other authors used the proof of > 20/30 PMNLs at the cervical smear for the definition of cervicitis. However, it has to be stated that even in cases of non-pathogenous cervicitis, PMNLs might be present, e.g. depending on the female cycle [Falk 2005], and indeed, staining of the cervical smear is no longer recommended by the CDC for diagnosis [Workowski 2021]. Other authors, like Ranjelovic et al. defined cervicitis with >30 PMNLs/HPF at the smear and the presence of cervical discharge, showing an excellent positive predictive value, however, they reported no sensitivity for relevant causative

agents, including NG, CT, MG, or TV [Randjelovic 2018]. In the case of staining, according to the latest recommendations of the CDC, an even lower cut-off value shall be considered to diagnose cervicitis (> 10 PMLS/HPF) [Workowski 2021].

Clinical criteria include mucopurulent discharge, friability (according to CDC), or oedematous ectopy [Workowski 2021, Brunham 1984, Marazzo 2007]. All of these might give a good hint for an underlying infectious disease, but in our point of view do not represent evidence-based 'hard criteria' for the diagnosis of STI-related cervicitis. Furthermore, they are not only subjective and therefore observer dependent. As an appropriate treatment must be based on the causative pathogen, NAATs are in our opinion the only meaningful method of choice for an accurate diagnosis of STI-related cervicitis.

Vaginal specimens might be used as first choice for the diagnosis of cervicitis [Bebear 2009, Lanjouw 2015]. If a purulent wet mount from the vagina is found, it is a hint for cervical inflammation, with a high predictive negative value, meaning if the wet mount is normal, the presence of cervicitis is highly unlikely [Randjelovic 2018].

For vaginal swabs (independent from who is taking the swab – self-assessment or medical staff), however, microscopy and culture are indeed not sufficient. According to the recent literature, cervical smears have much less sensitivity compared to urethral smears [Marazzo 2007, Bignell 2006, Unemo 2020].

We performed a smear in eight females and found signs of inflammation (20 PMNLs) in only two cases. However, according to highly regarded published reports we performed the recommended testing, using NAATs of either vaginal or cervical samples [Bignell 2006], and verified four STIs in those eight patients. A combined urethritis and cervicitis were found in two of these females.

In gonococcal cervicitis, the sensitivity of cervical smears ranges from 16% to 50% [Marazzo 2007, Bignell 2006, Unemo 2020]. Moreover, a cervical Gram-stain is not only not standardized, but even listed as unhelpful for diagnostics by the CDC [Randjelovic 2018]. According to the CDC, regarding urogenital infections, the optimal specimens for gonorrhea screening are vaginal swabs in women [Workowski 2021]. CT cervicitis can not be diagnosed via microscopy, nor clinical assessment since NAATs are necessary for diagnosis. The highest bacterial *chlamydia* load shows endocervical samples, followed by vaginal samples, followed by first void urine [Marazzo 2007 Bebear 2009, Lanjouw 2015]. Appropriate material for detecting CT infections in females therefore are vaginal or cervical swabs, or first void-urine, optimal are vaginal swabs [Workowski 2021].

We recommend additional microscopy and staining of the urethra in symptomatic females, and the usage of NAATs in all females at the urethra and from the vagina (better for TV/NG) or endocervix (better for CT) [Workowksi 2021].

Relevant information concerning the diagnosis of certain pathogens despite NG and CT

TV

In low TV prevalence countries, there are mainly two options for diagnosis. With a high specificity but low sensitivity (45-60%), microcopy can be performed. However, results of the wet mount must be assessed within 10 minutes or less after the take, due to the loss of motility of the protozoon [Sherrard 2018], whereby NAATs have the highest sensitivity (up to 97%) and specificity (up to 99%). [Kissinger 2015, Sherrad 2018]. According to the IUSTI guidelines, vaginal NAATs do represent the recommended test of choice, as endocervical samples have a clearly lower sensitivity (88%) [Kissinger 2015, Sherrard 2018]. Additionally, point-of-care tests (POCT) are available in some countries with high prevalence data [Sherrard 2018, Kissinger 2015].

MG

As mycoplasmae do not have a cell wall, the performance of Gram-staining is not feasible [Nenoff 2017]. A culturing of Ureaplasma is possible, but takes weeks to months, and moreover, they cannot be further differentiated [Horner 2018, Nenoff 2017, Workowski 2021]. In general, mycoplasma diagnostics is also based on NAATs [Nenoff 2017, Workowski 2021]. Until today, in Austria there is no possibility to check for MG resistance, however, that might become inevitable in the future, as high resistance profiles are known for that specific bacterium [Nenoff 2017].

It is recommended to test for MG in symptomatic males (mainly in NGU cases and probably epididymo-orchitis), as well as in females suffering from PID (and probably cervicitis), and proctitis in both sexes [Gnanadurai 2020, Jensen 2016]. The CDC even recommends MG testing only in men suffering from recurrent NGU, and in females with recurrent cervicitis and PID [Workowski 2021]. In a meta-analysis from Lis et al., 174 studies were analysed concerning the relationship between MG and cervicitis. The authors verified a significantly increased risk of cervicitis (pooled odds ratio 1.66 (95% CI, 1.35-1.04)) [Lis 2015].

A meta-analysis from 2015 reported a connection of MG with a just about 2-fold increased risk for cervicitis, PID, preterm delivery, spontaneous abortion, and infertility [Lis 2015]. Statistical significance could be found in MG and cervicitis, PID, preterm delivery, and spontaneous abortion [Lis 2015]. Interestingly, despite the high correlation between MG and cervicitis [Lis 2015], up until today there is no proved association with vulvovaginitis [Wiesenefeld 2017]. However, overall data in the literature seems to be inconsistent and further

analyses are necessary to fully elucidate the pathogenesis and pathogenicity of this bacterium [Weisenfeld 2017].

In our study, we observed no cases of MG infections at the vagina or the cervix.

A multitude of infected patients remain asymptomatic, clear infection spontaneously, and in those, testing/treatment is not recommended [Gnanadurai 2020, Workowski 2021]. Recent data even suggest that an asymptomatic infection might not be pathogenic [Gnanadurai 2020]. This hypothesis is of the outmost importance, as unnecessary treatment might result in higher antimicrobial levels and the threat of multi-resistant bacteria.

Moreover, in 2018 a position statement from the European STI guidelines editorial board was published, stating that MH, UP and UU should not be tested for and consequently not treated [Horner 2018]. Similarly, at present it is not recommended to detect or treat those bacteria, neither in asymptomatic, nor symptomatic patients [Horner 2016]. A colonization is possible, but there is no clear hint for any pathogenicity and development of the disease [Horner 2016]. The editorial board recommended only in the seldom cases of high UU load to bethink the option of treatment. Testing for UP, UU, MH, is not recommended by the CDC [Workowski 2021]. Our own results robustly underline the concept of non-pathogenic colonization, as in the vast majority of those individuals who had the above-mentioned bacteria, no symptoms, nor a urethritis was detected. For that reason, we did not prescribe any medication in those cases to prevent any unnecessary treatment, as mentioned above.

Diagnosis of Extragenital sites in both sexes

As depicted before, in extragenital sites, NAATs represent the recommended mode of detection, as microscopy has a low sensitivity and specificity in those sites, and moreover, the sensitivity of a culture is significantly lower [Unemo 2020, Workowski 2021].

A 10.6. TREATMENT

Neisseria gonorrheae

Treatment was performed due to the above-mentioned guidelines and consisted of ceftriaxone monotherapy or a combination of ceftriaxone and azithromycin. Antimicrobial testing was performed in 11 cases and showed sensitivity to ceftriaxone in every single patient. In the other nine cases of an NG infection, a culture was not created or did not grow. In five patients it was material from the oropharynx, in three the anorectal region, and in one the vaginal site. Interestingly, one patient showed a resistance towards azithromycin. This finding is not unexpected, since azithromycin seems to have higher resistance profiles in the treatment

of NG in latest tests. According to these, the ÖGSTD modified their latest recommendations (ÖGSTD), and changed the first line therapy to ceftriaxone monotherapy due to higher resistance profiles of azithromycin.

Treatment NGU

Some experts still recommend an ex juvantibus treatment of NGU cases in those individuals who have clinical symptoms and the diagnosis of urethritis, with either azithromycin 1 g orally as single dose or doxycycline 100 mg orally twice (BID) a day for a week [Bachmann 2017, Moi 2015]. If the diagnosis for urethritis can not be verified via microscopic analysis, no treatment should be initiated at all [Horner 2016]. This recommendation was in full concordance with our modus operandi.

Chlamydia trachomatis

The majority of our patients were treated with doxycycline 100 mg twice a day for 7 days, a smaller number of patients was treated with azithromycin 1 g p.o. as single dose. According to the guidelines both medications are legitimate options [Bebear 2009, Lanjouw 2015, ÖGSTD], however, doxycycline seems to be superior especially concerning extragenital site infections. Moreover, in the case of ascending infections the duration of treatment shall be prolonged, which was performed in one case of our patients, due to an epididymitis. All patients were encouraged to abstain from sexual intercourse for at least seven days after they and their partner(s) received treatment. A test of cure was offered to all patients after a minimum of three weeks after the end of treatment. It has to be emphasized that a test of cure is not routinely recommended if patients received the above first-line treatment [Lanjouw 2015]. However, a TOC is advised in case of (A) pregnant patients, (B) treatment other than first- line, (C) in the case of complicated infections, (D) recurrent or persisting symptoms, (E) if non-compliance is apprehended, or in (F) extra genital infections [Lanjouw 2015]. Additionally, a new test for CT (to detect possible re-infections) should be offered to young adults under the age of 25 years within the next three to six months after therapy [[Lanjouw 2015].

Mycoplasma genitalium

As mycoplasmae lack a cell wall, β-lactam antibiotics that affect cell wall synthesis, are not effective in this particular bacterium [Nenoff 2017].

In the case of the detection of MG one must keep in mind that doxycycline is only effective up to a third against these bacteria, whereas azithromycin might be effective up to ¾ of infected patients [Bachmann 2015]. As many studies report a very strong correlation of MG

with persistent NGU that initially has been treated with azithromycin, moxifloxacin 400 mg orally for one week and metronidazole 400 mg BID for seven days (which is the treatment for TV) should be administered [ÖGSTD Guideline 2018, Bachmann 2015]. Those who received doxycycline as initial therapy, should receive azithromycin 1g orally as single shot and metronidazole 400 mg BID for seven days [Bachmann 2015].

One must further discuss the fact that for NGU the European guidelines suggest doxycycline 100 mg bid for seven days as first-line therapy [Horner 2016]. This dose might be sufficient for uncomplicated CT infections, reduces the bacterial load and, even though it is not that effective towards MG, it seems not to support the development of tetracycline resistance [Horner 2017]. The reduction of bacterial load due to a 'pre-treatment' with doxycycline has also be mentioned by Gnanadurai et al. in 2020 [Gnanadurai 2020]. As stated, it is of outmost importance to underline that the goal in these cases is clinical-, and not microbiological cure [Gnanadurai 2020]. For this reason, Gnanadurai et al. do not emphasize a TOC for MG infections, whereas in the latest IUSTI Guideline by Jensen et al. it is highly recommended due to high resistance and shall be performed after about three weeks after the ending of therapy [Jensen 2016].

Trichomonas vaginalis

In case of recurrent or persisting TV urethritis, and if a re-infection and non-compliance can be ruled out, the treatment should be repeated (Metronidazole 400-500 mg BID for seven days) [Sherrad 2018]. In case of treatment failure, the dosage of metronidazole must be changed to metronidazole 2 g daily for five to seven days or to metronidazole 800 mg three times a day for one week [Sherrad 2018].

The two positive TV patients in our cohort had no pharyngeal complaints despite the fact that the potential infection was to be at the oropharyngeal region. In cases of MSM, an infection with TV is very unlikely and an administration of metronidazole can be abstained from [Bachmann 2017].

A 10.7. CONCLUSION

We conclude that the majority of urethritis and cervicitis infections would have remained undiagnosed, if testing would have been only performed in symptomatic patients at symptomatic sites, without finding any statistically significant differences across the group of asymptomatic versus symptomatic patients.

In addition, we conclude that three statistically significant risk factors are essential for the acquisition of a STI, namely an age younger than 25 years, non-heterosexual sexual orientation, and the presence of discharge. We believe that it would be inevitable to study the group of particularly adolescents in more detail, as they account for a large and eventually unknown number of STIs.

A 11. LIMITATIONS

By nature, our study is not devoid of important limitations. First, only patients from the STI outpatient's clinic of the department of dermatology and venereology of the Medical University of Graz were included into analyses, as we recruited individuals in the second largest city of Austria, which does not represent the largest STIs 'hot spot' of the country. Second, since another Austrian hospital that treats HIV+ patients, performs its own diagnostics and treatment, this patient population might be particularly underrepresented in our own study cohort. Furthermore, in Austria a lot of females present themselves at their gynecologist first und only for a TOC visit at our department. This is in line with a multitude of male patients presenting at their urologist first in the case of urethral symptoms. Moreover, due to the impacts of the COVID-19 pandemic during the recruitment period, an uncertain number of patients did presumably not present at our STI outpatients' clinic for various reasons. On the other hand, some patients presented themselves in threat of an STI without symptoms, and without a recent sexual intercourse. In addition, a remarkable number of patients who showed clinical signs of a STI, were finally not willing to participate in this study. Finally, we did not obtain information on the use of condoms, contraceptives or other protective methods, or the use of chem-sex.

PART B

Meta-analysis of a rare genital ulcer disease, named ulcus vulvae acutum Lipschütz

B 7. RATIONALE AND AIM

The second aim of this dissertation was to define an accurate and ready-to-use diagnostic algorithm for the rare and thus often underdiagnosed disease of UVAL. No specific guidelines for the diagnosis and treatment of this distinct and important disease are published so far. Therefore, we decided to perform a systematic literature review on UVAL in order to be able to formulate a diagnostic algorithm and appropriate treatment recommendations for this rare disease.

B 8. PATIENTS AND METHODS

According to the PRISMA criteria (Preferred Reporting Items for Systematic Reviews and Meta-Analyses), a systematic literature review was performed [Moher 2009]. PubMed and MEDLINE were sifted through for the following relevant search terms: (1) “ulcus vulvae acutum”, (2) “Lipschütz ulcer”, and (3) “acute genital ulcer AND vulva”. Papers were included into our dataset if they (1) presented case reports, case series or review articles on UVAL; (2) were published in 1990 or later; and (3) were written in English and published in a peer-reviewed journal. Publications were excluded if they (1) were published in another language than English, (2) discussed genital ulcers despite UVAL, or (3) focused on histopathology mainly. Based on our dataset, we extracted information reporting the quantity of cases, ‘type of article’, ‘subject’s age’, ‘count and localization of ulcer(s)’, ‘presence of influenza-like symptoms’, ‘sexual history’, ‘relevant diagnostic work-up’ (including histopathology, blood count, and serology), and ‘treatment/ management’. In addition, we manually browsed all relevant manuscripts listed in the references of the manuscripts included in our dataset, using the same search terms as mentioned above [Sadoghi 2020].

B 9. RESULTS

B 9.1. Analysis of dataset

Based on our inclusion and exclusion criteria 91 reports were selected. Sixty of those papers were excluded for the following reasons: seven, because they did not focus on UVAL,

14 because they were written in another language than English, 39 because they were published before 1990, and 8 because they were duplicates. Finally, twentyone manuscripts were included into our analysis dataset (Table 17). Of these 21 manuscripts, we extracted data of 60 patients, 33 from a case series [Viera-Baptista 2016] and 27 patients from other reports. Table 17 depicts each publication by first author and the year of publication (ranging from 2000 to 2017), type of article (case reports mainly, one case series), and the level of evidence (all level IV) [Sadoghi 2020].

Table 17- Characteristics of included published reports concerning UVAL [Sadoghi 2020]

	First author, year of publication	Type of article	Included cases	Level of Evidence
1	Delgado-Garcia 2014	CR*	1	IV
2	Hernandez Nunez 2008	CR	4	IV
3	Pelletier 2003	CR	1	IV
4	Fremlin 2017	CR	1	IV
5	Wolters 2017	CR	1	IV
6	Mourinha 2016	CR	1	IV
7	Garcia2016	CR	1	IV
8	Vieira-Baptista 2016	CS**	33	IV
9	Haidari 2015	CR	1	IV
10	Kinyo 2014	CR	2	IV
11	Burguete Archel 2013	CR	1	IV
12	Brinca2012	CR	1	IV
13	Truchuelo 2012	CR	2	IV
14	Chanal 2010	CR	1	IV
15	Ales- Fernandez 2010	CR	3	IV
16	Martin 2008	CR	1	IV
17	Sardy 2011	CR	1	IV
18	Wetter 2008	CR	1	IV
19	Trcko 2007	CR	1	IV
20	Svedmann 2004	CR	1	IV
21	Török 2000	CR	1	IV
	Publication years 2000-2017	**CR: n=20 CS: n=1**	**CR: n=27 CS: n=33**	**Level IV: n=21**

*CR: case report

** CS: case series

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The study identification for our systematic literature search is depicted in a flow diagram (Figure 6).

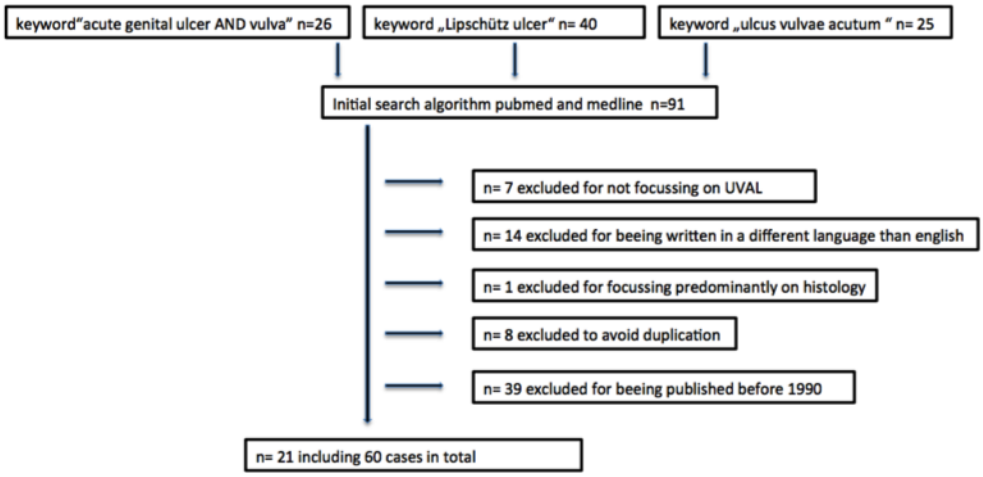

Figure 6- Flow diagram of study identification for literature on ulcus vulvae acutum Lipschütz (UVAL) [Sadoghi 2020]

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B 9.2. Meta-analysis of the literature

The results of our review and meta-analysis are summarized in tables 18, 19 and 20 [Sadoghi 2020]. Table 18 describes extracted data concerning the age of individuals, whether oral apthosis was present, if they had influenza-like symptoms, how many ulcers were found, and where they were localized, as well if individuals had a prior sexual contact (ever).

Table 18- Demographic and clinical data of included patients suffering from UVAL [Sadoghi 2020]

	age of patient	oral apthosis	Flu- like symptoms	amount of ulcers	localization of ulcers	prior sexual contact
1	13 years	-	+	1	LMA *	-
2	14 years	NA	+	4	LMI **	-
	14 years	NA	+	1	LMI	-
	12 years	NA	+	3	NA	-
	14 years	NA	+	1	LMA	-
3	25 years	-	+	1	LMI	-
4	14 years	NA	+	1	LMI	-
5	18 years	NA	+	3	LMI (2) urtehral orifice (1)	-
6	22 years	-	+	multiple	LMI, introitus	-
7	12 years	-	-	2	LMA	-
8	10–79 years	NA #	+ ##	11 patients: single ulcer	Vestibule (19), LMI (10), clitoris (1), interlabial sulcus (1), LMA (2)	NA###
9	15 years	-	+	3	LMI	-
10	10 years	NA	+	2	LMI	-
	25 years	+	+	3	LMI (2) et LMA (1)	NA
11	17 months	+	+	2	vaginal introit and perineum	-
12	30 years	-	+	2	LMI	+
13	11 years	-	-	1	LMI	-

	11 years	-	+	2	LMI	-
14	21 years	-	-	3	LMA and LMI	+
15	16 years	NA	+	1	LMI	-
	15 years	NA	+	1	LMI	-
	2 months	NA	-	1	LMI	-
16	16 years	NA	+	multiple	LMI	+
17	16 years	-	+	multiple	LMA and LMI	-
18	13 years	+	+	3	LMI	-
19	29 years	NA	-	2	LMI	+
20	14 years	+	+	1	LMA	-
21	17 years	-	+	4	LMI et LMA	-
	CR: **2 months- 30 years** **(mean: 15.5)** **CS:** **10-79 years, mean** **NA)**	**CR:** **yes: n=4** **no: n= 11** **NA: n= 12** **CS:** **yes: 4 (NA)** **no: NA** **NA: NA**	**CR:** **yes: n=22** **no: n= 5** **CS:** **yes: n=17** **no: n=NA**	**CR/ (CS):** **1: n= 10/(11)** **2: n= 6/(NA)** **3: n= 6/(NA)** **4 or more: n=5/(NA)**	**CR/ (CS):** **LMI: n= 21/(10)** **LMA: n= 8/(2)** **Introitus: n= 2/(NA)** **Urethral orificae: n= 1/(NA)** **Perineum: n= 1/(NA)** **Vestibule: n= NA/(19)** **Clitoris: n= NA/(1)** **Interlabial sulcus: n= NA/(1)**	**CR/ (CS):** **yes: n= 4/(NA)** **no: n= 22/(NA)** **NA: n= 1/(NA)**

Not defined whether oral aphtosis- but likely in 4 patients
Flu- like symptoms were described as fever in 11 patients; myalgia in 6 patients
Sexual debut 84.4%, but not defined whether ulcers occurred ‘simultaneously’
* labia majora (LMA)
** labia minora (LMI)
CR: case report
CS: case series

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Table 19 encompasses the individual diagnostic work up and management including histopathological analysis, blood count and serology.

Table 19- Individual diagnostic work-up of the different manuscripts in order to identify UVAL [Sadoghi 2020]

	histologic analysis (result, if applicable)		blood count		Serology		Serological results if positive	
			HSV	EBV	syphilis	HIV	others	
1	-	+	-	-	-	ND	-	
2	-	+	-	+	-	-	-	EBV IgG and IgM positive
	+ (unspecific)	+	-	-	-	-	-	
	-	+	-	-	-	-	-	
	-	+	-	-	-	-	-	
3	-	NA	+	+	-	-	-	EBV: IgG positive HSV: IgG positive
4	+ (unspecific)	NA	NA	NA	NA	NA	NA	
5	-	+	-	+	-	ND	-	EBV: IgM positive
6	-	NA	-	-	-	ND	-	
7	-	NA	-	-	-	-	-	
8	-	NA *	NA	+	NA	NA	NA	EBV: n=2 positive (not defined whether IgM or IgG)
9	-	NA	-	+	-	ND	-	EBV: IgG positive
10	-	+	-	-	-	ND	-	
	-	+	+	+	-	ND	+	EBV: IgG positive HSV: IgG positive

								Infection of influenza B &adenovirus
11	+ (unspecific)	+	-	+	-	-	-	EBV: IgM positive
12	+ (unspecific)	+	-	+	-	-	-	EBV: IgG positive
13	-	NA	ND	-	-	-	-	
	-	NA	ND	-	-	-	-	
14	-	+	-	-	-	-	+	Mumps: IgM & IgG positive
15	-	+	ND	-	-	-	-	
	-	+	ND	-	-	-	-	
	-	NA	NA	NA	NA	NA	NA	NA
16	-	+	-	+	-	-	+	EBV: IgG positive CMV: IgM& IgG positive
17	+ (unspecific)	+	-	+	-	-	-	EBV: IgG & IgM positive
17	-	NA	NA	NA	NA	NA	NA	
19	-	+	ND	-	-	-	-	
20	-	NA	ND	+	ND	ND	+	EBV: IgM positive
21	-	+	-	-	-	ND	-	
	CR / (CS): yes: n= 5/(0) no: n= 23/(33)	**CR / (CS): yes: n= 17/(NA) NA: n= 10/(NA)**	**+: n= 2 -: n= 16 ND: n=6 NA: n= 4**	**+: n= 10 (plus n=2 of CS) -: n= 14 ND: n= 0 NA: n=3**	**+: n= 0 -: n= 23 ND: n= 1 NA: n=4**	**+: n=0 -: n= 16 ND: n= 8 NA: n= 4**	**+: n=4 -: n= 20 ND: n= 0 NA: n= 4**	

NA: not available; ND: Not done * (blood count in case of severe systemic symptoms or delayed healing

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Table 20 summarizes management, therapeutic actions and outcomes (recurrence, relapse or sequela).

Table 20- Treatment protocols and outcomes for patients with UVAL [Sadoghi 2020]

	Treatment	resolved without sequelae after treatment	Recurrence
1	SA, OT	+	-
2	SS, SA	+	-
	SA	+	-
	OT	+	NA
	SS, TA	+	NA
3	SA	+	NA
4	SS	+	NA
5	SA	+	-
6	SA, TS	+	-
7	OT	+	-
8	NA	NA	NA
9	OT	NA	NA
10	SS, OT	+	NA
	SA, SS	NA	-
11	SA	+	-
12	SA	+	-
13	SH	+	-
	SH	+	-
14	OT	+	-
15	TA	+	-
	TA	+	-
	NA	+	-
16	TA, OT	+	-
17	SS, SA, OT	+	-
18	TS	+	+
19	SA, TA, SS	-	NA
20	SS	+	-
21	SA, OT	-	-
	CR/ (CS): **SA: n= 12 (NA)** **TA: n= 5 (NA)** **SS: n= 8 (NA)** **TS: n=2 (NA)** **SH: n=2 (NA)** **OT: n= 9 (NA)**	**CR / (CS):** **yes: n= 23 (NA)** **no: n= 2 (NA)** **NA: n= 2 (NA)**	**CR/ (CS):** **Yes: n=1 (NA)** **No: n= 19 (NA)** **NA n= 7 (NA)**

+: yes; -: no; TA: topical antibiotics TS: topical steroids SA: systemic antibiotics SS: systemic steroids OT: others SH: spontaneous healing; NA: not available

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We had no access to the raw data regarding the case series of the 33 patients published by Viera-Baptista in 2016, therefore it was not possible to fully evaluate the individual management of these patients. Thus, we decided to present data of the 20 individual case reports for 27 patients first, and the results from our analysis of the case series of 33 patients [Vieira Baptista 2016] second [Sadoghi 2020].

B 9.3. Twenty-seven individual case reports

The mean age of females ranged from two months to 30 years (mean 15.5 years). In four (14.8%) patients oral aphthosis was mentioned. In 22 (81.5%) females' flu-like symptoms were recorded. In ten (37.0%) patients a solitary ulcerous lesion was depicted. Two ulcers were found in six (22.2%) females, three ulcers in equally six patients, and more than four ulcers in five (18.5%) females. In most patients, labia minora (22 patients, 81.5%) and labia majora (7 patients, 26.0%) were affected. Moreover, the introitus vaginae was reported as affected region in three (11.1%) patients, urethral orifice in one (3.7%) patient, and perineum in equally one patient. A previous sexual contact was reported in four (14.8%) patients, Histology via biopsy was assessed in five (18.5%) patients. In most females, a blood count (17 patients, 62.9%) and serological testing (24 patients, 88.8%) was performed. Serology included testing of mainly viruses, such as herpes simplex virus (HSV), varicella zoster virus (VZV), Epstein barr virus (EBV), cytomegaly virus (CMV), hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), mumps, parvovirus B 19 (PVB19) influenza B, and adenovirus. Bacteria that were searched for were CT, chlamydia pneumoniae, TP, or mycoplasmae. The pathogen tested most frequently was EBV (24 patients, 88.8%). Treatment consisted of local and systemic antibiotics in 15 (55.5%) females, local and systemic steroids in seven (25.9%) patients, analgesics in six (22.2%) patients, and surgery in one (3.7%) patient. Complete healing was observed in 23 (85.2%) patients, healing with sequelae like scarring in two (7.4%) patients. In equally two females no outcomes were reported. A recurrence of the ulcer was described in one (3.7%) patient [Sadoghi 2020].

B 9.4. Case series

In the case series of Vieira-Baptista, the age ranged from ten to 79 years. In four (12.1%) females aphthosis was described, however, information about the exact localization was missing. In about the half, influenza-like symptoms were described (17 patients, 51.5%). A solitary ulcer was described in eleven (33.3%) patients, no occurrence of multiple ulcers was described in any patient. The ulcer occurred most frequently at the vestibule (19 patients,

57.6%), labia minora (10 patients, 30.3%), and labia majora (two patients, 6.0%). Moreover, in one female, the ulcer was described at the clitoris (3%), and one patient had the ulcer at the interlabial sulcus. In the majority of individuals (80%), a previous sexual contact was documented. However, no time frame was to be reported. In not a single case, a histologic report was available. If severe systemic symptoms or delayed heeling were reported, an analysis of blood count and serological testing were done. Of those, in two females a positive EBV results was described. Treatment and outcomes were not described in that case series [Viera-Baptista 2016, Sadoghi 2020].

B 9.5. Algorithm for diagnosis and treatment

Based on our systematic review, we propose the following algorithm and define two major and four minor criteria for a standardized diagnosis of UVAL. For the diagnosis of UVAL, two major criteria and ≥ two minor criteria must apply [Sadoghi 2020].

- Major criteria:

(1) Acute onset of one or more painful ulcerous lesions at the vulvar region

(2) Exclusion of infectious and other non-infectious possible causes for the ulcer (see Tables 21 and 22)

- Minor criteria:

(1) Localization of the ulcer(s) at vestibule or labia minora

(2) Virginity or no sexual intercourse within the last three months

(3) Presence of Flu-like symptoms

(4) Systemic infection within two to four weeks prior to the onset of the vulvar ulcer

Table 21- Recommended algorithm for exclusion of infectious diseases in order to identify UVAL [Sadoghi 2020]

Sexually-transmitted infections	- Exclusion of *Treponema pallidum* (dark field microscopy/ serological testing) - HSV Tzanck test and/ or PCR (swab) - In case of sexual intercourse check for gonorrhoea, *chlamydia trachomatis*, *haemophilus ducreyi* (if frequent in your country)
Other infectious conditions	- Bacteria culture - Microscopic analysis of fungi

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Table 22- The most common diseases mimicking UVAL [Sadoghi 2020]

DISEASE	Aid to differentiate
Infectious diseases	
Herpes genitalis	1.) HSV 1 or HSV 2 is to be found via swab and amplification technique (PCR) or Tzanck test 2.) Lesions are typically smaller 3.) Often typical history (previous or partners herpes infection) 4.) Often recurrent; history of labial herpes possible 5.) Sexually and non-sexually transmission possible
Primary syphilis lesion	1.) May be painful, but in the vast majority it is not painful 2.) In the majority of cases there is only one lesion, multiple are possible, but this is rare 3.) Lymphadenopathy is often predominant 4.) Serological tests or dark field microscopy will verify infection with *Treponema pallidum* 5.) A previous sexual contact is reported
Ulcus molle= Chancroid	1.) Rarely seen in western European countries 2.) Endemic in tropic/ subtropic regions 3.) Swab from ulcer: gram stain from ulcer: "school of fish"chain, culture, PCR
Lymphogranuloma venereum	1.) Mainly in MSM 2.) Check for chlamydia trachomatis (type L1-3) via PCR 3.) Mainly anorectal symptoms
Non - infectious systemic conditions	
Crohn's Disease	1.) Usually recurrent ulcers and erosions, mainly at the anal or perineal region, seldom at vulva and vagina 2.) Predominantly fistulas 3.) Ask for diarrhoea or gastrointestinal problems
Side effects of medication (e.g. methotrexate)	1.) Take medical history of medications on regular basis but also those on demand and check the correct intake/ dosage! 2.) Consider ulcerous variant of fixed drug eruption 3.) Consider variants of erythema multiforme variants and check integument
Topicals	Some patients may use inappropriate emollients
Behçet's disease	1.) History of recurrent oral aphthae and genital aphthae, as well as uveitis/ retinal vasculitis 2.) Mainly men – between 20 and 40 years 3.) Various other organs may be involved- skin,

	gastrointestinal tract, neurological, vascular disease, or arthritis. 4.) Positive pathergie test
Bullous diseases	1.) Indirect immunofluorescence 2.) Direct immunofluorescence 3.) Histopathology 4.) ANA; ENA
Traumatic cause	Sexual intercourse/ mechanic manipulation/ dermatitis factitia
Malignant tumors	1.) Exlusion via histological analysis 2.) Mainly in elderly 3.) Usually slow appearance over time

Reproduced from Sadoghi et al. *Ulcus vulvae acutum Lipschütz: a systematic literature review and a diagnostic and therapeutic algorithm [Sadoghi 2020]. Reproduced with permission of „JEADV".*

After exclusion of the most common infections or localized or systemic diseases that are able to mimic UVAL (Tables 21 and 22), symptomatic treatment to facilitate healing is recommended (Figure 7) [Sadoghi 2020].

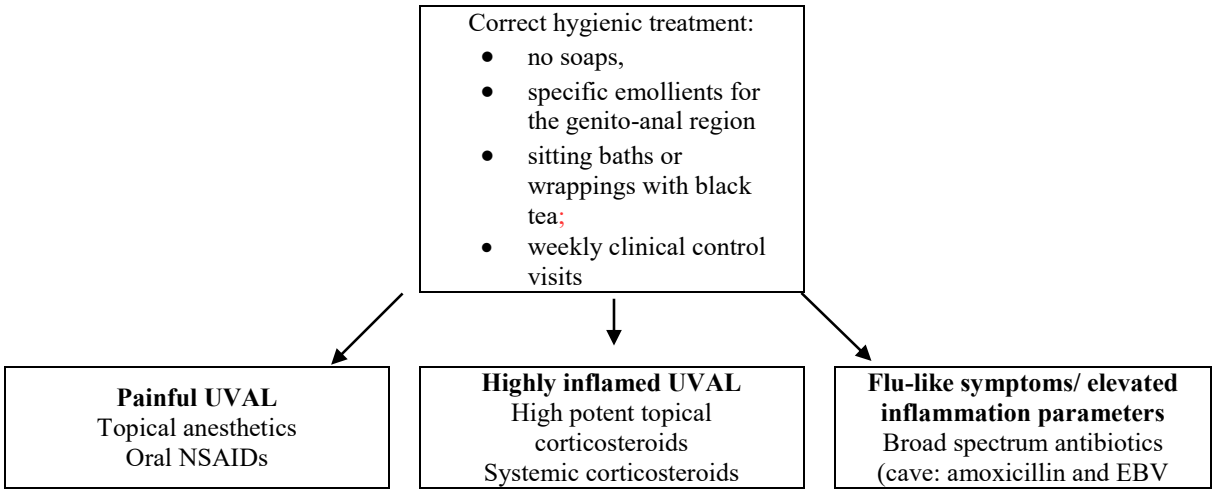

Figure 7- Proposed treatment algorithm for UVAL [Sadoghi 2020].

Reproduced from Sadoghi et al. *Ulcus vulvae acutum Lipschütz: a systematic literature review and a diagnostic and therapeutic algorithm [Sadoghi 2020]. Reproduced with permission of „JEADV".*

Interestingly, spontaneous healing might happen within a few weeks [Delgado 2014, Vieira-Baptista 2016, Maliyar 2019]. Specific emollients for the genital region should be recommended, as well as topical disinfectants or sitz baths [Maliyar 2019, Sadoghi 2020]. In the case of elevated laboratory parameters hinting towards a bacterial infection, broad-spectrum antibiotics shall be considered. To provide pain relief, topical anesthetic gels are suggested [Maliyar 2019, Huppert 2010, Wetter 2003], whereby NSAIDs shall be considered in every patient [Sadoghi 2020, Maliyar 2019]. High-potency topical corticosteroids shall be used in superficial ulcers, systemic corticosteroids in deep ulcerous disease [Wetter 2008, Brinca 2012, Delgado 2014]. Weekly control visits are highly recommended until subjective and objective improvement of the patient sets in [Huppert 2010].

B 10. Discussion

Genital ulcers occur due to various infectious and non-infectious etiologies. However, most genital ulcers are caused by a STI. As we recognized that most genital ulcers can be easily and clearly be identified via histology, serology or NAATs (see Table 21), which is not the case in UVAL, we decided to perform a systematic literature review and meta-analysis of this important topic.

Ulcus vulvae acutum Lipschütz describes a rare and suddenly occurring painful ulcer at the vulval region of unclear genesis [Ales Fernandes 2010, Truchuelo 2012, Delgado 2014, Hernandez-Nunez 2008, Vieira-Baptista 2016, Sadoghi 2020]. In its classic form, these ulcers present as single and large (>1 cm) lesions. However, it is not uncommon that up to four or so called 'kissing' ulcers are present [Sardy 2011, Huppert 2010, Sadoghi 2020].

In the systematic literature review and meta-analysis we found a single ulcer in ten females. Two ulcers were described in six patients, three ulcerous lesions were found in six patients as well, and ≥4 ulcers were reported in five patients [Sadoghi 2020]. Our findings are in line with a case series of Vieira-Baptista et al. They reported that multiple ulcers are more frequent (66.7% in their published UVAL case series including 33 patients) [Vieira-Baptista 2016]. We therefore proposed the compulsory major criteria number 1: 'acute onset of one or more painful ulcerous lesion at the vulvar region'.

Since the presence of a genital ulcer is a clear hint for a STI, it is of outmost importance to rule out classic STIs, including above all, primary syphilis and herpes genitalis, more seldom lymphogranuloma venereum (*Chlamydia trachomatis* L1-L3) or chancre (*Haemophilus ducreyi*) [Sadoghi 2020, Huppert 2010]. Therefore, a thorough report of a patients

comprehensive medical history, including sexual behavior must be performed. Non-infectious causes for GUD include mainly Behçet's disease, hidradenitis suppurativa, pyoderma gangraenosum, or malignant tumors among others [Roett 2012, Huppert 2010 Vieira-Baptista 2016, Kirshen 2015, Delgado 2014]. The necessary exclusion of the above-mentioned diseases (please s. Tables 21, 22) is recommended as major criteria no. 2: 'Exclusion of infectious and non-infectious causes for the ulcer.'

Clinically, deep ulcers with sharply demarcated borders and yellowish or greyish, fibrinous coatings are characteristic [Huppert 2010, Truchuelo 2012, Svedman 2004, Delgao 2014, Sadoghi 2020]. Due to our systematic literature review, the main location seems to be the labia minora (22/27; 81.5%) [Sadoghi 2020]. However, as described before, UVAL can be detected elsewhere [Vieira-Baptista 2016 Huppert 2010], like Vieira-Baptista and colleagues described the majority of UVAL being located on the vestibule (57.6%) and labia minora (30.3%) [Vieira-Baptista 2016]. Concluding the data, we decided to recommend those findings via as minor criteria number 1: 'Localization of ulcer(s) at vestibule or labia minora.'

Ulcus vulvae acutum Lipschütz is typically a disease in adolescents and young adults. Our results are in line with this fact, as most females evaluated in our systematic review were younger than the age of 16 years (19/27; 70.3%). Most of them had no sexual intercourse (23/27; 85.2%). Interestingly, Vieira-Baptista et al. described in their case series that 84.4% were no virgins [Vieira-Baptista 2016]. However, it has to be stated that is was not questioned how recently sexual intercourse had happened. Due to our findings, we advise that sexual activity shall be used as a minor criteria number 2: 'Virginity or no sexual intercourse within the last three months.'

Influenza-like symptoms, encompassing fever, chills, fatigue or malaise, were described in most patients (22/27; 81.5%) that were reviewed. This is in concordance with other authors [Huppert 2010, Ales Fernandes 2010, Truchuelo 2012, Hernendez-Nunez 2008]. Due to the fact that systemic symptoms are reported in most cases of UVAL, we recommend to use minor criteria number 3: 'Flu-like symptoms'.

The detailed etiopathogenesis of UVAL is still not fully elucidated yet. Several bacteria and viruses have been called causative for this disease. *Epstein Barr Virus, mycoplasma pneumoniae, cytomegalovirus, toxoplasmosis, influenza virus, mumps, salmonella* or *PVB19,* they all have been linked to UVAL [Wetter 2008, Sardy 2011, Chanal 2010, Huppert 2010, Truchuelo 2012, Pelletier 2003, Vieira-Baptista 2016]. Many authors performed serological

testing or used amplification techniques and had a blood count performed (17 of 27 cases (62.9%)).

The majority of publications reported serological testing for at least some infectious diseases (s. Table 19). The parameter, which was most frequently looked for, was EBV (24/27; 88.8%). However, an active infection with EBV was found in five patients only. Active infections were observed in one patient for influenza B, adenovirus [Kinyo 2014], mumps [Chanal 2010], CMV [Martin 2008], and salmonella parathyphii A [Pelletier 2003]. Interestingly, a case report emerged just recently, as Falkenhain-Lopez and colleagues handled an acute genital ulcer that occurred after a SARS-2 infection [Falkenhain-Lopez 2020]. It is not unlikely that UVAL might arise due to a SARS-2 infection, as the occurrence of UVAL is known for its infectious connexon [Sadoghi 2020, Maliyar 2019]. Furthermore, UVAL was also linked to an immunodeficiency in two girls with partial IgA deficiency [Kinyo 2014]. Due to the results of our meta-analysis, we decided to include an additional minor criteria number 4 for 'systemic infection within the last 2-4 weeks prior to onset of vulvar ulcer.'

Histological evaluation seems to be of no major help in the diagnosis of UVAL as it is non-specific [Vieira-Baptista 2016]. This is in line with our findings, as results from punch biopsy were done in 4/27 (14.8%) patients, and these results were also non-specific [Sadoghi 2020].

Based on our systematic literature review and meta-analysis, we recommend to use the above listed major and minor criteria for a standardized and accurate diagnosis of UVAL.

B 11. Limitations

This meta-analysis has several limitations. First, the included studies were mainly case reports and only one larger case series, and therefore had small sample sizes each. However, in studying rare diseases case reports are inevitable. Our results might be limited to results of the included studies, since studies in another language then English and all studies that were published before 1990 were excluded.

12 Overall conclusion

To the best of our knowledge, we were the first to perform a prospective study focusing on the prevalence, diagnosis, and treatment of STI related urethritis and cervicitis in the overall population of Austria. We identified a large number of STIs (close to 40%) in our study cohort, despite the fact that we observed more than 70% heterosexual individuals included. The prevalence of urethritis was 22.5%, and the prevalence of cervicitis was 20%. Helpful for any clinician working at adjacent specialties of STIs, we were able to verify three statistically significant risk factors for the acquisition of a STI [(Age younger than 25 years (p: 0.042), non-heterosexual sexual orientation (p: 0,027) and presence of discharge (p: 0,001)]. It seems to be a fact that a lot of patients do not present themselves at STI specialized clinics, as there are only a few existing in Austria. Moreover, according to their symptoms, most patients seem to present themselves at urologists or gynecologists first. Those specialists, however, seem to seldomly ask for a detailed sexual history. In any case, a correct diagnosis is critical for specific treatment, especially in times of high resistance profiles of some pathogens. Extragenital sites are for sure overlooked sites in most clinical settings. A lot of clinicians are confronted with probably STI related symptoms and education in this field is unquestionable necessary, as STIs are globally on the rise, and this might not be apparent to other specialists. Moreover, it is inevitable to understand local epidemiology, especially in the field of infectious diseases.

We recognized that most of the underlying diseases causing genital ulcers in Central European countries can be clearly identified via serology, histology, or molecular examinations, but this was not the case for UVAL. We therefore decided to perform a systematic literature review and worldwide meta-analysis, to be able to propose a clinically useful diagnostic algorithm including two major and four minor criteria. Our results were recently published in the European Journal of European Academy of Dermatology and Venereology (JEADV). We do hope that these results might provide several helpful tools for not only an accurate diagnosis, but also recommendations concerning the management of the often-underdiagnosed disease of UVAL.

13. References

Alés Fernandes M, Rodriguez Pichardo A, Garcia Bravo B, Ferrandiz Pulido L, Camacho Martinez FM: Three cases of Lipschutz vulval ulceration. *Int J STD AIDS* 2010; 21: 375-6.

Arabi M, Delavari M, Fakhrieh-Kashan Z, Hooshyar H. Review of *Trichomonas vaginalis* in Iran, Based on Epidemiological Situation *J Reprod Infertil* 2018; 19: 82-88.

AWMF Guideline. Gonorrhea- Diagnostik und Therapie der Gonorrhoe 2018. https://www.awmf.org/uploads/tx_szleitlinien/059-004l_S2k_Gonorrhoe-Diagnostik-Therapie_2019-03.pdf

AWMF Guideline. S2k Leitlinie. Infektionen mit *Chlamydia trachomatis.* https://www.awmf.org/uploads/tx_szleitlinien/059-005l_S2k_Chlamydia-trachomatis_Infektionen_2016-12-abgelaufen.pdf (13.01.2022)

Bachmann LH, Manhart LE, Martin DH, Seña AC, Dimitrakoff J, Jensen JS, Gaydos CA. Advances in the Understanding and Treatment of Male Urethritis. *Clin Infect Dis* 2015; 61: 763–9.

Bebear C, de Barbeyrac B. Genital Chlamydia trachomatis infections. *Clin Microbiol Infect* 2009; 15: 4–10.

Berntsson M, Tunbäck P. Clinical and microscopic signs of cervicitis and urethritis: correlation with chlamydia trachomatis infection in female STI patients. *Acta Derm Venereol* 2013; 93: 230-233.

Bing-jie Z, Yue-ping Y, Yan H et al. The prevalence of urethral and rectal Mycoplasma genitalium. *BMC Public Health* 2014; 14: 195-202.

Bignell C, Ison CA. Gonorrhoea. *Sex Transm Inf* 2006; 82: 6-9.

Birrell JM, Gunathilake M, Singleton S, et al. Characteristics and impact of disseminated gonococcal infection in the “top end” of Australia. *Am J Trop Med Hyg* 2019; 101: 753–760.

Brinca A, Canelas MM, Carvalho MJ, Vieira R, Figueiredo A. Lipschütz ulcer (ulcus vulvae acutum): a rare cause of genital lesion. *An Bras Dermatol* 2012; 87: 622-4.

Brunham RC, Paavonen J, Stevens CE, Kiviat N, Kuo CC, Critchlow CW, Holmes KK. Mucopurulent cervicitis--the ignored counterpart in women of urethritis in men. *NEJM* 1984; 311: 1-6.

Brunham RC, Gottlieb SL, Paavonen J. Pelvic Inflammatory Disease. *NEJM* 2015; 372: 2039-48.

Bulur I, Onder M. Behçet disease: New aspects. *Clinics in Dermatology* 2017; 35: 421–434.

Burguete Archel E, Ruiz Goikoetxea M, Recari Elizalde E, Beristain Rementería X, Gómez Gómez L, Iceta Lizarraga A. Lipschütz ulcer in a 17-month-old girl: a rare manifestation of Epstein-Barr primoinfection. *Eur J Pediatr* 2013; 172: 1121-3.

Carlin EM; Ziza JM, Keat A, Janier M. 2014 European Guideline on the management of sexually acquired reactive arthritis. *Int J STD Aids* 2014; 25: 901-912.

Chanal J, Carlotti A, Laude H, Wallet-Faber N, Avril MF, Dupin N. Lipschütz genital ulceration associated with mumps. *Dermatology* 2010; 221: 292-5.

CDC guideline
https://www.cdc.gov/std/treatment-guidelines/urethritis-and-cervicitis.htm
(checked at 3/12/2021)

European Centre for Disease Prevention and Control. Chlamydia infection. In: ECDC. Annual epidemiological report for 2018. Stockholm: ECDC; 2020.
https://www.ecdc.europa.eu/sites/default/files/documents/AER-for-2018-STI-chlamydia.pdf

Crucitti T, Jespers V, Mulenga C, Khondowe S, Vandepitte J, Buve A. Non- sexual transmission of Trichomonas vaginalis in adolescent girls attending school in Ndola, Zambia. *PLoS One*. 2011; doi.org/10.1371/journal.pone.0016310.

Davatchi A, Assaad-Khalil S, Calamia KT et al. International Team for the Revision of the International Criteria for Behçet's Disease (ITR-ICBD). The International Criteria for Behçet's Disease (ICBD): A collaborative study of 27 countries on the sensitivity and specificity of the new criteria. *JEADV* 2014; 28: 338-347.

Delgado-García S, Palacios-Marqués A, Martinez-Escoriza JC, Martin-Bayon TA. Acute genital ulcers. *BMJ Case Rep* 2014; doi:10.1136/bcr-2013-202504.

Dudareva-Vizule S, Haar K, Sailer A, Wisplinghoff H, Wisplinghoff F, Marcus U. Prevalence of pharyngeal and rectal Chlamydia trachomatis and Neisseria gonorrheae infections aming men who have sex with men in Germany. *Sex Trans Infect* 2014; 90: 46-51.

Falk L, Fredlund H, Jensen JS. Signs and symptoms of urethritis and cervicitis among women with or without Mycoplasma genitalium or Chlamydia trachomatis infection. *Sex Transm Infect* 2005; 81: 73–78.

Falkenhain-Lopez D, Agud-Dios M, Ortiz- Romero PL, Sanchez-Velazquez A. COVID-19-related acute genital ulcers. *JEADV* 2020; doi:10.1111/jdv.16740.

Farhi D, Wendling J, Molinari E, Raynal J, Carcelain G, Morand P, Avril MF, Francès C, Rozenberg F, Pelisse M, Dupin N. Non-sexually related acute genital ulcers in 13 pubertal girls: a clinical and microbiological study. *Arch Dermatol* 2009; 145: 38-45.

Fremlin AG, Irani S, Loffeld A. It's not always that simplex. *Arch Dis Child* 2018; doi: 10.1136/archdischild-2017-313749.

García JG, Pavón BM, Martín LM, Martínez BF, Bornielle CM, Caro FA. Lipschütz ulcer: A cause of misdiagnosis when suspecting child abuse. *Am J Emerg Med* 2016; doi: 10.1016/j.ajem.2015.12.027.

Gnanadurai R, Fifer H. Mycoplasma geniatlium: A review. *Microbiology* 2020; 166: 21–29.

Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. *Lancet* 2008; 371: 75–84.

Gottesmann T, Yossepowitch O, Samra Z, Rosenberg S, Dan M. Prevalence of mycoplasma genitalium in men with urethritis and in high risk asymptomatic males in Tel Aviv: a prospective Study. *Int J STD Aids* 2017; 28:127-132.

Haidari G, MacMahon E, Tong CY, White JA. Genital ulcers: it is not always simplex . . . . *Int J STD AIDS* 2015; 26:72-3.

Huppert JS. Lipschutz ulcers: evaluation and management of acute genital ulcers in women. *Dermatol Ther* 2010; 23: 533-40.

Hernandez-Nunez A, Cordoba S, Romero-Mate A, Minano R, Sanz T, Borbujo J. Lipschütz ulcers--four cases. *Pediatric Dermatol* 2008; 25: 364-7.

Horner PJ, Martin DH. Mycoplasma genitalium infection in men. *J Infect Dis* 2017; 216: 396–405.

Horner PJ, Blee K, Falk L, van der Meijden W, Moi H. 2016 European guideline on the management of non-gonococcal urethritis. *Int J STD AIDS* 2016; 27: 928–37.

Horner P, Doners G, Cusini M, Jensen JS, Unemo M. Should web e testing for urogenital Mycoplasma hominis, Ureaplasma parvum and Ureaplasma urealyticum in men and women? – a position statement from the European STI Gudielines Editorial Board. *JEADV* 2018; 32: 1845-1851.

Jadad A, Moore R, Carrol D, Jenkinson C, Reynolds D, Gavaghan D, et al. Assessing the quality of reports of randomized clinical trials: is blinding necessary? *Control Clin Trial* 1996; 17: 1-12.

Janier M, Unemo M, Dupin N, Tiplica GS, Potocnik M, Patel R. 2020 European guideline on the management of syphilis. *JEADV* 2020; doi: 10.1111/jdv.16946.

Jansen K, Steffen G, Potthoff A, Schuppe AK, beer D, Jessen H, Scholten S, Spornraft-Ragaller P, Bremer V, Tiemann C and MSM Screening Study group. STI in times of PrEP: high prevalence of chlamydia, gonorrhea, and mycoplasma at different anatomic sites in men who have sex with men in Germany. *BMC Infect Dis* 2020; doi: 10.1186/s12879-020-4831-4.

Jensen JS; Cusini M, Gomberg M, Moi H. 2016 European guideline on Mycoplasma genitalium infections *JEADV* 2016; doi: 10.1111/jdv.13849.

Jordan SJ, Toh E, Williams JA, Fortenberry L, LaPradd ML, Katz BP, Batteiger BE, Nelson DE, Batteiger TA. Aetiology and prevalence of mixed-infections and mono- infections in non-gonococcal urethritis in men: a case-control study. *Sex Transm Infect*. 2020 96: 306–311.

Kent CK, Chaw JK, Wong W, Liska S, Gibson S, Hubbard G, Klausner JD. Prevalence of rectal, urethral, and pharyngeal Chlamydia and Gonorrhea detected in 2 Clinical Settings among Men Who have Sex with Men: San Francisco, California, 2003; *Clin Infect Dis* 2005; 41: 67–74.

Khatib N, Bradbury C, Chalker V, Koh GCKW, Smit E, Wilson S, Watson J. Prevalence of Trichomonas vaginalis, Mycoplasma genitalium and Ureaplasma urealyticum in men with urethritis attending an urban sexual health clinic. *Int J STD AIDS* 2015; 26: 388-92.

Kinyo A, Nagy N, Olah J, Kemeny L, Bata- Csörgö Z. Ulcus vulvae acutum Lipschütz in two young female patients. *Eur J Dermatol* 2014; 24: 361-4.

Kirshen C, Edwards L. Noninfectious genital ulcers. *Semin Cutan Med Surg* 2015; 34:187-91.

Kissinger P. Trichomonas vaginalis: a review of epidemiologic, clinical and treatment issues; *BMC Infect Dis* 2015; doi: 10.1186/s12879-015-1055-0

Laetsch Semadeni B, Lautenschlager S. Differenzialdiagnose der genitalen Ulzera Teil 1. *Swiss Med Forum* 2009; 09: 45-49.

Lallemand A, Bremer V, Jansen K, Nielsen S, Münstermann D, Lucht A, Tiemann C. Prevalence of Chlamydia trachomatis infection in women, heterosexual men and MSM visiting HIV counselling institutions in North Rhine-Westphalia, Germany - should Chlamydia testing be scaled up? *BMC Infect Dis* 2016; 16: 610.

Lanjouw E, Ouburg S, de Vries HJ, Stary A, Radcliffe K, Unemo M. 2015 European guideline on the management of Chlamydia trachomatis infections. *Int J STD Aids* 2015; doi:10.1177/0956462415618837.

Lautenschlager S, Kemp M, Christensen JJ, Mayans MV and Moi H. 2017 European guideline for the management of chancroid. *Int J STD & AIDS* 2017; doi: 10.1177/0956462416687913

Lipschütz B. Über eine eigenartige Geschwulstform des weiblichen Genitales (Ulcus vulave acutum). *Arch Dermatolol Syph (Berlin)* 1913; 363-95.

Lis R, Rowhani-Rahbar A, Manhart LE. *Mycoplasma genitalium* infection and female reproductive tract disease: a meta-analysis. *Clin Infect Dis* 2015; 61: 418–26.

Lusk MJ, Konecny P. Cervicitis: A review. *Current Opinion in infectious diseases*. 2018; 21: 49-55.

Marazzo JM, Martin DH. Management of women with cervicitis. *CID* 2007: 44: 102-110.

Maliyar K, Mufti A, Syed M, Selk A, Dutil M, Bunce PE, Alavi A. Genital ulcer disease: a review of its pathogenesis and clinical features. J Cutan Med Surg 2019; 10.1177/1203475419858955.

Moher D, Liberati A, Tetzlaff J, Altman DG. The Prisma Group. Preferred reporting Items for systematic reviews and meta-analyses. *PLoS Med* 2009; 6: e 1000097.

Moher D, Liberati A, Tetzlaff J, Altman DG, Group P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *BMJ* 2009; 339: b2535.

Moher D, Cook DJ, Eastwood S, Olkin I, Rennie D, Stroup DF. Improving the quality of reports of meta-analyses of randomised controlled trials: the QUOROM statement. Quality of Reporting of Meta-analyses. *Lancet* 1999; 27:1896-900.

Moi H, Blee K, Horner PJ. Management of non-gonoccoal urethritis. *BMC Infect Dis* 2015; 15: 294.

Mourinha V, Costa A, Urzal C, Guerreiro F. Lipschütz ulcers: uncommon diagnosis of vulvar ulcerations. *BMJ Case Rep* 2016; doi: 10.1136/bcr-2015-214338.

Mroczkowski TF, Martin DH. Genital ulcer disease. *Dermatol Clin* 1994; 12:753-764.

Martin JM, Godoy R, Calduch L, Villalon G, Jordá E. Lipschütz acute vulval ulcers associated with primary cytomegalovirus infection. *Pediatr Dermatol* 2008; 25(1):113-5.

Nenoff P, Manos A, Ehrhard I, Krüger C, Paasch U, Helmbold P, Handrick W. Nichtvirale sexuell übertragene Infektionen- Epidemiologie, Klinik, Labordiagnostik und Therapie. *Hautarzt* 2017; 68: 43-58.

Nguyen VK, Greenwald ZR, Trottier H, Cadieux M, Goyette A, Beauchemin M, Charest L, Longpre D, Lavoie S, Tossa HG, Thomas R. Incidence of sexually transmitted infections before and after preexposure prophylaxis for HIV. *AIDS* 2018, 32: 523–530.

OEGSTD Guideline for treatment of STIs (german). (November 2018)
http://www.oegstd.at/web/images/doc/leitlinien/Buch_Therapieleitlinien_2018.pdf

OEGSTD update on gonorrhea (December 2021)
http://www.oegstd.at/web/images/doc/leitlinien/Leitlinien-Gonorrhoe_Update2021.pdf

Pelletier F, Aubin F, Puzenat E, Deprez P, Blanc D; Estavoyer JM, Humbert P. Lipschütz genital ulceration: a rare manifestation of parathyphoid fever. *Eur J Dermatol* 2003; 13:297-8.

Patel R, Kennedy OJ, Clarke E, Geretti A, Nilsen A, Lautenschlager S, Green J, Donders G, van der Meijden W, Gomberg M, Moi H, Foley E. 2017 European guidelines for the management of genital herpes . *Int J STD Aids* 2017; doi: 10.1177/0956462417727194.

Randjelovic I, Moghaddam A, Freiesleben de Blasio B, Moi H. The role of polymorphonuclear leukocyte counts from urethra, cervix, and vaginal wet mount in diagnosis of nongonococcal lower genital tract infection. *Infect Dis Obst Gyn* 2018; https://doi.org/10.1155/2018/8236575.

Ribeiro S, de Sousa D, Medina D, Castro R, Lopes A, Rocha M. Prevalence of gonorrhea and chlamydia in a community clinic for men who have sex with men in Lisbon, Portugal. *Int J STD & AIDS* 2019; 30: 951–959.

Rietmeijer CA, Mettenbrink CJ. Recalibrating the gram stain diagnosis of male urethritis in the era of nucleic acid amplification testing. *Sex Transm Dis* 2012; 39: 18–20.

Rowley J, Vander Hoorn S, Korenromp E, Low N, Unemo M, Abu-Raddad LJ, et al. Global and Regional Estimates of the Prevalence and Incidence of Four Curable Sexually Transmitted Infections in 2016. *WHO Bulletin.* 2019. https://www.who.int/bulletin/volumes/97/8/18-228486.pdf.

Roett MA, Mayor MT, Uduhiri KA. Diagnosis and management of genital ulcers. *Am Fam Physician* 2012; 85:254-62.

Rosen T, Brown TJ. Genital ulcers. Evaluation and Treatment. *Dermatol Clin* 1998; 16: 673-685.

Rossignol L, Feuillepain L, Ngangro NN, Souty C, Fournet N, Le Strat Y, Baroux N, Hanslik T, Lot F, Blanchon T. Estimate of male urethritis incidences in France between 2007 and 2017 with a specific focus on Neisseria gonorrhoeae, Chlamydia trachomatis, and Trichomonas vaginalis infections. *BMC Infect Dis* 2019; https://doi.org/10.1186/s12879-019-4202-1.

Sadoghi B, Stary G, Wolf P, Komericki P. Ulcus vulvae acutum Lipschütz (UVAL): a systematic literature review and a diagnostic and therapeutic algorithm. *JEADV* 2020; 10.1111/jdv.16161.

Sárdy M, Wollenberg A, Niedermeier A, Flaig MJ. Genital ulcers associated with Epstein Barr virus infection (ulcus vulvae acutum). *Acta Derm Venereol* 2011; 91: 55-9.

Shannon CL, Klausner JD. The Growing Epidemic of Sexually Transmitted Infections in Adolescents: A Neglected Population. *Curr Opin Pediatr* 2018; 30: 137–143.

Sherrad J, Wilson J, Donders G, Mendling W, Jensen JS. 2018 European (IUSTI/WHO) International Union against sexually transmitted infections (IUSTI) World Health Organisation (WHO) guideline on the management of vaginal discharge. *Int J STD & AIDS* 2018; 29: 1258–1272.

Skaletz-Rorowski A, Potthoff A, Nambiar S, Wach J, Kayser A, Kasper A, Brockmeyer NH. Age specific evaluation of sexual behavior, STI knowledge and infection among asymptomatic adolescents and young adults. *J Infect Public Health* 2020; 13: 1112–1117.

Smelov V, Thomas P, Ouburg S, A Morre S. Prevalence of genital Chlamydia trachomatis infections in Russia: systematic literature review and multicenter study. *Pathogen Dis* 2017; doi: 10.1093/femspd/ftx081.

Spornraft-Ragaller P, Dumke R. Prevalence and antibiotic resistance of rectal Mollicutes in HIV-infected men who have sex with men at the University Hospital of Dresden, Germany. *Infection* 2020; 48: 259–265.

Svedman C, Holst R, Johnsson A. Ulcus vulvae acutum, a rare diagnosis to keep in mind. *Eur J Obstet Gynecol Reprod Biol* 2004; 115: 104-5.

Tiplica GS, Radcliffe K, Evans C, Gomberg M, Nandwani R, Rafila A, Nedelcu L, Salavastru C. 2015 European guidelines for the management of partners of persons with sexually transmitted infections. *JEADV* 2015; 29: 1251–1257.

Trcko K, Belic M, Miljković J. Ulcus vulvae acutum. *Acta Dermatovenerol Alp Pannonica Adriat* 2007; 16:174-6.

Truchuelo MT, Vano-Galván S, Alcántara J, Pérez B, Jaén P. Lipschütz ulcers in twin sisters. *Pediatr Dermatol* 2012; 29: 370-2.

Török L, Domján K, Faragó E. Ulcus vulvae acutum. *Cutis* 2000; 65: 387-9.

Unemo M, Ross JDC, Serwin AB, Gomberg M, Cusini M, Jensen JS. 2020 European guideline for the diagnosis and treatment of gonorrhea in adults. *Int J STD AIDS* 2020; doi: 10.1177/0956462420949126.

Unemo M, Seifert HS, Hook EW; Hawkes S, Ndowa F, Dillon JAR. Gonorrhoea. *Nat Rev* 2019; https://doi.org/10.1038/ s41572-019-0128-6.

Van Gerwen OT and Muzny CA. Recent advances in the epidemiology, diagnosis, and management of *Trichomonas vaginalis* infection. *F1000Research* 2019; https://doi.org/10.12688/f1000research.19972.1.

Vieira-Baptista P, Lima-Silva J, Beires J, Martinez-de-Oliveira J. Lipschütz ulcers: should we rethink this? An analysis of 33 cases. *Eur J Obstet Gynecol Reprod Biol* 2016; 198: 149-52.

de Vries HJC, de Barbeyrac B, de Vrieze NHN, Viset JD, White JA, Vall-Mayans M, Unemo M. 2019 European guideline on the management of lymphogranuloma venereum. *JEADV* 2019; doi: 10.1111/jdv.15729.

Warner L, Newman DR, Austin HD, Kamb ML, Douglas Jr JM, Malotte CK, et al. Condom effectiveness for reducing transmission of gonorrhea and chlamydia: the importance of assessing partner infection status. *Am J Epidemiol* 2004; 159(3): 242–51.

Wetter DA, Bruce AJ, MacLaughin KL, Rogers RS 3rd. Ulcus vulvae acutum in a 13 year old girl after influenza A infection. *Skinmed* 2008; 7: 95-8.

Wiesenfeld HC，Manhart L. *Mycoplasma genitalium* in Women: Current Knowledge and Research Priorities for This Recently Emerged Pathogen. *J Infect Dis* 2017; 216: 389–95.

Witkin SS, Minis E, Athanasiou A, Leizer J, Linhares IM. *Chlamydia trachomatis*: the persistent pathogen. *Clin Vaccine Immunol* 2017; 24: e00203-17

Wolters V, Hoogslag I, van't Wout J, Boers K. Lipschütz ulcers: a rare diagnosis in women with vulvar ulceration. *Obstet Gynecol 2017*; 130: 420-2.

Workowski KA, Bachmann LH, Chan PA, Johnston CM, Muzny CA, Park I, Reno H, Zenilman JM, Bolan GA. Sexually Transmitted Infections Treatment Guidelines, 2021 *CDC: Morbidity and Mortality Weekly Report 2021*, Vol 70; No4.

World Health Organization. Tubal infertility: serologic relationship to past chlamydial and gonococcal infection. World Health Organization Task Force on the Prevention and Management of Infertility. *Sex Transm Dis* 1995; 22: 71–7.

Yu X, Zheng H. Syphilitic chancre of the lips transmitted by kissing: A case report and review of the literature. *Medicine (Baltimore*) 2016; 95: e3303.

Zouboulis CC, Bechara FG, Dickinson-Blok JL, Gulliver W, Horvath B, Hughes R, Kimball AB, Kirby B, Martorell A, Podda M, Prens EP, Ring HC, Tzellos T, van der Zee HH, van Straalen KR, Vossen ARJV, Jemec GBE. Hidradenitis suppurative/ acne inversa: a practical framework for treatment optimization- systematic review and recommendations from the HS Alliance working group. *JEADV* 2019; 33: 19-31.