Chlamydia trachomatis: Clinical, bacterial, and host aspects of a silent love bug

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SUMMARY

In this thesis factors related to the often asymptomatic character of *Chlamydia trachomatis* (CT) infections are presented and discussed with a focus on both clinical and more biological studies including host responses.
The 2015 European background review for the management of CT infections acts as a clinical overview of where we stand today in the burden of CT infections and presents updated information about epidemiology and clinical management.
As the natural course of a urogenital CT infection still remains partly unclear, we have focussed on two types of host responses that might contribute to the susceptibility of infections or to a prolonged, severe CT infection leading to tubal pathology in women. Firstly, we have investigated host serological responses induced by the pathogen CT during urogenital infections to elucidate serovar distributions in association with IgG serum concentrations. Secondly, we have investigated host genetic variation determining the host immunological responses which are influenced by genetic variation in the genes involved in recognition of CT.
We studied the abovementioned aspects of CT infections in a number of target groups in the Netherlands. Below, we summarise the major findings and conclusions per chapter.

In **chapter 1**, the introduction of this thesis, an overview of all major epidemiological and clinical aspects of CT infections are given. It is partly based on our European guideline for the management of urogenital CT infections.

In **chapter 2**, the background review for the 2015 European guideline on the management of CT infections is presented. This is a detailed description of background, evidence base of recommendations and discussions regarding our 2015 European guideline on the management of urogenital CT infections. European guidelines are produced to stimulate evidence based management by all professionals involved in sexual health care. The aim is to decrease transmission of CT infections and consequently prevent the rate of complications like PID and infertility.
The most important updates include: broader indications for testing and treatment of CT infections; clearer recommendation of using exclusively validated and quality assured highly sensitive and specific nucleic acid amplification tests for diagnosis; advice on (repeated) CT testing; recommendations of increased testing particularly at sexually transmitted infections and sexual health clinics to reduce the incidence of pelvic inflammatory disease and prevent exposure to infection; and recommendations to identify, verify and report CT variants. Details regarding the etiology, transmission, clearance, epidemiology and taxonomy of CT, clinical features, recommended diagnostics (including quality assurance), advice for CT infected patients, indications for therapy, recommended and alternative treatment regimens for urogenital and extragenital CT

infections, contact tracing and management, and the notification of CT cases are included.

In **chapter 3** we present a rare case of a young man with visual impairment and constipation with bloody anal discharge for 1 year. He reported to have had unprotected receptive anal sexual contacts with male partners. Physical examination revealed an annular plaque at the soft palate and perianal skin defects, including perianal fistulas. Further examination showed a positive NAAT on a rectal swab for N. gonorrhoeae and CT. CT genotyping demonstrated the L-serotype of CT confirming lymphogranuloma venereum (LGV). Further laboratory tests confirmed secondary syphilis which corresponded with the clinical picture of uveitis anterior and an annular structure at the palatum, resembling a condyloma latum. HIV and hepatitis C virus tests were negative. This advanced stage of LGV can mimic Crohn's disease. An adequate sexual history can be helpful in the differential diagnosis. In sexually active MSM with rectal complaints, LGV should always be considered, even if the HIV test is negative. With the increase of rectal LGV infections, especially in MSM, it is recommended that positive rectal specimens are subsequently genotyped for LGV.

In **chapter 4** we describe the results obtained by the Dutch *Chlamydia trachomatis* reference Laboratory in the period 2010-2015. This Laboratory is an initiative of the Epidemiology & Surveillance Unit, Centre for Infectious Disease Control, from the National Institute of Public Health & the Environment (RIVM) and the VU University Medical Center, Department of Medical Microbiology and Infection Control. The two main tasks of the Laboratory are: 1) providing CT surveillance in different geographical regions in the Netherlands by collecting CT-positive and CT-negative samples of men and women of all representative anatomical sites. This task has been initiated to monitor CT variant emergences, like the Swedish variant, to safeguard Dutch CT detection reliability and 2) functioning as a central CT Laboratory for questions regarding potential CT diagnostic difficulties including potential CT variants. For the first task we screened 25 CT- and 10 CT+ samples from 6 geographical locations each quarter of the year with a yearly minimum of 500 samples. 1.9% of the samples were discrepant as compared to the initial reported results. Sample degradation in combination with very low titres was the main reason for this. Seven samples contained a plasmid free strain identified via newly developed PCRs, resulting in an incidence of 0.2%. As this is a low percentage and most commercial tests use either RNA as a target or have a dual target system (plasmid and chromosomal), this discrepancy does not have a major effect on the detection rate. For the second task, the laboratory has been approached by different parties resulting in identification of a Swedish variant in the Netherlands and also the first rectal lymphogranuloma strain L2b in a female, a strain which was till then only identified in men having sex with men (MSM).

In summary the core tasks of the CT reference laboratory have resulted in the identification of different CT variants in the Netherlands. Hence it has proven its merit.

We evaluated in **chapter 5** the serological immunoglobulin (Ig) G serum concentrations in CT infected patients, since antibodies have been associated with clearing CT infections but also with the presence of tubal pathology in women. We have investigated urogenital swabs and serum samples from 718 Dutch positive CT patients attending a STI clinic in two major cities in the Netherlands (The Hague and Amsterdam). Detection of CT DNA in swabs was performed by two different PCR techniques. Genotyping of serovars was performed using the CT-DT assay (Labo Biomedical Products BV, Rijswijk, the Netherlands) which is based on the reverse hybridization assay (RHA) methodology and by means of PCR based Restriction Fragment Length Polymorphism (RFLP) analyses. Determination of CT IgG levels in serum of all patients was done by a specific Enzyme-Linked Immuno Sorbent Assay (pELISA) test generating quantitative results based on Optic Density (OD) values to further calculate IgG titres. Our results show that the most prevalent urogenital serovars from the B serogroup, serovars D and E, induce the highest IgG serum concentrations, and the least prevalent serovars from the C group induce the lowest concentrations in both men and women. These results provide more insight in understanding the immunological response against a CT infection at both serogroup and serovar level.

Genetic variations in genes encoding the host immune system are known to impact the course of infections in general. A single nucleotide polymorphism (SNP) is a genetic variation in a single nucleotide that occurs at a specific position in the genome, where each variation is present to some appreciable degree within a population (e.g. > 1%). Recent studies have shown a positive impact of vitamin D on the regulation of the immune system. The examination of polymorphisms within genes of the vitamin D biopathway in relation to CT infection has been described in **chapter 6** to further elucidate differences in susceptibility to urogenital CT infection in humans. An analysis of polymorphisms with either a proven or theoretical functional impact (either amino acid change, or impact on protein expression) and/or haplotype tagging (finding a set of so-called tagging SNPs) was eligible for inclusion in this study. We included SNPs from the following genes: VDR (rs1544410 G > A, rs2228570 C > T), CYP27B1 (rs10877012 G > T), DHCR7 (rs7944926 G > A, rs3829251 G > A), GC (rs3755967) and CYP2R1 (rs10741657 G > A, rs2060793 G > A). All polymorphisms were genotyped by LGC Genomics using their own form of competitive allele-specific PCR (KASP). In our STI cohort of Dutch Caucasian women (n=500), we did not observe statistically significant differences between the genotype distributions of the eight polymorphisms. For that reason, we assume that VDR, CYP27B1, DHCR7, GC, and CYP2R1 do not seem to play a major role in susceptibility to CT infections as they apparently do in other

diseases. However, genes in the vitamin D pathway exhibit a pleiotropic role in the immune system, therefore the role of vitamin D should not be disregarded yet for the entire clinical course of CT infections and requires further research with a focus to late complications including tubal factor infertility. This can be of value for implementing host genetic markers into clinical applications in the future to distinguish women prone for tubal pathology.

Chapter 7 is the discussion of the presented work. This thesis contributes amongst others in optimizing intervention strategies for the management of CT infections. Clinical guidelines are helpful for professionals in providing easily accessible (online) information for the management of urogenital CT infections. Improvement in both quality and quantity of CT-related clinical trials can further strengthen the evidence base. Secondly, the identification of women at risk for developing tubal pathology and thus infertility should be enhanced. Ideally, the best predictive tool for the triage of subfertile women with potential tubal pathology would be a dual assay composed of the most predictive serological and host genetic markers that are involved in the development of late complications in women after CT infection. Unnecessary, invasive and costly procedures like laparoscopies could in part be prevented.