INFLUENCE OF BACTERIAL INFECTIONS ON REPRODUCTIVE HEALTH

Ph.D Thesis

László Párducz, MD.

DEPARTMENT OF OBSTETRICS AND GYNAECOLOGY, KÁLMÁN PÁNDY MEMBER HOSPITAL,
CENTRAL HOSPITAL OF BÉKÉS COUNTY HOSPITALS

Doctoral School of Clinical Medicine Sciences,
Faculty of Medicine, University of Szeged, Hungary

Supervisor: Dezső Virok, MD, PhD

Department of Medical Microbiology and Immunobiology,
Faculty of Medicine, University of Szeged, Hungary

Szeged, 2017

Publications directly related to the thesis

I. Juhász E., Ostorházi E., Pónyai K., Silló P., **Párducz L.**, Marschalkó M., Rozgonyi F.: Ureaplasmas: from commensal flora to infectious diseases.
Rev. Med. Microbiol. 2011, ***22****: 73-83.****(IF:0.370)***

II. Farkas B, Ostorházi E, Pónyai K, Tóth B, Adlan E, **Párducz L**, Marschalkó M, Kárpáti S, Rozgonyi F. Az *Ureaplasma urealyticum* és a *Mycoplasma hominis* antibiotikumérzékenysége és gyakorisága szexuálisan aktiv egyének genitális mintáiban [Frequency and antibiotic resistance of *Ureaplasma urealyticum* and *Mycoplasma hominis* in genital samples of sexually active individuals].
Orv Hetil. 2011 Oct 16;152(42):1698-702.

III. Pónyai K, Mihalik N, Ostorházi E, Farkas B, **Párducz L**, Marschalkó M, Kárpáti S, Rozgonyi F. Incidence and antibiotic susceptibility of genital mycoplasmas in sexually active individuals in Hungary.
Eur J Clin Microbiol Infect Dis. 2013 Nov;32(11):1423-6. ***(IF:2.544)***

IV. Nemes-Nikodém E, Vörös E, Pónyai K, **Párducz L,** Kárpáti S, Rozgonyi F, Ostorházi E. The importance of IgM positivity in laboratory diagnosis of gestational and congenital syphilis.
Eur J Microbiol Immunol (Bp). 2012 Jun;2(2):157-60.

V. **Párducz L**, Eszik I, Wagner G, Burián K, Endrész V, Virok DP. Impact of antiseptics on Chlamydia trachomatis growth.
Lett Appl Microbiol. 2016 Oct;63(4):260-7. ***(IF:1.579)***

Publications with results related to the thesis

I. **Párducz L**., Ostorházi E., Pónyai K., Mihalik N., Párduczné Szöllősi A., Rozgonyi F.: A reprodukciót befolyásoló fiatalkori bakteriális STD fertőzések. *Nőgyógy. Szül. Továbbk. Szemle 2013,* ***15:*** *97-101.*

II. **Párducz L**.: Az alsó genitális tractus fertőzései. *In: A Nőgyógyászat kézikönyve* (Szerk.: Papp Z.) Medicina Könyvkiadó Zrt, Budapest, 2017. 836-856.

Introduction

Bacterial infections can influence the reproductive health by either as a direct infection of the reproductive organs or as a hematogenous dissemination of a distant/ generalised infection. Bacterial sexually transmitted diseases (STDs) are principally caused by the species of *Neisseria gonorrhoeae, Treponema pallidum, Haemophilus ducreyi, Gardnerella vaginalis,Streptococcus agalactiae, Chlamydia trachomatis, Mycoplasma genitalium, Mycoplasmahominis, Ureaplasma urealyticum* and *Ureaplasma parvum.* A number of studies focused on the STDs in Hungary in the past 20-30 years, however, the infections caused by the intracellular parasite *Mycoplasma* family have not been studied well. It could have been due to several factors, including their extreme sensitivity to environmental factors, difficult transport and culturing and the lack of specific serological tests. Because of the recent technical developments in sampling, cultivation and antibiotic sensitivity testing, we were able to culture *mycoplasmas in vitro* and perform antibiotic susceptibility testing of *Mycoplasma* and *Ureaplasma* isolates routinely. Since phenotypical characterization cannot distinguish between *U. parvum* and *U. urealyticum* and *M. genitalium* and *M. hominis* species, respectively, and genotyping was not available for us, we used the name *U. urealyticum* for both *Ureaplasma* species and *M. hominis* for both *Mycoplasma* species in the thesis. Various species in the class of *Mollicutes* have been shown to cause colonization and disease in the human body; as we mentioned before *M. hominis, M. genitalium, U. urealyticum* and *U. parvum* are urogenital pathogens. Female urogenital infections and its complications include urethritis, salpingitis, pelvic inflammatory disease, chorioamnionitis, premature birth, habitual abortion, postpartum sepsis, neonatal pneumonia and sepsis. In male patients acute and chronic urethritis, acute and chronic prostatitis, epididymitis, epididymoorchitis and oligoasthenospermia should be mentioned. It should be mentioned that genital *mycoplasmas/ ureaplasmas* can be found relatively frequently on the genital mucosa of sexually active people without any symptoms.

In the first part of the thesis, we discuss the incidence and antibiotic sensitivity of *U. urealyticum* cultured from the urogenital samples of sexually active Hungarian individuals visiting the outpatient service of the National STD Diagnostic and Therapeutic Center at the Department of Dermatology, Venereology and Dermatooncology, Semmelweis University, Budapest, Hungary.

The socioeconomical impact of venereal syphilis is significant. According to the Centers for Disease Control and Prevention the syphilis prevalence was approximately 400

cases/ 100.000 population in the 1940s, before the antibiotics era. The syphilis prevalence then steadily decreased and reached 2.1 cases per 100.000 population in 2000-2001. After 2001, the number of syphilis cases started to increase to 7.5 cases per 100.000 population by 2015. The prevalence of syphilis was correlated with sex and sexual behavior (highest among men, especially among men sex with men), age (highest prevalence age groups were 20–24 and 24–29 years). Syphilis prevalence was also different between races and regions. Importantly, parallel to the increase of the adult acquired syphilis cases, the congenital syphilis cases also increased during the 2000s, reaching 12.4 cases per 100.000 live births at 2015. The Hungarian epidemiology data show similar trends. In 2012 the number of syphilis cases increased by 10% compared to 2011, with more than three times more males than females. The highest syphilis prevalence was measured at the age group of 25-29 for males, and of 20-24 for females. The regional incidence was the highest in Budapest, and Bács-Kiskun county at the countryside.

In the second part of the thesis, we discuss syphilis seropositivity in 241 pregnant women of the 33.753 delivering serum samples at the Department of Dermatology, Venereology, and Dermatooncology of Semmelweis University, Budapest, Hungary from January 1, 2009 through December 31, 2011. We evaluate whether the *T. pallidum* IgM status of the mothers with syphilis indicate a risk of connatal syphilis better than Rapid Plasma Reagin (RPR) test, and evaluate the use of IgM immunoblots for the identification of infants with congenital syphilis.

C. trachomatis is an obligate intracellular bacterium with cell tropism to epithelial cells of the conjunctiva and the urogenital tract. The bacterium is particularly capable of establishing persistent infections and chronic inflammation. The local inflammation could lead to tarsus and oviduct fibrosis potentially leading to blindness and infertility. Sexually transmitted infections (STIs) caused by the urogenital tract pathogens *C. trachomatis* serovars D-K and L1-L3 are the most frequent STIs in the world. While antiseptics are not part of the antichlamydial chemotherapy, they have various intravaginal applications including the prevention of postoperative infections before cesarean section, trans-vaginal ultrasoundguided ovum pick-up, surgical treatment of HPV generated cervical lesions and other invasive procedures, and prevention of early-onset neonatal group B *Streptococcus* infection.

In the third part of the thesis we discuss the measurements of the antichlamydial effects of iodine aqueous solution, povidone-iodine, chlorhexidine and borax. These antiseptics are being used for the treatment of bacterial vaginosis, but their effect on *Chlamydia trachomatis* infections has not yet been investigated. Bacterial vaginosis is a

frequent dysbiosis, where the normal lactobacillus-dominated flora is replaced by an anaerob/ aerob polymicrobial flora. Bacterial vaginosis increases the risk of acquiring STIs including the frequent *C. trachomatis* infections. Intravaginal antiseptics are part of the bacterial vaginosis treatment, and ideally they should also inhibit the bacterial vaginosis-related STIs. Therefore, we tested the antichlamydial activity of four antiseptics: iodine aqueous solution, povidone-iodine, chlorhexidine and borax.

Aims

I. to determine the incidence of *U. urealyticum/ parvum* infections in sexually active Hungarian individuals according to ages.

II. to examine the age distribution of *Ureaplasma*-positive patients and the frequency of of symptoms.

III. to determine the antibiotic resistance spectrum of *U. urealyticum/ parvum* isolated from these patients.

IV. to evaluate the *T. pallidum* IgM immunoblots for the identification of newborns with congenital syphilis compared with RPR, *T. pallidum* particle agglutination (TPPA), and enzyme immunosorbent assay (EIA) tests.

V. to determine the antichlamydial effects of iodine aqueous solution, povidone-iodine, chlorhexidine and borax.

Materials and methods

Ureaplasma/ Mycoplasma detection and antibiotic sensitivity

To study the incidence and antibiotic susceptibility of Hungarian *Ureaplasma/ Mycoplasma* strains 4154 samples, 2114 from female and 2040 from male patients ranging between 15 and over 60 years of age, were obtained. For culturing the samples the Mycoplasma Duo kit (Bio-Rad, France) was used, with an incubation period of 48 hours, 37^{o}C, 5% CO_2. The determination of antibiotic sensitivity of the 373 *Ureaplasma* strains collected during a sixyear period was done in U9 medium with SIR Mycoplasma kit (Bio-Rad) under the same conditions.

Serological diagnosis of maternal and congenital syphilis

After prescreening 33.753 serum samples for syphilis serological positivity, we used RPR (Omega Diagnostics, UK), *T. pallidum*particle agglutination (TPPA) test (Fujirebio Inc., Japan), and enzyme immunosorbent assay test (Syphilis II-EIA, BioRad, France) to investigate 241 maternal serum samples and 242 serum samples of the babies born to these mothers. IgM immunoblots (MAST, UK) were prepared from all the 483 serum samples, but the IgM–IgG complex and the maternal IgG were evaluated with Mastsorb (MAST, UK) from the infants' serum. IgG immunoblots (MAST, UK) were prepared from the 241 maternal serum samples. One of the infants had symptoms like neurosyphilis; this diagnosis was confirmed with Venereal Disease Research Laboratory (VDRL) test (Omega Diagnostics, UK) and TPPA in the cerebrospinal fluid.

C. trachomatis propagation and HeLa cell culture

C. trachomatis serovar D strain (UW-3/CX, ATCC) was used, the strain was propagated and partially purified. HeLa 229 cells (ATCC) were incubated in 100 µl of minimal essential medium (MEM) with Earle salts supplemented with 10 % heat-inactivated fetal bovine serum.

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

MTT assay was performed to characterize the maximum non-toxic concentration of the antiseptics on HeLa cells. The average viability (OD 540) of three wells with untreated HeLa cells was considered the 100% viability. Viabilities of the treated cells were compared to the untreated controls as follows: Cell Viability (%) = (OD 540 of treated cells/ OD 540 of untreated cells) x 100.

Antiseptics used for *C. trachomatis* growth inhibition, *C. trachomatis* infection and DNA extraction

Iodine aqueous solution, povidone-iodine (Betadine, EGIS, Budapest, Hungary), chlorhexidine-digluconate (Chlorhexamed, GlaxoSmithKline, Brentford, UK) and borax were diluted in sucrose-phosphate-glutamic acid buffer (SPG). Concentration ranges of 100-0.78 μg/ml for iodine aqueous solution and borax, 390-3 μg/ml for povidone-iodine and 4-0.003 μg/ml for chlorhexidine with 2-fold dilutions were tested. All reagents were purchased from SIGMA, St. Louis, MO, USA, unless otherwise indicated.

Direct qPCR monitoring of the effects of the antiseptics on *C. trachomatis* growth

DirectqPCR was performed as described before in a Bio-Rad CFX96 real-time system, using the SsoFast EvaGreen qPCR Supermix (Bio-Rad) master mix and the *C. trachomatispykF* gene specific primer pairs. The qPCR ended with a melting curve analysis. Student's t-test has been used to compare the statistical differences of Ct values between two experimental conditions.

Immunofluorescent monitoring of the effects of the antiseptics on *C. trachomatis* growth

C. trachomatis growth was evaluated by immunofluorescent staining. Anti-chlamydia LPS antibody labeled with Alexa-647, was used for detection of chlamydial inclusions. Fluorescence signals were analyzed with an Axon GenePix Personal 4100A DNA chip scanner and GenePix Pro (version 6.1) software (Molecular Devices, USA) using the Cy5 channel and a 5 μm resolution. Inclusion counts were determined by the ChlamyCount software.

Results

Incidence, age distribution, symptoms and antibiotic resistance of genital *Ureaplasma* strains

Prevalence of *Ureaplasma* infection

Former microbiological examinations ruled out the possibility of *N. gonorrhoeae* and *C. trachomatis* infections in patients involved in this study. 2114 samples were collected from female patients. 2047 samples were cervix swabs and 11% of these samples were *Ureaplasma* positive by culture. 59 urethra samples and eight urine samples from females were also examined. 7.02% of the urethra specimens were *Ureaplasma*positive, while all the urine samples were negative. Based on these data it can be stated cervix swabs are the most suitable

samples for *Ureaplasma* culturing in female patients. 2040 samples were collected from male patients. The vast majority of these samples were obtained from urethra. 3.01% of the urethra samples turned out to be *Ureaplasma* positive by culture. Only 15 samples were glans swab, ejaculatum or urine. None of these were culture positive. Altogether 229 and 61 *Ureaplasma* strains were isolated in the study period from females and males, respectively. The total prevalence of *Ureaplasma* strains in the whole population examined was 6.98%. Subsequent study in the next two years slightly modified these tendencies showing an increase in the prevalence of *Ureaplasma* positive patients.

Age distribution and frequency of symptoms in *Ureaplasma* positive patients

Ureaplasma positive females and males covered a broad spectrum of ages from less than 16 to over 60-year old. *Ureaplasma*positive females from age 20 increased rapidly reaching a plateau at age 26, then gradually decreased from age 36 to the late 60s.The distribution of *Ureaplasma* positive males showed a rather different picture. It increased linearly from age 20 (5%) reaching a 25% peak at age 30, then linearly decreased to <5% at age 50, followed by a small rise between age 51-55, then a decrease to 0% over 60 years of age.

The ratio of symptom-free persons among *Ureaplasma* positive individuals was above 40% in both sexes. The dominant clinical symptoms in females were vaginal discharge (23.53%), genital pruritus (9.19%) and colpitis (11.76%). The dominant clinical symptoms in males were urethritis (35.48%), balanitis (13.97%) and urethral discharge (7.52%).

Antibiotic resistance of *Ureaplasma* strains

The distribution of antibiotic sensitivity of 373 *Ureaplasma urealyticum/ parvum* strains cultured from genital samples showed that no fully sensitive *Ureaplasma* strain was found. Extremely high incidence of resistance was detected in the *Ureaplasma* strains against erythromycin and clindamycin. However, cross resistance against the two macrolides, the older erythromycin and the newer azithromycin existed only in less than 10% of the strains. Every fourth strain was resistant to ofloxacin as representative of fluoroquinolons. Doxycycline proved to be the most effective agent with 97.32% susceptibility, followed by tetracycline (95.9%) and azithromycin (85.79%).

Serology of genstational and connatal syphilis

Clinical and serostatus of pregnant syphilis positive women and their children

Positive syphilis serology was noted in 241 pregnant women out of the 33.753 delivering serum samples during a three-year period. 230 mothers had adequate prenatal care. 217 of the cared mothers have already been adequatly treated against syphilis before pregnancy, they were advised to repeat therapy, according to the Hungarian guidelines. These women had RPR titer of 0-1:16, results of TPPA, EIA and IgG immunoblot tests were also positive, but IgM immunoblots results were negative. Children of these mothers had RPR titers of 0-1:8, equal or less than their mothers. All TPPA and EIA reactions were positive in these children, only IgM immunoblots were negative. These infants were uninfected, without any symptoms of connatal syphilis at birth. Eleven women, having received inadequate prenatal care, classified as *latent syphilis* patients, were first found to have reactive test results for syphilis at delivery. Seven mothers had the diagnosis of *syphilis latens tarda*, with negative RPR and IgM immunoblot results, but with positive TPPA, EIA and IgG immunoblot tests. At delivery the RPR test results of their infants were negative, however, TPPA and EIA were positive. None of these children had a positive IgM immunoblot result.The other four mothers, had the *syphilis latens recens* diagnosis at delivery, with an RPR titer at least 1:2 to a maximum of 1:256. TPPA, EIA and IgG immunoblot tests were all positive. One mother with an RPR titer of 1:64 has already had IgM negative immunoblot result. Her baby had the same serological results and had no clinical symptoms of syphilis, after adequate treatment the follow-up examinations and serological testing at 0, 3, 6, 9 and 12 months confirmed the absence of connatal syphilis infection. The last three women were found to have positive IgM results together with the other reactive syphilis tests at delivery. Remarkably, the first of them, an intravenous drug user, has had an RPR titer only of 1:2, but one of her twin sons has died immediately after birth. The RPR titer of this „A" infant was 1:8. RPR titer of „B" infant was 1:2, his treponemal tests, including IgM immunoblot, were all positive. The second motherchild pair had the same RPR titer of 1:256, all treponemal tests, including IgM immunoblot were positive in both of them, but the infant had no symptoms of connatal syphilis. The last mother had an RPR titer of 1:64, TPPA, EIA and IgG, IgM immunoblots were positive. The premature daughter had RPR titer of 1:64, positive results of TPPA, EIA and IgM immunoblot. Prematurity was the only non-specific clinical manifestation of connatal syphilis in this case.

Impact of antiseptics on C. trachomatis growth
Cell viability of HeLa cells incubated with antiseptics

We tested the long term impact of the antiseptics on the viability of the HeLa human cervical epithelial cells. HeLa cell viability was determined by MTT assay after 48 h incubation. The maximum non-toxic concentrations were 390 μg/ml, 200 μg/ml, 2 μg/ml and 25 μg/ml for povidon-iodine, iodine aqueous solution, chlorhexidine and borax, respectively. The maximum non-toxic concentration for borax was 25 μg/ml, but the cell viability for borax reached 80% at 400 μg/ml.

Direct qPCR measurement of the impact of antiseptics on *C. trachomatis* growth

The direct qPCR method was used to determine the antichlamydial activity of antiseptics**.** HeLa cells were infected with *C. trachomatis* at multiplicity of infection 8 (MOI 8) after preincubation (37 ^{o}C, 1 h) with serial 1:2 dilutions of povidone-iodine, chlorhexidine, iodine aqueous solution and borax starting with the maximum non-toxic concentrations. The antiseptics' MICs were calculated as it was described before. Briefly, the chlamydial DNA concentrations (threshold cycle (Ct) values) measured in the three parallel wells of a given antiseptic concentration were compared with the Ct values measured in the three parallel wells of the highest antiseptic concentration (we considered it as the inoculum) using Student's t-test. The lowest antiseptic concentration, where the Ct values did not change significantly compared with the inoculum was considered the MIC value. The MIC value of povidone-iodine was 97 μg/ml, the MIC value of chlorhexidine was approximately 4 μg/ml. The iodine aqueous solution and the borax did not show antichlamydial activity in the tested concentration range.

Estimation of the qPCR inhibitory activity of the antiseptics

Since the growth related chlamydial DNA synthesis was measured by a qPCR method, we tested whether the applied antiseptics had a direct inhibitory impact on the qPCR. This effect could appear as a false-positive antichlamydial activity. The Ct levels of the povidone-iodine, chlorhexidine, iodine aqueous solution and borax mixtures were only 1.33, 0.79, 0.85 and 1.28cycles higher than the untreated *C. trachomatis* infected cell lysate's, therefore the observed antichlamydial effect of the antiseptics were not due to the inhibition of the qPCR.

ChlamyCount immunofluorescent measurement of the impact of antiseptics on *C. trachomatis* growth

To validate the qPCR results with an independent chamber slide infection method, we performed *C. trachomatis* infections (MOI 8) in the presence of the antiseptics with the highest concentrations used for qPCR. ChlamyCount inclusion number data showed that the povidone-iodine and chlorhexidine treatment decreased the chlamydial inclusion number approximately 94 % and 94 %, respectively, while the iodine aqueous solution and borax decreased the number of chlamydial inclusions by 13 % and 43 %, respectively.

Discussion

Incidence and antibiotic susceptibility of *U. urealyticum/parvum*

Ureaplasmas are common bacteria of the human urogenital tract. Screening of asymptomatic men showed that 11% of them were colonized. *Ureaplasma* was detected in children (5%), in sexually inactive women during reproductive age (40%), in sexually active women (60-80%) and also in postmenopausal women (25%). In pregnancy the detection rate can be as high as 82% and 24% in the puerperium. Based on our results we can state that *U. urealyticum/ parvum* strains have high prevalence in both sexes in the Hungarian population. We were able to detect *U. urealyticum/ parvum* in all sexually active age groups; the highest number of isolates were from age group of 21-40 years in both men and women. Concering the symptoms. The ratio of symptom-free carrierswas above 40% in both sexes. Comparing our resistance results with those published in international journals, we found similar tendencies regarding certain antibiotics such as macrolides, clindamycin and tetracycline, as well as the outstandingly high erythromycin and clindamycin resistance and cross-resistance, while we found deviations in cases regarding fluoroquinolone resistance. Regarding erythromycin and clindamycin resistance, the examination method we applied showed only constitutive cross-resistance. According to literature data, azithromycin and josamycin are the most effective antibiotics against *Ureaplasma*, but resistant strains have already been isolated. Our data showed, that azithromycin was effective against Hungarian *Ureaplasma* strains. In our study fluoroquinolons were represented by ofloxacin with 25.2% resistance. The Hungarian situation is significantly more favourable than those in other countries. Tetracycline was effective in 95.9% on Hungarian *Ureaplasma* strains. Doxycycline seems to be the drug of first choice in case of *Ureaplasma*infections with only 2.41% resistance. In case of pregnancy, infancy and allergy the medication to be chosen is azithromycin. In case of suspected *U. urealyticum/ parvum*infection we highly recommend to test the antibiotic

resistance after the first unsuccessful antibiotic therapy. The most up-to-date procedure is, however, to perform antibiotic sensitivity test prior to commencing therapy.

Serological identification of gestational and congenital syphilis in the Hungarian population

Globally, nearly two million pregnant women are infected with *T. pallidum* each year. Approximately 50% of women with untreated syphilis have been transmitting the infection to their newborn child, resulting in profound adverse outcomes including an estimated 440.000 perinatal deaths each year. Since 1994, almost every year a congenital syphilis case has been observed in Hungary. In 2007 and 2008, one and two cases were observed, respectively. RPR test used for screening has the advantage of being inexpensive, widely available, and usable for determination of treatment efficacy. Limitations of this non-treponemal test include the lack of sensitivity in primary and late syphilis and the possibility of a prozone reaction or false-positive results. Treponemal tests like EIA or TPPA are technically more difficult to perform and more expensive, but they remain reactive for years with or without treatment. Several antigens that elicit high antibody titers during *T. pallidum* infection and are not crossreactive with serum from patients with other common spirochetal diseases have been identified. TPPA and EIA tests were developed using these recombinant antigens.

From the 241 mothers with positive syphilis screening tests, 217 were successfully treated before pregnancy. The next 13 pregnant women diagnosed with syphilis during pregnancy opted for treatment. The remaining 11 mothers were diagnosed with syphilis only at delivery. All four connatal syphilis cases were from mothers without undergoing prenatal care and syphilis screening. The success rate for mother-to-child transmission intervention (number of successful interventions/ number of syphilis positive women who received intervention) was 100% in our cases. *Rawstron et al*. determined maternal IgM status to be better indicator for a risk of connatal syphilis than a maternal RPR titer ≥1:16. However, they described that neither a titer ≥1:16 nor TP IgM reactivity identified all mothers who delivered infected infants; they found babies with congenital syphilis whose mothers had negative TP IgM and RPR titers ≤1:8. In our study, the mother of the twins with connatal syphilis had only an RPR titer of 1:2. None of the mothers of the infected children was IgM negative in our cases. The infant of the untreated mother, who had an RPR titer of 1:64, but was negative for IgM, was uninfected. Infected infants can produce IgM *in utero* after 3 months. Previous studies using either ELISA or *T. pallidum* IgM Western-Blot have similarly found that IgM antibodies cannot be detected in all babies with congenital syphilis. Serodiagnosis of

congenital syphilis is difficult because of the transfer of the IgG antibodies from mother to fetus. Fetus produces IgM antibodies (rheumatoid factor= RF), against maternal IgG. IgG–RF complex reacts in IgM immunoblot, or maternal IgG compete with fetal IgM for the Agbinding position, resulting in false-positive or false-negative tests, respectively. We found reactivity to antigens of 45, 17, and 15 kDa only in one case. Our observations confirm that antenatal syphilis screening with the parallel use of treponemal (EIA, TPPA) and nontreponemal (RPR) tests facilitates detection and treatment of syphilis during pregnancy. Successful treatment offsets vertical transmission. The use of IgM immunoblot examination allows the identification and treatment of high-risk newborns.

Measurement of the antimicrobial activities of various antiseptics against *C. trachomatis*

We tested the cytotoxic- and antichlamydial effects of various antiseptics that can be used intravaginally. The cytotoxicity tests revealed that iodine and borax did not influence the viability of the HeLa cervical epithelial cell lines at the tested concentration range, while the povidone-iodine and chlorhexidine showed a concntration-dependent toxicity after 48 hours of incubation, the time of the developmental cycle of *C. trachomatis*. In the case of these two antiseptics we used the maximum non-toxic concentration for further tests of antichlamydial capacity. We used a preincubation of the chlamydial elemntary bodies with the antiseptics, to mimic the effect of these compounds on the extracellular infectious form of *C. trachomatis.* However it cannot be excluded that these antiseptics can be transported into the host cells and may have an effect on the intracellular development of the bacterium. Further studies needed to measure the intracellular effects of the antiseptics.

Instead of measuring the chlamydial growth inhibition by immunofluorescent staining of the so-called inclusions of *C. trachomatis*, we used the recently developed qPCR based chlamydial DNA accumulation measurement as a readout of chlamydial replication. Our qPCR data showed that the chlorhexidine and povidone-iodine had an antichlamydial effect, while the iodine aqueous solution and borax did not possess any antichlamydial activity in the tested concentration range. Chlorhexidine had the lowest antichlamydila MIC, but the MIC was close to the maximum non-toxic concentration, therefore its therapeutic index was low. Our data indicates that chlorhexidine could be an effective antichlamydial agent *in vivo*, but may be applied as a short term rinsing, rather than as a long-term vaginal gel. On the other hand, the povidone-iodine had a MIC of 97 μg/ml, while its maximum non-toxic concentration was 390 μg/ml, suggesting that this antiseptic can be applied long-term intravaginally.

Also, to validate the qPCR-based growth measurements, we used the immunofluorescence based ChlamyCount growth measurement system. ChlamyCount data validated the qPCR measurements in the case of povidone-iodine and chlorhexidine, although the extent of detected growth reduction was lower than that determined by qPCR. The reason could be that the dynamic range of our immunofluorescent ChlamyCount method was about 2 $\log_{10}$, while the qPCR method's dynamic range was ~5 $\log_{10}$. Different from the qPCR results, the ChlamyCount method also showed a limited chlamydial growth inhibition in the case of iodine aqueous solution and borax. The fact that the chlamydial DNA synthesis remained constant after the application of these latter two compounds but the inclusion numbers slightly decreased, might indicate that small portion of the iodine aqueous solution and borax treated chlamydial EBs become persistent, maintaining the chlamydial DNA synthesis, but formed smaller/ less intense inclusions that was not detected by the ChlamyCount method.

Altogether, our results showed, that povidone-iodine had the widest antichlamydial therapeutic index and could maintain an antichlamydial effect when used intravaginally. Since *C. trachomatis* infection could be linked to bacterial vaginosis, povidone-iodine may treat/ limit these two clinical entities at the same time.

New results of the thesis

1. We showed that the prevalence of *U. urealyticum/ parvum* species is high in sexually active female and male populations in Hungary.

2. We revealed that in the Hungarian population the age distribution was different between the *Ureaplasma* positive females and males.

3. We performed a large-scale antibiotic resitance screen of *Ureaplasma* isolates in Hungary. We showed that the erythromycin and clindamycin resistances were extremely high, but the cross-resistance between the two macrolides, the older erythromycin and the newer azithromycin was infrequent. Every fourth strain was resistant to ofloxacin as representative of fluoroquinolons. Doxycycline proved to be the most effective agent with 97.3% susceptibility, followed by tetracycline and azithromycin.Because of the extremely high ratio of erythromycin and clindamycin resistant strains, the present guideline to treat *ureaplasma* infection have to be change

by replacing erythromycin with azithromycin. Moreover, administering erythromycin has the danger to further select the resistant strains.

4. We studied the incidence of gestational and connatal syphilis in the Hungarian population. We concluded, that maternal IgM immunoblot results identify mothers at risk of delivering babies with connatal syphilis better than the height of maternal RPR titer. Our data of Hungarian newborns, supports the previous results in connatal syphilis serology. In newborns, IgM test which depends on the infant's response has more specificity in diagnosing connatal syphilis than IgG that can transfer across the placenta.

5. We tested the antichlamydial effect of four vaginal antiseptics. We showed that povidone-iodine and chlorhexidine had a marked antichlamydial activity when applied at concentrations that was not toxic to the host epithelial cells. The therapeutical index of povidone-iodine was higher than that of chlorhexidine, indicating a higher clinical safety *in vivo*.

Acknowledgements

Special thanks to Professor Dr. Sarolta Kárpáti, former director and chairwoman of the Department of Dermatology, Venereology and Dermatooncology, Semmelweis University, Budapest, Hungary, to admit me, and to Professor Emeritus Dr. Ferenc Rozgonyi, former leader of the National STD Microbiology Diagnostic Laboratory, Department of Dermatology, Venereology and Dermatooncology, Semmelweis University, Budapest, to govern me in the *Ureaplasma* studies, and to the co-authors of my publications for involving me in these studies.

I am grateful for the help of Dr. Dezső Virok, Department of Medical Microbiology and Immunobiology, University of Szeged, Szeged, Hungary to direct the *Chlamydia* experiments and to overview my PhD studies. The skilful technical assistance of the laboratory staffs in both Budapest and Szeged is also highly appreciated. I also highly appreciate the help of Ágnes Tölgyesi from the library of Kálmán Pándy Békés County Municipal Hospital, Gyula in thesis preparation.

Thanking for the helping hand from Professor Dr György Bártfai and Dr Gábor Németh Director of the Department of Obstetrics & Gynecology at Szeged University Clinics and also special thanks for being persistent & attentive within the professional cooperation.

Special thanks to Dr Tamás Major for the helping hand given within the professional cooperation.

I am also very grateful for the patience, encouragement and continuous help of my wife and our children, which were absolutely necessary to prepare this work.